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

C. GENOUĐ, H. SALLANÓN, 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 macronutrients inhibit the lateral root formation. This inhibitory effect is a result of unbalanced SO$_2$ 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 (Capeillades et al. 1990, Sallanon et al. 1991), poorly developed vascular systems, and low photosynthetic activity (Pospšílova et al. 1988, 1989, 1992, Kozai 1991, Sallanon et al. 1997; for reviews see Pospšílova 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.

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Fax: (33) 4 71 45 57 51 ; e-mail: huguette.sallanon@u-clermont1.fr
Materials and methods

Rose (Rosa hybrida cv. Deladal) 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 cm² glass vessels closed with a polycarbonate lid contained 120 cm³ 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 KNO₃, 1.5 mM MgSO₄·7 H₂O, 3.47 mM Ca(NO₃)₂·4 H₂O, 1.73 mM NaH₂PO₄, 0.15 mM NaCl, 13.19 μM MnSO₄·4 H₂O, 1.03 μM ZnSO₄·7 H₂O, 30 μM H₃BO₃, 0.96 μM CuSO₄·5 H₂O, 0.18 μM CoSO₄, 0.03 μM Mn(C₂H₃O₂)₂·4 H₂O, 26.3 μM EDTA-Fe) in 25 000 cm³ polycarbonate box closed with polycarbonate lids, containing 4 000 cm³ of medium and 100 plantlets. The boxes were flushed at a flow rate of 5 cm³ min⁻¹ L⁻¹ with ambient air (CO₂ concentration of 340 cm³ m⁻³, AC-plants) or CO₂ enriched air (2 500 cm³ 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 %.

Results

Rooting phase: During rooting, the number of leaves per shoot and the leaf area were the same regardless of 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. 1A,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. 1D), 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. 1D,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. 1F).

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. 2A,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 gs was similar up to the day 7 in darkness and under light (Fig. 3A,B,C). On day 14, it was 3 times higher under light than in darkness for AC- and EC-plants (Fig. 3B,C).

Sucrose accumulated from day 7 at all growth conditions (Fig. 4A). 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. 4B).

From day 0, the PEPC activity rose from 17.7 μmol(CO₂) kg⁻¹(protein) s⁻¹ to maxima reached on day 7, of 97.0 μmol(CO₂) kg⁻¹(protein) s⁻¹ in control and 59.7 μmol(CO₂) kg⁻¹(protein) s⁻¹ in plantlets cultured in liquid media. The enzymatic activity gently decreased until day 10 and it stabilised afterwards (Fig. 4C).

The initial RuBPCO activity in control plantlets regularly grew from 1.3 μmol(CO₂) kg⁻¹(protein) s⁻¹ to 15.8 μmol(CO₂) kg⁻¹(protein) s⁻¹ (Fig. 4D). In plantlets from liquid media, it remained stable for the first 7 d, then increased to 113.8 μmol(CO₂) kg⁻¹(protein) s⁻¹ in AC-plantlets and to 128.0 μmol(CO₂) kg⁻¹(protein) s⁻¹ in both EC- and AC-plantlets.

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 microscopy. gs 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 Albuizio (1978) was used to determine RuBPCO and phosphoenolpyruvate carboxylase (PEPC) activities. CO₂ exchanges were measured as described by Genoud-Gourichon et al. (1993).

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