BIOLOGIA PLANTARUM 38 (4): 605-609, 1996

BRIEF COMMUNICATION

Hardening by abscisic acid of tobacco plantlets grown *in vitro*

J. POSPÍŠILOVÁ

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

Abstract

Tobacco plantlets were grown *in vitro* on Murashige and Skoog's medium with 2 % of saccharose. Addition of 0.01 mM abscisic acid (ABA) into the medium decreased stomatal conductance of the adaxial epidermis and especially the abaxial epidermis without negative effects on growth parameters. As a result the rate of water loss from ABA-treated plantlets taken out of cultivation vessels was slower than that of control plantlets. This could help their acclimation after transplantation to *ex vitro* conditions.

Additional key words: *Nicotiana tabacum*, stomatal conductance, transpiration curves.

The widespread use of micropropagation is restricted by a high percentage of plants lost or damaged in consequence of wilting during acclimation to greenhouse or field conditions. Special conditions during *in vitro* cultivation result in the formation of plants with abnormal morphology, anatomy and physiology (for review see, *e.g.*, Pospíšilová *et al.* 1992, 1996, Buddendorf-Joosten and Woltering 1994, Kozai and Smith 1995). Difficulties after transplanting plantlets *ex vitro* are especially caused by delay in the development of cuticle, epicuticular waxes and functional stomatal apparatus, and thus by high stomatal conductance and high stomatal and cuticular transpiration rates of leaves of plantlets taken out of the cultivation vessels. Improvements can be achieved by a decrease in air humidity (*e.g.*, by using lids

---

Received 7 May 1996, *accepted* 16 June 1996.

Abbreviations: ABA - abscisic acid; BAP - 6-benzylaminopurine; g_s - stomatal conductance; E - transpiration rate.

Acknowledgements: The author thanks Mrs. L. Kolčabová and Mrs. M. Koubová for skilled assistance. The work was supported by grant No. 501/95/1303 of the Grant Agency of the Czech Republic.

permeable to water vapour or by bottom cooling), by an increase in irradiance, or by forced ventilation during *in vitro* cultivation (Wardle *et al.* 1983, Ziv 1986, Short *et al.* 1987, Capellades *et al.* 1990, Roberts *et al.* 1990, Smith *et al.* 1990, Ghashghaie *et al.* 1992, Deng and Donnelly 1993, Yue *et al.* 1993, Cassells and Walsh 1994). However, most of the procedures for lowering air humidity are not practical as they lead to a quick drying of the cultivation medium and to a decrease in plantlet growth (Wardle *et al.* 1983, Ghashghaie *et al.* 1992, Sallanon and Maziere 1992, Solárová *et al.* 1996). The relative water loss from detached leaves of *in vitro* grown plantlets could be reduced by application of paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol], indolebutyric acid or 6-benzyl-aminopurine (BAP) (Smith *et al.* 1992, Pospíšilová *et al.* 1993, Eliasson *et al.* 1994) or by decreased osmotic potential of medium by polyethylene glycol (Zaid and Hughes 1995).

It was found in previous experiments that a period of decreased air humidity is important for the development of functional stomatal apparatus (Pospíšilová 1996). The similarities between some effects of water stress and of exogenously applied abscisic acid have often been observed (for review, *e.g.*, Davies and Jones 1991). Therefore, the aim of this paper was to follow the effect of adding ABA to the cultivation medium on the development of functional stomatal apparatus.

Plantlets of tobacco (*Nicotiana tabacum* L. cv. SR1) were cultivated for 3 weeks on the medium of Murashige and Skoog (1962) containing 2 % saccharose and 0 or 0.01 mM abscisic acid, at a 16-h photoperiod, an irradiance (400 - 700 nm) of 200 μmol m^{-2} s^{-1} and day/night temperature of 25/20 °C. Stomatal conductances (g_s) of abaxial and adaxial leaf epidermes were measured with a diffusion porometer *Delta-T* (type *Mk3, Delta-T Devices,* Kingston upon Thames, UK) at a temperature of 25 °C, irradiance of 860 μmol m^{-2} s^{-1}, and relative air humidity of 40 - 50 %.

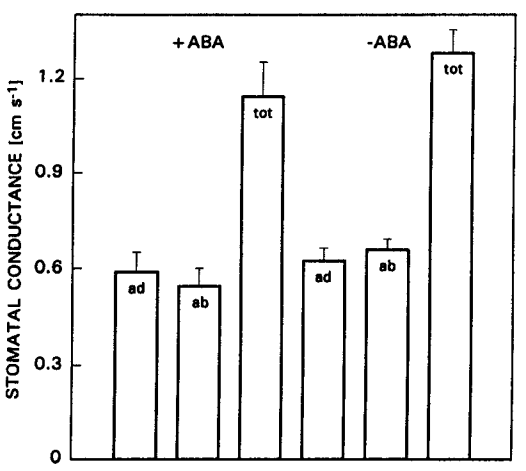

Fig. 1. Adaxial (ad), abaxial (ab) and total (tot) stomatal conductances of tobacco plantlet leaves as affected by application of 0.01 mM ABA into cultivation medium (means of 18 leaves ± SE).

From each plantlet the 2nd, 3rd and 4th leaves from the top were measured, six plantlets at each treatment. Under the same conditions, the transpiration rate (E) was determined from water loss curves measured gravimetrically on leaves originally fully turgid (10 leaves per treatment). The E was calculated from the slope of the water loss curve (as the first derivative) in the first minutes after cutting, as it is supposed that in this period the rate of water loss corresponds to the E *in situ* under the same conditions. Irradiance and air temperature and humidity were measured with the *LI 185B* radiometer with quantum sensor (*Li-Cor*, Lincoln, USA) and with the *JUMO Humitherm TDAc-70* (*M.K. Juchheim*, Fulda, Germany), respectively. Dry mass was determined in samples oven-dried at 90 °C to constant mass. The experiments were repeated twice with similar results.

After 3 weeks, application of 0.01 M ABA to the cultivation medium caused a decrease in stomatal conductance of tobacco plantlet leaves. Abaxial conductance was decreased more than adaxial (Fig. 1). As a consequence, the rate of water loss from leaves of ABA treated plantlets was slower than that of control plants. The shape of water loss curves indicated that stomata of ABA treated plantlets closed sooner and to a greater degree with increased leaf water deficit (Fig. 2). The maximum E was hardly affected (0.23 and 0.24 mg(H_2O) g^{-1}(H_2O) s^{-1} for control and ABA treated leaves, respectively). In contrast, application of 1.0 mg dm^{-3} BAP into the cultivation medium induced a decrease in the rate of water loss of tobacco plantlets (Pospíšilová *et al.* 1993) which was due to a decrease in maximum E but not to stomatal closing.

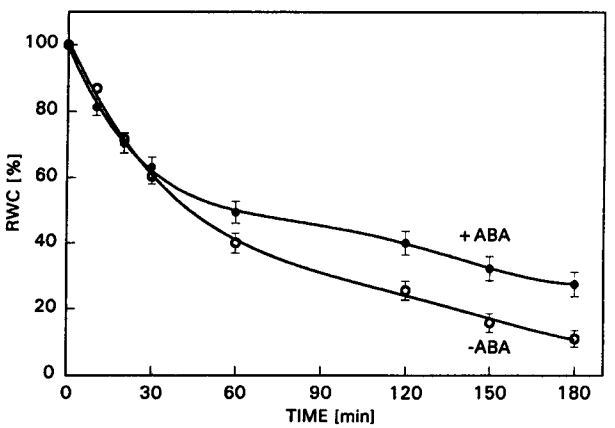

Fig. 2. Transpiration curves (changes in relative water content, RWC, after cutting of fully water saturated leaves) of tobacco plantlets as affected by application of 0.01 mM ABA into cultivation medium (means of 10 leaves ± SE).

ABA is known as a growth retardant. Nevertheless, its application at the low concentration used (0.01 mM) had no negative effect on plantlet growth (fresh and dry masses of leaves, stems and roots - data not shown). Application of ABA to the cultivation medium has been used for enhancement of maturation and desiccation tolerance of somatic embryos (*e.g.* Emons *et al.* 1993, Etienne *et al.* 1993, Misra

et al. 1993) or adaptations of callus cultures to salinity and osmotic shock (Eberhardt and Wegmann 1989). These experiments have shown that ABA could be suitable for hardening of tobacco plantlets *in vitro*. On the other hand pretreatment of *Aronia arbutifolia* plantlets with ABA provided no physiological advantage that would facilitate *ex vitro* acclimation (Colonguasp *et al.* 1996). Experiments on hardening of plantlets the transfer of which to *ex vitro* conditions is difficult (*e.g.*, transgenic tobacco plants with introduced *ipt*-gene and so an elevated content of endogenous cytokinins) are now in progress.

References

Buddendorf-Joosten, J.M.C., Woltering, E.J.: Components of the gaseous environment and their effects on plant growth and development *in vitro*. - Plant Growth Regul. **15**: 1-16. 1994.

Capellades, M., Fontarnau, R., Carulla, C., Debergh, P.: Environment influences anatomy of stomata and epidermal cells in tissue-cultured *Rosa multiflora*. - J. amer. Soc. hort. Sci. **115**: 141-145, 1990.

Cassells, A.C., Walsh, C.: The influence of gas permeability of the culture lid on calcium uptake and stomatal function in *Dianthus* microplants. - Plant Cell Tissue Organ Cult. **37**: 171-178, 1994.

Colonguasp, W., Nell, T.A., Kane, M.E., Barrett, J.E.: Effects of abscisic acid on *ex vitro* acclimatization of *Aronia arbutifolia* (L.) Pers. - J. amer. Soc. hort. Sci. 121: 101-104, 1996.

Davies, W.J., Jones, H.G.: Abscisic Acid. Physiology and Biochemistry. - BIOS Scientific Publishers, Oxford 1991.

Deng, R., Donnelly, D.J.: *In vitro* hardening of red raspberry through CO_2 enrichment and relative humidity reduction on sugar-free medium. - Can. J. Plant Sci. 73: 1105-113, 1993.

Eberhardt, H.-J., Wegmann, K.: Effect of abscisic acid and proline on adaptation of tobacco callus cultures to salinity and osmotic shock. - Physiol. Plant. **76**: 283-288, 1989.

Eliasson, M.K., Beyl, C.A., Barker, P.A.: *In vitro* responses and acclimatization of *Prunus serotina* with paclobutrazol. - J. Plant Growth Regul. **13**: 137-142, 1994.

Emons, A.M.C., Samallo-Droppers, A., Van der Toorn, C.: The influence of sucrose, mannitol, L-proline, abscisic acid and gibberellic acid on the maturation of somatic embryos of *Zea mays* L. from suspension cultures. - J. Plant Physiol. **142**: 597-604, 1993.

Etienne, H., Montoro, P., Michaux-Ferriere, N., Carron, M.P.: Effect of desiccation, medium osmolality and abscisic acid on the maturation of *Hevea brasiliensis* somatic embryos. - J. exp. Bot. **44**: 1613-1619, 1993.

Ghashghaie, J., Brenckmann, F., Saugier, B.: Water relations and growth of rose plants cultured *in vitro* under various relative humidities. - Plant Cell Tissue Organ Cult. **30**: 51-57, 1992.

Kozai, T., Smith, M.A.L.: Environmental control in plant tissue culture - general introduction and overview. - In: Aitken-Christie, J., Kozai, T., Smith, M.L. (ed.): Automation and Environmental Control in Plant Tissue Culture. Pp. 301-318. Kluwer Academic Publishers, Dordrecht - Boston - London 1995.

Misra, S., Attree, S.M., Leal, I., Fowke, L.C.: Effect of abscisic acid, osmoticum, and desiccation on synthesis of storage proteins during the development of white spruce somatic embryos. - Ann. Bot. **71**: 11-22, 1993.

Pospíšilová, J.: Effect of air humidity on the development of functional stomatal apparatus. - Biol. Plant. **38**: 197-204, 1996.

Pospíšilová, J., Čatský, J., Šesták, Z.: Photosynthesis in plants cultivated *in vitro*. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 525-540. Marcel Dekker, New York 1996.

Pospíšilová, J., Čatský, J., Synková, H., Macháčková, I., Solárová, J.: Gas exchange and *in vivo* chlorophyll fluorescence in potato and tobacco plantlets *in vitro* as affected by various concentrations of 6-benzylaminopurine. - Photosynthetica **29**: 1-12, 1993.

Pospíšilová, J., Solárová, J., Čatský, J.: Photosynthetic responses to stresses during *in vitro* cultivation. - Photosynthetica **26**: 3-18, 1992.

Roberts, A.V., Smith, E.F., Mottley, J.: The preparation of micropropagated plantlets for transfer to soil without acclimatization.- In: Pollard, J.W., Walker, J.M. (ed.): Methods in Molecular Biology. Vol. 6. Plant Cell and Tissue Culture. Pp. 227-236. Humana Press, Clifton 1990.

Sallanon, H., Maziere, Y.: Influence of growth room and vessel humidity on the *in vitro* development of rose plants. - Plant Cell Tissue Organ. Cult. **30**: 121-125, 1992.

Short, K.C., Warburton, J., Roberts, A.V.: *In vitro* hardening of cultured cauliflower and *Chrysanthemum* plantlets to humidity. - Acta Hort. **212**: 329-334, 1987.

Smith, E.F., Roberts, A.V., Mottley, J.: The preparation *in vitro* chrysanthemum for transplantation to soil. 3. Improved resistance to desiccation conferred by reduced humidity. - Plant Cell Tissue Organ Cult. **21**: 141-145, 1990.

Smith, E.F., Gribaudo, I., Roberts, A.V., Mottley, J.: Paclobutrazol and reduced humidity improve resistance to wilting of micropropagated grapevine. - HortScience **27**: 111-113, 1992.

Solárová, J., Součková, D., Ullmann, J., Pospíšilová, J.: *In vitro* culture: environmental conditions and plantlet growth as affected by vessel type and stopper material. - Zahradnictví **23**(2): 51-58, 1996.

Wardle, K., Dobbs, E.B., Short, K.C.: *In vitro* acclimatization of aseptically cultured plantlets to humidity. - J. amer. Soc. hort. Sci. **108**: 386-389, 1983.

Yue, D., Gosselin, A., Desjardins, Y.: Effect of forced ventilation at different relative humidities on growth, photosynthesis and transpiration of geranium plantlets *in vitro*. - Can. J. Plant Sci. **73**: 249-256, 1993.

Zaid, A., Hughes, H.: Water loss and polyethylene glycol-mediated acclimatization of *in vitro*-grown seedlings of 5 cultivars of date palm (*Phoenix dactylifera* L.) plantlets. - Plant Cell Rep. **14**: 385-388, 1995.

Ziv, M.: *In vitro* hardening and acclimatization of tissue culture plants. - In: Withers, L.A., Alderson, P.G. (ed.): Plant Tissue Culture and its Agricultural Applications. Pp. 187-196. Butterworths, London - Boston - Durban - Singapore - Sydney - Toronto - Wellington 1986.