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Growth and Development Temperature Influences Level of Tolerance to High Light Stress[1]

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ABSTRACT

The influence of growth and development temperature on the relative tolerance of photosynthetic tissue to high light stress at chilling temperatures was investigated. Two tuber-bearing potato species, *Solanum tuberosum* L. cv Red Pontiac and *Solanum commersonii* were grown for 4 weeks, at either 12 or 24°C with 12 hours of about 375 micromoles per second per square meter of photosynthetically active radiation. Paired leaf discs were cut from directly across the midvein of leaflets of comparable developmental stage and light environment from each species at each growth temperature treatment. One disc of each pair was exposed to 1°C and about 1000 micromoles per second per square meter photosynthetically active radiation for 4 hours, and the other disc was held at 1°C in total darkness for the same duration. Photosynthetic tissue of *S. tuberosum*, developed at 12°C, was much more tolerant to high light and low temperature stress than tissue developed under 24°C conditions. Following the high light treatment, 24°C-grown *S. tuberosum* tissue demonstrated light-limited and light-saturated rates that were approximately 50% of their paired dark controls. In contrast, the 12°C-grown tissue from *S. tuberosum* that was subjected to the light stress showed only a 18 and 6% reduction in light-limited and light-saturated rates of photosynthetic oxygen evolution, respectively. Tissue from 24°C-grown *S. commersonii* was much less sensitive to the light stress than was tissue from *S. tuberosum* grown under the same conditions. The results presented here demonstrate that: (a) acclimation of *S. tuberosum* to lower temperature growth conditions with a constant light environment, results in the increased capacity of photosynthetic tissue to tolerate high light stress at chilling temperature and (b) following growth and development at relatively high temperatures *S. commersonii*, a frost- and heat-tolerant wild species, has a much greater tolerance to the high light stress at chilling temperature than does *S. tuberosum* cv Red Pontiac, a frost-sensitive cultivated species.

An excess of incident light energy can result in injury to photosynthetic systems (10). Injury to the photosynthetic apparatus is proposed to occur whenever rates of light energy harvest and trapping exceed rates of light energy utilization in metabolic processes (9). A pronounced interaction has been demonstrated between tissue temperature and the threshold of incident light energy at which light-dependent injury occurs in photosynthetic systems (photoinhibition). A number of studies have shown that a sharp decrease in tissue temperature results in a dramatic increase in the susceptibility of photosynthetic processes to injury by a given incident light level (8, 11). In previous work (14), we have demonstrated a direct relationship in potato species between the relative capacity to increase frost tolerance during exposures to low, nonfreezing temperatures and the relative degree of tolerance to light stress at low temperature.

The capacity of photosynthetic systems to adjust light harvesting efficiency in response to the light environment is well known (1, 12). The development of photosynthetic leaf tissue under low, frost-acclimating temperatures results in significant structural alterations in a number of key proteins or protein complexes including those involved in both light energy harvest and light energy utilization (2, 3). We have evidence that photosynthetic tissue adapted to a lower growth temperature and a constant light level has a decreased efficacy of light energy harvest and trapping as well as an increased capacity to utilize trapped light energy relative to tissue developed at a higher growth temperature (15).

Collectively these findings suggested, although it has never been demonstrated, that acclimation to lower growth temperatures would result in an increased capacity to tolerate high light stress at chilling temperatures. Our evidence also suggests that a potato species such as *S. commersonii*, which is able to frost-harden at low, nonfreezing temperatures, would have a greater tolerance to the high light stress than would the cultivated species *S. tuberosum*, which cannot frost-harden. To test these hypotheses, we utilized a treatment chamber to provide a high light and low temperature stress to leaf tissue from two potato species which differ in cold-tolerance characteristics and were developed under two different growth temperature conditions.

MATERIALS AND METHODS

Plant Material

Two tuber-bearing *Solanum* species, the cold-tolerant *S. commersonii* (PI 472834) and the cold-sensitive *S. tuberosum* cv Red Pontiac, were used in these studies. Clonal lines were propagated from a single seed of each species in order to obtain genetically homogeneous plant material. Three-week-old rooted stem cuttings were transplanted to 1:1 (v/v) peat and vermiculite (Jiffy Mix, JPA, West Chicago, IL) in 8 L pots. Plants were allowed to establish for 2 weeks at 18°C, 70 to 80% relative humidity and a 12 h photoperiod of about 375 $\mu mol \cdot s^{-1} \cdot m^{-2}$ photosynthetically active radiation (PAR) at the canopy level. After the establishment period, plants

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were transferred to growth temperatures of either 12 or 24°C for 4 weeks with all other environmental conditions held constant. At the end of the treatment period, terminal leaflets which were expanded in area to 80 to 90% of the largest leaflets on that particular plant and exposed to 375 ± 25 PAR, were selected for use in assays.

Light and Low Temperature Stress Treatment

Leaf tissue was subjected to low temperature and either high light or darkness in a chamber described previously (16). One pair of leaf discs (13 mm diameter) was cut from directly across the midvein in the center of each leaflet, with one disc used in the light stress treatment and the other as the dark control. Leaf discs were floated in wells on a buffer maintained at 1°C consisting of 0.5 mM $CaSO_4$, 20 mM $NaHCO_3$, 50 mM Hepes-KOH at pH 7.2. One leaf disc of each pair was placed into the light compartment at an incident light intensity ranging from 750 to 1250 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR for 4 h and the other disc placed into the dark compartment also for 4 h. Variations in light stress levels were distributed equally over all treatments. Following the 4 h treatment all discs were held on ice in the dark until assays could be conducted. Previous studies had shown that injured or healthy leaf tissue, when held in the dark at 0 to 4°C, maintained its initial capacity to fix carbon dioxide for several hours (13).

Respiratory and Photosynthetic Assays

Respiratory O_2 uptake and light-limited and light-saturated photosynthetic O_2-evolution were assayed at 18°C in an oxygen electrode chamber containing saturating levels of bicarbonate (14). First, O_2 uptake was measured in the darkened electrode chamber for 8 min, followed by measurements of light-limited photosynthetic O_2 evolution at 114–121 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR for 7 min and then light-saturated photosynthetic oxygen evolution at 1500 to 1750 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR for 18 min. Light level was regulated with neutral density gradients. The photosynthetic light response curve for our system and the plant tissue utilized was linear within the range of 22 to 203 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR. Oxygen uptake and evolution rates from light-treated tissue were expressed as a percentage of paired dark control tissue. The results presented here are from a single experiment and substantiated results obtained during preliminary tests.

Chl Determination

Following assays, Chl was extracted with 96% ethanol, and then was quantitated spectrophotometrically (17).

RESULTS

Photoinhibitory treatments had dramatically different effects on leaf tissue of *Solanum tuberosum* developed at the two different temperatures (Table I). For example, tissue of *S. tuberosum* developed at 12°C showed about a 18 and 6% inhibition of light-limited and light-saturated photosynthesis, respectively, while tissue developed at 24°C showed an approximately 50% inhibition of both light-limited and light-saturated photosynthesis as compared to the dark control. By contrast, leaf tissue from *S. commersonii* grown at either 12 or 24°C showed a similar light-limited and light-saturated photosynthetic response to the light and low temperature stress (Table I). The high light treatment had no significant inhibitory effect on the respiratory oxygen uptake rates of leaf tissue from any of the species developed at either of the growth temperature treatments and, in fact, appeared to be slightly stimulatory (Table I).

Light-limited assays of photosynthetic oxygen evolution gave a more sensitive indication of the light-dependent inhibition of photosynthetic function. Light-stressed tissue which showed 6 to 10% inhibition of light-saturated photosynthesis showed a 18 to 29% inhibition of light-limited photosynthesis as compared to dark controls, with the exception of *S. tuberosum* grown at 24°C (Table I).

In both species, light-saturated photosynthetic capacity was greater in tissue developed at the lower growth temperature. In tissue grown at 12°C, relative to 24°C-grown tissue, there was a 43% increase in *S. tuberosum* and a 90% increase in *S. commersonii* (Table I). Conversely, both species demonstrated a decline in light-limited photosynthetic capacity (efficiency) in tissue developed at the lower growth temperature. This

Table I. *Effect of Photoinhibitory Treatment (average PAR of 1000 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at 1°C for 4 h) on Respiratory Oxygen Uptake and Gross Light-Limited (114–121 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR) and Light-Saturated (1500–1750 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR) Photosynthetic Oxygen Evolution*

Leaf tissue used in stress treatment was from *S. commersonii* and *S. tuberosum* grown at either 12 or 24°C for 4 weeks. Assays were conducted at 18°C, calculated on a Chl basis and rates from light-stressed leaf discs were expressed as a percentage of their paired dark control and shown as means ± SE, $n = 3$.

Species	Growth Temperature	Respiration	Gross Light-Limited Photosynthesis	Gross Light-Saturated Photosynthesis
	°C		*% of dark control*	
S. tuberosum	12	97 ± 1.8 (9)[a]	82 ± 3.6 (22)	94 ± 1.7 (93)
	24	112 ± 14.2 (5)	51 ± 3.4 (23)	53 ± 13.2 (65)
S. commersonii	12	105 ± 1.1 (11)	79 ± 5.2 (25)	90 ± 6.3 (99)
	24	105 ± 8.2 (5)	71 ± 9.6 (31)	93 ± 4.1 (52)

[a] Actual dark control rates (μmol oxygen/mg Chl h^{-1}) are shown in parentheses.

relative decline, in 12°C relative to 24°C-grown tissue, was 4% in *S. tuberosum* and 19% in *S. commersonii* (Table I).

The exposure of leaf tissue to high light levels had no effect on Chl content per area as compared to tissue from the same leaflet which was held in the dark (Table II). In general, Chl content per area, was about 10% less in 12°C-grown than 24°C-grown tissue. Also, *S. commersonii* had about 10% less Chl than *S. tuberosum*, at each growth temperature treatment.

DISCUSSION

The data presented here show, for the first time, that the acclimation of photosynthetic tissue to low temperature results in increased tolerance to high light stress, relative to tissue developed at a higher growth temperature. This was seen in *S. tuberosum* where tissue developed at 12°C for four weeks had a much higher level of tolerance to the high light treatment than the tissue developed at 24°C (Table I). Very little attention has been paid to a light stress component during acclimation of cold- and particularly frost-tolerant plants to low, nonfreezing temperatures. We have demonstrated that a pronounced light-dependent injury does occur in frost-tolerant potato species during standard acclimation procedures in which temperature is shifted from 20 to 5°C with light level held constant (14). This is not surprising when one considers that the utilization of trapped light energy is temperature dependent in such processes as electron transport and carbon metabolism, while the harvest and trapping of light energy is essentially temperature independent (9). Clearly, dramatic adjustments must occur within the photosynthetic apparatus if it is to maintain a balance in the flux of light energy through the system. In many respects, a plant exposed to either a sudden decrease in temperature or a sudden increase in light intensity faces the same problem: chloroplasts with an overdeveloped light-trapping capacity with respect to their capacity to metabolically utilize and thus dissipate trapped light energy (15). Plant adaptations which would reduce light trapping capacity and/or increase the capacity for light energy utilization would appear to provide greater protection of the photosynthetic apparatus during sudden changes in the light and/or temperature environment. The results presented here also suggest that, in fact, both types of adjustments occur (Table I). Both species showed a substantial increase in light-saturated photosynthetic capacity (light energy utilization) and *S. commersonii* demonstrated a marked decrease in light-limited photosynthesis (light harvesting efficiency).

The present work also shows that light-limited photosynthesis is a more sensitive indicator of initial photoinhibitory injury than is light-saturated photosynthesis. In tissue of *S. commersonii* grown at 12 or 24°C and tissue of *S. tuberosum* grown at 12°C, there is very little inhibition of light-saturated photosynthesis (less than 10%) but an approximately 20% inhibition of light-limited photosynthesis in the same tissue (Table I). This is in agreement with what has been reported in other studies of short-term photoinhibitory stress (8) and is indicative of the inactivation of photosynthetic reaction centers (4, 6, 7). The fact that respiration is not affected by the light stress suggests that the light-dependent injury may be localized to the chloroplast and does not directly effect other cellular compartments.

A greater tolerance in tissue from *S. commersonii* developed at 24°C compared to similar tissue from *S. tuberosum* is also apparent (Table I). The results show that the threshold at which massive inhibition of photosynthetic processes occurs has not yet been reached in tissue of *S. commersonii* with the stress treatments used here. *S. commersonii* is much more tolerant of freezing stress in the unacclimated state (*i.e.* grown at 20°C) and dramatically increases its freezing tolerance during low temperature acclimation, whereas *S. tuberosum* is unable to increase its frost tolerance (5). Another frost-tolerant wild species which is able to acclimate, *S. acaule*, has been shown to be more tolerant of a light-stress component during low temperature acclimation than is *S. tuberosum* (14). When considering tissue development at 12°C relative to 24°C-grown tissue, the magnitude of the photosynthetic adjustment is greater in tissue from *S. commersonii* than *S. tuberosum*, with a 90 *versus* 43% increase in light-saturated photosynthesis and a 19 *versus* 4% decline in light-limited photosynthesis. However, in tissue developed at 24°C, the actual values for the species appear slightly more favorable for *S. tuberosum* which has the greater light-saturated photosynthetic capacity and a lesser light-limited efficiency than *S. commersonii*.

Differences in photosynthetic parameters at the two growth temperatures appear to be general responses with differences between the species due, in part, to the magnitude of the response. Clearly other protective mechanisms are involved, possibly both at the tissue and molecular level. The work presented here emphasizes that plant responses to the light and temperature environments are interdependent and that the ability of photosynthetic tissue to acclimate to low temperature may depend to some extent upon its ability to respond to the associates changes in the light energy 'budget.'

Table II. *Chl Content of Leaf Tissue from Two Different Potato Species Grown at Two Growth Temperatures and Subjected to High Light Stress at 1°C*

Species	Growth Temperature	Chl Content Following Light Stress Treatment	
		Light	Dark
	°C	μg/cm²	
S. tuberosum	12	41 ± 2.9[a]	41 ± 3.2
	24	43 ± 2.1	45 ± 2.6
S. commersonii	12	36 ± 0.6	36 ± 1.5
	24	40 ± 2.3	40 ± 1.0

[a] Means ± SD, $n = 3$.

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