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## Temperature dependence of chlorophyll fluorescence induction in plant leaves

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## Summary

Growth and production of crops is preceded by a light dependent process converting carbon dioxide ( $\text{CO}_2$ ) from the air and water to sugars and oxygen ( $\text{O}_2$ ): photosynthesis. The first reactions of photosynthesis occur in two protein-pigment complexes, the photosystems I and II. These complexes are situated in the membranes of the chloroplast: the thylakoids. The absorption of light energy in the photosystems induces chemical reactions resulting in the fixation of  $\text{CO}_2$ . Both growth and photosynthesis are affected by temperature. This thesis evaluates the possibility to assess limitation of growth by low temperatures at the level of the photosystems. Therefore, temperature effects on the functioning of the photosystems were studied and related to genetic variation in low temperature sensitivity of plants.

Part of the absorbed light energy is lost for the plant by heat and chlorophyll fluorescence production. The amount of emitted chlorophyll fluorescence is a measure for the functioning of the photosystems, at room temperature primarily for the functioning of photosystem II. Less efficient chemical fixation of light energy results in more emission of chlorophyll fluorescence.

Temperature effects on the functioning of the photosystems were studied by briefly inducing chlorophyll fluorescence by low light and at different temperatures. The maximum fluorescence of induction curves ( $F_p$ ) was determined for leaf discs.  $F_p$  reflects a maximal reduction of the quinone electron acceptor of photosystem II,  $Q_a$ . This acceptor is reduced by photosystem II activity and oxidised by photosystem I activity. The temperature dependence of both processes determines the extent of  $Q_a$  reduction and thus the level of  $F_p$ . Between  $0^\circ\text{C}$  and  $30^\circ\text{C}$  two discontinuities were found in  $F_p$  versus temperature curves: a low temperature break (*LTB*) and a high temperature break (*HTB*).  $F_p$  decreased from  $0^\circ\text{C}$  to the temperature of *LTB*, and from the temperature of *HTB* to  $30^\circ\text{C}$ .

*LTB* was absent when photosynthetic electron transport was inhibited, while *HTB* remained. *LTB* also depended on the light intensity used to induce chlorophyll fluorescence. Increasing the light intensity from  $10$  to  $150\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  resulted in the disappearance of *LTB*. These results were found for cucumber and tomato (chapters 4 and 5) and indicate a limitation in electron transport beyond photosystem II at temperatures below the *LTB* temperature and a decrease of the efficiency of light energy capture of photosystem II at temperatures above the *HTB* temperature. This interpretation was sustained for tomato by an analysis of other fluorescence parameters (chapter 6).

In addition, the temperature dependence of net  $\text{CO}_2$  fixation of cucumber and tomato leaf discs was determined (chapters 4 and 5). Net  $\text{CO}_2$  fixation rates exceeded 90% of the maximal value found between the temperatures of *LTB* and *HTB*. The temperatures of *LTB* and *HTB* indicate the temperature range for optimal functioning of photosynthesis, although photosynthetic activity at steady state is not limited by electron transport.

The limitation in photosynthetic electron transport at temperatures below the *LTB* temperature may be caused by local phase transitions in the lipid matrix of the thylakoid membrane by the transition of domains from the liquid-crystalline to the rigid gel phase. Gel domains in the thylakoid membrane will limit lateral diffusion in the membrane of the electron carrier plastoquinone and thus inhibit photosynthetic electron transport. This interpretation is sustained by the similar temperatures found for *LTB* and for phase transitions of lipids, reported in the literature (chapter 1, 4 and 5).

The decreased efficiency of light energy capture of photosystem II at temperatures above the *HTB* temperature corresponded with alterations in photosystem II heterogeneity (chapter 6). The fraction reaction centers with a relatively small antenna increased at the expense of reaction centers with a relatively large antenna.

The perspectives for the use of chlorophyll fluorescence measurements of leaves to assess low temperature sensitivity of plants was studied. Differences in *LTB* and *HTB* temperatures between species (chrysanth, spinach, cucumber and tomato) and between genotypes of one species (cucumber and tomato) were related to differences in low temperature sensitivity (chapter 3). The low temperature tolerant chrysanth showed no *LTB* between 0° and 30°C. The *LTB* temperatures of the remaining species were lower in accordance with low temperature sensitivity. For tomato, a lower growth temperature resulted in a lower *LTB* temperature, while the *HTB* temperature remained constant. The temperature of *HTB* was lower when the light intensity during growth was decreased (chapter 3).

If less low temperature sensitive genotypes would be available energy costs for the cultivation of horticultural crops in greenhouses could be saved. For the improvement of breeding such genotypes a fast method to screen genotypes on their low temperature sensitivity is very desirable. Chapter 2 describes equipment, developed during the present investigations, to measure the temperature dependence of chlorophyll fluorescence induction in a relatively short time and for a large number of samples. Chapter 7 describes an experiment to test the application of the temperature dependent chlorophyll fluorescence method to select less low temperature sensitive tomato genotypes. Chlorophyll fluorescence measurements could discriminate between 8 genotypes with regard to their temperature sensitivity of the thylakoid membrane. This discrimination did not correspond with genetic differences in net CO<sub>2</sub> fixation rates of leaves, nor with genetic differences in relative growth rates at low temperature and low light. A final conclusion regarding the application of the method could not yet be given based on the results of this experiment due to a limited genetic variation in growth of the studied tomato genotypes and due to difficulties in assessing genetic differences in low temperature sensitivity.