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Plastome from high-altitude *Lycopersicon hirsutum* does not improve low-temperature tolerance of cold-sensitive *Lycopersicon esculentum*.

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Introduction

Chloroplasts have conserved their own genome (plastome) during evolution. The plastome consists of approximately 100 genes, encoding photosystem PSI and PSII proteins, components of the photosynthetic electron transport machinery, subunits of the ATP-synthase complex, the large subunit (LSU) of ribulose-1,5-bisphophate carboxylase/oxygenase (Rubisco) and its own replication, transcription and translation system (Hall and Rao 1994). Compared with the domestic tomato (*Lycopersicon esculentum* Mill.), chloroplast functioning of high-altitude accessions of *L. hirsutum* Humb. & Bonpl. is more tolerant to chilling in the dark (Walker et al. 1990; Venema et al. 1999a). Also, photosynthetic performance of *L. hirsutum* recovered faster after exposure to chilling (Yakir et al. 1986; Venema et al. 1999b). Due to the inability of *L. esculentum* pollen to fertilise ovules of wild *Lycopersicon* species (unilateral incongruity) hybrids derived from crosses of tomato and related wild species contain chloroplasts from the *L. esculentum* parent (Wolters et al. 1994). Derks (1992) circumvented this problem by fusion of protoplasts of a cytoplasmic albino mutant with gamma-irradiated protoplasts from a high-altitude accession of L. hirsutum (cybridisation). The aim of the present study was to investigate the role of the chloroplast in suboptimal-temperature tolerance by comparing growth and photosynthesis of an alloplasmic tomato cybrid of *L. esculentum* c.v. LRC with chloroplasts from a more cold-tolerant *L. hirsutum* accession (LA 1777) with the performace of both euplasmic parents.

Materials and methods

Plant Material

An alloplasmic line (AH47) with the nuclear genome of the chilling-sensitive cytoplasmic albino mutant of *L. esculentum* Mill. cv. Large Red Cherry (LRC) and the plastid genome (plastome) from the more chilling-tolerant *L. hirsutum* Humb. & Bonpl. LA 1777 (Derks 1992) was used and compared with the euplasmic parents. Seeds of genotypes were kindly provided by Plant Research International, Wageningen, The Netherlands.

Growth conditions

Seedlings were grown in a glasshouse at a 25/20°C day/night temperature regime. After 18 days for the euplasmic and alloplasmic tomato, and after 32 days for the slower-germinating *L. hirsutum*, plants were randomly distributed over two adjacent glasshouses with an optimal (25/20°C) or suboptimal (16/14°C) day/night temperature regime. Plants were grown under natural light supplemented with light from 400 W Philips HPI-T lamps, at a minimum photosynthetic photon flux density of 225 μmol m^{-2} s^{-1} during a 12-h photoperiod.

CO_2 exchange

Light response curves of CO_2 exchange were measured on attached leaves in a leaf chamber (LSC-1, Analytical Development Co., Hoddesdon, Herts, UK), by infrared gas analysis (model 225 MK3, ADC) in an open system with 350 µl l^{-1} CO_2 at 16 and 25°C. White light was provided by a projection lamp. Neutral-density filters created different PPFDs.

Measurement of photosynthetic oxygen evolution

Oxygen evolution was measured on two 11 mm diameter leaf discs in a temperature-controlled leaf chamber with a Clark-type electrode according to Delieu and Walker (1981). Light- and CO_2-saturated oxygen evolution (P_{max}) was determined at a saturating PPFD of 1200 µmol m^{-2} s^{-1} at 5, 15 and 25°C.

Determination of susceptibility to photoinhibition

Photoinhibition treatments were performed under a moderate PPFD (300 µmol m^{-2} s^{-1}) at low temperature (2.5 ± 0.5°C) (van Wijk and van Hasselt 1993). Chlorophyll *a* fluorescence of leaf discs (11 mm diameter) was measured using a pulse-amplitude fluorometer (PAM-101/-103, H. Walz GmbH, Effeltrich, Germany). Chilling-induced photoinhibition was analysed as the time-dependent loss of maximum quantum efficiency of PSII, (F_v/F_m) at 20°C after 15 min of dark adaptation.

Results and discussion

The cybrid line AH47 was phenotypically similar to *L. esculentum* and displayed no visible *L. hirsutum* traits. Shoot biomass production showed similar patterns in the euplasmic

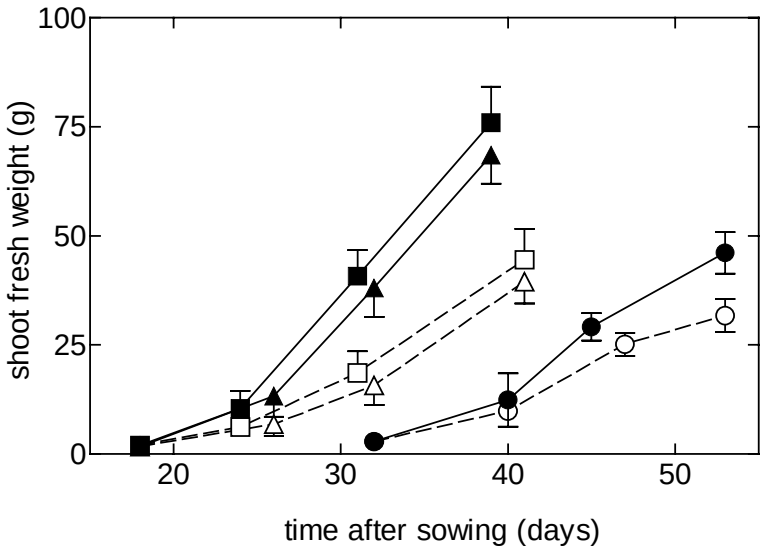

Figure 1. Time-dependent increase of shoot fresh weight of *L. esculentum* cv. LRC (■, □) cybrid AH47 (▲, Δ) and *L. hirsutum* LA 1777 (●, ○) grown in an optimal (25/20°C; closed symbols) and suboptimal (16/14°C; open symbols) day/night temperature regime. Data represent means ± SD, n = 3

L. esculentum and alloplasmic tomato line AH47. At the final harvest, shoot FW was not significantly different between euplasmic and alloplasmic tomato, either at optimal, or at suboptimal temperature. Shoot biomass production of the plastome donor *L. hirsutum* was less inhibited by suboptimal temperature.

The light response curve of net CO_2 uptake in leaves of the cybrid resembled the curve obtained from leaves of *L. esculentum* but in *L.hirsutum* saturation occurred at a higher light intensity irrespective of growth temperature. (Fig. 2A, B).

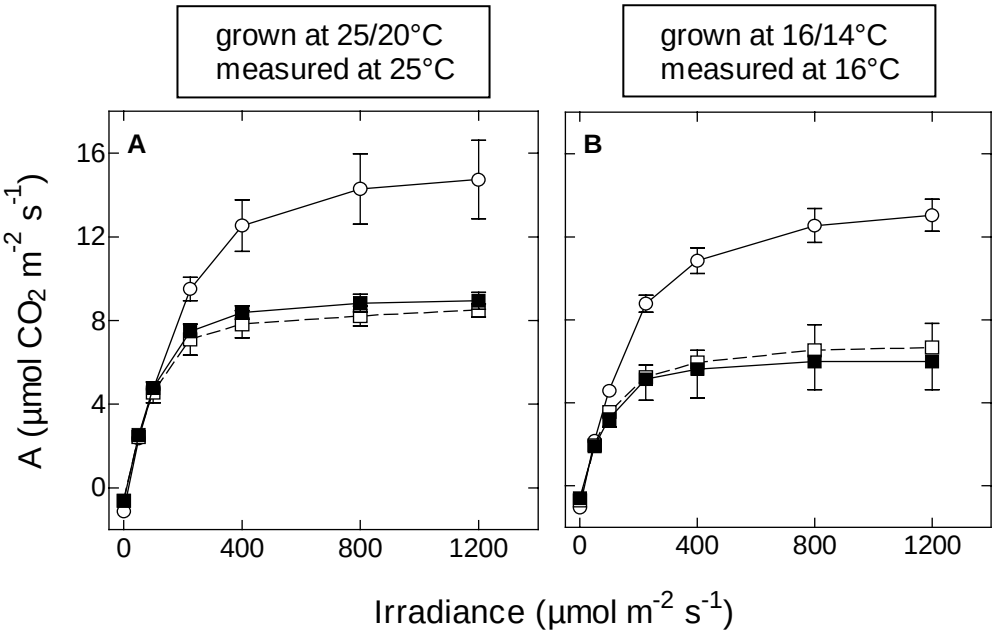

Figure 2. Light-response curves of youngest fully expanded leaves of *L. esculentum* cv. LRC (■), cybrid AH47 (□) and *L. hirsutum* LA 1777 (○) grown in an optimal and suboptimal temperature regime. Measurements on plants grown atoptimal and suboptimal temperature were performed at 25 and 16°C, respectively. Data represent means ± SD, n = 3.

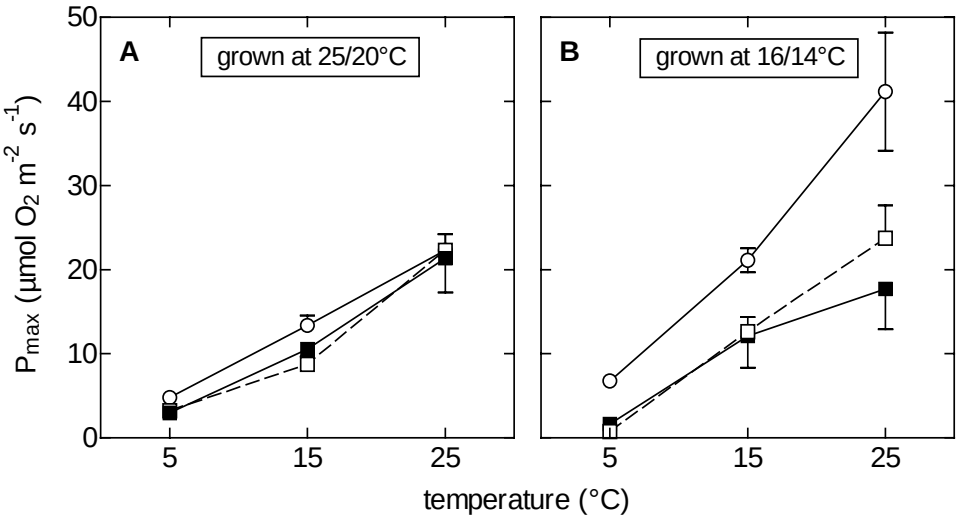

Figure 3. Temperature-dependence of the maximum photosynthetic capacity (P_{max}) of fully expanded leaves of *L. esculentum* cv. LRC (■), cybrid AH47 (□) and *L. hirsutum* LA 1777 (○) grown at optimal (A) and suboptimal (B) temperature regime. Values represent means ± SD, n = 3.

Leaves of euplasmic and alloplasmic tomato plants grown at optimal temperature showed comparable temperature dependencies of P_{max}. In *L. hirsutum* grown at optimal temperature, higher P_{max} values ($P \leq 0.05$) were apparent at 15°C and at 5°C than in *L. esculentum* and the cybrid (Fig. 3A). *L. hirsutum* showed significantly higher P_{max} values ($P \leq 0.05$) at the three assay temperatures in leaves of plants grown at suboptimal (Fig. 3B) compared to optimal temperatures (Fig. 3A). This acclimation effect was absent in *L. esculentum* and the cybrid.

The time course of the decrease of F_v/F_m during chilling in the light (Fig. 4) showed that the alloplasmic tomato was more susceptible to chill-induced photoinhibition than both its nuclear and plastome parent, which showed a similar susceptibility.

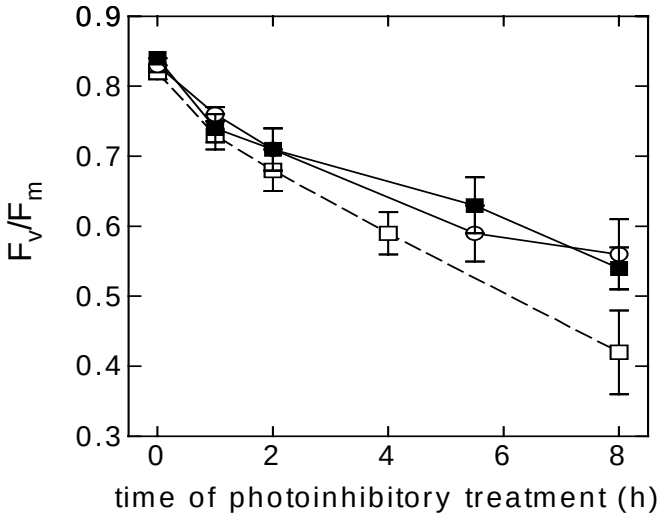

Figure 4. Time-dependent decrease of the maximum quantum yield of PSII electron transport (F_v/F_m) during illumination with 300 µmol m^{-2} s^{-1} at 2.5 ± 0.5°C in discs punched from the youngest fully expanded leaves of *L. esculentum* cv. LRC (■), cybrid AH47 (□) and *L. hirsutum* LA 1777 (○) grown in an optimal (25/20°C) day/night temperature regime. Data represent means ± SD, n = 5.

Discussion

The results of this study show that the *L. hirsutum* plastome did not affect low temperature tolerance of photosynthesis in the tomato cybrid. This indicates that low temperature tolerance of photosynthesis is controled by the nuclear genome, which encodes 80–90% of the chloroplast proteins. In agreement with our results, plastid exchange had no marked effect on chloroplast functioning in *Oenothera* cybrids before and after long-term chilling (Daubhorn and Brüggemann 1996).

The observation that the cybrid was more susceptible to chill-induced photoinhibition of PSII than *L. esculentum* and *L. hirsutum*, suggested that cybridisation rendered the chloroplast more susceptible to light stress. Enhanced sensitivity to high-light stress has been reported also for cybrids of *Nicotiana tabacum* (Peter et al. 1999). In contrast to LRC and the cybrid, P_{max} of *L. hirsutum* was significantly higher in response to growth at suboptimal temperature. Cold hardening of spring and winter wheat cultivars also resulted in a comparable differential acclimation potential of P_{max} (Hurry et al. 1995).

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