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Evaluation of cold stress of young industrial chicory (*Cichorium intybus* L.) plants by chlorophyll *a* fluorescence imaging. I. Light induction curve

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Abstract

Industrial chicory, *Cichorium intybus* L., is cultivated for the production of inulin. Most varieties of industrial chicory exhibit rather poor early growth, which limits further yield improvements in their European cultivation area. The poor early growth could be due to suboptimum adaptation of the gene pool to growth at low temperatures, sometimes in combination with high light intensities, which is typical of early-spring mornings. We have used chlorophyll (Chl) *a* fluorescence to evaluate the response of young plants of the cultivar 'Hera' to low temperatures and high light intensities. Plants were grown at three temperatures: 16°C (reference), 8°C (intermediate), and 4°C (cold stress). Light-response measurements were carried out at different light intensities in combination with different measurement temperatures. Parameters that quantify the photosystem II (PSII) operating efficiency (including PSII maximum efficiency and PSII efficiency factor) and nonphotochemical quenching (NPQ) are important to evaluate the stress in terms of severity, the photosynthetics processes affected, and acclimation to lower growth temperatures. The results clearly demonstrate that in young industrial chicory plants the photosynthetic system adapts to lower growth temperatures. However, to fully understand the plant response to the stresses studied and to evaluate the long-term effect of the stress applied on the growth dynamics, the subsequent dark relaxation dynamics should also be investigated.

Additional key words: chilling; low temperature; nonphotochemical quenching; photochemical quenching; photoinhibition; screening.

Introduction

Industrial chicory, *Cichorium intybus* L., is cultivated for the production of inulin, a linear fructose polymer with a terminal glucose molecule. The inulin is commercialized as such or, after partial hydrolysis, as a syrup of fructose and glucose. Inulin is a prebioticum and is often used as a dietary soluble fibre that stimulates growth of the beneficial intestinal flora. It can also be used as a low-calorie sweetener after partial hydrolysis, and it enhances calcium absorption in the intestine. Chicory is a biannual species and early sowing increases the risk of bolting (becoming generative during the first season), through the exposure to vernalization temperatures immediately after germination. For this reason, major breeding efforts have recently been dedicated to the development of varieties which are less prone to bolting (Baert and Van Bockstaele 1999) and which can be sown earlier. Early

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Abbreviations: *ANOVA* – analysis of variance; Chl – chlorophyll; EC – electrical conductivity; F_0 – the minimum Chl fluorescence in dark-adapted state; F_0' – the minimum Chl fluorescence in light-adapted state; F_m – the maximum Chl fluorescence in dark-adapted state; F_m' – the maximum Chl fluorescence in light-adapted state; F_q' – the difference between F_m' and F' (measured immediately before application of the saturation pulse used to measure F_m'); F_q'/F_m' – the operating quantum efficiency of PSII photochemistry; F_q'/F_v' – the PSII efficiency factor; F_v – the variable (differential) fluorescence in dark-adapted state ($F_m - F_0$); F_v' – the variable fluorescence in light-adapted state ($F_m' - F_0'$); F_v/F_m – the maximum quantum efficiency of PSII photochemistry in dark-adapted state; F_v'/F_m' – the maximum quantum efficiency of PSII photochemistry in light-adapted state; $F_v/(F_m \cdot F_0)$ – the fraction of PSII centers that are capable of photochemistry; GT – growth temperature; ML – measurement light intensity; MT – measurement temperature; NPQ – nonphotochemical quenching of the Chl fluorescence signal; PAM – pulse amplitude modulated; PAR – photosynthetic active radiation; PSII – photosystem II; q_N – nonphotochemical quenching coefficient of the Chl fluorescence signal; SE – standard error.
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sowing allows for a longer growth season and can contribute to improve yield (Baert 1997). However, the yield potential of the currently available bolting-resistant varieties has not yet been completely exploited due to the slow youth growth that most chicory varieties display at low temperature (Baert 1997).

It is well known that growth is a temperature-driven process and that low temperatures can affect the growth rate of young plants (Wolfe 1991, Venema *et al.* 2000). Furthermore, a previous study (Devacht *et al.* 2009) demonstrated that chicory plants grew more slowly at 4°C than at 16°C. The negative effect of low temperatures can be strengthened when combined with high light intensities, causing photoinhibition of the photosystem II (PSII). Photoinhibition can result into (*1*) an irreversible inhibition of the photosynthetic apparatus (*i.e*., chronic photoinhibition) or (*2*) a slow, reversible reduction of the photosynthetic efficiency (*i.e*., dynamic photoinhibition) (Osmond 1994, Alves *et al.* 2002). In dynamic photoinhibition, the decrease in efficiency is due to damage and breakdown of the PSII reaction centre protein D1 (Kyle *et al.* 1984). The intensity of the stress applied has a significant influence on the recovery rate: the higher the stress, the slower the recovery rate of PSII. It has been demonstrated that responses to photoinhibitory stress conditions are species- and genotype-dependent (Madhava Rao 2006).

This combination of low temperature and high light intensity can be particularly prevalent during early-spring days across chicory's main European cultivation range (Northern France, Belgium, the Netherlands and Germany). It is therefore possible that the observed slow growth of young chicory plants is mediated by the negative influence of low temperature, on its own or in combination with high light intensities, on the photosynthetic apparatus. Direct estimation of growth rates of young plants in a nondestructive manner is desirable but not easy. Possible proxies are parameters correlated to early vigour, such as maximum net photosynthesis and energy input in the photochemical processes (Fracheboud *et al.* 1999, Lootens *et al.* 2004, Devacht *et al.* 2009). These data can be used to identify genotypes with improved early growth at low temperatures.

In this study we evaluated the stress response of young chicory plants using Chl fluorescence imaging and Chl fluorescence parameters related to the induction kinetics. Chl *a* fluorescence has long been recognized as a suitable tool to noninvasively monitor photosynthetic activity and has been extensively used to assess plant responses to a variety of environmental stresses (Leipner *et al.* 2001, Sayed 2003). The Chl *a* fluorescence signal is quenched either photochemically or nonphotochemically. The quantity of energy dissipated by either process depends on the nature and intensity of the stress applied and on the plant's response. To determine the influence of the stress applied on the efficiency of PSII, the induction and relaxation kinetics of the Chl *a* fluorescence signal can be studied. Furthermore, recent developments in imaging of Chl *a* fluorescence offer the possibility to evaluate the efficiency of PSII in tissues, individual cells and even chloroplasts *in situ* (Leipner *et al.* 2001). This method is quick and inexpensive and can be used for large-scale screening (Ehlert and Hincha 2008) as several leaves/plants can be measured simultaneously. The main objective of the experiments described here is to better understand the response of young chicory plants to low temperature and/or high-light-intensity stress.

Materials and methods

Plant materials and growth conditions: All the experiments were carried out using plants of the industrial chicory variety 'Hera'. This variety is known to possess a good early vigour (Devacht *et al*. 2007) and displays a high level of genetic variability. Seeds were sown in perforated multi-well plates (4 × 6) filled with a mixture of peat and perlite (EC = 250 µS cm^{-1}) and placed in trays with an irrigated underlay. These trays were first kept for 6 days at 16°C to allow germination. They were then transferred to the corresponding growth temperature (GT). For the reference situation, the seedlings were further grown during 4 days at 16°C. For the stress situations the seedlings were kept either at 8°C or 4°C during 10 days. It was necessary to keep the plants for a longer period at 8°C and 4°C before screening to allow them to reach the same developmental stage (cotyledons) as was reached after 4 days of growth at 16°C. The choice of these growth temperatures was based on the average temperatures during early spring, as extracted from the Typical Reference Year tables for Belgium (Dogniaux *et al*. 1978). In all cases, the air relative humidity was set at 60% (the vapour pressure deficit was not monitored) and the light intensity at 220 µmol m^{-2} s^{-1} for 16 hours per day (*TL-D 58W/840*, *Philips*, the Netherlands). All plants were grown in growth chambers (*1600US*, *Weiss*, Reiskirchen, Germany).

Chl *a* fluorescence: The induction and relaxation kinetics of the Chl *a* fluorescence were estimated at different measurement light intensities (ML) (50, 100, 200, 400, 800, and 1,200 µmol m^{-2} s^{-1}) in combination with various measurement temperatures (MT) (2–16°C, at intervals of 2°C). The MT was maintained throughout the whole procedure. A Chl fluorescence imaging system (*CFImager, Technologica*, UK) was used. The measurement procedure lasted 2.5 h (Fig. 1). In a first step, the plants were dark-adapted for 30 min. F_0 was measured using a measurement light level of 0.52–0.85 µmol m^{-2} s^{-1}. F_m was measured with a saturation pulse of 4,947 µmol m^{-2} s^{-1}. After 20 s, the plants were exposed to actinic

light at the corresponding ML for 1 h. During this light period a saturation pulse of 4,947 µmol m^{-2} s^{-1} was given for 800 ms every 2 min for the quenching analysis. For each of the 48 measurement conditions (combination of ML and MT), a different multi-well plate was screened. Within each plate 14 plants were selected at random. This corresponds to the maximum number of traces that this Chl fluorescence imaging system can record simultaneously. Chl fluorescence measurements were done on the adaxial side of the mature cotyledons. The Chl *a* fluorescence parameters of the induction curve were automatically generated by the Chl fluorescence imaging system and are based on Baker and Oxborough (2004). The following parameters were estimated: F_0 – the minimum Chl fluorescence in dark-adapted state, F_0' – the minimum Chl fluorescence in light-adapted state [based on the calculation described by Oxborough and Baker (1997)], F_v/F_m – the maximum quantum efficiency of PSII photochemistry in dark-adapted state, F_v'/F_m' – the maximum quantum efficiency of PSII photochemistry in light-adapted state, $F_v/(F_m \cdot F_0)$ – the fraction of PSII centers that are capable of photochemistry, F_q'/F_m' – the operating quantum efficiency of PSII photochemistry (Genty *et al.* 1989), and F_q'/F_v' – the PSII efficiency factor. With F_m – the maximum Chl fluorescence in dark-adapted state, F_m' – the maximum Chl fluorescence in light-adapted state, F_v – the variable (differential) fluorescence in dark-adapted state ($F_m - F_0$), F_v' – the variable fluorescence in light-adapted state ($F_m' - F_0'$), and F_q' – the difference between F_m' and F' measured immediately before application of the saturation pulse used to measure F_m'. We also estimated the nonphotochemical quenching of the Chl fluorescence signal as NPQ = $(F_m - F_m')/F_m$ (Bilger and Björkman 1990). NPQ was used instead of nonphotochemical quenching coefficient (q_N), because NPQ is unaffected by changes in the rate constant of PSII photochemistry and this in contrast with q_N. These changes might occur under photoinhibitory conditions such as investigated in this study (Adams III and Demmig-Adams 2004, Krause and Jahns 2004, Oxborough 2004).

Statistical analysis: The effect of the GT, MT, and ML on the different Chl *a* fluorescence parameters was analyzed by one-way and factorial analysis of variance (*ANOVA*; significance level $p \leq 0.05$). To determine whether the different combinations of GT, MT, and ML had a significant effect on the Chl *a* fluorescence parameters a *Duncan* Post-Hoc test was performed. All calculations were conducted using the statistical software package *Statistica v. 9* (*Statsoft*, USA).

Results

Effect of light and temperature on the fluorescence induction curve: The fluorescence induction curves are shown for plants grown at 16°C and measured at 16°C or 2°C in combination with ML of 100 or 800 µmol m^{-2} s^{-1} (Fig. 1). The curve at MT = 16°C in combination with ML = 100 µmol m^{-2} s^{-1} (Fig. 1*A*) can be considered to be the reference situation, without temperature or light stress. After the actinic light was switched on, the fluorescence signal quickly decreased due to the generation of NPQ (Lambrev *et al.* 2007). After about 20 min in actinic light, a steady state was reached. Lowering the MT to 2°C (Fig. 1*B*) resulted in a lower fluorescence signal upon illumination. NPQ was generated more slowly than when the measurements were carried out at 16°C. Ten minutes after the actinic light was switched on, the photochemical quenching was lower than at a MT of 16°C. This was probably caused by the temperature effect on the dark reactions.

Comparison of Fig. 1*A* with 1*C* and Fig. 1*B* with 1*D* illustrates that at 800 µmol m^{-2} s^{-1} the photochemical signal during the actinic light period was lower than at 100 µmol m^{-2} s^{-1}. This implies that at 800 µmol m^{-2} s^{-1} NPQ increased significantly to dissipate the excess of energy not used in the dark reactions. At 2°C in combination with a high actinic light intensity of 800 µmol m^{-2} s^{-1} (Fig. 1*D*), the photochemical signal upon illumination was extremely low, and the steady state was hardly reached at the end of the actinic light period (after 1 h).

However, caution has to be taken during the the dark adaptation in the first 30 min of the procedure. The curves showed that the measuring light (0.52–0.85 µmol m^{-2} s^{-1}) used to visualize the dark adaptation period was strong enough to induce photosynthesis (data not shown). This part of the curve should be horizontal. However, a "miniature version" of a Kautsky curve was detected. This means that PSII was not fully oxidized during the dark adaptation period. As a consequence, F_0 was slightly overestimated and the estimated F_m value was not the absolute maximum. This means that the different parameters of NPQ were underestimated in this study, but this has no influence on the main conclusions presented.

PSII efficiency factor and nonphotochemical quenching of the fluorescence signal: Both F_q'/F_v' and NPQ were significantly affected by the GT, MT, and ML applied (Figs. 2, 3). NPQ increased significantly with increasing ML and decreasing MT (Figs. 2*B,* 3*C*), whereas F_q'/F_v' showed the opposite trend and decreased significantly with increasing ML and decreasing MT (Figs. 2*A*, 3*A*). For MT between 16 and 8°C, NPQ rose first fast and decreased after the first five min of the induction curve. At lower MT (4 and 2°C), NPQ did not decrease and even increased with time (Fig. 2*B*).

The minimum value of F_q'/F_v' and the maximum value

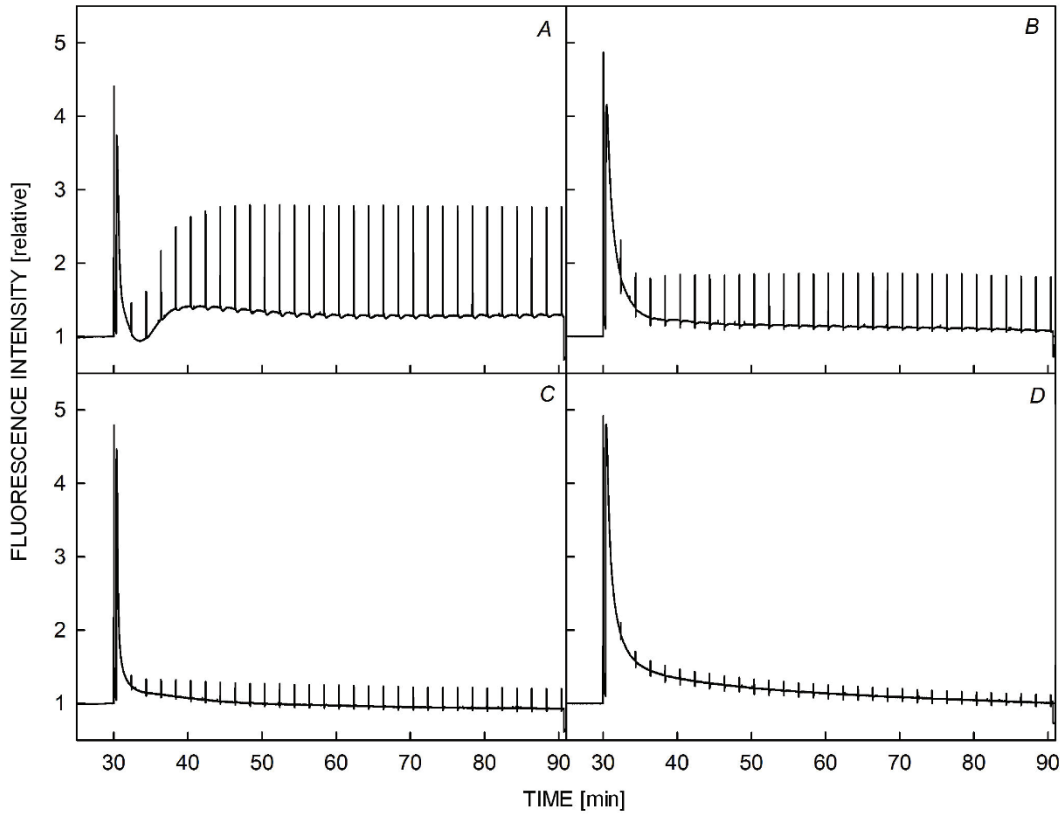

Fig. 1. Chl fluorescence signal registered with a Chl fluorescence imaging system for industrial chicory plants (variety 'Hera') grown at 16°C (reference situation) and measured under different conditions: (*A*) 16°C and 100 μmol m^{-2} s^{-1}, (*B*) 2°C and 100 μmol m^{-2} s^{-1}, (*C*) 16°C and 800 μmol m^{-2} s^{-1}, and (*D*) 2°C and 800 μmol m^{-2} s^{-1}. The fluorescence intensity has been normalized to the initial fluorescence intensity (F_0). The curves represent the average curves of 14 plants. For details on the measurement procedure, *see* text.

of NPQ during the light induction period for plants grown at 16°C (Fig. 3*A,C*, respectively) revealed that the ML and MT applied had a significant effect on both parameters. The minimum F_q'/F_v' was the highest at low ML and declined with increasing ML. This contrasts with the behaviour of maximum NPQ, which was the lowest at low ML and increased with increasing ML. At lower MT, the minimum F_q'/F_v' decreased and the maximum NPQ increased even further. This implies that at higher light levels and lower temperatures more excess energy had to be dissipated.

The graphs for F_q'/F_v' and NPQ (Fig. 2) also show that the induction time of NPQ (time point at which maximum NPQ was reached) depends on the measurement conditions (Fig. 2*B*), in contrast to the induction time of F_q'/F_v' (time point where minimum F_q'/F_v' was reached, this is the start of the dark reactions), which was induced after 2 min regardless of the stress condition applied (Fig. 2*A*). However, this result should be interpreted with care as it could be biased by the measurement procedure: the kinetics of F_q'/F_v' were not recorded in detail during the first two minutes of the induction curve.

The time shift in the induction of the maximum NPQ is clear: at an MT of 2°C, the maximum NPQ is reached more slowly than at 16°C, even at the lowest ML (Fig. 3*E*), whereas at a higher MT, near the GT, the induction of NPQ was much faster for ML below 400 μmol m^{-2} s^{-1}. The activation of NPQ was also delayed as the ML increased. This implies that the activation of NPQ is not only a temperature-bound process, but also a light-dependent process. The activation process of NPQ does not work as efficiently at low temperatures and at higher light intensities. Furthermore, as the measurements were limited to 1 h, a value of 60 min for the induction time of NPQ in the graphs could in some cases indicate that the maximum activation of NPQ was not yet reached. Consequently, the induction time of NPQ could even exceed 60 min in some cases. However, this does not have any influence on the interpretation of the effect of the conditions applied on the nonphotochemical process.

The temperature at which the plants were grown had also an effect on the minimum F_q'/F_v' and the maximum NPQ values. The values obtained for plants grown at different temperatures are presented in Fig. 3*B*, *D*, respectively. In this case only the results measured at 4°C in combination with varying ML are shown. Similar tendencies were also found at other MT. The effect of GT on the minimum F_q'/F_v' was small. For the maximum NPQ on the other hand, plants grown at 16°C displayed

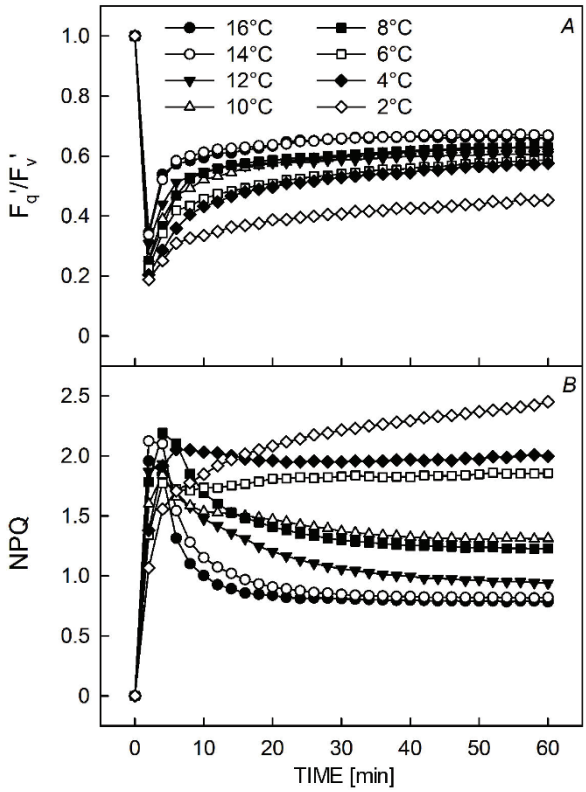

Fig. 2. The induction curve of (*A*) the PSII efficiency factor (F_q'/F_v') and (*B*) the nonphotochemical quenching (NPQ) for plants of the industrial chicory plants (variety 'Hera') grown at 16°C and measured at various measurement temperatures (MT) in combination with a light intensity of 200 µmol m^{-2} s^{-1} (n = 14).

significantly higher values compared to plants grown at 8°C and 4°C (Fig. 3*D*). Furthermore, for plants grown at 16°C, NPQ was induced more slowly than for plants grown at 8°C or 4°C (Fig. 3*F*). The induction of NPQ was slower even at ML of about 50 µmol m^{-2} s^{-1}. In plants grown at 4°C and measured at 4°C, NPQ was induced much faster at ML of about 200 µmol m^{-2} s^{-1}, probably due to the acclimation to lower GT.

The light induction parameters: The effect of a particular stress condition on the efficiency of PSII was estimated using the maximum quantum efficiency of the PSII photochemistry in the dark-adapted state, F_v/F_m. This parameter was significantly affected by the GT but not by the MT (Fig. 4).

In order to determine how efficient PSII photochemistry still was under the stress conditions applied, the following parameters were studied: F_q'/F_m', F_v'/F_m' and F_q'/F_v'. The results of this analysis for plants grown at 16°C, 8°C, or 4°C and measured at the respective GT (16°C, 8°C, or 4°C), and at 2°C are presented in Fig. 5.

As described earlier (Genty *et al.* 1989), the PSII operating efficiency decreased with increasing PAR in a nonlinear manner, and is related to changes in both PSII maximum efficiency and the PSII efficiency factor. For all GT and MT considered, the PSII efficiency factor showed a more pronounced decrease than the PSII maximum efficiency as the ML intensity increased (steeper curves). The PSII maximum efficiency was the highest at ML below 200 µmol m^{-2} s^{-1}. At higher light levels the decrease was slower or leveled out when the MT was lower than the GT (Fig. 5*B,D,F*). Decreasing the MT to 2°C affected all parameters but not to the same extent in all conditions tested (Fig. 5*G,H,I*). Lowering the MT from 16°C (Fig. 5*A*) to 2°C (Fig. 5*B*) decreased the PSII efficiency factor much more at light intensities above 100 µmol m^{-2} s^{-1} than at lower light intensities (Fig. 5*G*). The changes observed in PSII maximum efficiency when plants were investigated at a MT of 16°C (Fig. 5*A*) compared to a MT of 2°C (Fig. 5*B*) were similar at the different light intensities tested (Fig. 5*G*). The relative differences between the response estimated at a MT equal to the GT and at a MT of 2°C were less pronounced when the plants had been grown at a GT of 8°C (Fig. 5*H*) or 4°C (Fig. 5*I*), compared to the plants that had been grown at a GT of 16°C (Fig. 5*G*). Finally, the decrease in the PSII operating efficiency observed for plants grown at 4°C and measured 2°C at higher ML was mostly due to a decrease in the PSII maximum efficiency.

A clear acclimation effect was detected in this study (Fig. 6). When the response of plants grown and measured at 4°C was compared to that of plants grown and measured at 16°C, an average decrease of the PSII operating efficiency of 26% was observed irrespective of the ML (Fig. 6*A*). This was caused by an average decrease of the PSII maximum efficiency with 18% and an average decrease of the PSII efficiency factor of 10%. In contrast, when plants grown at 4°C and measured at 2°C were compared to plants grown 16°C and measured at 2°C, light-dependent temperature acclimation became apparent (Fig. 6*B*). At ML higher than 100 µmol m^{-2} s^{-1}, an increasing PSII operating efficiency was found for plants grown at 4°C compared to plants grown at 16°C (both measured at 2°C). This was mainly caused by a strong increase in PSII efficiency factor at GT of 4°C even though in the meantime the average PSII maximum efficiency decreased with 33%, almost irrespective of the light level.

Finally, analysis of $F_v/(F_m \cdot F_0)$, indicated that the number of reaction centers that are still capable of performing photochemistry declined with increasing ML and with decreasing MT (Fig. 7). Consequently, high ML and low MT cause more severe stress to PSII reaction centers. For plants grown at 4°C (Fig. 7*B*), the number of active reaction centers capable of photochemistry was higher at MT. Plant acclimation can explain this effect. No significant difference was found between the GT of 8°C and 4°C (Fig. 7*B*).

Discussion

Chl *a* fluorescence is a nondestructive method to evaluate the efficiency of PSII photochemistry, which is essential for biomass production (Fracheboud and Leipner 2003, Baker and Rosenqvist 2004, Govindjee 2004). Chl fluorescence parameters can be used for high throughput screening and early detection of both biotic and abiotic stress (Gray *et al.* 2003, Nedbal and Whitmarsh 2004, Ehlert and Hincha 2008).

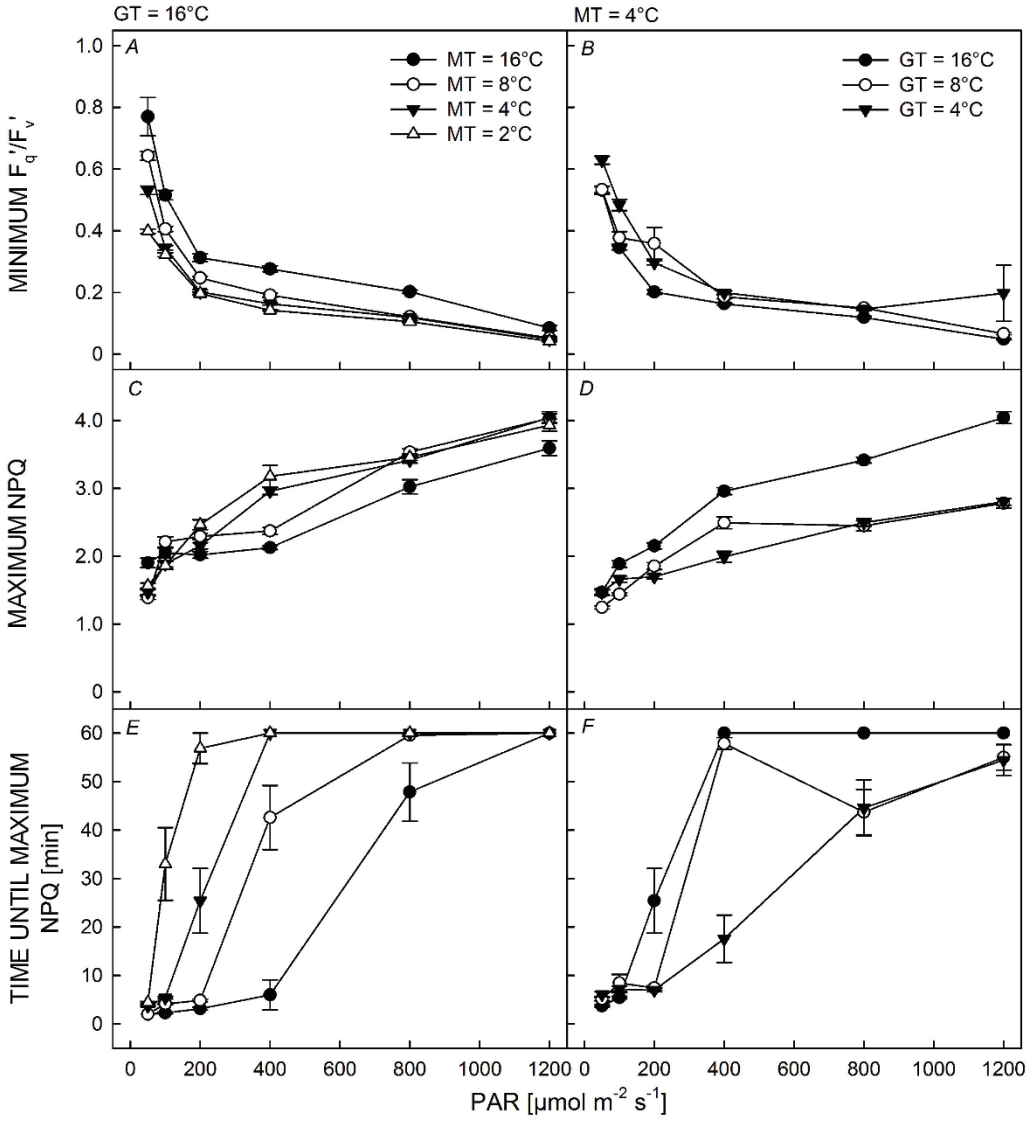

Fig. 3. The light-response curve of (*A*) the minimum value of F_q'/F_v' (GT = 16°C, MT = 2–16°C), (*B*) the minimum value of F_q'/F_v' (MT = 4°C, GT = 4–16°C), (*C*) maximum value of NPQ (GT = 16°C, MT = 2–16°C), (*D*) maximum value of NPQ (MT = 4°C, GT = 4–16°C), (*E*) time until maximum NPQ is reached (GT = 16°C, MT = 2–16°C), and (*F*) time until maximum NPQ is reached (MT = 4°C, GT = 4–16°C) for industrial chicory plants (variety 'Hera'). GT – growth temperature, MT – measurement temperature. n = 14, mean ± SE.

Fluorescence induction curve: The quenching of the fluorescence signal during light induction depends on the generation of NPQ (Lambrev *et al.* 2007). According to our results, the response of young industrial chicory plants is strongly dependent on the stress condition applied. At low MT, a reduction of the fluorescence signal was observed as a consequence of the increase in nonphotochemical energy-dissipation processes. This reduction of the fluorescence signal became even more apparent when low temperatures were combined with high ML. Lambrev *et al.* (2007) attributed this temperature dependence of NPQ to the fact that temperature affects almost all reaction rates in the photosynthetic apparatus, such as the proton transfer across the thylakoid

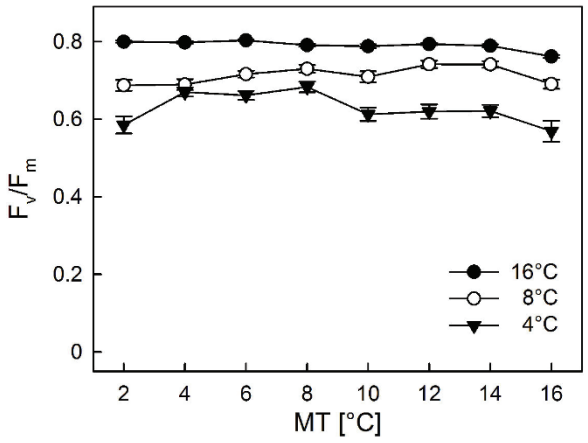

Fig. 4. The temperature-response curve of the maximum quantum efficiency of PSII photochemistry in the dark-adapted state (F_v/F_m) of industrial chicory plants (variety 'Hera') grown at 16°C, 8°C or 4°C. GT – growth temperature for the various measurement temperatures (MT). n = 14, mean ± SE.

membrane and the de-epoxidation of violoxanthin to form zeaxanthin. Our results also demonstrate that the activation of NPQ is not only a light-dependent process (since the function of NPQ is to dissipate excess light energy), it is also strongly affected by temperature. The combination of light and temperature stress affected the PSII efficiency more than each condition on its own.

Photochemical and nonphotochemical processes: The photochemical (PSII efficiency factor) and the non-photochemical processes (NPQ) were significantly affected by the GT, MT, and ML applied. Lowering the MT and increasing the ML resulted in opposite reactions of the PSII efficiency factor F_q'/F_v' and NPQ, namely a decrease and an increase, respectively as also described in literature. This implies that these conditions cause such a severe stress to PSII that most of the energy is considered to be excessive and needs to be dissipated through non-photochemical processes. Also the pattern of development changed for PSII efficiency factor and NPQ when the MT was lowered, especially for NPQ. This could be related to the conformational changes in the thylakoid membrane necessary to activate the non-photochemical quenching processes. These changes are temperature-dependent. At low temperatures, the activation of NPQ is probably slowed down and needs to be produced throughout the whole measurement procedure to cope with the excess energy present. In contrast, photochemical quenching remains low during the entire procedure at low temperatures.

The maximum NPQ increased as the MT decreased and the ML increased as was also found for *Silene dioica* (Baker 1991), for soybean at high light (Lichtenthaler and Burkhart 1999) and for grapevines at low temperatures (Hendrickson *et al.* 2003). This can be due to a downregulation of photosynthesis by a partial blockage of the photosynthetic apparatus, the quantum conversion and the electron transport, or to a photosynthetic control to minimize the damage to the D1 protein of the PSII reaction center at high ML. At low MT and low ML, the maximum NPQ value remained low indicating that no photoprotection was needed under those conditions or that other processes may contribute to the energy dissipation at low MT (Hendrickson *et al.* 2003). This could also be an advantageous trait as it will allow the plant to withstand this low-temperature condition and still perform efficiently.

The minimum PSII efficiency factor, on the other hand, decreased with decreasing MT and increasing ML. Brüggeman *et al.* (1992) described the same phenomenon for young tomato plants exposed to chilling. They suggested that the decrease of the PSII efficiency factor could be linked to limited dark reactions, as NADPH accumulates and thus limits the electron transport. Indeed, as dark reactions are temperature-dependent processes (Kingston-Smith *et al.* 1997), it is expected that at lower MT the dark reactions are slowed down. Lichtenthaler and Burkhart (1999) also showed that the minimum PSII efficiency factor decreased with increasing ML.

However, at a GT of 4°C, the minimum PSII efficiency factor levels were not different from the minimum PSII efficiency factor levels at 16°C and 8°C. This contrasts with the maximum NPQ, which showed lower levels at lower GT. Industrial chicory plants acclimated to low GT seem to sustain the stress conditions better than plants grown at higher (normal) temperatures.

Also the time required to achieve the maximum NPQ depended highly on the stress conditions applied. Lambrev *et al.* (2007) attributed retardation in NPQ induction to the fact that activation of NPQ requires structural reorganization of one or more proteins in the thylakoid membrane. Factors which limit this structural change, such as low temperatures, may limit the NPQ transition. This can explain the slow rate of NPQ formation at low temperatures. A similar reaction was also found for chicory plants measured at temperatures near the GT in combination with high ML. Apparently, for industrial chicory, higher ML cause the same impairment in the reorganization of the proteins to activate NPQ as low temperatures do.

The formation rate of NPQ depended also on the GT. For plants grown at lower temperatures a faster induction of NPQ was recorded when low MTs were applied. Most probably the plants acclimated to lower GT, allowing them to protect their internal processes faster and more efficiently than plants grown at 16°C. This effect could be mediated a higher capacity to de-epoxidate violaxanthin to form zeaxanthin, by a swifter proton transfer over the thylakoid membrane, or by a faster reorganization of proteins in the thylakoid membrane (Lambrev *et al.* 2007).

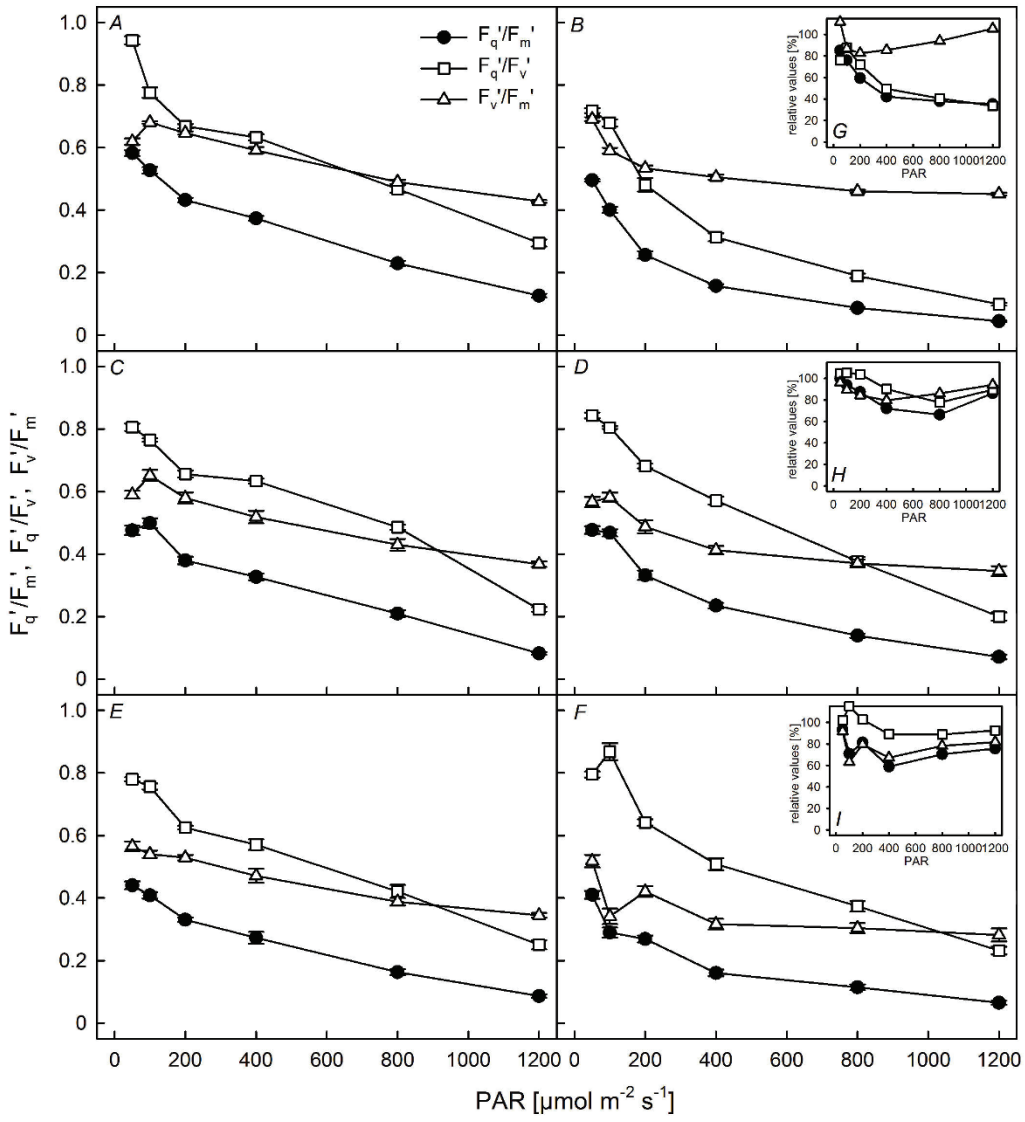

Fig. 5. The light-response curve of the operating quantum efficiency of PSII photochemistry (F_q'/F_m'), the maximum quantum efficiency of PSII photochemistry in light-adapted state (F_v'/F_m'), and the PSII efficiency factor (F_q'/F_v'), for industrial chicory plants (variety 'Hera') grown and measured at 16°C (*A*), grown at 16°C and measured at 2°C (*B*), grown and measured at 8°C (*C*), grown at 8°C and measured at 2°C (*D*), grown and measured at 4°C (E), grown at 4°C and measured at 2°C (*F*). (*G*), (*H*), (*I*) show the relative changes observed at a MT = 2°C with respect to MT = GT. n = 14, mean ± SE.

All the data together illustrate that young industrial chicory plants are much affected as the ML rises above 200 µmol m^{-2} s^{-1} and the MT declines below 8°C. Only these "severe" conditions have a significant effect on the PSII efficiency. The damage that these conditions cause to PSII should be analyzed by relaxation dynamics studies.

Light induction parameters: The lower the GT, the lower the PSII efficiency was in darkness. This slowing down of the PSII efficiency at lower GT is probably due to a decreased activity of the photochemical quenching processes or to photodamage under these conditions (Baker and Rosenqvist 2004).

It has been described (Genty *et al.* 1989, Baker and Oxborough 2004) that the PSII operating efficiency (F_q'/F_m') can be calculated as the product of the PSII maximum efficiency (F_v'/F_m') and the PSII operating efficiency (F_q'/F_v'). As the ML increased, we found that the PSII operating efficiency was much more influenced by changes in the ability of processes downstream of PSII to use the products of electron transport than by the PSII maximum efficiency. This finding is in accordance with the data of Lawson *et al.* (2002). The effect of an MT lower than the GT differed in dependence of the GT. The PSII operating efficiency of plants grown at 16°C was lower when measured at 2°C than when measured at 16°C. This change was mainly due to changes in the

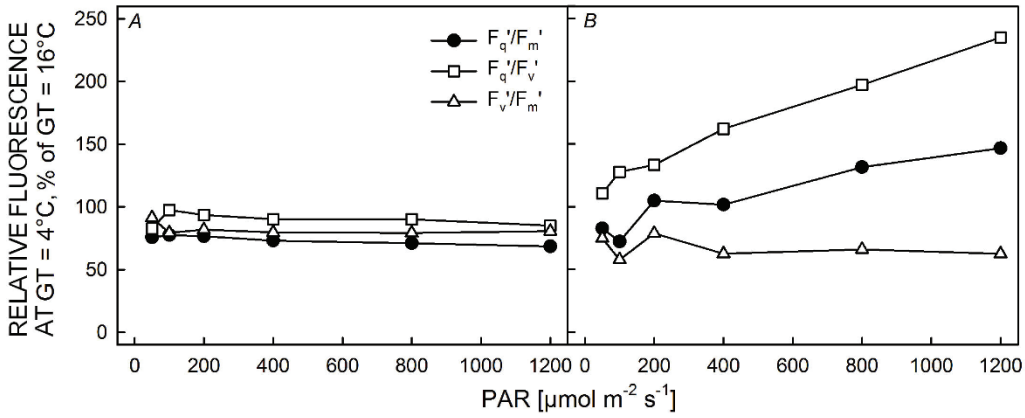

Fig. 6. Relative difference of the light response of the fluorescence parameters [operating quantum efficiency of PSII photochemistry (F_q'/F_m'), the maximum quantum efficiency of PSII photochemistry in light-adapted state (F_v'/F_m'), and the PSII efficiency factor (F_q'/F_v')] of industrial chicory plants (variety 'Hera') (*A*) grown at GT = 4°C (and measured at MT = 4°C) and plants grown at GT = 16°C (and measured at MT = 16°C), and (*B*) plants grown at GT = 4°C (and measured at MT = 2°C) and plants grown at GT = 16°C (and measured at MT = 2°C).

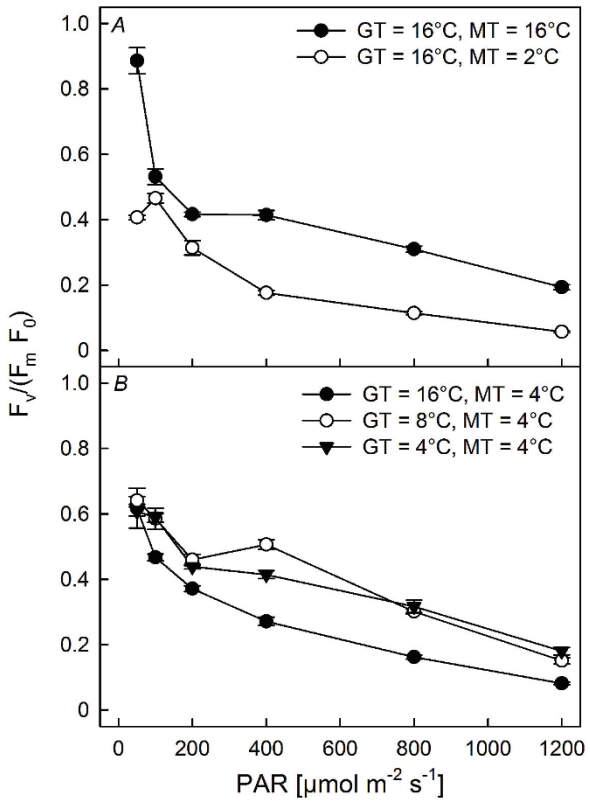

Fig. 7. The light-response curve of $F_v/(F_m \cdot F_0)$ (related to the number of reaction centers still capable to perform photochemistry) of plants of the variety 'Hera' (*A*) grown at 16°C and measured at 16°C or 2°C, and (*B*) grown at 16°C, 8°C or 4°C and measured at 4°C. GT – growth temperature, MT – measurement temperature. n = 14, mean ± SE.

ability of processes downstream of PSII to utilize the products of electron transport. Probably low temperatures affect the rate of the dark reactions, causing a large decrease in the capacity to consume ATP and NADPH. For plants grown at 8°C and measured at 2°C, the changes in the PSII operating efficiency were caused by a processes downstream of PSII and a downregulation of PSII. For plants grown at 4°C and measured at 2°C, the lower MT caused a decrease in the PSII operating efficiency mainly due to a downregulation of PSII. This shift from a downstream reaction to a downregulation of PSII was very clear as GT was lowered. Furthermore, an acclimation effect was found when plants grown at 4°C and measured at 2°C were compared to plants grown at 16°C and measured at 2°C. For ML higher than 100 µmol m^{-2} s^{-1}, the ability of processes downstream of PSII to use the products of the electron transport was much improved in plants that had been grown at low GT. This was combined with a downregulation of PSII irrespective of the ML. It seems that the plants adjusted both processes: light capture became less efficient and dark reactions or other users of ATP and NADPH were stimulated. An increase in the activity of the Calvin cycle enzymes as result of a cold acclimation has also been found for herbaceous species (Koroleva *et al.* 1994). A possible mechanism to improve the efficiency of the dark reactions is to increase the proportion of unsaturated fatty acids in the membranes of cell organelles, like the chloroplasts. The consequently less rigid membranes could allow a better functioning of the embedded enzymes for the dark reactions (Palta *et al.* 1993, Kodama *et al.* 1995). However, in this study the saturation level of the fatty acids in the membrane was not measured.

$F_v/(F_m \cdot F_0)$ declined with decreasing MT and increasing ML in industrial chicory. The effect of temperature on this parameter was previously reported by Govindachary *et al.* (2004) for cucumber. Our results also demonstrate that the acclimation to lower GT diminishes the effect of the applied stress conditions on the capability of the reaction centers to perform photochemistry.

In general, the light induction parameters investigated

here indicate how the conditions applied affected PSII photochemistry. However, the analysis of the subsequent relaxation parameters is necessary to determine the damage to the PSII efficiency.

Conclusion: The main objective of this study was to better understand the response of young industrial chicory plants to low temperature and/or high light intensity stress using the variety 'Hera'. We used Chl *a* fluorescence imaging to evaluate the tolerance of young chicory plants to the particular stress conditions investigated. In this article, the parameters related to the light induction kinetics of the Chl *a* fluorescence process were analyzed and discussed in detail.

We found that parameters such as the PSII operating efficiency (including PSII maximum efficiency and PSII efficiency factor) and NPQ are important to evaluate the effect of stress in terms of severity, processes that are affected, and acclimation to lower GT. The results clearly demonstrated that young industrial chicory plants can adapt to lower GT. Despite the light induction parameters, PSII efficiency factor and non-photochemical quenching processes indicating the immediate effect of the applied stress on the PSII efficiency, a detailed analysis of the subsequent relaxation kinetics in darkness is necessary to fully understand the plant responses (Lootens *et al*. 2011).

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