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Photosynthetic response of different pea cultivars to low and high temperature treatments

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Abstract

The thermo-sensitivity of two new pea (*Pisum sativum* L.) cultivars—Afila (mutant in the gene transforming leaves into mustaches) and Ranen (mutant for early ripening)—as compared to the control cultivar Pleven-4 to either low (4 °C, T_4) or high temperature (38 °C, T_{38}) was investigated by means of chlorophyll (Chl) fluorescence kinetics. The low temperature treatment decreased the photosynthetic activity, measured *via* a decline of the Chl fluorescence decrease ratios $R_{Fd}690$ and $R_{Fd}735$, and this was mainly due to a decline of the Chl fluorescence decrease parameter F_d and maximum Chl fluorescence F_m. In the new cv. Ranen the R_{Fd} ratios at first decreased and increased again after 24-h exposure to 4 °C, indicating its good acclimation ability to low temperature. The cold-induced changes in the photosynthetic performance of all cultivars were reversed after transferring plants back to 23 °C for 48 h. In the Chl and carotenoid (Car) contents no or little changes occurred during the T_4 treatment, except for a slight but clear increase of the ratio Chl *a/b* and a decrease in the ratio Chl/Car. In contrast to this, the T_{38} treatment for 72 h decreased the R_{Fd} ratios more strongly than the T_4 exposure did. In fact, an irreversible injury of the photosynthetic apparatus was caused in the control pea cv. Pleven-4 by a 48-h T_{38} exposure and for the new cv. Afila after a 72-h T_{38} exposure. In contrast, the cv. Ranen was less and little sensitive to the T_{38} exposure. In the heat-sensitive cvs. Pleven-4 and Afila, the decrease in R_{Fd} values at T_{38} was associated with a strong decline of the Chl *a+b* and total Car contents. The Chl *a+b* decline could also be followed *via* an increase of the Chl fluorescence ratio F_{690}/F_{735}. Parallel to this, a strong decline of Chl *a/b* from *ca*. 3.0 (range 2.85–3.15) to *ca*. 1.9 (range 1.85–1.95) occurred indicating a preferential decline of the Chl *a*-pigment proteins but not of the Chl *a/b*-pigment protein LHC2. In the relatively heat-tolerant cv. Ranen, however, the ratio Chl *a/b* declined only partially. After the T_4 treatment the stress adaptation index Ap was higher in cv. Ranen than in controls and reached in heat-treated Ranen plants almost the starting value indicating a cold and heat stress hardening of the treated plants. The Chl fluorescence parameters and pigment contents were influenced by T_{38} and T_4 treatments in various ways indicating that the mechanisms of low and high temperature injury of the photosynthetic apparatus are different. The new cv. Ranen exhibited a cross tolerance showing a fairly good acclimation ability to both T_4 and T_{38}, hence it is a very suitable plant for outdoor growth and for clarification of the acclimation mechanisms to unfavourable temperatures.

Additional key words: chlorophyll fluorescence; chlorophyll fluorescence decrease ratio; cultivar differences; *Pisum*; vR_{Fd}-values; stress adaptation index; thermo-sensitivity.

Introduction

When plants are exposed to stress conditions, *e.g.* to a temperature above or below the normal physiological range, they exhibit various responses and their photosynthetic performance becomes affected as well (Lichtenthaler 1996). There is general agreement that the primary site of damage to the photosynthetic apparatus caused by either low or high temperature exposure is associated with components of the photosystems located in

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Abbreviations: Ap – stress adaptation index; Car – carotenoid; Chl – chlorophyll; cv. – cultivar; F_m – maximal Chl fluorescence; F_s – steady state Chl fluorescence; F_{690} – red Chl fluorescence band near 690 nm; F_{735} – far-red Chl fluorescence band near 735 nm; F_{680}/F_{735} – ratio of red to far-red Chl fluorescence; PS – photosystem; R_{Fd} – Chl fluorescence decrease ratio, measured at red ($R_{Fd}690$) and far-red Chl fluorescence maximum ($R_{Fd}735$), respectively, T_4 and T_{38} – low and high temperature treatments at 4 and 38 °C, respectively.
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thylakoid membranes, most probably with photosystem 2 (PS2) 2 (Havaux and Strasser 1992, Mamedov *et al.* 1993), whereas photosystem 1 (PS1) activity is more stable (Sayed *et al.* 1994). There is general consensus that the optimum temperature for photosynthesis exhibited by a plant species reflects the environmental temperature range to which the species has genetically and physiologically been adapted (Berry and Björkman 1980). Yet, plants can exhibit a high degree of plasticity with respect to the temperature response of photosynthesis.

Chlorophyll (Chl) fluorescence measurements are widely used as an indicator of the functional state and damage of the photosynthetic apparatus under stress constraints. At room temperatures and physiological conditions the Chl fluorescence originates primarily from Chl *a* of PS2 (Papageorgiou 1975, Gitelson *et al.* 1998) and reflects the primary processes of photosynthesis, such as photon absorption, distribution and transport of excitation energy, and the photochemical reaction in PS2 (Fork and Satoh 1986, Krause and Weis 1991, Govindjee 2004). Under these conditions, there exists only a very small contribution of PS1 to the overall Chl fluorescence emission (Pfündel 1998, Franck *et al.* 2002). Due to the functional relation of PS2 to the other components of the photosynthetic apparatus, Chl fluorescence yield and particular Chl fluorescence parameters can serve as an indirect indicator for photosynthetic quantum conversion and the condition of the integral photosynthetic process (Schreiber *et al.* 1986, Lichtenthaler *et al.* 1992, 2005a, Roháček 2002, Govindjee 2004).

From the slow component (min range) of the Chl fluorescence induction kinetics of pre-darkened leaves the ratio of Chl fluorescence decrease to the steady state Chl fluorescence ($R_{Fd} = F_d/F_s$) can be determined. This Chl fluorescence decrease ratio, R_{Fd}, covers the whole process of photosynthesis, including the full induction period, the transition of the photosynthetic apparatus from the non-functional state 1 to its functional state 2, and also the photosynthetic CO_2 fixation (Lichtenthaler and Rinderle 1988, Lichtenthaler and Miehé 1997). In fact, the values of the R_{Fd} ratio are higher for sun leaves than shade leaves and are linearly correlated to the net CO_2 fixation rates, P_N, of leaves (Lichtenthaler and Babani 2004). Thus, R_{Fd}-values permit a fast screening of the photosynthetic activity and vitality of plants also under stress. The comparative registration of the red and far-red Chl fluorescence bands F_{690} and F_{735} (near 690 and 735 nm, respectively) provides more information than measuring at just one wavelength region alone (Lichtenthaler and Rinderle 1988). Moreover, from the ratios $R_{Fd}690$ and $R_{Fd}735$ one can determine the stress adaptation index, Ap (Strasser *et al.* 1987). This index is a measure of how a leaf can reorganise the structure of the photosynthetic apparatus for best adaptation to the applied stress conditions, whereby sun exposed leaves (sun leaves) and water stressed leaves exhibit higher Ap-values and can tolerate more heat, irradiance, and water stress than leaves of low-irradiance (*e.g.* shade leaves) and well watered plants (Lichtenthaler and Rinderle 1988). The fact, that the red Chl fluorescence F_{690} in the 690 nm range, when emitted deeper inside the leaf tissue, is partially reabsorbed by the absorption bands of the *in vivo* Chl forms, whereas the far-red band F_{735} is little affected by re-absorption (Gitelson *et al.* 1998), causes increasing F_{690} re-absorption with increasing Chl content of leaves, whereby the values of the ratio red to far-red Chl fluorescence bands F_{690}/F_{735} decline. Thus, measurements of the red and far-red Chl fluorescence also allow determining the ratio of the two Chl fluorescence bands, $F_{690}/F_{735,}$ which is an excellent inverse indicator (curvilinear relationship) of changes in the Chl content of leaves under stress conditions (Lichtenthaler 1987a, Lichtenthaler and Rinderle 1988, Lichtenthaler and Babani 2004).

Our previous Chl fluorescence investigations of pea plants have shown that some cultivars can preserve the physiological state and activity of PS2 in a wide temperature range of 10–35 ºC, whereas temperatures above 40 ºC result in an irreversible damage of the photosynthetic apparatus (Georgieva *et al.* 1992, Georgieva and Yordanov 1993). In thermo-sensitive cultivars the changes in the photosynthetic activity induced by cold (2 ºC) or heat (35 ºC) treatments were partially reversible when the plants were placed back to normal room temperature (Georgieva and Lichtenthaler 1999). The aim of the present investigation was to apply Chl fluorescence to characterize and compare the thermo-sensitivity of photosynthetic activity of two new pea cultivars with a known control when exposed to both relatively low (4 ºC, T_4) and high (38 ºC, T_{38}) temperatures. A major point was to find out not only differences in their cold and heat sensitivity, but also to check whether cold tolerant cultivars would exhibit a cross tolerance to higher temperatures. Another accent was not only to follow the changes in photosynthetic performance during the induction of stress and damage, but to check a possible regeneration of the photosynthetic activity of pea plants when the stress temperature factors were removed, a knowledge that is essential for field growth of new pea cultivars.

Materials and methods

Plant growth and temperature treatment: The thermosensitivity of three pea cultivars—Pleven-4 (control), Afila (mutant in the gene transforming leaves into mustaches), and Ranen (mutant for early ripening)—was investigated. Experiments were carried out with 10 d-old plants from all three cultivars, grown on peat-soil in a phyto-chamber at 23 °C and a photosynthetic photon flux density of 180 µmol m^{-2} s^{-1} (12/12 h day/night cycle). The 10 d-old plants were exposed to either T_4 or T_{38} treatments. The functional state of the photosynthetic apparatus was investigated during 72 h of treatment at the respective temperature, and then also after a 48-h recovery period at control conditions of 23 °C.

Chl fluorescence induction kinetics (Kautsky effect, slow component, minute range) of pre-darkened leaves (20 min dark adaptation) were measured in the red (near 690 nm) and far-red (near 735 nm) bands of the Chl fluorescence emission spectrum using the Karlsruhe laser-induced two-wavelength Chl fluorometer (*LITWaF* – excitation He/Ne laser, 632.8 nm, 10 mW, photon flux density *ca*. 650 µmol m^{-2} s^{-1} at the leaf level). Measurements were carried out at room temperature with leaf discs (diameter 9 mm); in the case of T_4 and T_{38} plants, the leaf discs were re-adapted in the dark for 20 min at room temperature. Chl fluorescence was excited and sensed from the adaxial (upper) leaf side. From the fluorescence kinetics measured at the Chl fluorescence bands F_{690} and F_{735}, the Chl fluorescence decrease ratios, $R_{Fd}690$ and $R_{Fd}735$, were calculated. R_{Fd} is defined as ratio of fluorescence decrease (F_d) to the steady state Chl fluorescence (F_s): $R_{Fd} = (F_m - F_s)/F_s = F_d/F_s$ (Lichtenthaler and Miehé 1997, Lichtenthaler *et al.* 2005a). From $R_{Fd}690$ and $R_{Fd}735$ the stress-adaptation index Ap (Strasser *et al.* 1987, Lichtenthaler and Rinderle 1988) was determined as: $Ap = 1 - (1 + R_{Fd}\,735)/(1 + R_{Fd}\,690)$. The Ap is also equivalent to the equation: $Ap = 1 - (F_m/F_s$ at 735 nm$)/(F_m/F_s$ at 690 nm).

Pigment determinations: Chls and carotenoids (Cars) were extracted in 100 % acetone and determined by means of a spectrophotometer *Shimadzu UV 200* using the re-determined coefficients and equations given by Lichtenthaler (1987b) which allow determining the pigments in the same extract (see also Lichtenthaler and Buschmann 2001).

Results

T_4: The R_{Fd} values of the leaves of control plants of the three pea cultivars grown at 23 °C showed the normal values of 3.0–3.4 for $R_{Fd}690$ and 2.0–2.4 for $R_{Fd}735$ usually found in plants grown at low to medium irradiance. In the pea plants that were kept at 23 °C for the full length of the experiment (controls), the values of the R_{Fd} ratios and the Ap stress index did not change during the following 72 h *plus* the additional 48 h of the experiment. In fact, in control plants all values remained within the variation range of the standard deviation of *ca*. ±5 %.

When exposed to T_4, the Chl fluorescence signatures and the ratios of the three pea cultivars showed a different behaviour. The $R_{Fd}690$ and $R_{Fd}735$ of cv. Pleven-4 were initially (up to 10 h) the same as in the controls but then declined by about 20 % after 72 h (Fig. 1*A*). The values of Ap (0.230 in controls) changed hardly or not at all except for a small significant decline only after 72 h of T_4 exposure (Table 1). Upon transfer of the Pleven-4 plants back to room temperature the values of the R_{Fd} ratios and the Ap index recovered to values which were significantly higher (by 25 and 13 %, respectively) than the respective control values. In contrast, the R_{Fd} values measured in the new cv. Ranen initially decreased up to 24 h of the cold treatment and were then by 25 % ($R_{Fd}690$) and 20 % ($R_{Fd}735$) lower than in the controls (Fig. 1*B*). This reduction of the R_{Fd}-values at the first hours of the T_4 treatment was primarily due to a decline of the Chl fluorescence decrease (F_d690 and F_d735), seen also in a decline of the corresponding F_m values, whereas the values of the steady-state Chl fluorescence (F_s690 and F_s735) were little affected. However, after 24 h, the values of $R_{Fd}690$ and $R_{Fd}735$ started to increase again, and after 72 h of low temperature treatment they almost reached the level of control Ranen plants kept at room temperature. The decline and subsequent increase in R_{Fd} values were accompanied by similar changes in the Ap values (Table 1), showing that pea plants from the new cv. Ranen were able to acclimate to T_4 during the treatment. After a 48 h recovery time of the cv. Ranen plants at room temperature the R_{Fd} values were even *ca*. 25 % higher than in the control plants. The Ap index of the cvs. Pleven-10 and Ranen then exhibited significantly higher values (0.260 and 0.254, respectively) than in the corresponding control plants (0.230 and 0.210, respectively). In fact, the significantly higher stress adaptation index after the cold treatment in the cvs. Pleven-4 and Ranen indicated that a certain cold hardening of the photosynthetic apparatus took place in these cultivars.

The third pea cultivar, the new cv. Afila, was the most sensitive to T_4 exposure (Fig. 1*C*). The values of $R_{Fd}690$ and $R_{Fd}735$ continuously declined and were about 30 % lower after 24 h at T_4 and *ca*. 35 and 30 %, respectively, lower after 72 h at T_4 than in the corresponding controls. When the plants were brought back to room temperature, the R_{Fd} values, however, recovered to the values of control plants. The values of Ap were reduced by 22 and

14 %, respectively, after 48 and 72 h at T_4 (Table 1) and recovered after 48 h at room temperature to the control values, but were not increased as in cvs. Pleven-4 and Ranen.

Chl fluorescence ratio F_{690}/F_{735} showed in control plants of cv. Pleven-4 the values of 0.45±0.02 typical for green leaves when measured at maximum Chl fluorescence F_m and 0.36±0.03 at the steady state Chl fluorescence F_s. This decline in F_{690}/F_{735} from F_m to F_s by *ca*. 20 to 25 %, first described by Buschmann and Schrey (1980) and Kocsányi *et al*. (1988), is typical for green photosynthetically active leaves. During the 72 h T_4 treatment the values of F_{690}/F_{735} did not change significantly in the cv. Pleven-4. The ratio varied in the range of controls by ±5 % measured at F_m and F_s, thus indicating that major changes in the Chl content did not occur during the cold treatment.

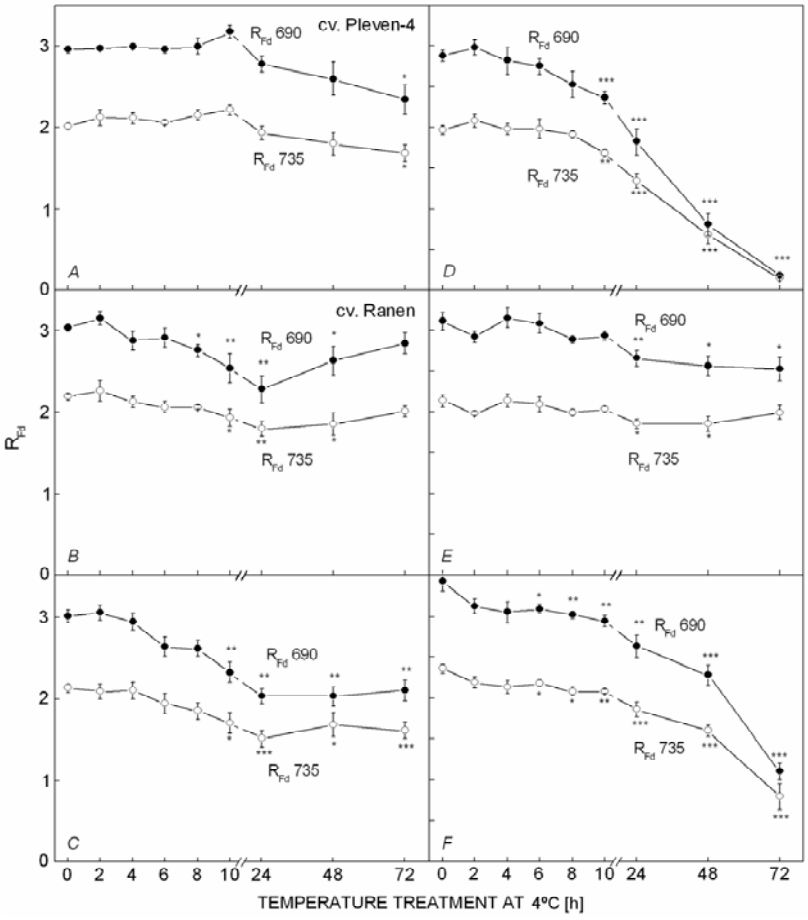

Fig. 1. Influence of low temperature (4 °C) (*left*) and high temperature (38 °C) treatments (*right*) of pea plants from cultivars Pleven-4 (*A*,*D*), Ranen (*B*,*E*), and Afila (*C*,*F*) on the variable Chl fluorescence ratios $R_{Fd}690$ and $R_{Fd}735$. Each point is the mean of 6 replications from 2 separate cultivations of pea plants. A significant decrease of R_{Fd} values measured at 4 °C or 38 °C as compared with control plants kept at 23 °C is indicated by: $^{*}p<0.05$; $^{**}p<0.01$, and $^{***}p<0.001$.

Table 1. Changes in the stress adaptation index Ap of pea plants from cultivars Pleven-4, Ranen, and Afila measured after different times of cold treatment at 4 °C and after 48 h recovery at 23 °C. Means of 6 replications from 2 separate cultivations of pea plants. The standard deviations are given in parenthesis. A significant decrease or increase of the Ap as compared to controls is indicated by $^{*}p<0.05$ and $^{**}p<0.01$.

Variant	cv. Pleven-4	cv. Ranen	cv. Afila
Control	0.230 (0.008)	0.210 (0.005)	0.217 (0.014)
2 h	0.212 (0.008)	0.214 (0.012)	0.236 (0.016)
4 h	0.219 (0.014)	0.216 (0.010)	0.213 (0.011)
6 h	0.223 (0.005)	0.189 (0.010)	0.181 (0.020)
8 h	0.209 (0.016)	0.182 (0.009)	0.208 (0.013)
10 h	0.230 (0.009)	0.168^{*} (0.018)	0.184 (0.016)
24 h	0.223 (0.006)	0.155^{**} (0.012)	0.168^{*} (0.018)
48 h	0.225 (0.010)	0.215 (0.007)	0.169^{*} (0.010)
72 h	0.189^{*} (0.017)	0.216 (0.011)	0.187^{*} (0.015)
Recovery 48 h	0.260^{**} (0.010)	0.254^{**} (0.008)	0.210 (0.006)

In cv. Ranen the ratio F_{690}/F_{735} amounted to 0.52±0.02 at F_m and 0.41±0.02 at F_s in the control plants at room temperature and showed after 24 h of cold treatment a tendency for a small insignificant decline of less than 7 % at F_m and F_s. But thereafter, with the adaptation of plants to low temperature and during the 48 h recovery time at room temperature, the values of F_{690}/F_{735} were the same as in the room temperature controls.

In the cold-sensitive cv. Afila the F_{690}/F_{735} values of the control plants (room temperature) were 0.54±0.02 at F_m and 0.42±0.01 at F_s and similar the ones of the cold-tolerant new cv. Ranen. During a 24 h cold treatment at 4 ºC the values showed a tendency for a decline by about 5–8 % at F_m and F_s, but during the subsequent 48 h of regeneration at room temperature (23 ºC) the values in cv. Alfila were similar to those of control plants. Thus, an increase in F_{690}/F_{735} that would indicate a partial Chl breakdown during the T_4 treatment did not occur in any of the three pea cultivars.

Chl and Car contents: The three pea cultivars had slightly differential Chl (*a*+*b*) and Car (x+c) contents per leaf area unit. In the control plants the highest pigment contents per leaf area unit were found in cv. Pleven-4 with 475 mg(Chl *a*+*b*) m^{-2} and 92 mg(Car x+c) m^{-2} (Table 2), whereas the cvs. Ranen and Afila had somewhat lower contents of both prenyl pigment classes (Table 2). For all three cultivars the values of Chl *a/b* were in the same range of 2.86–2.93 and the ratio of Chls/Car in the range of 4.94–5.04. These are typical pigment ratios for green leaf tissues (Schindler *et al.* 1994, Babani and Lichtenthaler 1996, Lichtenthaler and Babani 2004).

During the T_4 treatment for 72 h the Chl and Car contents per leaf area unit of cv. Pleven-4 did initially not change. However, after 48 and 72 h the Chl content was *ca.* 10 % lower, whereas the total Car content remained unchanged. It is also seen in the pigment ratios that a certain rearrangement of Chl and Car contents, together with some minor Chl breakdown, occurred during the T_4 treatment in cv. Pleven-4: the values for Chl *a/b* continuously increased from 2.89 to 3.08 and the mass ratio Chl/Car decreased in parallel from 5.04 to 4.43 after 72 h. The changes in both pigment ratios indicate that a small amount of the light-harvesting pigment complex of PS2 (LHC2) was broken down during the cold treatment. Details in the relationship between LHC2 amounts and pigment ratios are found in Lichtenthaler *et al.* (1982a,b), and were reviewed by Lichtenthaler and Babani (2004). In the subsequent 48-h recovery time of plants at 23 °C the leaves of cv. Pleven-4 increased their Chl and Car contents above that of controls (Table 2).

In the pea cvs. Ranen and Afila significant changes in the pigment contents during the 72-h T_4 treatment and the subsequent 48 h recovery time of the plants at 23 ºC, as compared to the controls, did not occur. Also the pigment ratios did not change, except for a small significant decrease of Chl/Car in cv. Ranen from 4.94 to 4.57 (Table 2).

Table 2. Contents of chlorophylls (*a*+*b*) and total carotenoids (x+c) [mg m^{-2}(leaf area)] and pigment ratios Chl *a/b* and chlorophylls/carotenoids (*a*+*b*)/(x+c) of different pea cultivars during temperature treatment at 4 ºC and after recovery (48 h at 23 ºC). Means of 6 replications from 2 cultivations. The standard deviation amounted to >6 % for pigment contents and to >4 % for pigment ratios. A significant decrease or increase as compared to controls is indicated by $^{*}p<0.05$.

Variant	cv. Pleven-4				cv. Ranen				cv. Afila			
	a+*b*	x+c	*a/b*	(*a*+*b*)/(x+c)	*a*+*b*	x+c	*a/b*	(*a*+*b*)/(x+c)	*a*+*b*	x+c	*a/b*	(*a*+*b*)/(x+c)
Control	454	90	2.89	5.04	390	79	2.86	4,94	376	76	2.93	4.95
2 h	438	90	2.96	4.87	358	74	2.94	4.84	362	72	2.98	5.03
4 h	452	93	2.94	4.86	372	74	2.86	5.03	377	74	2.98	5.09
6 h	455	96	2.94	4.74	393	79	2.83	4.97	369	70	2.82	5.27
8 h	438	92	3.02	4.76	355	72	2.94	4.93	391	77	2.88	5.08
10 h	428	89	3.05	4.81	410	83	2.85	4.94	382	74	2.76	5.16
24 h	448	94	3.05	4.77	368	78	2.91	4.72	364	72	2.75	5.06
48 h	409*	89	3.12*	4.60*	394	86	2.86	4.58*	371	76	2.83	4.88
72 h	403*	91	3.10*	4.43*	359	78	2.90	4.60*	357	76	2.90	4.70
Recovery	487	103	3.11*	4.73*	373	82	2.87	4.57*	387	78	2.98	4.94

T_{38}: High temperature treatment of pea plants strongly decreased the Chl fluorescence decrease ratios $R_{Fd}690$ and $R_{Fd}735$ of cvs. Pleven-4 and Afila (Fig. 1*D*,*F*). In contrast to T_4, this T_{38}-induced reduction of R_{Fd}-values was due to an increase in F_s and a decline in the Chl fluorescence decreased F_d and with it also in F_m. After 72 h of T_{38} exposure the R_{Fd}-values dropped down to only 4 % in cv. Pleven-4 and to 32 % in cv. Afila as compared to the starting values and to those of the room temperature controls. Exposure of these two pea cultivars to T_{38} apparently caused irreversible injury of the photosynthetic apparatus. The leaves did not recover during the subsequent 48 h at 23 °C; in fact, the plants of these two cultivars died off. Such stress-induced decline of R_{Fd}-values to less than 1.0 indicates an irreversible damage to the photosynthetic apparatus (Lichtenthaler and Rinderle

1988). Parallel observations showed that the irreversible injury of the photosynthetic activity of pea plants of cv. Pleven-4 was already caused by 48 h at 38 °C and in the slightly less cold sensitive cv. Afila after a 72 h exposure at 38 °C. These results were confirmed by the strong decline of the Ap index in both cultivars (Table 3). The values of the Ap were reduced by *ca*. 90 and 50 % after 72 h at T_{38} in the cvs. Pleven-4 and Afila, respectively. This decline in photosynthetic activity was associated with a breakdown of Chls. The Chl content decreased by 51 and 36 % after 72 h of T_{38} treatment in cvs. Pleven-4 and Afila, respectively (see below and Table 4).

In contrast to cvs. Pleven-4 and Afila, the new cv. Ranen was rather tolerant to high temperature (Fig. 1, *right*). Both $R_{Fd}690$ and $R_{Fd}735$ only decreased by *ca*. 15 % after 24 h and at the end of the heat treatment they were only 19 and 7 % lower than in the control plants. Moreover, both ratios recovered during the subsequent 48 h at 23 °C. The values of Ap hardly changed up to 24 h at T_{38}, but thereafter the Ap values were significantly lower than in the controls (Table 3). During the subsequent 48 h at normal room temperature the relatively heat-tolerant plants from the new cv. Ranen and their Ap index recovered.

Table 3. Changes in the stress adaptation index Ap of pea plants of cultivars Pleven-4, Ranen, and Afila measured after different times of an exposure to 38 °C and after a 48 h recovery period at 23 °C. Means of 6 replications from 2 separate cultivations of pea plants. The standard deviations are given in parenthesis. A significant decrease of Ap as compared to controls is indicated by $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$. [a]The leaves wilted and did no longer show Chl fluorescence induction kinetics.

Variant	cv. Pleven-4	cv. Ranen	cv. Afila
Control	0.236 (0.010)	0.237 (0.010)	0.238 (0.011)
2 h	0.222 (0.003)	0.238 (0.011)	0.227 (0.002)
4 h	0.217 (0.017)	0.245 (0.006)	0.225 (0.007)
6 h	0.207 (0.017)	0.229 (0.009)	0.222 (0.006)
8 h	0.215 (0.008)	0.229 (0.007)	0.232 (0.010)
10 h	0.220 (0.005)	0.230 (0.007)	0.217 (0.005)
24 h	0.162^{**} (0.020)	0.219 (0.007)	0.210^{*} (0.009)
48 h	0.068^{***} (0.007)	0.171^{**} (0.010)	0.204^{**} (0.011)
72 h	0.027^{***} (0.006)	0.137^{***} (0.010)	0.121^{***} (0.020)
Recovery	no[a]	0.214 (0.020)	no[a]

Table 4. Contents of chlorophylls (*a*+*b*) and total carotenoids (x+c) [mg m^{-2}(leaf area)] and pigment ratios Chl *a*/*b* and chlorophylls/carotenoids (*a*+*b*)/(x+c) of different pea cultivars during temperature treatment at 38 °C and after recovery (48 h at 23 °C). Means of 6 replications from 2 cultivations. The standard deviation amounted to >6 % for pigment contents and to >3 % for pigment ratios. A significant decrease as compared to controls is indicated by $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$. [a]The leaves wilted and died off; pigments could no longer be determined.

Variant	cv. Pleven-4				cv. Ranen				cv. Afila			
	a+*b*	x+c	*a*/*b*	(*a*+*b*)/(x+c)	*a*+*b*	x+c	*a*/*b*	(*a*+*b*)/(x+c)	*a*+*b*	x+c	a/b	(*a*+*b*)/(x+c)
Control	504	106	3.02	4.76	434	88	3.15	4.93	401	81	2.85	4.95
2 h	501	104	2.91	4.82	423	84	3.05	5.04	421	86	2.78	4.90
4 h	451	94	2.75^{*}	4.80	410	83	3.04	4.94	413	82	2.63^{*}	5.04
6 h	450	90	2.76^{*}	5.02	426	85	2.98	5.01	389	79	2.64^{*}	4.92
8 h	418^{*}	85^{*}	2.63^{**}	4.92	433	88	2.94	4.92	368	75	2.59^{*}	4.91
10 h	413^{*}	87^{*}	2.80^{*}	4.75	406	80	2.91	5.08	376	79	2.79	4.76
24 h	375^{**}	86^{*}	2.66^{**}	4.36^{*}	441	91	2.90	4.85	329^{*}	73	2.64^{*}	4.51*
48 h	292^{***}	70^{**}	1.91^{***}	4.17^{**}	387^{*}	83	2.64^{*}	4.66^{*}	325^{*}	76	2.11^{**}	4.27**
72 h	247^{***}	67^{**}	1.85^{***}	3.69^{**}	383^{*}	91	2.29^{*}	4.21^{**}	257^{**}	61^{*}	1.95^{***}	4.21**
Recovery	no[a]	no[a]	no[a]	no[a]	385^{*}	92	2.31^{**}	4.18^{**}	219^{***}	53^{**}	1.97^{***}	4.13**

Chl fluorescence ratio F_{690}/F_{735} and pigment contents

Pleven 4: In the heat-sensitive cv. Pleven-4 the ratio F_{690}/F_{735} (in controls 0.47±0.02 at F_m and 0.36±0.01 at F_s) had successively and significantly increased by 25 % during the 72 h heat exposure ($p<0.01$) when measured at F_m and by 59 % when measured at F_s ($p<0.001$) indicating Chl breakdown. No regeneration of the plants during the subsequent 48 h at room temperature of 23 °C occurred, since the plants died off.

The breakdown of total Chl (and also total Cars) in cv. Pleven-4 during T_{38} treatment was confirmed by determination of the pigment contents of the leaves. After a 72 h exposure to T_{38} the Chl *a*+*b* content was significantly reduced by 51 % and the Car content by 37 % (Table 4). The preferential breakdown of Chls as compared to Cars was indicated by a successive decline of the Chl/Car mass ratio from 4.76 (controls) to 3.69 in the 72-h heat exposed cv. Pleven-4. During this 72-h heat exposure, Chl *a* was broken down faster than Chl *b* which is documented by a progressive decline of the Chl *a*/*b* ratio from 3.02 (controls) to 1.85 (Table 4).

Afila: In the also heat-sensitive cv. Afila F_{690}/F_{735} (in controls 0.50±0.02 at F_m and 0.38±0.01 at F_s) increased by 10 % during the 72 h of F_{38} exposure when measured at F_m and by 31 % when measured at F_s, indicating a Chl breakdown during the heat treatment. This Chl breakdown was also documented by means of spectrophotometric pigment determination. During the 72 h of T_{38} treatment the Chl *a*+*b* content was decreased by 36 %

and declined further to –45 % as compared to controls during the subsequent 48-h period at normal room temperature (Table 4). The breakdown of total Cars proceeded more slowly with *ca*. –25 % after 72 h heat treatment and *ca*. –34 % after the additional 48 h at room temperature. The preferential breakdown of Chls as compared to Cars was also shown in the mass ratio of Chl/Car which gradually declined from 4.95 to 4.21 after 72 h of T_{38} exposure and further to 4.12 during the following 48 h (Table 4). Similar to the heat-sensitive cultivar Pleven-4, in the new cv. Afila Chl *a* was broken down faster than Chl *b* as documented in a progressive decline of the Chl *a/b* ratio of 2.85 (in controls) to 1.95 after the 72-h T_{38} exposure as well as the subsequent 48 h at room temperature of 23 ºC.

Ranen: In the relatively heat-tolerant cv. Ranen the ratio F_{690}/F_{735} increased during the T_{38} exposure to the same level as in the heat-sensitive cvs. Pleven-4 and Afila. The values of F_{690}/F_{735} (in controls 0.52±0.01 at F_m and 0.40±0.01 at F_s) were augmented by *ca*. 15 % at F_m and by *ca*. 29 % at F_s during the 72 h heat exposure and remained at that level during the subsequent 48 h at room temperature. Although an increase in F_{690}/F_{735} usually indicates a decrease in the Chl content, this could hardly be observed in cv. Ranen. After 72 h of heat treatment the Chl content had declined by *ca*. 12 % and remained at that level during the subsequent 48 h at room temperature of 23 ºC (Table 4). However, the total Car content did not change. As a consequence the ratio Chls/Cars declined from 4.92 to 4.21 after 72 h at T_{38} and to 4.18 during the subsequent 48 h at room temperature.

From the corresponding control pea plants of the T_4 and T_{38} experiments we calculated the mean Chl contents as well as the ratio F_{690}/F_{735} for the three pea cultivars. The Chl contents were 479, 412, and 389 kg m^{-2}(leaf area) and the corresponding F_{690}/F_{735} ratios measured at the upper leaf side at F_m were 0.46, 0.51, and 0.53 and at F_s 0.36, 0.39, and 0.41 for the cvs. Pleven-4, Ranen, and Afila, respectively. These data correlate well with the inverse curvilinear relationship of Chl content and the ratio F_{690}/F_{735} established for other plants (Lichtenthaler and Babani 2004). The ratios F_{690}/F_{735}, when measured at the lower leaf side of bifacial leaves, were higher by *ca*. 20–30 %, yet the curvilinear relationship of this ratio to the Chl content also exists in this case (data not shown). Although the values of F_{690}/F_{735} provide a good gross estimate of Chl contents, only the direct spectrophotometric determination of the Chl contents in leaf extracts provides the correct Chl amounts. The latter proved to be more sensitive to smaller changes in Chl contents than the ratio F_{690}/F_{735}.

Discussion

The $R_{Fd}690$ and also $R_{Fd}735$ are good criteria for the potential photosynthetic activity of a leaf, and $R_{Fd}690$ values higher than 2.5 indicate a very good photosynthetic activity (Lichtenthaler and Rinderle 1988, Babani and Lichtenthaler 1996). This is further demonstrated by the fact that the $R_{Fd}690$ values are linearly correlated with the photosynthetic CO_2 fixation rates as shown for various sun and shade leaves (Lichtenthaler and Babani 2004, Lichtenthaler *et al.* 2005b). The initial values for $R_{Fd}690$ of the investigated pea cultivars were similar and indicated a well functioning photosynthesis performance and good photosynthetic rates per leaf area unit.

T_4 treatment decreased the photosynthetic activity, as determined *via* a decline of the $R_{Fd}690$ and $R_{Fd}735$ values, which was mainly due to a decline of F_d and a slight relative increase of F_s, since the overall fluorescence yield at F_m had also decreased. This indicated a reduction of the photosynthetic quantum conversion process at T_4 exposure. The photochemical reduction of the primary stable quinone acceptor of PS2, Q_A, is controlled by two factors: (*1*) the rate of electron transport affecting the photochemical reaction of the thylakoid electron carrier pool, and (*2*) the quantum distribution of the excitation photons within the photosynthetic apparatus affecting the balance of PS1 and PS2. As reported by Öquist *et al.* (1993), frost-hardening decreased the reduction state of Q_A and decreased the sensitivity of winter rye to photoinhibition of photosynthesis. Maciejewska and Bauer (1993) have found in rice plants a marked decrease in the maximum Chl fluorescence and a smaller one in the initial fluorescence F_0 during the first two days of cold treatment. Thus, our results in pea cultivars on the changes in F_s, F_m, and F_d as well as in R_{Fd} are in agreement with such observations. Also Janssen *et al*. (1992) have shown a limitation of the photosynthetic electron transport at low temperature which restricted NADPH and ATP supply for CO_2 fixation.

The cold induced changes in the photosynthetic activity were fully reversible after transferring the plants back to 23 ºC for 48 h. Moreover, *via* determination of R_{Fd}, we found a higher photosynthetic activity when the plants were transferred for 48 h to room temperature. This remarkably fast recovery of photochemical quantum conversion as seen in the R_{Fd} values was mainly due to the re-increase of F_d and with it F_m, whereas F_s remained almost the same as after 72 h of T_4 treatment. Some recovery of the photochemical activity of the pea cv. Ranen already began after the plants had been T_4-exposed for 24 h. In fact, plants from this cultivar were the most adaptive to low temperature.

The T_4 exposure of the three pea cultivars had practically little or no effect on the Chl and Car contents of the cvs. Ranen and Afila, and caused only a small decline in the Chl content in cv. Pleven-4. In the latter,

the slight continuous increase of the Chl *a/b* ratio (up to *ca*. 7.3 %) and the decrease in the mass ratio of Chl/Car (up to *ca*. 12 %) showed that the cold treatment caused a re-orientation of the Chl *a* and Chl *b* contents also with respect to total content of Cars. Such pigment ratio changes were apparently caused by a slight decline in the content of LHC2, since only a decline in the latter pigment-protein can result in such pigment changes (see Lichtenthaler *et al.* 1982a,b). Whether such a slight decrease of the LHC2 might have also occurred in cv. Ranen, as seen from the decrease of Chl/Car (see Table 4), is not clear, since this decline was not accompanied by a corresponding increase in Chl *a/b*.

T_{38}: In contrast to the T_4 treatment, we found that the first parameter influenced by T_{38} in pea plants was F_s which started to increase at the first hours of heat treatment when the F_m values remained close to those of the control. But this already caused some decline in F_d, which is the difference of $F_m - F_s$. The longer T_{38} treatment decreased F_m and strongly increased F_s, consequently leading to a strong reduction in F_d and R_{Fd}. The results with leaves of cv. Pleven 4 showed that F_d was the most sensitive parameter at the T_{38} exposure. During the Chl fluorescence induction kinetics the steadily increasing F_d increasing from F_m to F_s parallels the oxygen evolution (Lichtenthaler and Rinderle 1988). Moreover, among the different reactions of PS2 the O_2-evolving process is particularly sensitive to heat (Havaux 1993, Nishiyama *et al*. 1993). The inactivation of O_2-evolution at higher temperatures has been ascribed to the release of Mn atoms and the 33 kDa protein from PS2 complex (Enami *et al*. 1994). When plants are exposed to high temperature, photosynthetic rates of their leaves sharply decrease. Zhang *et al*. (1995) have observed that at the start of heat stress the reduction of photosynthetic rates can also be attributed to a certain limitation of CO_2 supply due to a decreased stomata conductance, associated with a partial closure of stomata, whereas at later stages of high temperature exposure this was attributed to an inhibition of photosynthetic activity.

Our results with the T_{38} treatment of the three pea cvs. show that of the three pea plants only the new cv. Ranen is able to acclimate to 38 °C for an exposure time of 72 h implemented in this study. Since the cv. Ranen is also cold-tolerant to 4 °C, it possesses a cold-heat cross tolerance, and is an excellent new cultivar for outdoor cultivation. The exact mechanism, by which the photosynthetic thermo-stability of leaves of cv. Ranen exposed to cold and heat stress conditions is manifested, requires further research. Fast adaptive changes in the PS2 complex such as conformational changes in PS2 or changes in the surroundings of the thylakoid membranes, as suggested by various *in vivo* and *in vitro* studies in other plants (Havaux 1994), may also be the cause for the cross tolerance in the new cv. Ranen. This cv. is a very suitable pea plant for outdoor cultivation and for investigating the nature of acclimation processes of plants to low and high temperatures.

Table 5. Overview on the differences in the range of R_{Fd} values and the stress adaptation index Ap between sun leaves and leaves of high irradiance (HI) plants as compared to shade leaves or leaves from low irradiance (LI) plants. The values shown were calculated from the data given by Lichtenthaler and Rinderle (1988) and several other not yet published data sets.

Parameter	Sun leaves + HI leaves	Shade leaves + LI leaves
$R_{Fd}690$	2.6–4.6	1.6–2.8
$R_{Fd}735$	2.1–2.9	1.1–2.4
Ap index	0.204–0.335	0.111–0.225

The Ap index was in the new cv. Ranen, which is fairly tolerant to both cold and heat, higher after the T_4 treatment and it recovered to almost the same starting value after the T_{38} treatment, whereas in the two heat-sensitive cvs. Pleven-4 and Afila the Ap fully declined together with the R_{Fd} values. The Ap increase after the stop of the T_4 treatment indicates a partial cold hardening of the photosynthetic apparatus particularly in the new cv. Ranen and also in Pleven-4. To judge the Ap index one has to consider that its height is determined by the difference between the height of the R_{Fd} values measured in the red Chl fluorescence band (F_{690}) and those measured in the far-red band (F_{735}). In fully photosynthetically active plant tissue the values of $R_{Fd}690$ are in the average of 20–70 % higher than those of the ratio $R_{Fd}735$. The essential and actual Chl fluorescence emission band is the red band F_{690} (Gitelson *et al*. 1998). Hence, the changes in PS2 photochemistry and photosynthetic quantum conversion are reflected in this F_{690} band to a higher degree than in the F_{735} band. This is why the R_{Fd} values measured at F_{690} are higher than those determined at F_{735}. Moreover, in sun leaves and leaves of plants exposed to high irradiance the R_{Fd} values are considerable higher ($R_{Fd}690$: 2.6–4.6; $R_{Fd}735$: 2.1–2.9) than in shade leaves or leaves of plants grown at low irradiance ($R_{Fd}690$: 1.6–2.8; R_{Fd} 735: 1.1–2.4) as was calculated from data given by Lichtenthaler and Rinderle (1988) and other unpublished data sets. In sun and high irradiance (HI) leaves the values of $R_{Fd}690$ were in the average 40–70 % higher than those of $R_{Fd}735$, which is paralleled by higher Ap values (Table 5). In contrast, in shade and low irradiance (LI) leaves the $R_{Fd}690$ values were only 18–40 % higher than the $R_{Fd}735$ values, and the Ap values were correspondingly lower in the range of 0.120–0.195. With increasing water stress in beech and tobacco the R_{Fd} values declined relatively fast, *e.g*. by 55 and 80 %, whereas the Ap value declined at the same time only by 10 and 30 %, respectively (Lichtenthaler and Rinderle 1988). A slower decline of the Ap as compared to R_{Fd} values under stress is an indication that the respective plant can stand some stress and will recover when the stress factors are removed. However, with increased

aging of leaves in intact tobacco plants, the R_{Fd} and Ap values declined in parallel, and Ap even faster than R_{Fd}, as shown in the paper cited above. This decline in Ap indicates that older senescing leaves are more stress sensitive than fully functional green leaves and can no longer recover. The general decline of Ap under stress and in aging leaves as well as the lower Ap values in LI leaves are caused by the fact that the differences between the R_{Fd} values measured at F_{690} and F_{735} become smaller under stress and at LI growth. At a progressed damaging stress or age senescence the differences between the R_{Fd} values measured at F_{690} and F_{735} become very small and are hardly detectable. The exact reason for the changing differences during stress or leaf development and aging in the relative height between the $R_{Fd}690$ and $R_{Fd}735$ values (and this is in general independent of the actual height of the R_{Fd} values), which are reflected in either rising or decreasing Ap values, is not yet elucidated and needs further research. Yet the results presented here demonstrate the good applicability of the stress adaptation index Ap in stress and eco-physiological research, since plants with higher Ap values, such as sun or HI leaves, can endure a higher stress load. The stress adaptation index Ap has also successfully been applied to quantify stress adaptation and damage of plants to petrol engine exhaust pollutants and drought (Subhash *et al*. 2004), and permitted to differentiate more stress sensitive plants from more stress tolerant ones.

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