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BRIEF COMMUNICATION

Influence of heat shock on chlorophyll fluorescence of white oak (*Quercus pubescens* Willd.) leaves

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Abstract

Chlorophyll fluorescence parameters of *Quercus pubescens* Willd. as response to heat shock (HS) by immersing leaves for 5 and 15 min in water of temperatures between 38 and 59 °C were examined. Fluorescence was measured after different periods of recovery (15, 30, 90, 210, and 1 440 min at 24/26 °C night/day temperature and 100 % humidity). The effective quantum yield of photosystem 2 (*Y*) in control and HS-treated leaves was always measured after previous 15 min irradiation. Under a 5 min HS, *Y* did not change after using temperatures below 44 °C, was rapidly restored after HS of moderate temperatures (44–48 °C), and progressively decreased and recovered eventually to the initial value after HS of high temperatures (48–52 °C). *Y* did not recover after HS with temperatures higher than 52 °C. Increase in the duration of HS from 5 to 15 min lead to change of the initial *Y* at each HS temperature, but the recovery processes were similar to those characteristic after 5 min incubation. The processes of recovery may depend mainly on the specificity of injuries caused by different heat shock temperatures. Thus *Q. pubescens* is able to preserve and recover the functional potential of its photosynthetic apparatus in response to HS up to 52 °C.

Additional key words: photosystem 2; temperature.

The growth and survival of plants can be determined by the thermotolerance capabilities of photosynthesis (Sharkey 2000). The composition of thylakoid membranes of chloroplasts, which contain the light-absorbing system, electron transport chain, and ATP synthase, is important in determining a plant's ability for growth and photosynthesis at high temperatures (Murakami *et al.* 2000). Heating followed by cooling causes deleterious changes in many physiological processes and disrupts the intricate organization of the thylakoid membrane, which has a high content of unsaturated fatty acids (Berry and Björkman 1980). These membranes must be sufficiently fluid to allow the "spinning" of ATP synthase, yet solid enough to produce the proton-motive force needed to propel this spinning (Gounaris *et al.* 1984). The thylakoid membrane is heterogeneous, and the lipids must be kept properly dispersed to prevent them from concentrating and forming non-bilayer structures.

We examined the influence of the temperature of HS and the period of recovery after shock on the fluorescence of *Quercus pubescens* leaves. The experiment was set up in a completely randomized block design with 6 replicates. Leaves were collected in August 2005 from the white oak trees that grow in forests of the Leova area of Moldova. As humidity strongly influences the sensitivity of plants to the growing temperature, we incubated the isolated leaves in glass crystallizers on wet filter paper, at a relative humidity of 100 %. The crystallizers were exposed to 60 PAR from luminescent lamps with 16 h daylength, and 26/24 °C day/night temperature for the duration of the experiments. In order to assess the effect of temperature on the photosynthetic machinery, leaves were pre-treated by immersion for 5 or 15 min in distilled water at various temperatures between 25–60 °C. The

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Abbreviations: F_m' – maximal fluorescence of leaves in light; F_t – fluorescence under steady-state irradiation; HS – heat shock; PAR – photosynthetically active radiation; *Y* – yield.

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evolution of effective quantum yield of photosystem 2 (PS2) under given irradiance [$Y = (F_m' - F_t)/F_m'$] was measured with a modulated chlorophyll fluorometer *PAM-2100* (*H. Walz*, Germany), after irradiating the control and HS-treated leaves at photosynthetically active radiation (PAR) of 20 µmol m^{-2} s^{-1} for 15 min at 26 °C before each measurement. F_m' is the maximal fluorescence yield reached with a saturating pulse when the leaf is pre-irradiated, and F_t represents the measured fluorescence yield at any given time at steady-state conditions. Minimum fluorescence (F_0) was excited at 655 nm with 600 Hz modulation frequency, and maximum fluorescence (F_m) was measured with 20 kHz modulation frequency. The F_m was elicited by saturating flashes of 0.8 s duration from a built-in miniature halogen lamp.

The photosynthetic yield recovered quickly after HS at temperatures below 48 °C (Fig. 1*A*). A period of 270 min was not long enough to detect the recovery of *Y* after HS with temperature between 48–53 °C, but *Y* was maintained at a high enough level in this temperature range to recover after a longer period after the HS. Exposure to temperatures higher than 53 °C caused a dramatic decrease of *Y* immediately after HS.

Increasing the period of HS incubation of white oak leaves from 5 to 15 min at different temperatures increased the inhibitory effect on effective quantum yield, but this effect was only quantitative (Fig. 1*A*). The distribution of temperatures by their qualitative effects on *Y* was not influenced by this factor. For example, after incubation for 15 min at 52 °C, *Y* initially dropped significantly lower than after incubation for 5 min, but later its level became comparable to that observed for the 5-min treatment.

In order to compare the specificity of recovery after HS with different temperatures, we measured *Y* at different periods after HS (Fig. 1*B*). Immediately after HS, exposure of leaves at temperatures lower than 44 °C did not cause significant changes in the effective quantum yield of PS2, whereas shock with temperatures of 44–48 °C significant lowered the quantum yield, which was even more pronounced using temperatures of 48–55 °C. Further increase of the temperature of HS led to further reduction of *Y*. The recovery of quantum yield of PS2 after HS was dependent on the temperature of HS. *Y* recovered quickly immediately after HS with temperatures of 44–48 °C. The recovery of quantum yield was not visible during the first 270 min after HS with 49–51 °C, but later a recovery occurred and *Y* recovered almost completely at 1 440 min (24 h) after HS. In general, increasing the period of recovery led to an improved effective quantum yield of PS2, a measure of injury to photosynthesis under HS. The recovery only occurred when the temperature during HS was lower than 52 °C and its kinetics depended on the temperature of HS.

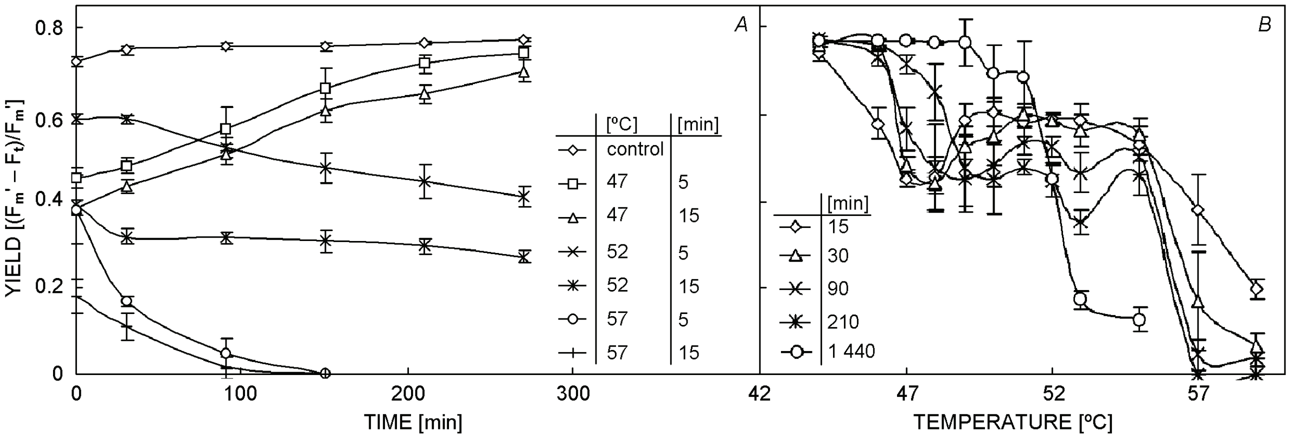

Fig. 1. Evolution of effective quantum yield of photosystem 2 of *Quercus pubescens* Wild isolated leaves, pre-incubated at various temperatures for 5 and 15 min, depending on (*A*) the time of exposure or (*B*) heat shock (HS) temperature, after HS at PAR of 50 µmol m^{-2} s^{-1}, 26 °C, and relative humidity of 100 %. Immediately before the measurement of *Y*, the leaves were exposed for 15 min at PAR of 20 µmol m^{-2} s^{-1}. Means from six independent measurements. *Bars* represent standard deviation.

Quantum yield is a sensitive indicator of changes in thylakoid membrane integrity caused by environmental stresses. Results from both field and controlled environment experiments demonstrate that the decline of *Y* caused by high temperature depends on genotype and other factors (Berry and Björkman 1980, Sharkey 2000). *Y* depends on the nature and level of injuries, as well as on the results of competition between the processes of maintenance and development of heat injuries with those of their recovery. The result of this competition strongly depends on the temperature of HS and on the time period after HS. An increase of the duration of HS from 5 min up to 15 min influenced the injuries only quantitatively, because the effectiveness of recovery was mainly influenced by the value of temperature (Fig. 1*A*). The processes of recovery dominated immediately after HS for temperatures of 44–48 °C, but initially after HS with higher temperatures the processes of amplification of injuries dominated (Fig. 1*B*). The domination of recovery from HS with temperatures higher than 48 °C was observed only for periods longer than 270 min after transfer of leaves to normal temperature (24/26 °C, night/day) and if the temperature of HS was lower than 52 °C (Fig. 1*B*). The conclusion that not only the level of injury, but also differences in their nature after HS with different temperatures influenced the effectiveness of the recovery process is supported by the data in Fig. 1*B*. Augmentation of the level of injury by increasing the period of HS did not influence qualitatively the recovery of injury after HS. Hence the injuries caused by HS of 48 °C recovered more slowly (or did not recover at all) in comparison with those induced by lower temperatures.

Immediately after 5 min of HS with temperatures of 48–55 °C the values of *Y* were significantly higher than in samples exposed at lower temperatures (Fig. 1*B*). It is difficult to suppose that after the same period of HS, exposure to higher temperatures would cause fewer injuries than exposure to the lower ones. Probably in these particular cases, the higher levels of *Y* injury after exposure to higher temperatures were caused by the interaction of many events (Pearcy 1978, Berry and Björkman 1980, Nash *et al.* 1985). We found the same type of HS-temperature dependence of *Y* also in leaves of other plant species (*Quercus robur* L., *Solanum tuberosum* L., *Buxus sempervirens* L. *etc.*).

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