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Photosynthetic Decline from High Temperature Stress during Maturation of Wheat[1]

I. Interaction with Senescence Processes

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ABSTRACT

Photosynthetic capacity decreases rapidly when temperate species are exposed to heat stress during reproductive development. We investigated whether injury in wheat (*Triticum aestivum* L.) resulted from general acceleration of senescence processes or specific heat-induced lesions. *In situ* photosynthetic capacity of leaf discs and thylakoid reactions were measured using flag leaf tissue from two cultivars maintained at 20 and 35°C during maturation. Photosynthetic rates of leaf discs decreased faster at 35 than at 20°C and were more photolabile in cv Len than in cv Waverly at high temperature. Patterns of thylakoid breakdown also differed in the two wheat genotypes at 20°C: intersystem electron transport and photosystem II activity decreased linearly during postanthesis development in Len wheat, whereas coupling of photophosphorylation to electron transport declined late during senescence in Waverly wheat. Heat stress induced early loss of intersystem electron transport followed sequentially by decreased silicomolybdic acid, + 3-(3,4-dichlorophenyl)-1-dimethylurea-mediated photosystem II activity and 2,5-dichloro-*p*-benzoquinone-mediated photosystem II activity in Len. Stress accelerated the uncoupling process, but loss of intersystem electron transport and photosystem II activities was slower in Waverly than in Len. We conclude that high temperature initially accelerated thylakoid component breakdown, an effect similar to normal senescence patterns. Thylakoid breakdown may induce a destabilizing imbalance between component reaction rates; an imbalance between photosystem II and cytochrome *f*/b_6-mediated activities would be particularly damaging during heat stress.

High temperatures occur frequently during reproductive growth of temperate species and strongly influence many plant processes. Senescence symptoms of wheat, for instance, are delayed until physiological maturity when plants are grown below 20°C, but degradative processes accelerate and photosynthetic activities decline as the temperature rises (1). Even mildly elevated temperatures during reproductive growth are injurious in this respect and seriously reduce economic yield.

Numerous chloroplast components and processes are altered by senescence at normal temperatures (10–13, 15, 16, 19). Loss of content and/or activity of soluble chloroplast enzymes and uncoupling of photophosphorylation decrease photosynthesis in wheat (4). Intersystem electron transport decreases before PSI or PSII activity during senescence in bean (*Phaseolus vulgaris* L.) (15) and barley (*Hordeum vulgare* L.) (11).

High temperature stress adversely affects many photosynthetic processes in immature plants (2, 6, 20, 22, 27). In C_3 monocots, activity of RuBPCase[3] (EC 4.1.1.39) is notably reduced as are chloroplast enzymatic processes in general (9, 17, 20). Photosynthetic membranes appear to be especially sensitive to high temperatures whether the stress is applied to isolated organelles (2, 6, 20, 22, 27) or to intact tissues (9, 20). The rapid decline of photosynthesis from high temperatures occurs in whole plants (1, 2) and detached leaves (9). It is not clear whether these are general or specific responses, however, because interpretation is hampered by possible artifacts from detaching leaves, incubation in darkness, and other perturbations.

Our previous investigations have concerned maturing plants, in which high temperature stress responses are superimposed on normal senescence processes (1, 2, 18). Whole-chain electron transport decreased more rapdily than RuBPCase activity under those condtions (1). Objectives of the present investigation were to identify electron transport component reactions that are altered by high temperature stress during reproductive development of wheat and to determine whether injury results from general acceleration of senescence processes or specific, heat-induced lesions.

MATERIALS AND METHODS

Plant Material

Seeds of two spring wheat (*Triticum aestivum* L.) cultivars, Len from the northern Great Plains and Waverly from the

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[3] Abbreviations: RuBPCase, ribulose-1,5-bisphosphate carboxylase/oxygenase; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (dibromothymoquinone); DCBQ, 2,5-dichloro-*p*-benzoquinone; DPIP, 2,6-dichlorophenolindophenol; FeCy, ferricyanide; MA, methylamine; MET, metronidizole; PQ, plastoquinone; SiMo, silicomolybdic acid; SOD, super-oxide dismutase; TMBZ, 3,3′,5,5′-tetramethyl-benzidine; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine.

Pacific Northwest, were germinated in vermiculite moistened with distilled water. Seedlings were transplanted to 2-L containers of continuously aerated Hoagland solution, which was replaced weekly. Plants were grown until anthesis in controlled environment chambers at 20°C/15°C day/night with 450 μmol m^{-2} s^{-1} PAR (400–700 nm) as measured with a Li-188B quantum meter (Li-Cor, Inc., Lincoln, NE), a 16/8-h light/dark regime, and 40% RH.

Tillers were individually labeled by date of anther extrusion, and differential temperature treatments were initiated when about 75% reached anthesis. Conditions were maintained as before for control plants, and temperatures were increased to 35°C/25°C shoot/root during the day and 25°C/15°C shoot/root during darkness for heat-stressed plants. Roots were maintained at the temperatures indicated to avoid the effects of high root temperatures on senescence of shoots (18). Temperature treatments were maintained until plants senesced completely.

Leaf Photosynthesis *in Situ*

Flag leaf blades, three from stressed plants and three from control plants, were collected at approximately 2-d intervals after differential temperature treatments were imposed. The epidermis was readily removed by skimming a razor blade over the blades while they were immobilized by double sticky transparent tape in a trough formed by glass microscope slides and cover slips.

Photosynthesis of peeled 25-mm^2 leaf discs was measured at 30°C in a Hansatech DW1 oxygen electrode (Hansatech Ltd., King's Lynn, UK) that was modified to produce stable O_2 recorder traces. The stir bar at the bottom of the reaction vessel was protected from the leaf disk by nylon mesh inserted into the chamber 5 mm above the electrode surface. The mesh was perforated with a hot needle to allow circulation of reaction medium (100 mM sucrose, 1 mM KH_2PO_4, 5 mM Na-EDTA, 5 mM KCl, 1 mM $MgCl_2$, 15 mM $NaHCO_3$, 25 mM Tricine-KOH [pH 7.8]) throughout the vessel. Nylon filament inserted through the reaction vessel plunger held the leaf disc on the nylon mesh. Measurements were made on four discs from each leaf at two light intensities, 200 and 400 μmol m^2 s^1 PAR. Radiation from a 250-W quartz-tungsten-halogen lamp (Oriel Corp., Stafford, CT) was filtered through 10 cm of water and one (400 μmol) or two (200 μmol) layers of blue acetate transparency film. Peeled leaf discs were used because epidermal tissue prevented bathing of the mesophyll tissue by the surrounding reaction medium.

Leaf Transpiration and Temperature

Transpiration rates and temperature were ascertained in preliminary experiments using a Li-Cor 1600 porometer. The middle third of flag leaves was measured.

Thylakoid Reactions

Thylakoids were isolated from segments of the middle two-thirds (0.4–0.6 g) of flag leaf blades by a modified method (14) at 2-d intervals after initiation of temperature treatments. Blade segments were immersed in 15 mL of medium (100 mM sucrose, 0.5 mM Na-EDTA, 1 mM $MgCl_2$, 30 mM NaCl, 2 mM Na-isoascorbate, 1 mg/mL BSA, 50 mM Tricine-KOH [pH 8.2]) and cooled to 0°C in a salt water bath. The samples were then immediately homogenized with a Polytron (Brinkman Instruments, Westbury, NY) at a setting of 6. The homogenate was filtered through two layers of Miracloth (Calbiochem, San Diego, CA), centrifuged at 800*g* for 20 s to remove cell debris, and centrifuged at 10,000*g* for 5 min to pellet thylakoids. Thylakoids were resuspended in homogenizing medium minus Na-EDTA and isoascorbate, stored on ice under darkness, and used for assays within 75 min.

Activities of thylakoids containing 10 to 20 μg Chl mL^{-1} (3) were measured at 30°C using an unmodified Hansatech DWI oxygen electrode and 750 μmol m^{-2} s^{-1} PAR from the source used for intact leaf photosynthesis. The thylakoid reactions monitored were (a) whole chain transport, $H_2O \rightarrow$ FeCy with and without DBMIB to estimate short-circuiting by the direct oxidation of PSII by FeCy; (b) $H_2O \rightarrow$ FeCy with ADP and Pi, which repeats reactions (a) but includes photophosphorylation; (c) $H_2O \rightarrow$ FeCy uncoupled with MA, which also repeats reaction (a) under uncoupled conditions to better estimate electron transport capacity; (d) $H_2O \rightarrow$ PSII $\rightarrow$ DCBQ, which measures PSII activity; (e) $H_2O \rightarrow$ PSII $\rightarrow$ SiMo, which measures PSII activity under conditions where DCMU must be removed from the plastoquinone-binding protein before activity occurs; (f) ascorbate-DPIP-Cyt *f* $\rightarrow$ MET, which measures transport from a point at or very near Cyt *f* through PSI; and (g) ascorbate-TMPD $\rightarrow$ PSI $\rightarrow$ MET, which measures electron transport mediated by PSI (14). Reactions (a) to (d) and (f) to (g) were with basic reaction medium (100 mM sucrose, 10 mM KH_2PO_4, 2 mM $MgCl_2$, 0.5% BSA, 40 mM Hepes-KOH [pH 7.6]) with additional modifications shown in Table I. Electron acceptors were recrystallized as recommended (28).

SDS-PAGE of Thylakoid Proteins

Polyacrylamide gel electrophoresis was performed as described by Chua (5). Gels were stained with TMBZ to detect heme (26).

Experimental Designs and Data Analyses

Temperature treatments and sampling dates were arranged in completely randomized designs with three replications over time. Activities of leaf discs and thylakoid preparations from control plants were regressed on age (days after anthesis), and regressions were used to normalize activities in control leaves at ages corresponding to those of leaves from stressed plants. Results are expressed as mean percentage differences between activities of leaves from stressed plants and age-normalized activities of control leaves for each sampling date. Standard error of the mean differences between control and stressed plants were calculated for each date shown in figures.

RESULTS

Mean *in situ* photosynthetic rates of leaves from non-stressed plants grown at 20°C in a system that eliminated stomatal resistance and photorespiration as factors were sim-

Table I. *Photochemical Activities of Thylakoids Isolated from Normally Senescing Flag Leaves of Wheat Plants Grown at 20°C/15°C after Anthesis and Correlation Coefficients of Regressions of Activity Loss on Time (days)*

The reaction mixture for SiMo-mediated PSII activity contained basic reaction medium without BSA and with 2 μM DCMU (14). The TMPD (2.5 mM) and DPIP (0.2 mM) assay mixtures contained basic reaction medium including 20 mM MA, 0.4 mg SOD mL^{-1}, 2 μM DCMU, 20 mM MET, and 2.5 mM ascorbate; DCBQ assays contained basic media plus 0.5 mM DCBQ.

Reaction	O_2 Evolution		% Activity Loss[a]			
	Waverly	Len	Waverly		Len	
	μmol O_2 mg^{-1}	Chl h^{-1}	% d^{-1}	r	% d^{-1}	r
Leaf disc						
$H_2O \rightarrow CO_2$	107.0	121.0	—[b]	—	1.0	
Isolated thylakoids						
Whole chain						
$H_2O \rightarrow$ FeCy (basal)	34.5	55.5	—	—	1.1	−0.77**[c]
$H_2O \rightarrow$ FeCy (+ADP)	88.1	144.9	—	—	1.1	−0.86**
$H_2O \rightarrow$ FeCy (uncoupled)	139.6	186.4	—	—	1.1	−0.86**
PSII-dependent						
$H_2O \rightarrow$ DCBQ	277.2	356.00	—	—	0.5	−0.69*
$H_2O \rightarrow$ SiMo	63.0	96.6	—	—	—	—
PSI-dependent						
DPIP → MET	84.0	89.5	—	—	1.2	−0.86**
TMPD → MET	109.1	105.0	—	—	—	—
Coupling ratio	2.6	2.7	0.6	−0.59*	—	—

[a] % of original activity lost per day. [b] Significant correlation of daily activity loss is denoted. [c] * Significant at P = 0.05 level; ** significant at P = 0.01 level.

ilar in Len and in Waverly wheat (Table I). Rates in leaves from plants stressed at 35°C declined rapidly in Len to 40 to 50% below rates in nonstressed plants (Fig. 1). Photosynthesis in stressed Waverly leaves decreased initially and then leveled off at 75% the control rates. There was no significant loss of Chl during the measuring period (data not shown).

Plant daytime leaf temperatures averaged 3°C below the 20°C air temperature in control plants and 5°C below the 35°C air temperature in stressed plants during the first 14 and 10 d after anthesis, respectively (data not shown). The mean temperature differential decreased to approximately 2 and 3°C, respectively, until rapid Chl loss occurred after 28 d at 20°C and 21 d at 35°C, when it fell to 1°C for both cultivars under both regimes. Transpiration rates decreased steadily from 9 and 30 μg H_2O cm^{-2} s^{-1} in control and stressed plants, respectively, at the beginning to 4 μg H_2O cm^{-2} s^{-1} after 28 and 21 d and declined further to nearly 0 μg H_2O cm^{-2} s^{-1} at the end of the experiment.

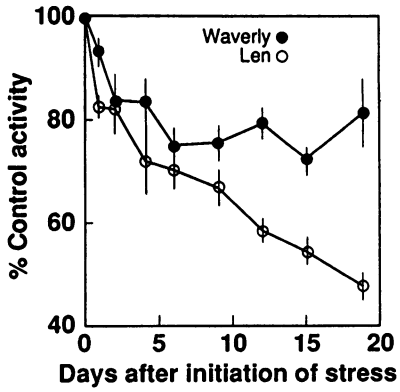

Figure 1. *In situ* photosynthetic rates of leaf discs from Len and Waverly wheat plants grown at 35°C/25°C as percentage of plants grown at 20°C/15°C after anthesis. Each point represents the mean of four measurements on six flag leaves from three experiments. Vertical bars are standard error.

Whole-chain electron transport activities in normally senescing leaves were considerably higher in Len than in Waverly cultivars (Table I). Most activities decreased gradually during postanthesis development in Len wheat and there was a significant linear component in regressions of activity loss on time (Table I). Thylakoid activities remained relatively constant until the last few days of postanthesis development in Waverly wheat. As a result, there was not a significant linear regression of activity loss on time for most activities. Intersystem reactions declined about 1.1%/d in Len but stayed nearly constant in Waverly during the period. Stress increased basal electron transport and decreased ADP enhancement of transport in Waverly; the ratio of those reactions, an index of coupling of electron transport to photophosphorylation, declined over time (Fig. 2). The change in reaction rates was nearly parallel in Len wheat, however, so that the coupling ratio was more stable.

PSII activities were substantially lower in Waverly than in Len wheat and fell only 0.2 and 0.5%, respectively, per day in the two cultivars during normal senescence at 20°C (Table I). High temperature stress initially decreased DCBQ-mediated PSII activity and increased SiMo-mediated PSII activity in Waverly and less so in Len (Fig. 3A and B). Rate of the

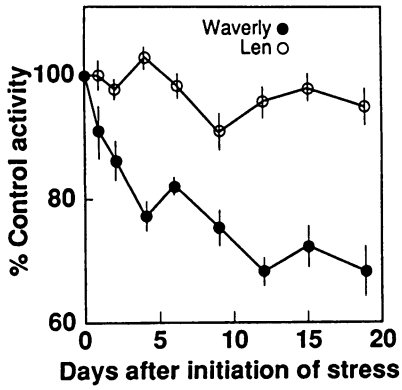

Figure 2. Coupling of photophosphorylation to electron transport in Len and Waverly wheats grown at 35°C/25°C as percentage of activities in plants grown at 20°C/15°C. Each point represents the mean age-normalized activity of four to six flag leaves. Vertical bars are standard error.

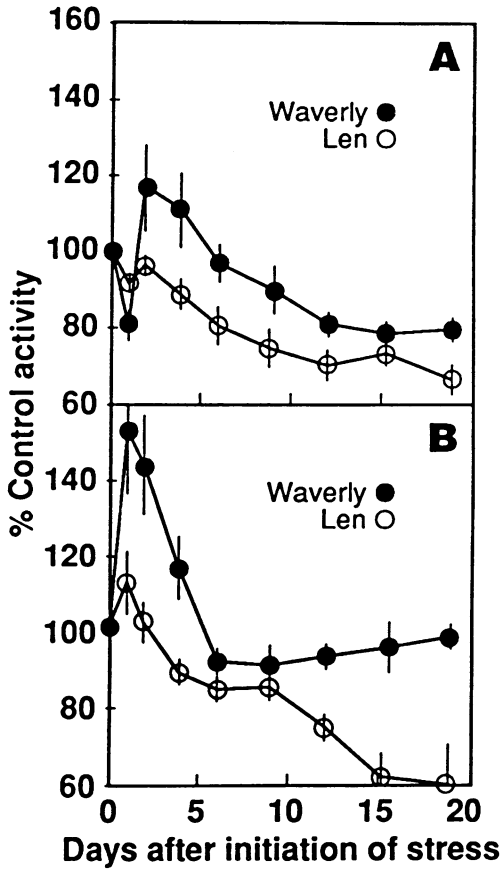

Figure 3. DCBQ-mediated PSII (panel A) and SiMo-mediated PSII (panel B) activities in Len and Waverly wheats grown at 35°C/25°C as percentage of activities in plants grown at 20°C/15°C. Each point represents the mean age-normalized activity of four to six flag leaves. Vertical bars are standard error.

DCBQ reaction returned to control levels or higher and then fell during the balance of the measuring period. Following the initial increase, PSII activity mediated by SiMo declined below controls in Len wheat. Intersystem transport mediated by DPIP decreased faster than PSII activities during normal senescence in Len (Table I). Both TMPD- and DPIP-mediated reactions increased after several days of stress (Fig. 4A and B). The increase in the DPIP-mediated reaction followed a sharp initial decrease of that reaction in stressed Len (Fig. 4B). The concentration of TMBZ-detectable Cyt *f*, which receives electrons from DPIP (14, 25), decreased more rapidly in heat-stressed than in control plants during senescence (Fig. 5).

Thylakoid activities were not correlated with *in situ* photosynthetic rates of nonstressed Waverly wheat during maturation (Table II). Most thylakoid measurements and photosynthesis were highly correlated in Len wheat, however, suggesting that photosynthesis was limited by electron transport capacity during normal senescence. High temperature stress in Waverly wheat induced a significant correlation between decreasing photochemical activities, including coupling, and photosynthesis. This contrasted with nonstressed plants, in which photochemical activities did not decrease linearly during maturation and did not correlate with photosynthesis. Decreasing intersystem electron transport and PSII activities

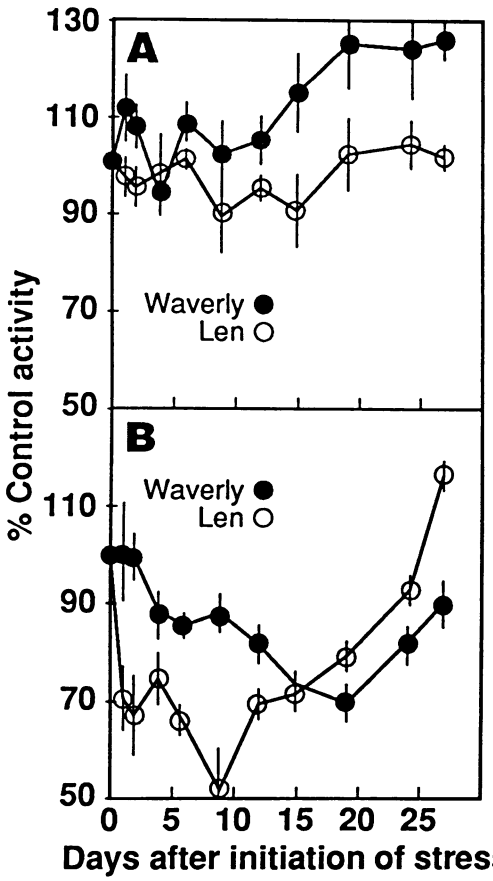

Figure 4. TMPD-mediated PSII activity (panel A) and DPIP-mediated intersystem electron transport (panel B) in Len and Waverly wheats grown at 35°C/25°C as percentage of activities in plants grown at 20°C/15°C after anthesis. Each point represents the mean age-normalized activity of four to six flag leaves. Vertical bars are standard error.

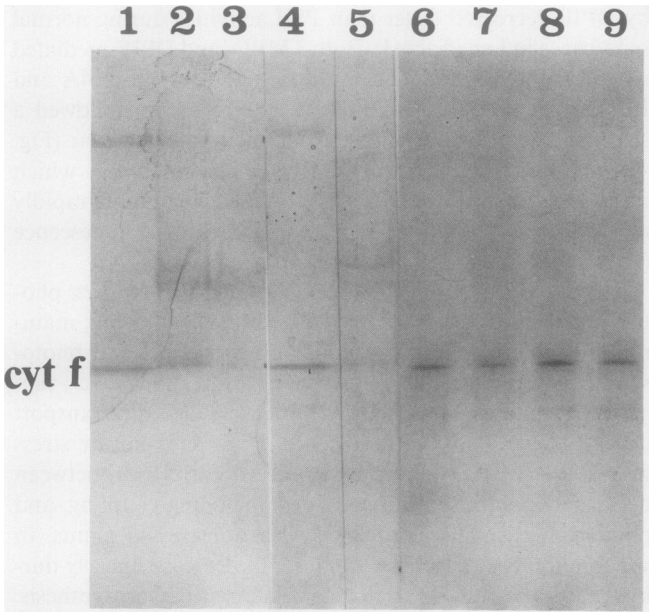

Figure 5. Composite TMBZ-stained SDS-PAGE of thylakoid membrane proteins showing changes in Cyt *f* concentration. Len wheat at initiation (lane 1), 1 week (lane 2), and 3 weeks (lane 3) of heat stress; Waverly wheat at initiation (lane 4) and 3 weeks (lane 5) of heat stress; control plants of Len at dates corresponding to 1 (lane 6) and 3 (lane 7) weeks of stress; control plants of Waverly corresponding to 1 (lane 8) and 3 (lane 9) weeks of stress.

Table II. *Simple Correlation Coefficients between Thylakoid Partial Reactions and Photosynthesis in Senescing Flag Leaves of Wheat Plants Grown at 20°C/15 and 35°C/25°C after Anthesis*

Photochemical Activity	Correlation with Leaf Disc Photosynthesis			
	Waverly		Len	
	20°C	35°C	20°C	35°C
	r			
PSII → DCBQ	NS[a]	0.74**	0.78**	0.54*
PSII → SiMo	NS	NS	NS	0.67**
DPIP → PSI (intersystem)	NS	0.49*	0.67**	0.61[b]
H_2O → FeCy (phosphorylating)	NS	NS	0.79**	NS
Coupling ratio	NS	0.56**	NS	NS

[a] NS, not significant; * significant at 5% level; ** significant at 1% level. [b] D 1–9 only because activity increased after d 9 (see text).

paralleled decreases in *in situ* photosynthesis in Len wheat at 20°C. High temperature decreased the correlation between intersystem electron transport and *in situ* photosynthesis. PSII activity mediated by SiMo in the presence of DCMU correlated with photosynthesis in stressed plants but not in nonstressed plants of Len wheat.

Heat stress lowered the average ratio of DCBQ-mediated to SiMo-mediated PSII activity in Waverly from 4.5 to 3.7, whereas it increased the ratio in Len wheat from 2.9 to 3.7. The decrease in Waverly was initially from 4.5 to 2.4 due to a very large increase of SiMo-mediated activity (Fig. 3B). There was no loss of photosynthetic response to increasing light intensity at that time. The increased ratio in Len was associated with a smaller initial increase of SiMo-mediated activity and a greater loss of SiMo activity later during stress in that cultivar. In general, an intrinsically high PSII activity coupled with a high ratio of DCBQ- to SiMo-mediated PSII activity was associated with decreased response to higher irradiance.

DISCUSSION

Lability of PSII is a primary limitation of photosynthesis in young plants and their organelles at high temperatures (2, 13, 23, 27). Direct high temperature injury to PSII may result from components at the oxidizing side, perhaps the oxygen-evolving complex (2, 27). Light harvesting pigments may also separate from PSII reaction centers, leading to loss of thylakoid function (23). In most instances, however, photosynthetic electron transport reactions are stable at moderately elevated temperatures and become labile at temperatures greater than 40°C for C_3 monocots (2, 13, 17, 20, 22, 24). Our studies show that electron transport activities other than PSII become thermolabile during reproductive development. Injury clearly depends on senescence pattern during reproductive growth as shown by comparison of Len and Waverly wheats, each exhibiting distinct senescence and injury patterns. An imbalance in component reaction rates was initiated by differential decrease of component activities during senescence. The effect of high temperature was to exacerbate such imbalances, leading in Len wheat to a large divergence between PSII capacity and intersystem electron transport capacity early during the stress period.

Cyt f/b_6 complex content may limit intersystem transport in senescing barley (11) and decreases before other thylakoid components in senescing oat (*Avena sativa* L.) (10). Changes at Cyt *f* were probably injurious throughout the stress period in Len wheat, but substantial loss was not detected by TMBZ stains of SDS-PAGE gels until after the first week of stress.

Our assumption that DPIP-mediated reaction reflects transport through Cyt *f* is based on previous reports (11, 21, 25) that ascorbate-reduced DPIP donates its electrons very closely to or directly to Cyt *f*. Direct membrane damage at Cyt f/b_6 results from heat, which changes the site of the DPIP electron donation to the Cyt f/b_6 complex in pea (*Pisum sativum* L.) chloroplasts (25). A conformational change exposes new DPIP acceptor sites and increases DPIP-mediated activity after brief incubation at high temperatures. The increase in DPIP activity observed in Len wheat after 11 d resembled the heat-induced increase in pea (25).

The possibility that a conformational change affected PSII activity was inferred from the differential response to heat stress of two PSII reactions. Silicomolybdate mediates the same PSII reaction as DCBQ and also removes DCMU from the 32-kD binding protein of PSII (8). Heat stress increased the average ratio of DCBQ- to SiMo-mediated activity (DCBQ/SiMo) after the first day of stress in Len but decreased it in Waverly wheat. The data indicate that DCMU was more tightly bound by the 32-kD protein in Len wheat as a result of heat stress. The change in binding of DCMU in Len implied by the ratio (DCBQ/SiMo) occurred shortly after the sharp

DPIP activity decrease. High temperature tolerance of five weed species differing in triazine herbicide resistance correlated with decreased triazine binding (7). Heat stress may likewise cause tighter *in vitro* binding of DCMU in Len wheat if there is an association between 32-kD protein binding characteristics and thermotolerance. The change in DCMU binding was consistent with a physiological change near PSII that reduced photosynthetic efficiency at high light intensity.

The nature of heat injury in senescing tissue differs from that reported in studies employing seeding tissue (9), *in vitro* heated membranes (2, 6, 22, 27), or very high temperatures (6, 24). The supraoptimal temperatures we used largely seem to alter regulation of senescence processes and thereby reduce photosynthesis and leaf area duration during the critical grain filling period. Heat stress appears to disrupt coordination of those activities, leading to a large imbalance in the ratio of PSII/intersystem transport capacity and accelerating leaf deterioration in the process.

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