PHOTOSYNTHETICA 38 (4): 629-634, 2000

Growth, stomatal conductance, photosynthetic rate, ribulose-1,5-bisphosphate carboxylase/oxygenase and phosphoenolpyruvate carboxylase activities during rooting and acclimatisation of *Rosa hybrida* plantlets

C. GENOUD*, H. SALLANON*, A. HITMI*, Y. MAZIERE**, and A. COUDRET*

*Laboratoire de Biotechnologies, Environnement-Santé, Université d'Auvergne, I.U.T. de Clermont-Fd, 100 rue de l'Egalité, F-15000 Aurillac, France**
*Laboratoire de Culture in vitro, Pépinières et Roseraies G. Delbard, F-03600 Commentry, France***

Abstract

The rooting of shoots of micropropagated *Rosa hybrida* cv. Madame Delbard was conducted on MS medium with 30 kg m^{-3} sucrose or on hydroponic medium (containing less mineral salts), under higher photosynthetic photon flux density (PPFD) (100 in comparison with 45 μmol m^{-2} s^{-1}) and flushed by ambient air [AC, 340 μmol(CO_2) mol^{-1}] or by CO_2-enriched air (EC, 2 500 μmol mol^{-1}) and lower relative humidity (80-90 % *vs.* 96-99 %). This cultivation led to plantlets with longer roots and adventitious root formation. Net photosynthetic rate and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activities, RuBPCO/phosphoenolpyruvate carboxylase activities ratio, and starch accumulation increased under these conditions. After 14 d, plantlets had functional stomata and could be acclimated on open benches without gradual decrease in relative humidity. The percentage of survival was higher when the rooting took place in EC than in AC. However, the advantage acquired during rooting phase by plantlets cultured in liquid medium was not maintained after 4 weeks of acclimatisation.

Additional key words: mineral salts; relative humidity; rose.

Introduction

Commercial laboratories micropropagate roses on medium with sucrose gelified with agar. We previously reported that *Rosa hybrida* could be acclimatised well even if the explants which did not exhibit roots were cultured on sugar-free Murashige-Skoog (MS) rooting medium under high photon flux density (PPFD) and CO_2 concentration (Genoud-Gourichon *et al.* 1996). Such culture conditions enabled root initiation. Many studies (Collet 1985, Vinterhalter and Vinterhalter 1992) already point out that a decrease of the inorganic salts in the media improves the root formation and the lateral roots development while adversely the macroelements inhibit the lateral root formation. This inhibitory effect is a result a unbalanced SO_4^{2-} with NO_3^- and NH_4^+. The MS medium was developed for heterotrophic growth of tissues and organs (cf. Genoud *et al.* 1999), but Kozai *et al.* (1988, 1991) built up a medium which is optimal for photoautotrophic tissue cultures.

The special conditions during *in vitro* cultivation can result in reduced leaf epicuticular wax (Dhawan and Bajwani 1987), increased stomatal conductance (Capellades *et al.* 1990, Sallanon *et al.* 1991), poorly developed vascular systems, and low photosynthetic activity (Pospíšilová *et al.* 1988, 1989, 1992, Kozai 1991, Sallanon *et al.* 1997; for reviews see Pospíšilová *et al.* 1997a,b). In particular, the high relative humidity (RH) is involved in abnormal stomatal functioning due to the guard cell walls Ca^{2+} content (Ziv *et al.* 1987, Sallanon *et al.* 1991).

The aim of our experiments was to investigate the effects of a modified inorganic salt composition, RH, and CO_2 concentration on rooting and acclimatisation of the rose.

Received 23 September 1999, *accepted* 17 April 2000.
Fax: (33) 4 71 45 57 51 ; e-mail: huguette.sallanon@u-clermont1.fr

Materials and methods

Rose (*Rosa hybrida* cv. Deladel) from the nursery G. Delbard (Malicorne, France) was *in vitro* multiplied on Murashige and Skoog (1962) (MS) medium as described by Sallanon and Mazière (1992). For rooting, control plantlets were grown on a solid medium containing MS salts, 30 kg m^{-3} sucrose, 2.85 mM indol-3-yl-acetic acid (IAA), and 7 kg m^{-3} agar. Each 850 cm^3 glass vessels closed with a polycarbonate lid contained 120 cm^3 of medium and 30 plantlets. Culture conditions were 16-h photoperiod, PPFD of 45 µmol m^{-2} s^{-1}, day/night temperature 23±1/19±1 °C, RH inside the growth vessels 96±2 %.

Plantlets were cultured for 14 d on aerated hydroponic nutrient solution (HM: 5 mM KNO_3, 1.5 mM $MgSO_4 \times 7\ H_2O$, 3.47 mM $Ca(NO_3)_2 \times 4\ H_2O$, 1.73 mM NaH_2PO_4, 0.15 mM NaCl, 13.19 µM $MnSO_4 \times 4\ H_2O$, 1.03 µM $ZnSO_4 \times 7\ H_2O$, 30 µM H_3BO_3, 0.96 µM $CuSO_4 \times 5\ H_2O$, 0.18 µM $CoSO_4$, 0.03 µM $Mo_7(NH_4)_6O_2 \times 4\ H_2O$, 26.3 µM EDTA-Fe) in 25 000 cm^3 polycarbonate box closed with polycarbonate lids, containing 4 000 cm^3 of medium and 100 plantlets. The boxes were flushed at a flow rate of 5 cm^3 m^{-3} s^{-1} with ambient air (CO_2 concentration of 340 cm^3 m^{-3}, AC-plants) or CO_2 enriched air (2 500 cm^3 m^{-3}, EC-plants). Culture conditions were 16-h photoperiod, PPFD of 100 µmol m^{-2} s^{-1}, day/night temperature 23±1/19±1 °C, and RH 80±5/90±5 %.

After 14 d, plantlets were transferred to greenhouse, potted in standard fertilised peats, and acclimated under PPFD of 100 µmol m^{-2} s^{-1} and temperature of 21±3 °C. One half of plantlets was acclimated as usual for *in vitro* cultures (progressive decrease of RH), while the other half was directly transplanted (day/night RH was 40±10/60±10 %).

Shoot length, number of leaves per shoot, leaf area, roots per shoot, and root length were measured. Stomatal characteristics (index, density, and shape) were determined on imprints by optical microscope. g_s was measured with an automatic porometer (*Delta-T-Devices*, England). Saccharide contents were determined as recommended by *Boehringer* (Mannheim, Germany): the NADPH production obtained was measured at 340 nm. The Chen *et al.* (1971) method modified by Passera and Albuzio (1978) was used to determine RuBPCO and phosphoenolpyruvate carboxylase (PEPC) activities. CO_2 exchanges were measured as described by Genoud-Gourichon *et al.* (1993).

All parameters, except g_s, were measured on 0-, 3-, 7-, 10-, and 14-d-old plantlets during the rooting phase and on 4-week-old acclimatised plantlets. g_s was measured on days 0, 7, and 14 during the root formation. Day 0 was the day of transplanting to rooting media.

Results

Rooting phase: During rooting, the number of leaves per shoot and the leaf area were the same regardless the growth conditions. The shoot length of 14-d-old AC-plantlets cultured on HM medium was the highest while there was no difference in the other conditions (Fig. 1*A,B,C*). All the treatments induced roots development on days 3 to 7. The number of roots at days 7 and 10 was the highest on the MH medium for the EC-plants (Fig. 1*D*), but on day 14 it was the same for all plantlets. However, roots of control plants were 1.8 fold shorter than those of EC-plants and the latter were 1.5 time smaller than in AC-plants (Fig. 1*D,E*). Moreover, plantlets raised on HM medium had adventitious roots. CE-plants rooted earlier (day 7), but on day 14 the rooting rates were similar (Fig. 1*F*).

Stomata were located only on the abaxial leaf surface. Under all growth conditions, the stomatal index increased while stomatal density decreased along the rooting phase, and both parameters reached the values of the acclimatised plantlets (Fig. 2*A,B*). Stomata had more round shape at the beginning of the culture and they became more elliptical at the end. The shape of epidermal cells changed in the course of cultivation and they exhibited more sinuous undulations at the end, especially for CA-plants. In all growth conditions also g_s was similar up to the day 7 in darkness and under light (Fig. 3*A,B,C*). On day 14, it was 3 times higher under light than in darkness for AC- and EC-plants (Fig. 3*B,C*).

Sucrose accumulated from day 7 at all growth conditions (Fig. 4*A*). On day 14, EC-plants contained 8.4 g kg^{-1}(FM) of sucrose which is about twice the content of the other plantlets. Starch accumulation was higher in plantlets raising from liquid media than in controls (Fig. 4*B*).

From day 0, the PEPC activity rose from 17.7 µmol(CO_2) kg^{-1}(protein) s^{-1} to maxima reached on day 7, of 97.0 µmol(CO_2) kg^{-1}(protein) s^{-1} in control and 59.7 µmol(CO_2) kg^{-1}(protein) s^{-1} in plantlets cultured in liquid media. The enzymatic activity gently decreased until day 10 and it stabilised afterwards (Fig. 4*C*).

The initial RuBPCO activity in control plantlets regularly grew from 1.3 µmol(CO_2) kg^{-1}(protein) s^{-1} to 15.8 µmol(CO_2) kg^{-1}(protein) s^{-1} (Fig. 4*D*). In plantlets from liquid media, it remained stable for the first 7 d, then ineased to 113.8 µmol(CO_2) kg^{-1}(protein) s^{-1} in AC-plantlets and to 128.0 µmol(CO_2) kg^{-1}(protein) s^{-1} in both EC- and AC-plantlets.

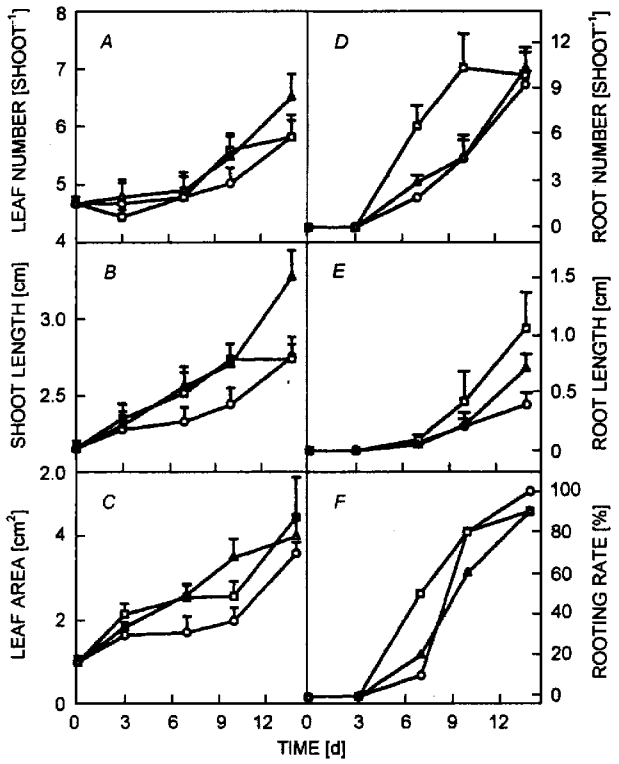

Fig. 1. Number of leaves per shoot (*A*), shoot length (*B*), leaf area (*C*), number of roots per shoot (*D*), root length (*E*), and rooting rate (*F*) of *Rosa* plantlets grown under different treatments. Bars represent SD. O control plantlets; □ plantlets cultured on HM medium flushed with CO_2-enriched air (EC-plants); Δ plantlets cultured on HM medium flushed with ambient air (AC-plants). Means of 10 measurements.

Net photosynthetic rate (P_N) of control plantlets slightly increased during the culture period, while in EC-plants it raised from 75 to 198 μg(CO_2) m^{-2} s^{-1} between days 7 and 10 and in AC-plants from 31 to 155 μg(CO_2) m^{-2} s^{-1} (Fig. 5).

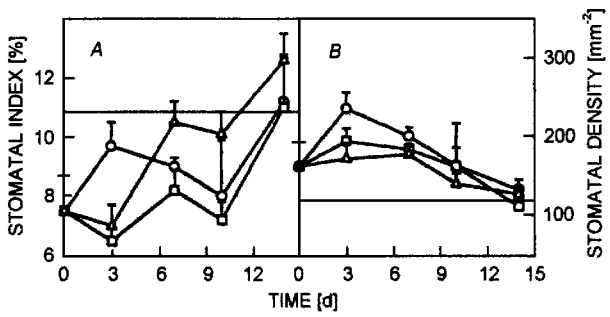

Fig. 2. Stomatal index (*A*) and stomatal density (*B*) of *Rosa* plantlets grown under different treatments. Bars represent SD. O control plantlets; □ plantlets cultured on HM medium flushed with CO_2-enriched air (EC-plants); Δ plantlets cultured on HM medium flushed with ambient air (AC-plants); *the line* — represents values observed in acclimatised plants. Means of 10 measurements.

Acclimatisation phase: All control plantlets were lost unless they were acclimatised for two weeks during which RH was gently reduced. On the opposite, the plantlets from liquid media were able to survive at a convenient rate when transferred into greenhouse with low RH (Table 1). 15 % of the control plants died during the first two weeks of acclimatisation. After 4 weeks of acclimatisation, whatever the origin were, all the plantlets had a similar morphology and similar enzymatic and photosynthetic abilities (values not shown).

Discussion

The root length of plantlets grown on hydroponic media (HM) was higher than on MS medium, and lateral roots developed. The consistent differences in nitrogen composition of the media could be an explanation since Vinterhalter and Vinterhalter (1992) suggested that the NO_3^-/NH_4^+ balance and thus the resulting pH of the medium might be a major factor of root growth regulation. Hyndman *et al.* (1982) and Collet (1985) also evidenced improvements of root establishment on low nitrogen concentration media. Probably, the lateral roots we observed on some treatments made the adaptation of plantlets to *ex vitro* conditions easier and helped their further growth in greenhouse. Of course, lateral roots increased total root area and thus might improve nutrient and water uptake. A high osmotic potential of medium promotes also root formation as shown by Vandemoortele *et al.* (1993) in *Brassica oleracea* L. This is consistent with our results since osmotic potential of the hydroponic medium, measured by the psychrometric method, was higher than that of MS medium (-0.05 MPa and -0.25 Mpa, respectively).

The medium aeration can explain the best root elongation as previously mentioned by Gebhart (1985) and Jay-Allemand *et al.* (1992) who correlated the initiation and the development of adventitious roots to O_2 uptake and to the activation of the alternative pathway in the respiratory metabolism (Hase 1987). When plantlets were grown on HM medium and flushed with EC, roots appeared more readily. The supply of CO_2 could stimulate photosynthesis and growth resulting in a better root formation.

The hydroponic medium and the cultivation in large polycarbonate boxes also affected PEPC and RuBPCO activities, starch accumulation, and sucrose content. PEPC activity of control plantlets was always higher than RuBPCO activity similarly as during multiplication of *Juglans regia* (Sallanon *et al.* 1995, 1997). The stimulation of PEPC activity by sucrose is concomitant

with a high respiratory activity of the cells (Nato and Vidal 1983). The drop of the PEPC activity and the raise of RuBPCO activity between days 7 and 14 indicate a progressive transition from heterotrophic to autotrophic metabolism. The CO_2 fixation linked to PEPC scored for 90 % of the whole CO_2 fixation until day 7. This is a general feature of actively dividing cells (Coudret and Ducher 1993). During the second part of cultivation, 57 % of the CO_2 fixation is linked to RuBPCO. Although RuBPCO is synthesised in light, it starts to work after a lag phase (Arron and Bradbeer 1975). So this lag phase would be characterised by an asynchrony in the rates of synthesis of the two subunits and/or in the rates of their binding in the assembled functional RuBPCO.

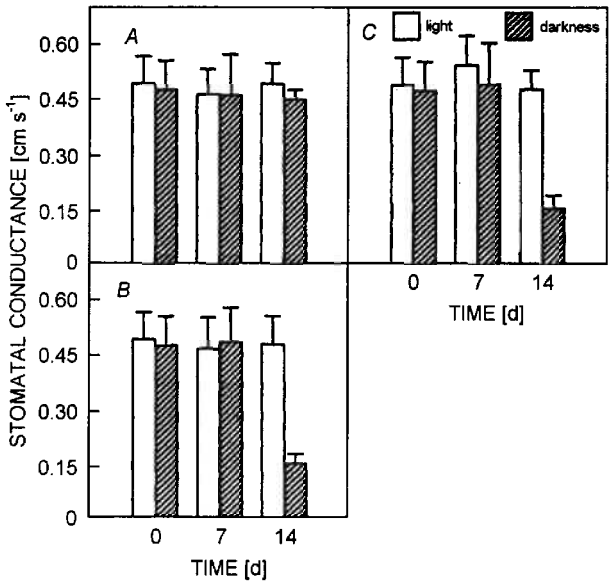

Fig. 3. Stomatal conductance in light and darkness of *Rosa* plantlets grown under different treatments. Bars represent SD. (*A*) control plantlets; (*B*) plantlets cultured on HM medium flushed with CO_2-enriched air; (*C*) plantlets cultured on HM medium flushed with ambient air. Means of 10 measurements.

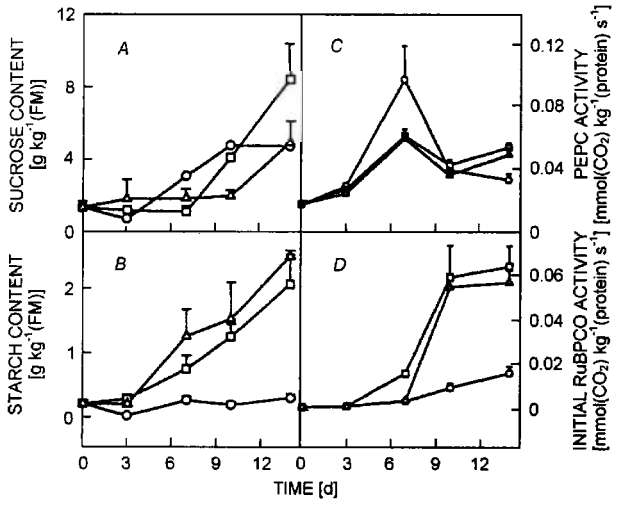

Fig. 4. Sucrose (*A*) and starch (*B*) contents, and activities of phosphoenolpyruvate carboxylase (PEPC, *C*) and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO, *D*) in *Rosa* plantlets grown under different treatments. *Bars* represent SD. O control plantlets; □ plantlets cultured on HM medium flushed with CO_2-enriched air (EC-plants); Δ plantlets cultured on HM medium flushed with ambient air (AC-plants). Means of 4 independent experiments.

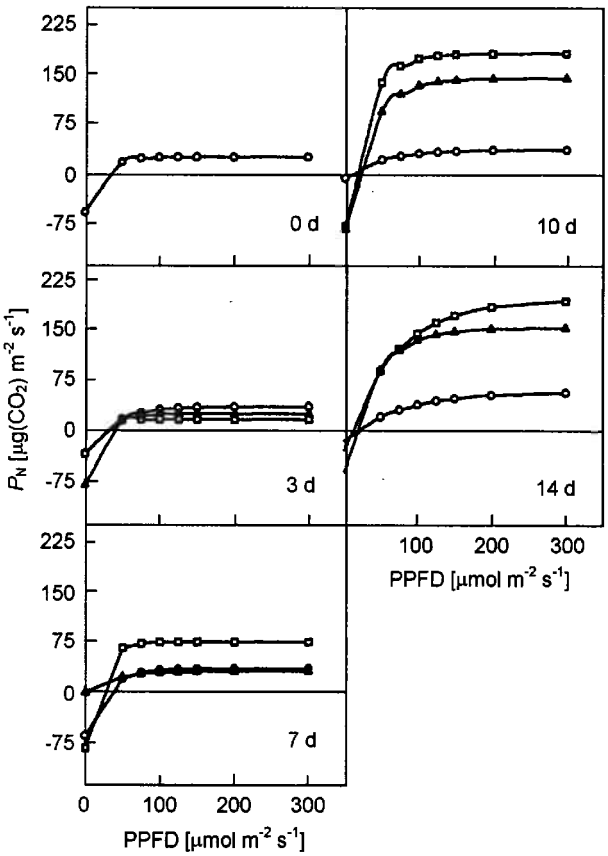

Fig. 5. Net photosynthetic rate, P_N, of *Rosa* plantlets grown for 0, 3, 7, 10, and 14 d under different treatments as a function of PPFD. O control plantlets; □ plantlets cultured on HM medium flushed with CO_2 enriched air (EC-plants); Δ plantlets cultured on HM medium flushed with ambient air (AC-plants). Means of 3 independent experiments.

The RuBPCO activity of plantlets grown on liquid media remains low beside that one of mature leaves (in which CO_2 fixation linked to RuBPCO accounted for 90 % of the total CO_2 fixation). This difference may be explained by the sustained growth of our plantlets while the leaves used for comparisons were mature. Such results were already reported by Eckermann and Bauman (1995).

Our results show that during the first half of the rooting phase, plantlets have not very functional photosynthetic apparatus (reduced photosynthetic capacities, weak enzymatic activities, and low chlorophyll

content, values not shown). During the second part of the culture, the photosynthetic apparatus is formed (increase of RuBPCO activity and chlorophyll content, values not shown). Hdider and Desjardins (1994) reported similar results for strawberry.

The g_s of 14-d-old EC- and AC-plants decreased in darkness, which is the evidence of stomata functioning. Moreover, stomata are more elliptical and the epidermal cell shape shows more sinuous undulations. These plant leaves exhibited anatomical characteristics similar to those grown in greenhouse. Capellades *et al.* (1990) showed similar results on *Rosa multiflora*. They indicated that *in vitro* pre-treatment (high irradiance and low RH) reduced transpiration plant losses and shortened the acclimatisation period in greenhouse. Indeed, the decrease of RH induces an increase of transpiration rate and so an increase of Ca^{2+} absorption. Sallanon *et al.* (1991) showed on *Rosa hybrida* that calcium content was 10 times lower in stomata of *in vitro*-grown plants than in stomata of greenhouse-grown plant and that stomata dysfunctioning in *in vitro* plants could be linked to low Ca^{2+} content. So, the stomata of CA- and CE-plants are functional and do regulate water losses and these plants may be acclimatised without a gradual decrease of RH.

The high P_N of plantlets grown on liquid medium allowed a better use of radiant energy during acclimatisation. Sucrose and starch accumulation, linked to a better photosynthesis functioning, may be used by the *in vitro* shootlets during the acclimatisation period (Capellades *et al.* 1991) and may explain the good survival rate in acclimatisation.

Table 1. Survival rate [%] after 2 and 13 weeks of acclimatisation in greenhouse for control plants, plants cultured on HM medium flushed with CO_2-enriched air (EC-plants) and plants cultured on HM medium flushed with ambient air (AC-plants). N: no particular RH; acclimatisation: acclimatisation with gradual decrease in RH.

Growth type	Survival rate [%] after 2 weeks acclimat.	N	13 weeks acclimat.	N
control	85	0	85	0
EC-plants	93	82	78	73
AC-plants	55	61	48	57

The development advantage acquired by plantlets cultured on liquid medium, was not maintained *ex vitro*. Navarro *et al.* (1994) obtained similar results on banana plants. However, the new cultivation procedure (hydroponic medium and low RH due to flushing with AC or EC) permits to produce plants without particular acclimatisation phase. The CO_2 enrichment was important during the rooting phase but it was not obligatory.

References

Arron, G.P., Bradbeer, J.W.: The use of two-dimensional polyacrylamide gel electrophoresis in the study of the biosynthesis of ribulosebiphosphate carboxylase. - In: Avron, M. (ed.): Proceedings of the Third International Congress on Photosynthesis. Vol. III. Pp. 2081-2088. Elsevier Scientific Publ., Amsterdam – Oxford – New York 1975.

Capellades, M., Lemeur, R., Debergh, P.: Effects of sucrose on starch accumulation and rate of photosynthesis in *Rosa* cultured *in vitro*. – Plant Cell Tissue Organ Cult. **25**: 21-26, 1990.

Chen, N.T.M., Brown, R.H., Black, C.C., Jr.: Photosynthetic $^{14}CO_2$ fixation products and activities of enzymes related to photosynthesis in bermudagrass and other plants. – Plant Physiol. **47**: 199-203, 1971.

Collet, G.F.: Enracinement amélioré lors de la production *in vitro* de rosiers. – Rev. suisse Vitic. Arbor. Hort. **17**: 259-263, 1985.

Coudret, A., Ducher, M.: Les carboxylations dans les systèmes cellulaires de type C3. – Acta bot. Gall. **139**: 397-411, 1993.

Dhawan, V., Bhojwani, S.: Hardening *in vitro* and morpho-physiological changes in the leaves during acclimatization of micropropagated plants of *Leucaena leucocephala* (Lam.) de Wit. – Plant Sci. **53**: 65-72, 1987.

Eckermann, N., Baumann, G.: Enzymatic changes in callus cultures of sugar beet during the transition from photoheterotrophic to photoautotrophic growth. – Photosynthetica **31**: 163-175, 1995.

Gebhart, T.K.: Development of a sterile cultivation system for rooting of shoot tip cultures (red raspberries) in duroplast foam. – Plant Sci. **39**: 141-148, 1985.

Genoud, C., Coudret, A., Amalric, C., Sallanon, H.: Effects of micropropagation conditions of rose shootlets on chlorophyll fluorescence. – Photosynthetica **36**: 243-251, 1999.

Genoud-Gourichon, C., Sallanon, H., Coudret, A. : Effects of sucrose, agar, irradiance and CO_2 concentration during the rooting phase on the acclimation of *Rosa hybrida* plantlets to *ex vitro* conditions. – Photosynthetica **32**: 263-270, 1996.

Genoud-Gourichon, C., Veisseire, P., Coudret, A.: Effects of pre-treatment temperature and cap closure on photosynthesis potentialities of potato cultivated *in vitro*. – Photosynthetica **29**: 73-79, 1993.

Hase, A.: Changes in respiratory metabolism during callus growth and adventitious root formation in Jerusalem artichoke tuber tissues. – Plant Cell Physiol. **28**: 833-841, 1987.

Hdider, C., Desjardins, Y.: Changes in ribulose-1,5-bisphosphate carboxylase/oxygenase and phosphoenolpyruvate carboxylase activities and $^{14}CO_2$ fixation during the rooting of strawberry shoots *in vitro*. – Can. J. Plant. Sci. **74**: 827-831, 1994.

Hyndman, S.E., Hasegawa, P.M., Bressan, P.A.: Stimulation of root initiation from cultured rose shoots through the use of reduced concentrations of mineral salts. – HortScience **17**: 82-83, 1982.

Jay-Allemand, C., Capelli, P., Cornu, D.: Root development of

in vitro hybrid walnut microcuttings in a vermiculite-containing gelrite medium. – Scientia Hort. **51**: 335-342, 1992.

Kozai, T., Iwabuchi, K., Watanabe, K., Watanabe, I.: Photoautotrophic and photomixotrophic growth of strawberry plantlets *in vitro* and changes in nutrient composition of the medium. – Plant Cell Tissue Organ Cult. **25**: 107-115, 1991.

Kozai, T., Kubota, C., Watanabe, I.: Effects of basal medium composition on the growth of carnation plantlets in auto- and mixo-trophic tissue culture. – Acta Hort. **230**: 159-166, 1988.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassay with tobacco tissue cultures. – Physiol. Plant. **15**: 473-497, 1962.

Nato, A., Vidal, J.: Phosphoenolpyruvate carboxylase activity in relation to physiological processes during the growth of cell suspension cultures from *Nicotiana tabacum*. – Physiol. vèg. **21**: 1031-1039, 1983.

Navarro, C., Teisson, C., Côte, F., Ganry, J.: Effects of light intensity and CO_2 concentration on growth of banana plants (*Musa* AAA, cultivar "Petite Naine") *in vitro* and subsequent growth following acclimatization. – Scientia Hort. **60**: 41-54, 1994.

Passera, C., Albuzio, A.: Effect of salinity on photosynthesis and photorespiration of two wheat species (*Triticum durum* cv. PEPF 2122 and *Triticum aestivum* cv. Marzotto). – Can. J. Bot. **56**: 121-126, 1978.

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 – Basel – Hong Kong 1997a.

Pospíšilová, J., Čatský, J., Solárová, J., Šesták, Z., Synková, H., Wilhelmová, N.: Photosynthesis and water relations in plantlets cultivated *in vitro*. – Acta Univ. Carol. Biol. **41**: 177-194, 1997b.

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

Pospíšilová, J., Solárová, J., Čatský, J., Ondřej, M., Opatrný, Z.: The photosynthetic chararacteristics during the micropropagation of tobacco and potato plants. – Photosynthetica **22**: 205-213, 1988.

Pospíšilová, J., Tichá, I., Čatský, J., Solárová, J., Ondřej, M.: Photosynthesis in regenerants of tobacco transformed by plasmids of *Agrobacterium*. 3. Transplanted plants. – Photosynthetica **23**: 130-135, 1989.

Sallanon, H., Dimon, B., Carrier, P., Chagvardieff, P.: Effects of CO_2 concentration and irradiance on growth and photosynthesis of *Juglans regia* plantlets grown *in vitro*. – Photosynthetica **31**: 241-249, 1995.

Sallanon, H., Isaka, H., Dimon, B., Ravel, C., Chagvardieff, P.: CO_2 exchanges and nutrient uptake during multiplication and rooting of micropropagated *Juglans regia* plantlets. – Plant Sci. **124**: 107-116, 1997.

Sallanon, H., Laffray, D., Coudret, A.: Ultrastructure and functionning of guard cells of *in vitro* cultured rose plants. – Plant Physiol. Biochem. **29**: 333-339, 1991.

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

Sallanon, H., Tort, M., Coudret, A.: The ultrastructure of micropropagated and greenhouse rose plant stomata. – Plant Cell Tissue Organ Cult. **32**: 227-233, 1993.

Vandemoortele, J.L., Billard, J.P., Boucaud, J., Gaspar, T.: Effet de l'osmolarité et de la composition du milieu sur la callogénèse, la caulogenèse et la rhizogénèse de fragments d'hypocotyles de *Brassica oleracea* L. var. *botrytis*. – Biol. Plant. **35**: 17-24, 1993.

Vinterhalter, D., Vinterhalter, B.: Effect of inorganic nutrition on the formation of lateral roots in *Dracaena fragans* Ker-Gawl cultured *in vitro*. – Plant Cell Tissue Organ Cult. **28**: 267-274, 1992.

Ziv, M., Schwartz, A., Fleminger, D.: Malfunctioning stomata in vitreous leaves of carnation (*Dianthus caryophyllus*) plants propagated *in vitro*; implications for hardening. – Plant Sci. **52**: 127-134, 1987.