Photosynthetic Decline from High Temperature Stress during Maturation of Wheat

II. Interaction with Source and Sink Processes

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ABSTRACT

High temperature stress reduces grain growth in wheat (Triticum aestivum L.) by altering source activity and sink capacity. The impact of stress on source and sink interactions in two wheat cultivars of differing source thermostolerance was monitored by analysis of chlorophyll fluorescence transients, Fv (variable fluorescence) and PSM (peak, stationary, maximum), of attached flag leaves on intact and decapitated tillers grown at optimum (20°C) and stress (35°C) temperatures after anthesis. The thermodetolerant cultivar Waverly had reduced Fv and PS quenching and a large increase in SM during heat stress. The less thermodetolerant cultivar, Len, exhibited increased Fv and PS quenching and a small increase of SM. Fluorescence induction was similar in intact and decapitated tillers of Len, indicating diminished sink-source interaction during heat stress. The present results and previous observations of photosynthetic activities indicate that cyclic electron transport and photophosphorylation in flag leaves of the thermodetolerant cultivar were stimulated by sink demand (increased SM in intact plants). Reduced grain development in the thermodetolabile cultivar resulted from limited capacity to support cyclic electron transport and photophosphorylation (slight increase in SM of intact plants and large reduction of Cytochrome f/b6-mediated electron transport capacity). It was concluded that heat stress injures the photosynthetic apparatus during productive growth of wheat and that diminished source activity and sink capacity may be equally important in reducing productivity.

High temperature stress after anthesis severely reduces reproductive development in wheat (1, 25) and other species (6). Whether stress-induced yield reduction is primarily due to diminished source or altered sink activity is often difficult to discern. It is apparent that stress reduces yield by decreasing source activity (1, 12, 23), which is especially critical because most grain mass is from current photosynthesis after anthesis (7). Substantial evidence also indicates, however, that stress limits yield by reducing sink capacity for development (6, 15, 22, 25).

Source activity is damaged by heat because both leaf area duration (10, 22, 23) and photosynthesis (1, 12) are reduced. Injury near PSII has frequently been identified in heat-treated membranes (14) and intact tissues (21). Heat injury limits sink growth potential particularly when stress is imposed during early sink developmental stages (15). At later developmental stages, stress reduces sink ability to absorb assimilate (6, 22).

Source and sink activities are related by direct feedback mechanisms (16) during normal monocarpic senescence and by pathways in source tissue that mitigate effects of feedback regulation (5). Effects of stress on feedback regulation depend partly on relative sensitivity of source and sink activities to the stress. Source and sink interactions may be regulated by a common factor, e.g., cytokinin from roots, which influences sink activity (10, 13) and stability of chloroplast ultrastructure (4). Heat injury to roots also may interfere with synthesis or transport of cytokinin and alter sink and source activities simultaneously (13).

Fluorescence induction is useful for evaluating thermodetolerance of plants (21). High temperature injury to the oxidizing side of PSII affects Fv3 yield, and adaptive mechanisms that protect PSII during stress modify fluorescence yield during induction (14, 21). High temperature influences the relative contribution of several nonphotochemical components of fluorescence quenching (14). Effects of high temperature on the subsequent slow fluorescence transients, SMT, which are associated with induction of photophosphorylation (3, 8, 14) and Calvin cycle activity (8, 11, 19, 20), apparently have not been investigated. These activities, however, are highly temperature sensitive (1, 12, 14).

We used stress-induced changes in fluorescence induction, including the SMT transient, to identify cultivar-specific changes in thylakoid activity and source-sink interactions during reproductive development. Fluorescence monitoring in this manner permits analysis of the responsiveness of leaf activity to sink demand during grain development in plants subjected to sustained high temperature stress and adds a dimension to use of fluorescence as a diagnostic tool.

1 Abbreviations: Fv, variable fluorescence; DCBQ, 2,5-dichloro-p-benzoquinone; HES, light-induced high energy state of thylakoid membranes; PSM, peak, stationary, and maximum transients of fluorescence induction curve (OPSMT); Qe, fluorescence quenching due to high energy state of thylakoids; SiMo, silicomolybdic acid.
MATERIALS AND METHODS

Plant Material

Two spring wheat (Triticum aestivum L.) cultivars, Waverly, which is adapted to the Pacific Northwest, and Len, which is adapted to the northern Great Plains, were used. These cultivars were selected for comparison because previous studies showed that high temperature stress caused substantially different changes in thylakoid activities, fluorescence transients, and grain yields (9). Seeds of the two cultivars were germinated in vermiculite, and seedlings were transferred to 2-L containers of continuously aerated Hoagland solution holding six plants each. Plants were grown in controlled environment chambers at 20°C day (16 h) and 15°C night (8 h) cycles until anthesis. Light intensity was maintained at 450 mol m⁻²s⁻¹ PAR (400–700 nm) at midcanopy throughout development, and RH was 40%.

Stress Treatment

Differential temperature treatments were initiated when at least 36 tillers of similar anthesis date (within 3 d) were available for each cultivar. Stressed plants were transferred to chambers maintained at air temperatures of 35°C day (16 h)/25°C night (8 h). Plant roots were kept at 20°C day/15°C night by immerging the containers in water maintained at the desired temperature by coils controlled by a temperature regulator (9, 13). Nonstressed control plants were maintained at 20°C day (16 h)/15°C (8 h) night temperatures until they matured.

Thirty-six tillers of uniform age for each cultivar were selected for fluorescence monitoring. Eighteen tillers were decapitated at the start of the stress treatment, and additional tillers were decapitated at various times during the course of senescence (12). This enabled us to observe whether presence of the sink caused irreversible changes in fluorescence pattern that would indicate an effect of sink on rate of thylakoid senescence. At least 10 control tillers were similarly monitored for each cultivar. The experiment was repeated twice.

Fluorescence Measurements

Fluorescence data were collected with an SF-20 fluorometer (Richard Branker Ltd. Ottawa, Canada). Measurements on all plants were made at 25°C following at least a 5-h dark period. Variable fluorescence was read from the digital display of the instrument. Induction curves for measurement of the PSM transients were obtained with a strip chart recorder. Three curves were collected from the abaxial surface of the middle third of flag leaves and data were averaged for each transient for each date. Data are graphed as changes in amplitude of the P to S and S to M transients as well as the rate of quenching (the slope) of the P to S transient over treatment duration.

The SF-20 fluorometer does not strictly measure constant fluorescence or 0, and values of Fv should be considered indexes instead of absolute relative units.

Yield Determination

Spikes of intact tillers were individually threshed when they matured. Total kernel weight was obtained for each tiller of control and stressed plants after drying the grain to constant weight at 65°C.

RESULTS

Representative fluorescence induction curves are shown in Figure 1, and the fluorescence transients (Kautsky effect) are labeled in panel A. In addition to the changes in fluorescence intensity labeled O, P, S, and M, R, the slope of the P to S transient, was also measured. Induction curves of nonstressed plants were similar for both cultivars (Fig. 1, panels A and B). The onset of stress changed the fluorescence transients, more so in intact Waverly than in Len plants, and led to very distinctive induction patterns for the two cultivars (Fig. 1, panels C versus D and E versus F).

Fluorescence transients of Len and Waverly wheats were nearly indistinguishable during grain development at 20°C/15°C control temperatures (Fig. 2). Decapitating nonstressed plants did not significantly change the parameters Fv, P to S, R, or S to M in either cultivar (data not shown). Variable fluorescence, however, increased in intact plants of both cultivars as grain filling progressed and flag leaves senesced. The amplitude of fluorescence quenching during the P to S transient (PS) was stable until the fifth week of grain filling, after which quenching decreased in both cultivars. The rate of quenching during the P to S transient (R) increased slightly relative to the amplitude shortly before quenching diminished.

The rise to M decreased gradually in cv Len during matu-
25°C and then increased gradually between the first and third week (Fig. 3A). Variable fluorescence of intact Len plants decreased upon initiation of heat treatment (Fig. 3B). After the initial decrease, Fv returned to the prestress level and then decreased gradually through the first 2 weeks of stress. Thereafter, Fv began to increase and then ultimately decreased rapidly in all stressed leaves once the final stages of leaf senescence were reached. Data of final changes to Fv are not shown in these figures because the PSM transient disappeared simultaneously.

The amplitude (PS) and rate (R) of quenching decreased rapidly in stressed Waverly wheat (Fig. 3C). In contrast, P to S declined gradually during the first 2 weeks in Len wheat (Fig. 3D). During the period of decline, R decreased more slowly than the amplitude of quenching.

The P to S transient was restored in Waverly wheat after about 2 to 3 weeks of stress (Fig. 3C). The most conspicuous feature of resumed quenching was the sharp increase of rate relative to the amplitude. Restoration of P to S in Len wheat was similarly delayed and was again accompanied by a large increase in R. Awns began senescing at the time that PS increased in individual tillers, indicating the approach of physiological maturity, although flag leaves remained green.

The rise from S to M increased in both cultivars within hours of the initiation of heat stress (Fig. 3E and 3F). During subsequent days, M declined gradually in Waverly and continued to increase in Len. The increase of M above control levels was twice as great in Waverly as in Len. After 2 weeks of stress, M had decreased from 4-fold greater to 3.5-fold greater than in controls in Waverly wheat. In Len, M was initially twice as high in stressed plants than in controls and increased to 2.5 times control levels (Fig. 1 and 3).

The Fv decreased in decapitated Waverly and remained constant in decapitated Len tillers during the first 10 days of stress treatment (Fig. 4A and B). Thereafter, Fv stabilized at the diminished level in Waverly wheat but continued to increase in decapitated Len.

The P to S transients were nearly identical in flag leaves of decapitated plants and intact plants in heat-stressed Len (Figs. 4D and 3D). In Waverly wheat, however, P to S decreased less in decapitated plants than in intact plants after an initial rise at the onset of stress (Figs. 3C and 4C). As was the case in intact plants, P to S and R increased at about the 20th d of stress. The rise to M was similar in intact and decapitated Len wheat during stress (Figs. 3F and 4F), but, in heat-stressed Waverly, the immediate increase of M that occurred previously was also observed in decapitated plants. The M transient decreased rapidly through d 7, however, and increased gradually again to a peak at about 2.5 weeks (Figs. 3E and 4E).

Mean grain yields per intact tiller over all experiments were 1.3 ± 0.3 g and 0.6 ± 0.8 g for Len wheat and 1.7 ± 0.3 g and 1.4 ± 0.2 g for Waverly wheat under control and stress conditions, respectively. Len wheat completed grain growth 42 d and Waverly wheat 49 d after anthesis under control conditions. Grain growth was complete in both cultivars after 24 d of stress.

**DISCUSSION**

The relationship of heat injury at the suborganelle level to economic yield of plants is not well understood. It is clear,
however, that stress during reproductive growth has a large effect on plant productivity (1, 12). Characterization of stress effects in young plant material has limited application to interpreting stress response in mature tissue (9). Advances in interpreting fluorescence induction transients (2, 3, 8, 14, 17, 18) and instruments for noninvasive fluorescence measurements now permit experiments to relate changes in source organs to development of sink organs. Diagnostic use of fluorescence measurements to date has emphasized detection of injury to the photosynthetic apparatus, particularly PSII (21). Our results show that it is also possible to detect sink effects on leaf photosynthesis under the conditions of altered demand imposed by heat stress. Fluorescence changes due to inflorescence removal indicated that sink stimulation of source activity during stress was stronger in Waverly wheat, the more thermotolerant cultivar of the two we studied.

Sink removal effects on the PSM fluorescence transient suggested that photophosphorylation mediated by linear or cyclic electron transport was responsive to sink demand during reproductive growth at high temperatures. This interpretation relies on reports that the fluorescence rise from S to M is due to dissipation of the transthylakoid proton gradient during ATP synthesis and decrease of Qe (8, 11, 17). In addition, high temperatures increase the influence of nonphotochemical quenching, including Qe, on fluorescence (14). When ATP is not being rapidly synthesized, Qe would reduce fluorescence yield more in heat-stressed than nonstressed tissue. Loss of Qe due to rapid ATP synthesis would be expected to increase M more in stressed than nonstressed tissue.

The sharp decrease of PS to R and increase of M in Waverly accompanied a more stable photosynthesis during stress (9). Increased amplitude of the PS transient and the decrease of M after inflorescence removal support the suggestion that sink demand induced a high requirement for ATP at the expense of Qe during induction of photosynthesis. Physiological maturity, signalled by drying of awns, clearly increased amplitude and rate of PS in intact tillers and, to a lesser degree, in decapitated tillers of both cultivars. The sudden, similar PS increase in both instances may result from a cessation of sink demand, reducing the requirement for ATP synthesis. Feedback inhibition of photosynthesis due to decreased availability of Pi results from accumulation of sucrose in the cytoplasm (16). Photosynthetic ATP synthesis is then reduced, leading to enhancement of HES formation and rapid fluorescence quenching due to Qe enhanced by high temperature (14). The effect indicates that sink demand terminated before loss of leaf photosynthetic activity, which continued for several days (9).

Cyclic photophosphorylation is a PSII-mediated process that also requires functional Cyt F645/680 for activity. Early stress reduction of electron transport through Cyt f in Len wheat (9) may have restricted sink stimulation of cyclic activity, thus reducing the effect on M. Intact and decapitated plants had similar M transients, indicating smaller sink effects than in Waverly. The M peak and slower decline of Cyt f activity in Waverly wheat distinguished that cultivar from Len wheat as grain filling proceeded (9), suggesting a relationship among grain filling, cyclic transport, and Cyt f. The decrease of Fv in Waverly but not in Len wheat was indicative of increased distribution of light energy to the low fluorescing PSII for the purpose of cyclic electron transport. Stimulation of PSII-mediated transport by high temperatures has been reported and involves changes at Cyt f (24).

This study and previous investigations (9) demonstrate genotypic differences in the senescence pattern for thylakoid membranes during heat stress. Similar genetic variation at separate loci undoubtedly influence sink growth at high temperatures. Measurement of fluorescence induction should prove useful in elucidating environmental effects on assimilate allocation in maturing plants.

LITERATURE CITED