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BRIEF COMMUNICATION

Analysis of the gas exchange components in chilled tomato plants

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Abstract

A positive linear relationship between the net CO_2 exchange rate (P_N) and the leaf stomatal conductance (g_s) under an optimal temperature, and even more distinct one after a short-term chilling (CH, 15-17 h, 2 °C in darkness), that was found in two tomato cultivars (sensitive to a low temperature cv. Robin and tolerant cv. New Yorker) suggested a partial stomatal limitation of photosynthesis. The CH treatment of cv. Robin resulted in an intercellular CO_2 concentration (C_i) increase because of which a negative correlation between C_i and P_N was observed. In cv. New Yorker a positive correlation was observed. Detrimental effect of the low temperature in cv. Robin was more evident in plants with a relatively small root system (SR), but drought-hardening positively affected the response to CH only in the plants with bigger roots (BR). On the contrary, in cv. New Yorker the favourable effect of such pre-treatment was more evident in SR than in BR plants.

Additional key words: drought acclimation; intercellular CO_2 concentration; low temperature stress; *Lycopersicon esculentum;* net photosynthetic rate; stomatal conductance.

The chilling (CH) affects photosynthesis of a sensitive plant in a complex way. In tomato plants, the inhibition of photosynthesis is an early response even to a short-term low temperature exposure, with different recovery times proportional to the stress detrimental effects (Martin and Ort 1985) and pre- and post-chilling conditions (Starck *et al.* 1994a,b). The CH in the dark appears less damaging and more reversible than it does in the light (Martin and Ort 1982, 1985) where the

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photoinhibition of photosynthesis and photooxidative impairment can be induced. Moreover, the dark CH probably leads toward inhibition of the PS2 water-splitting reaction due to the loss of extrinsic membrane proteins and manganese (Shen *et al.* 1990). These reactions are different in various genotypes (Janssen *et al.* 1995).

Because the low temperature affects chloroplasts directly, stomatal closure is only partly responsible for the P_N decrease. The response of stomata to CH depends on other environmental factors including water availability, air humidity, irradiance, and time of the day (Guye and Wilson 1987, King and Reid 1987, McKersie and Leshem 1994). The effect of CH can rarely be separated from the shoot water deficit due to impairment of the root water uptake (Wilson 1976, McWilliam *et al.* 1982). However, after several hours the plants are able to rehydrate. The turgor recovery is associated perhaps with ABA accumulation and with stomatal closure (Pardossi *et al.* 1992). Moreover, the CH effect seems to be alleviated by a moderate drought acclimation (Wilson 1976, Irigoyen *et al.* 1994, Starck *et al.* 1994a).

Under a mild CH, relatively more of dry matter is allocated to the root, similarly as in a drought stress (Stamp 1994). Nevertheless, it is still not clear whether this change reflects a prerequisite for acclimation to CH. However, the CH treatment of a tomato cultivar less sensitive to CH (New Yorker) causes an increase of biomass partitioning to the roots especially in plants with SR system (Starck *et al.* 1994a,c). On the contrary, after CH the root growth was drastically reduced in the more sensitive tomato cv. Robin. CH also drastically influences root functions concerning the nutrient uptake and transport (Engels *et al.* 1992, Engels and Marschner 1992), thus the level of low temperature sensitivity could be related to the root size and activities.

Our previous results (Starck *et al.* 1994a) showed that P_N drastically decreased just after CH, especially in the cv. Robin, but it almost recovered after 24 h already. In chilled Robin plants with SR, the P_N was completely inhibited but in the cv. New Yorker it was less affected. The favourable effect of drought-hardening on the CH susceptibility was observed only in plants with BR (cv. Robin) or SR (cv. New Yorker). In the present paper an attention was paid to detailed analysis of the relationship between P_N, g_s and C_i to find the factor limiting photosynthesis in consequence of the CH.

Tomato cultivars Robin and New Yorker were grown during springtime under the greenhouse supplemental lighting (high pressure sodium vapour lamps, irradiance about 350 μmol m^{-2} s^{-1}). They were grown in pot-rings with fertilised and optimally watered peat. During 3 or 4 (cv. Robin and cv. New Yorker, respectively) weeks, the roots were permitted to protrude through the peat to the pot, with Hoagland solution changed once a week. Afterwards, plants with protruded root system were sorted into SR and BR groups. One half of the SR or BR plants of both cultivars was hardened by pre-treatment with limited water deficit during five successive days. Drought stress was imposed by ceasing peat watering in parallel with keeping protruded roots 2 h daily out of the solution. After the treatment, non-hardened (NH) and hardened (H) plants were transferred into a dark phytotron chamber with a high humidity for 15 or 17 h (Robin and New Yorker, respectively), either to the temperature of 16 °C (non-chilled - NCH) or 2 °C (CH).

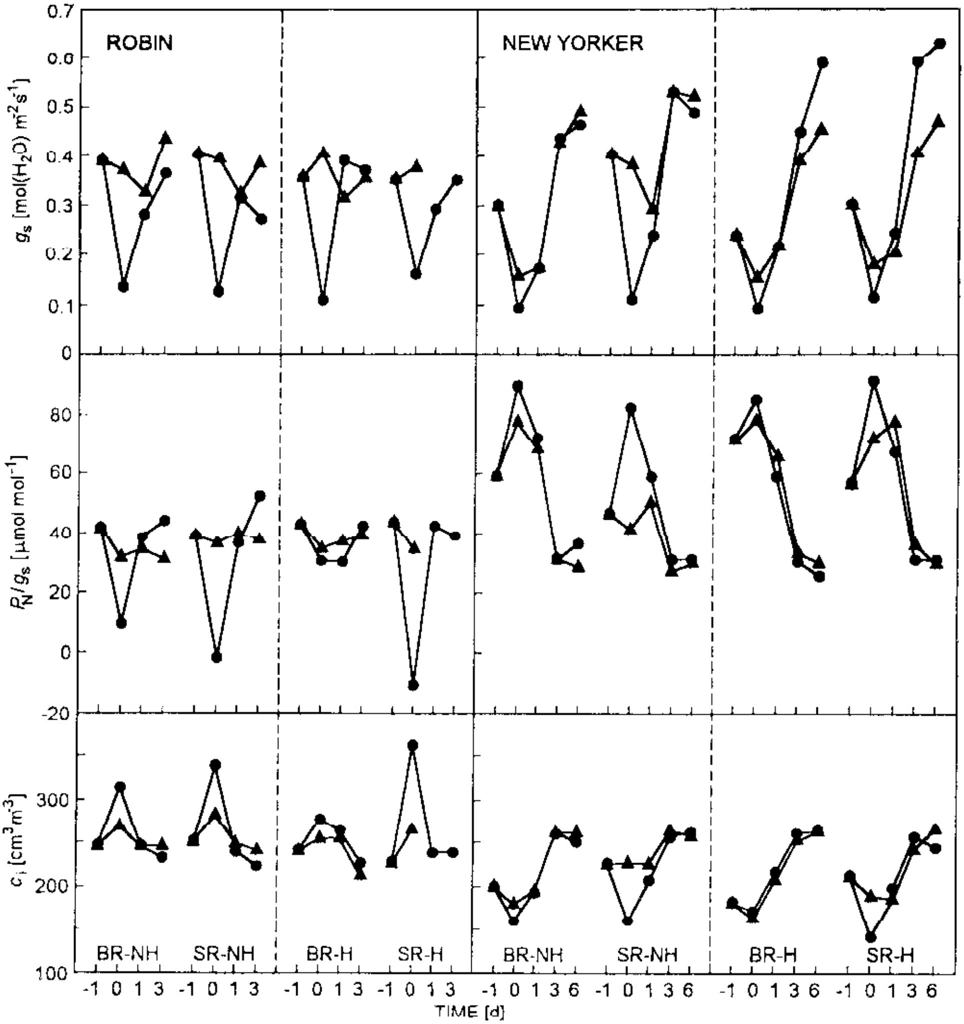

Fig. 1. The time course of leaf stomatal conductance (g_s), net photosynthetic rate to g_s ratio (P_N/g_s) and intercellular CO_2 concentration (C_i) of tomato cultivars Robin and New Yorker under normal conditions (*triangles*) and after a chilling treatment (*circles*). NH - non hardened; H - hardened by limited water deficit; BR - big root size; SR - small root size.

The P_N, g_s, and C_i values were measured by a Portable Photosynthesis System *Li-Cor* 6200 (Lincoln, USA). An attached leaflet, near to the terminal one (leaf below the cluster) was placed into a 250 cm^3 chamber and irradiated with combined natural and artificial radiation (400 W high pressure sodium lamps; 400-650 μmol m^{-2} s^{-1} PAR). The measurements were taken at a leaf temperature of 20-27 °C and 320-350 cm^3(CO_2) m^{-3}. Gas exchange parameters were estimated in each case on the same leaflets: at the end of water hardening, but one day before low temperature treatment (day -1), 2 h after chilling treatment (day 0), after one and three days of recovery in the greenhouse (days 1 and 3), and also after 6 d (only cv. New Yorker).

One day before CH there was no significant effects neither of drought-hardening nor of root size on g_s in cv. Robin (Fig. 1, *top*) but the response of cv. New Yorker was remarkable: BR and H plants had significantly lower g_s than the respective SR and NH plants. In CH plants compared to NCH plants of both cultivars, the CH induced a considerable reduction in g_s. Under NCH conditions, it was also darkness (17 h) that caused a decrease of g_s in cv. New Yorker (but with the exception of NH-SR plants). These results confirm the supposition of McKersie and Leshem (1994) that "if the chilling treatment is imposed when the stomata are closed, they tend to remain closed". The partial stomatal closure is a general CH-induced effect in tomato (Brüggemann *et al.* 1992a, Starck *et al.* 1994b), but in some cases a locking-open phenomenon is observed (King and Reid 1987). In our experiment, only 1 d after the CH, the g_s of Robin plants almost completely recovered, but in cv. New Yorker it was steadily increasing with the time during recovery in both NCH and CH plants. The maximal g_s values estimated during the recovery time were about 0.6 mol m^{-2} s^{-1} for CH New Yorker plants and only 0.4 mol m^{-2} s^{-1} for cv. Robin ones (Fig. 1, *top*). Higher maximal g_s values of cv. New Yorker are rather a result of greater stomatal opening than due to another component because this cultivar has less stomata per unit leaf area on both upper (5.7 mm^{-2}) and lower (35.7 mm^{-2}) epidermal surfaces than the cv. Robin (11.6 and 42.7 mm^{-2}, respectively). Thicker cuticle or many epidermal hairs observed in cv. Robin plants may contribute to a low g_s confirming higher drought-tolerance of this cultivar.

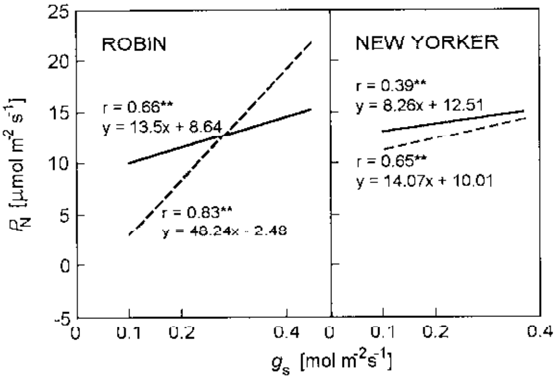

Fig. 2. Relationship between P_N and g_s under non-chilling conditions (——) and after chilling treatment (----) of two tomato cultivars.

Linear regression analysis confirmed a significant, positive correlation between P_N and g_s in both cultivars (Fig. 2) under an optimal temperature, but only a slight one in cv. New Yorker (r^2 = 14 %). In CH plants, P_N was more determined by g_s (r^2 = 69 % and 42 % for cvs. Robin and New Yorker, respectively). According to Wong *et al.* (1979), the positive linear relationship between P_N and g_s does not necessarily indicate that the stomatal aperture determines the P_N. In cucumber, an impairment of the stomatal control is responsible only for 20 % of the CH-induced inhibition of photosynthesis (Shen *et al.* 1990). P_N in tomato after a CH night is affected mainly at the level of the chloroplast, and the effect caused by reduced g_s is only small (Ort and Martin 1983). In contrast to the above-mentioned facts, our results suggest that stomatal-limitation as a post-effect of CH is important for regulation of P_N in both tomato genotypes.

According to Morgan *et al.* (1993) the P_N/g_s gives a rough evaluation of water use efficiency. The CH-caused significant drop in this ratio in cv. Robin (Fig. 1, *middle*) is due to a proportionally larger reduction of P_N (Starck *et al.* 1994a) than of g_s. This effect was strongly expressed in SR plants (significant interaction: R × CH). Hence the impairment of chloroplast reactions dominated over the chilling-induced decrease of g_s. In cv. Robin the lowest negative effect of CH on both the P_N/g_s and P_N was found in plants adapted to a water stress and with BR. The P_N/g_s of this cultivar fully recovered one day after the CH, independent on root size and drought-hardening. On the contrary, just after the CH a significant increase of P_N/g_s was observed in CH plants of cv. New Yorker (Fig. 1, *middle*). This effect was slightly more expressed in plants with SR (significant interaction: R × CH). In cv. New Yorker this ratio was steadily decreasing throughout the recovery times in both NCH and CH plants coincidentally to the increased g_s (Fig. 1). According to Martin and Ort (1985), full P_N recovery in dark-CH tomato plants required 12-48 h. They found an independent response of P_N and g_s during the first hour after CH and concluded that stomatal opening could not be a significant factor of the recovery process. Our results did not confirm this idea. As a consequence of a long-term CH under a low irradiance, the photosynthetic capacity as well as g_s of mature tomato leaves did not recover during one week after the stress (Brüggemann *et al.* 1992a,b). Starck *et al.* (1994b) concluded that different time scales of tomato plants recovery after a CH were determined by irradiance and temperature before and after the stress period.

The CH caused accumulation of intercellular CO_2 in cv. Robin and decline in cv. New Yorker (Fig. 1, *bottom*). The opposite effects were strongly expressed in SR plants of both cultivars. There was a negative correlation between C_i and P_N in CH cv. Robin plants (r = -0.83**). In cv. New Yorker the decline in P_N after CH was positively related to the decrease in C_i (r = 0.42**). Accumulation of intercellular CO_2 after CH in the sensitive cultivar may suggest either an impairment of the chloroplast reactions or stimulation of respiration processes (Sasson and Bramlage 1981, Markhart 1986).

Drought-hardening positively affected the response to CH in cv. Robin BR plants and cv. New Yorker SR plants. Hence the root functions in initiating acclimation reactions of a shoot. One of the root roles may comprise a quick communication with the shoot through a hormonal and/or hydraulic signal (Janowiak and Dörffling 1994). A low temperature reduces the hydraulic conductance of the root membranes, thus diminishing the water uptake (McWilliam *et al.* 1982). The reduced flow of water into the plant through roots and the simultaneous delay of stomata closure may bring about a rapid decline in the leaf water potential. On the other hand, the ability of roots to affect the nutrient uptake and transport is changed just as well (Engels and Marschner 1992, Engels *et al.* 1992). This negative effect of CH on the root activities is associated with a low rate of root respiration and decreased activity of the plasma membrane ATPase. Therefore, one of the adaptive responses of CH-tolerant tomato plants is increased biomass allocation towards the roots (Starck *et al.* 1994a,c) that ameliorates the negative consequences of CH on the root functions.

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