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Effect of air humidity on the development of functional stomatal apparatus

J. POSPÍŠILOVÁ

Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Na Karlovce 1a, CZ-160 00 Praha 6, Czech Republic

Abstract

Phaseolus vulgaris L. seedlings were grown under different air humidities simulating conditions during micropropagation (very high humidity during *in vitro* cultivation and low air humidity after transfer *ex vitro*). The functional stomatal apparatus developed after a short period of growth at low air humidity at the beginning of plant ontogeny or after transfer from high to low air humidity, but not in plants grown steadily under high air humidity. The ability of stomata to regulate gas exchange was not persistent and disappeared after transfer of plants from low to high humidity.

Additional key words: abscisic acid, bean, *Phaseolus vulgaris*, stomatal conductance, transpiration rate, wilting.

Introduction

During *in vitro* cultivation plantlets grow under very special conditions: low irradiance, low air turbulence, high air humidity, saccharides as carbon and energy sources, large doses of growth regulators, *etc.* (for review see Pospíšilová *et al.* 1992, 1996). These special conditions result in the formation of plants with abnormal morphology, anatomy and physiology.

The transpiration rate of plantlets *in situ* is extremely low and the leaf water potential corresponds to the water potential of the medium. The development of cuticle and epicuticular waxes is retarded. Stomatal density in plantlet leaves may be greater or smaller than in leaves of comparable plants grown *ex vitro*. Plantlet stomata are often large and have a changed shape and structure. Guard cells in plantlet leaves have thinner cell walls and contain more starch and chloroplasts (Marín *et al.* 1988) and less calcium (Sallanon *et al.* 1991) than those in leaves of *ex vitro* grown plants. The stomata are often not able to close even when subjected to

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extreme levels of closing stimuli, *e.g.* dark, high concentration of CO_2, low air humidity and abscisic acid (ABA), or closure of stomata is not complete or is extremely slow (Wardle *et al.* 1979, Brainerd *et al.* 1981, Wardle and Short 1983, Ziv *et al.* 1987, Marín *et al.* 1988, Pospíšilová *et al.* 1988, Frommel *et al.* 1991, Preece and Sutter 1991, Diettrich *et al.* 1992, Sallanon *et al.* 1993, Santamaria *et al.* 1993). The cause may be lack of ATPase activity detected at the plasmalemma membrane of plantlet guard cells by Sallanon *et al.* (1991). Stomatal conductance, and stomatal and cuticular transpiration rates of leaves of plantlets taken out of the cultivation vessels are very high (*e.g.* Conner and Conner 1984, Pospíšilová *et al.* 1987, 1988, 1989, 1992, Sutter 1988, Diettrich *et al.* 1992, Santamaria *et al.* 1993, Santamaria and Kerstiens 1994) and this causes difficulties after transplanting plantlets *ex vitro*.

On the other hand, the photosynthetic apparatus is usually not expressivelly modified and the net photosynthetic rate of autotrophically grown plantlets is mainly restricted by the low CO_2 concentration during the light period in the fairly air tight cultivation vessels (*e.g.* Solárová 1989, Pospíšilová *et al.* 1992, 1996, Buddendorf-Joosten and Woltering 1994, Solárová *et al.* 1995).

The widespread use of micropropagation is restricted by high percentage of plants lost or damaged in consequence of wilting during the acclimation to greenhouse or field conditions. As air humidity seems to be the main limiting environmental factor, it is of special importance to study in detail acclimation of plants to different levels of air humidity.

Model experiments with bean plants grown under conditions simulating the *in vitro* cultivation (Čatský *et al.* 1995) proved that the development of functional photosynthetic apparatus requires in addition to light also a short period of increased CO_2 concentration at the beginning of plant ontogeny. Simultaneously the model plants grown under high air humidity had high conductances of adaxial and abaxial epidermes and high transpiration rate similarly as the plantlets grown *in vitro*.

The aim of further experiments was to find *1*) if a period of decreased air humidity is essential for the development of functional stomatal apparatus, *2*) how long this period has to be, and *3*) how steady is the developed stomatal apparatus. The results are presented in this paper.

Materials and methods

Seeds of French bean (*Phaseolus vulgaris* L. cv. Jantar) were germinated in Petri dishes on filter paper soaked with distilled water. After 4 d, seedlings were placed into holes in porcelain holders in 4500-cm^3 glass vessels, each containing 1000 cm^3 of the Hewitt nutrient solution that was changed every third day. The plants were grown in a growth cabinet at an irradiance of 450 $\mu mol\ m^{-2}\ s^{-1}$, photoperiod 12 h. During the further 4 d, the plants were grown either in two closed vessels under high relative humidity of air (92 - 94 %), temperatures of 27 - 29 °C, and CO_2 concentration decreasing with the development of photosynthetic activities during plant ontogeny (from 1200 to 300 mg m^{-3}) or in two open vessels under CO_2

concentrations of *ca.* 700 mg m^{-3}, relative air humidity 30 - 35 % and temperature 25 - 26 °C. After this period one of the closed vessels was opened and one of the opened vessels was closed. Under these conditions the plants were grown for further 7 d. A longer treatment was not possible as after this period some signs of senescence of plants grown steadily under high humidity were observed. In addition the secondary leaves started to shade primary leaves.

Stomatal conductances of abaxial and adaxial epidermes of primary leaves were measured by a diffusion porometer *Delta-T* (type *Mk3, Delta-T Devices,* Cambridge, UK) at a temperature of 25 °C, irradiance of 860 μmol m^{-2} s^{-1}, and relative air humidity of 40 - 50 %. At least 6 leaves from 3 plants were measured at each treatment and time and experiments were repeated six times. Under the same environmental conditions water loss curves were measured gravimetrically on 30 leaves that were originally fully turgid. These measurements were done for comparing wilting of plants growing in conditions simulating the *in vitro* culture and of plantlets *in vitro* (leaves of which were often not suitable for porometric determination of stomatal conductance).

Results and discussion

Bean seedlings were grown on mineral medium in environment simulating the conditions during micropropagation. Plants grown under high air humidity roughly corresponded to the plantlets cultivated *in vitro* and the transfer from high to low humidity simulated the transfer from *in vitro* to *ex vitro* conditions. Plants grown under low humidity corresponded to seedlings grown *ex vitro*. The transfer from low to high humidity was done with the aim to determine the stability of developed stomatal apparatus.

Transpiration rate of young seedlings grown for 4 d under high as well as low air humidity was rather high when measured under irradiance 860 μmol m^{-2} s^{-1}, temperature 25 °C and relative air humidity 40 %. However, during further growth the transpiration rate of plants grown under low air humidity considerably decreased while that of plants grown under high air humidity increased (Fig. 1). Consequently the leaves of plants grown steadily under high air humidity quickly wilted similarly as the leaves of plantlets grown *in vitro*. The shape of water loss curves of leaves of bean plants grown steadily under high humidity was similar to that of water loss curves of leaves of tobacco plantlets grown *in vitro*. The shape of water loss curves of leaves of bean plants grown steadily under low humidity corresponded to that of water loss curves of leaves of tobacco seedlings grown under low air humidity in air-conditioned chamber or of leaves of tobacco plantlets acclimated (for 3 weeks) to low air humidity after transplantation (Pospíšilová *et al.* 1987, 1988). It is possible to suppose that high transpiration rate of plants grown under high air humidity was caused by retardation in development of cuticle as well as ability of stomata to regulate gas exchange as it was supposed for leaves of plantlets grown *in vitro*.

Adaxial and abaxial stomatal conductances (g_{ad} and g_{ab}) of leaves of bean plants grown continually under low air humidity increased at the beginning of plant

ontogeny, reached maximum 7 d after the beginning of treatment (11 d from the beginning of germination) and then slightly decreased (Fig. 2). The ontogenetic

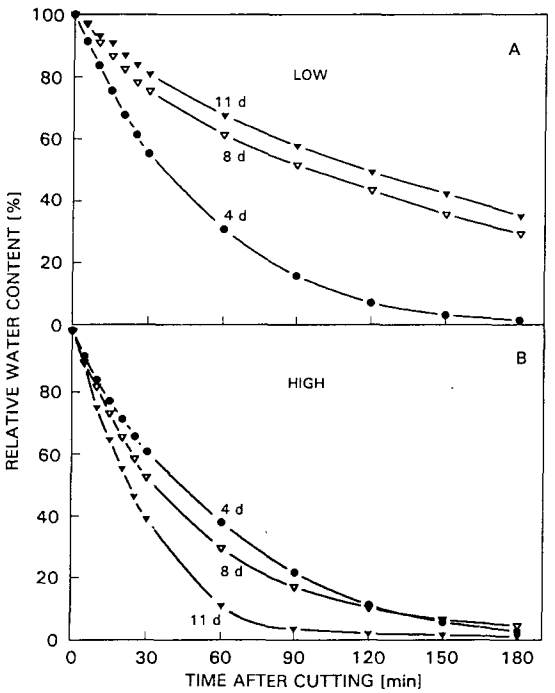

Fig. 1. Comparison of transpiration curves (changes in relative water content after cutting of fully water saturated primary leaves) of bean plants grown for 4, 8 and 11 d at low (*top*) or high (*bottom*) air humidity. Transpiration curves were measured under irradiance 860 μmol m^{-2} s^{-1}, temperature 25 °C and relative air humidity 40 %. Means of 30 leaves, S.E. were from 0.1 to 2.6 %.

courses of g_{ad}, g_{ab} or total epidermal conductance (g_{ep}) (Fig. 3) were in a good agreement with the ontogenetic course of g_{ep} observed in bean plants grown in air-conditioned chamber or in greenhouse (for review see Čatský *et al.* 1985). Similar ontogenetic course of g_{ab} of leaves of bean plants grown continually under high humidity was also found but all the values of g_{ab} were much higher. Much higher were also values of g_{ad} which rose during the whole treatment period. Plants transferred from high air humidity to low humidity gradually acclimated to low humidity: g_{ad} and g_{ab} gradually decreased and after 7 d they were similar to those of plants grown continually under low air humidity. This acclimation was in agreement with the acclimation of plantlets transplanted from *in vitro* culture to *ex vitro*, *e.g.* of *Brassica* (Grout and Aston 1977, Wardle *et al.* 1979), *Leucaena* (Dhawan and Bhojwani 1987), *Nicotiana* (Pospíšilová *et al.* 1988), *Prunus* (Drew *et al.* 1992), *Saintpaulia* (Short *et al.* 1984) and *Solanum* (Conner and Conner 1984, Baroja Fernandez 1993) but acclimation of these plants was slower than that of bean plants. The rate of acclimation was species specific. In addition, bean seedlings were very young (8 d) when they were transferred and so the period of growth under high

humidity was much shorter than in the case of *in vitro* cultivated plantlets. When plants grown at the beginning of their ontogeny under low air humidity were transferred to high air humidity, their g_{ad} and g_{ab} increased (Fig. 2). The acclimation from low to high humidity lasted similar period as acclimation from high to low air humidity.

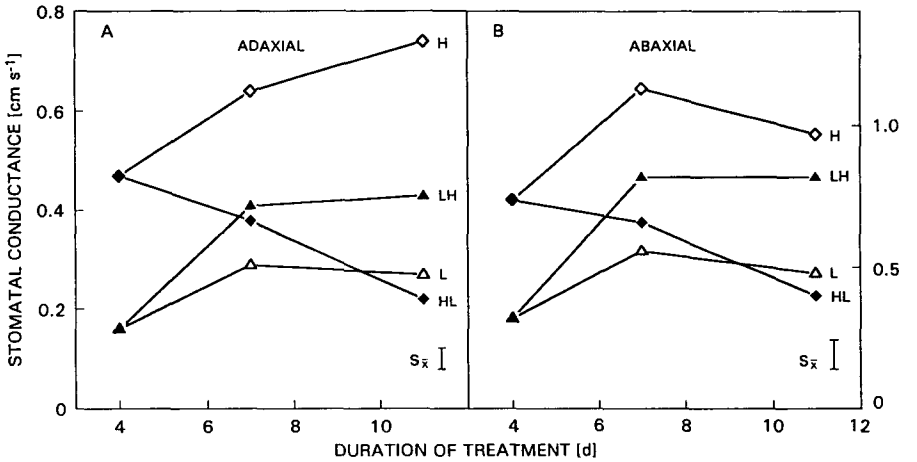

Fig. 2. Conductances of adaxial and abaxial epidermes of primary leaves of bean plants grown for 4, 7 and 11 d at high (*H*) or low (*L*) air humidity, for 4 d at high and for further 3 or 7 d at low humidity (*HL*) or for 4 d at low and for further 3 or 7 d at high humidity (*LH*). Irradiance during measurement 860 μmol m^{-2} s^{-1}, temperature 25 °C and relative air humidity 50 %. In bean leaves abaxial conductance is much higher than adaxial conductance (see *y-axis* scale). *Bars* indicate average S.E.

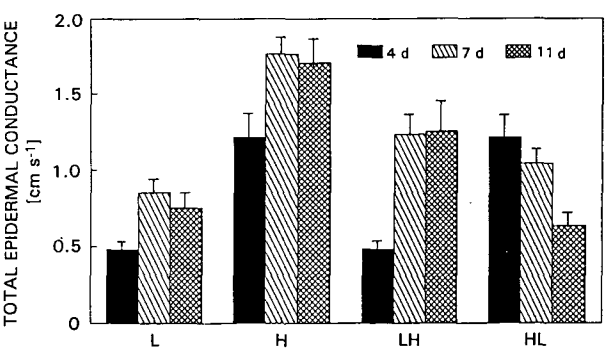

Fig. 3. Total epidermal conductance of primary leaves of bean plants grown for 4, 7 and 11 d at high (*H*) or low (*L*) air humidity, for 4 d at high and for further 3 or 7 d at low humidity (*HL*) or for 4 d at low and for further 3 or 7 d at high humidity (*LH*). Irradiance during measurement 860 μmol m^{-2} s^{-1}, temperature 25 °C and air humidity 50 %. *Bars* indicate S.E.

The ability of stomata to regulate water loss was further tested by dipping petioles of cut leaves into 0.1 mM ABA solution for 1 h. In all cases g_s after treatment with ABA was lower but the percent decrease was much higher in leaves grown continually under low air humidity than under high humidity and intermediate in

plants grown under low→high or high→low humidities (Fig. 4). The low sensitivity of bean plants grown under high air humidity to ABA is also in good agreement with low sensitivity to ABA of plantlets grown *in vitro*.

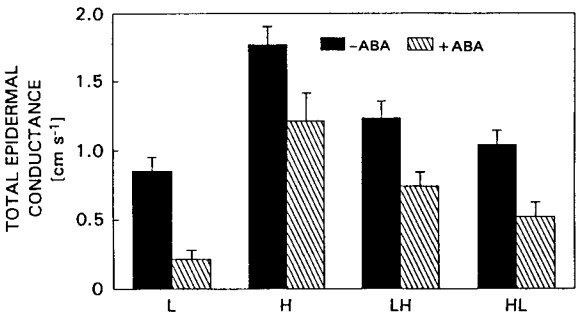

Fig. 4. Changes in total epidermal conductance of primary leaves of bean plants grown for 11 d at high (*H*) or low (*L*) air humidity, for 4 d at high and for further 7 d at low humidity (*HL*) or for 4 d at low and for further 7 d at high humidity (*LH*) induced by dipping leaf petioles in 0.1 mM solution of abscisic acid for 1 h. *Bars* indicate S.E.

However, the effect of air humidity can be indirect. Stomata respond rather to the rate of transpiration than to air humidity *per se* (Mott and Parkhurst 1991, Monteith 1995). Therefore the cause of retardation of development of stomatal apparatus under high air humidity may be the very low transpiration rate.

In conclusion, the functional stomatal apparatus develops after a short period of treatment with low air humidity (a short period of increased transpiration rate). However, the ability of stomata to regulate gas exchange is not persistent and disappears again after transfer of plants into high air humidity. On the other hand, the already developed photosynthetic apparatus remains functional in plantlets growing under low ambient CO_2 concentration (Čatský *et al.* 1995).

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