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Effects of micropropagation conditions of rose shootlets on chlorophyll fluorescence

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Abstract

Rosa hybrida plantlets were rooted on solid sucrosed medium (MS) under an irradiance (PPFD) of 45 μmol m^{-2} s^{-1} or on liquid hydroponic solution (MH) at 100 μmol m^{-2} s^{-1}. Then all plantlets were acclimated without sucrose under 100 μmol m^{-2} s^{-1} PPFD. After 7 d in rooting stage, the ratio of variable over maximal chlorophyll fluorescence (F_v/F_m) was significantly higher for plants grown in MH than in MS and hence the higher irradiance at this stage of growth had no photoinhibitory effect. The radiant energy was used by the photochemical process and also by photoprotective mechanisms of photosystem 2, expressed by increases in the rates of electron flux, net photosynthesis, and non-photochemical quenching. This effect on F_v/F_m was maintained during three weeks in acclimation phase. The resistance of plantlets increased as new leaves formed, and after six weeks in acclimation, there was no difference between the two conditions. The study under higher irradiance (100, 150, or 300 μmol m^{-2} s^{-1}) indicated that photoinhibition might take place at 300 μmol m^{-2} s^{-1} whatever the growth conditions.

Additional key words: acclimation; F_v/F_m; irradiance; net photosynthetic rate; nonphotochemical quenching; rooting; *Rosa hybrida*.

Introduction

Micropropagated plantlets are typically grown under special climatic conditions of high relative humidity (RH) and low irradiance; these conditions can result in reduced leaf epicuticular wax (Dhawan and Bhojwani 1987), increased stomatal conductance (Cappelades *et al.* 1990, Sallanon *et al.* 1991), low photosynthetic activity and poorly developed vascular systems (Pospíšilová *et al.* 1988, 1989, 1992, Pospíšilová 1996, Kozai 1991, Sallanon *et al.* 1997a,b; for review see Pospíšilová *et al.* 1997). These changes are the suggested causes for the susceptibility of plantlets

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to water stress during acclimation. Gradual hardening-off with periods of decreasing humidity are necessary for plantlets to survive the transition from culture to the greenhouse or the field.

In addition to water stress, factors affecting photosynthesis are important for the acclimation of time-cultured plants. If high quantities of sugar are present in the cultivation medium, photosynthetic capabilities are not essential to growth (Kozai 1991, Sallanon *et al.* 1995, Synková 1997). On leaving the *in vitro* conditions in the acclimation phase, these plants are suddenly placed in autotrophic conditions in a dryer atmosphere (80 %) and under higher photosynthetic fluxes (at least 100 μmol m^{-2} s^{-1}). Hardening-off plantlets *in vitro*, by reducing the humidity (Maene and Debergh 1987, Vanderschaeghe and Debergh 1987) and increasing the irradiance during the rooting phase, reduces losses because plantlets are less fragile when removed from culture (Short *et al.* 1987). Autotrophy was promoted in several species when the sugar in the medium was reduced or eliminated and when the carbon dioxide concentration and the irradiance were increased (Pospíšilová *et al.* 1992).

Moreover, excessive radiation can possibly lead to photoinhibition and water stress affects photosynthesis indirectly *via* stomata closure (Cornic *et al.* 1987). Chlorophyll (Chl) fluorescence is now used as a non-destructive and highly sensitive method for the determination of various aspects of photosynthetic metabolism (Lichtenthaler and Rinderle 1988) and processes of photoinhibition in *in vitro* plantlets (Cappelades *et al.* 1990, Pospíšilová *et al.* 1993, Sallanon *et al.* 1997a,b).

In this work we evaluated the effect of hardening-off plantlets by reducing the humidity and increasing the irradiance on photosynthesis and Chl fluorescence parameters during the rooting and acclimation of *Rosa hybrida* plantlets.

Materials and methods

A rose tree (*Rosa hybrida* Mme Delbard deladel) was micropropagated *in vitro* during the multiplication phase on Murashige and Skoog (1962) medium (MS) according to the method of Sallanon and Mazière (1992). During the rooting phase, plantlets were cultivated using two methods:

(*1*) MS conditions: Plants were grown on a solid medium containing MS salts with 30 kg m^{-3} of sucrose, 2.85 mM indole-3-acetic acid (IAA), and 7 kg m^{-3} of agar. Each 850 cm^3 glass vessel closed with a polycarbonate lid contained 120 cm^3 of medium and 30 plantlets. Relative humidity (RH) in the containers was 96 ± 2 %. The cultures were maintained in a growth room at a photosynthetic photon flux density (PPFD) of 45 μmol m^{-2} s^{-1} (*Mazdafluor TF65,* 58 W) for 16 h. Day/night air temperature was 23/19 ± 1 °C.

(*2*) MH conditions: The plants were grown on hydroponic nutrient solution, flushed with water-saturated ambient air at a flow of 5 cm^3 s^{-1}. Each 25 000 cm^3 polycarbonate box closed with a polycarbonate lid contained 4 000 cm^3 of medium and 100 plantlets. The RH in the containers was 80 ± 5 %. The cultures were

maintained in a growth room at a PPFD of 100 µmol m^{-2} s^{-1} (*Mazdafluor TF65*, 58 W), for 16 h. Day/night air temperature was 23/19 ± 1 °C.

Two weeks after transplantation, plantlets had roots and were able to survive in a greenhouse. During the acclimation phase, the RH was progressively decreased and the PPFD was 100 µmol m^{-2} s^{-1}.

Chl fluorescence emission from the upper surface of the leaves was measured with a pulse amplitude modulation fluorometer (system *MK II MFMS/2T Hansatech Instruments,* Norfolk, U.K.). The initial Chl fluorescence, F_0, was obtained after 30 min-dark adaptation. The maximal Chl fluorescence level, F_m, was obtained with a saturating flash (1 s, 5000 µmol m^{-2} s^{-1}, *FLS1* radiation source, *Hansatech*). After 200 s, actinic irradiation was initiated with a *LH7* source (*Hansatech*) at 100 µmol m^{-2} s^{-1}. Saturating flashes were fired every 50 s to determine maximal fluorescence during actinic exposure (F_m'). These conditions were maintained until a steady state of variable fluorescence, F'. The F_0' was measured after light extinction and far-red irradiation. Maximal photochemical efficiency in dark-adapted leaves (F_v/F_{mf}, where $F_v = F_m - F_0$), actual quantum efficiency of photosystem 2 (PS2) electron transport under actinic irradiation [$F2 = (F_m'-F')/F_m'$], the rate of electron flow ($J_f = \phi 2 \times I \times a \times f$, where I is the irradiance incident on the leaf, a the fractional absorbance of radiation by the leaf, f is the absorbance by PS2 divided by the absorbance of PS2+PS1; a = 0.8 and f = 0.5) and the non-photochemical quenching {$q_N = 1 - [(F_m' - F_0')/(F_m - F_0)]$} were calculated according to Schreiber (1986) and Genty *et al.* (1989).

Photoinhibition was imposed on plantlets by exposing them for 5 h to 100, 150, and 300 µmol m^{-2} s^{-1}. Net photosynthetic rate (P_N) of plantlets and photosynthetic irradiance saturation curves were measured as described by Genoud-Gourrichon *et al.* (1993).

Measurements were done on days 7 and 14 during the rooting phase, then after 21 and 42 d of acclimation. All experimental values were averages of four independent experiments. Means were compared by LSD (Least Significant Difference) at the 0.05 confidence level using the Student's *t*-test.

Results

Evolution of Chl fluorescence parameters and photosynthetic capacities dependent on culture conditions (MS or MH)

Chl fluorescence measurements: During the rooting phase and after 21 d of acclimation, the ratio F_v/F_m was higher for plantlets cultivated under MH conditions than under MS conditions (Table 1). After 42 d of acclimation, no difference appeared between plantlets rooted under these two conditions (Table 1). For the MS-plantlets, the rate of electron flow (J_f) was stable during the rooting phase, then it increased during the first 3 weeks of acclimation, and afterwards became stable (Table 1). In the MH-plantlets it increased during the second half of the rooting phase and after 21 d of acclimation. At this stage, J_f was similar for the two growth

conditions. The non-photochemical quenching (q_N) followed a similar evolution as J_f, after 14 d of rooting it was higher for MH-plantlets, and during acclimation it was similar for both conditions.

Table 1. Evolution of the ratio F_v/F_m, the rate of electron flow (J_f), and the nonphotochemical quenchinq (q_N) for plantlets cultivated under MS and MH conditions, during the rooting and the acclimation phase. Means followed by the same letter in columns are not significantly different at $p = 0.05$, with the Mann and Withney's test.

Growth phase, plantlet age	Condition	F_v/F_m	J_f	q_N
rooting phase (7 d)	MS	0.643 ± 0.002 (a)	4.72 ± 0.75 (a)	0.578 ± 0.074 (a)
	MH	0.664 ± 0.004 (b)	4.80 ± 0.54 (a)	0.557 ± 0.004 (a)
rooting phase (14 d)	MS	0.648 ± 0.008 (a)	4.85 ± 0.25 (a)	0.549 ± 0.019 (a)
	MH	0.675 ± 0.004 (b)	5.48 ± 0.48 (b)	0.636 ± 0.002 (b)
acclimation (35 d)	MS	0.606 ± 0,009 (c)	6.20 ± 0.09 (c)	0.718 ± 0.046 (c)
	MH	0.669 ± 0.008 (b)	6.62 ± 0.76 (c)	0.708 ± 0.054 (c)
acclimation (56 d)	MS	0.663 ± 0,016 (b)	6.00 ± 0.14 (c)	0.718 ± 0.049 (c)
	MH	0.666 ± 0.017 (b)	6.03 ± 0.14 (c)	0.713 ± 0.048 (c)

Photosynthesis measurements: In both growth conditions micropropagated plantlets showed a positive net photosynthetic rate (P_N) from 50 µmol m^{-2} s^{-1} PPFD (Fig. 1). With sugar in the medium, the respiration rate was increased. Under saturating irradiance (PPFD 150 to 200 µmol m^{-2} s^{-1}) during rooting, the P_N of 14-d-old MH-plantlets was significantly higher than that of the MS-plantlets. During acclimation no difference in P_N was found between the growth conditions (Fig. 1). All these values were higher than those obtained during rooting.

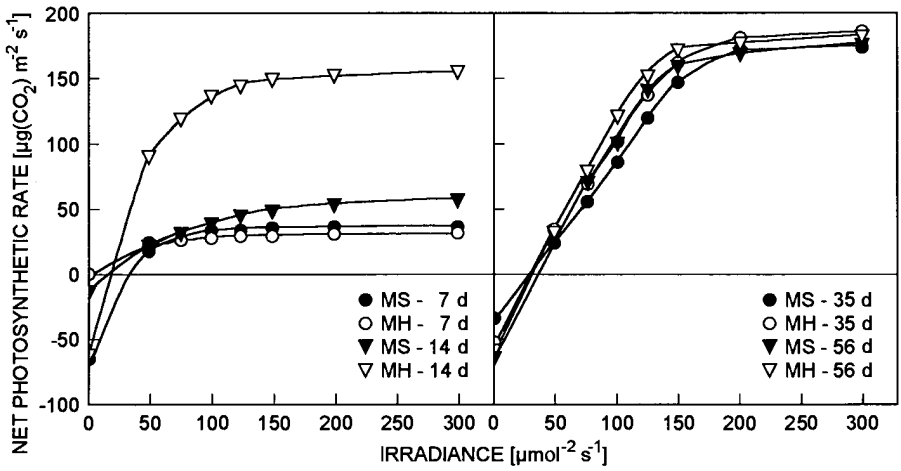

Fig. 1. Photosynthetic response to irradiance of plants 7- and 14-d-old and grown under MS or MH conditions during rooting (*left*) and plants 35- and 56-d-old in acclimation (*right*) (these plants had been rooted in MH or MS conditions).

Chl fluorescence parameters at different irradiances

In MS-plantlets (Fig. 2*A,B*) F_v/F_m decreased when the irradiance increased from 45 to 300 μmol m^{-2} s^{-1}, the trend being similar for 7- and 14-d-old plantlets. During the first weeks of acclimation (Fig. 2*C*), F_v/F_m decreased when the irradiance increased from 45 to 300 μmol m^{-2} s^{-1} but after 42 d of acclimation (Fig. 2*D*) the ratio was not statistically different after irradiations of 45, 100, or 150 μmol m^{-2} s^{-1}.

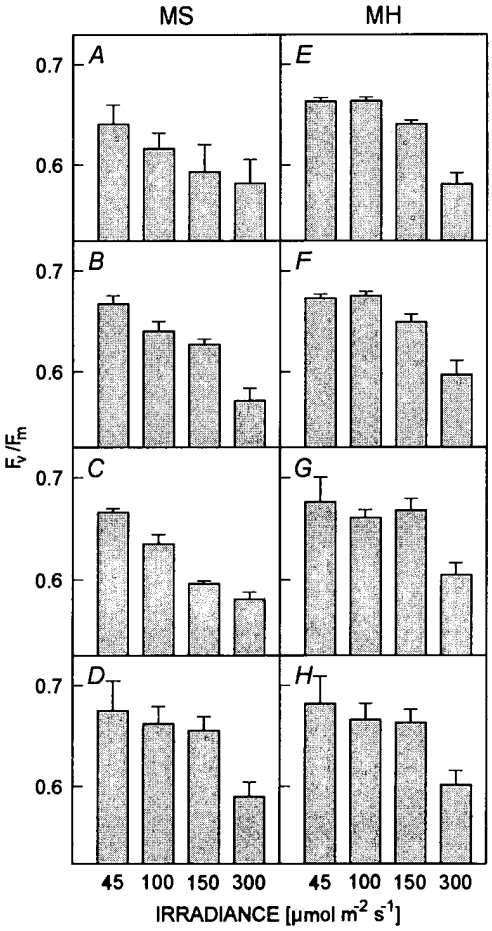

Fig. 2. Variation of the variable chlorophyll florescence ratio F_v/F_m under different irradiances (45, 100, 150, and 300 μmol m^{-2} s^{-1}) for plantlets cultivated under MS conditions (*A* to *D*) or MH conditions (*E* to *H*). *A, E*: 7-d-old plantlets in rooting phase; *B, F*: 14-d-old plantlets in rooting phase; *C, G*: 35-d-old plantlets in acclimation; *D, H*: 56-d-old plantlets in acclimation.

In MH-plantlets we did not find any significant difference under irradiances of 45 or 100 μmol m^{-2} s^{-1}. The F_v/F_m decreased by 4 % at PPFD of 150 μmol m^{-2} s^{-1}, and a further decrease of 10 % was observed after irradiation of 300 μmol m^{-2} s^{-1} (Fig. 2*E,F*). During 21 and 42 d of acclimation, the response was similar (Fig. 2*G,H*): the F_v/F_m was stable until 150 μmol m^{-2} s^{-1} and then decreased (by 10 %) under 300 μmol m^{-2} s^{-1}.

q_N of plantlets rooted under MS conditions (Fig. 3*A,B*) increased by 44 % when plantlets were irradiated by 100 μmol m^{-2} s^{-1}. During the acclimation phase, there

was no variation of q_N with the increase in irradiance (Fig. 3*C,D*). In MH-plantlets (Fig. 3*E,F*), the increase of q_N from 45 to 100 µmol m^{-2} s^{-1} was about 21 %. There was no difference of q_N between 100 and 300 µmol m^{-2} s^{-1}. During the acclimation (Fig. 3*G,H*) no variation was observed.

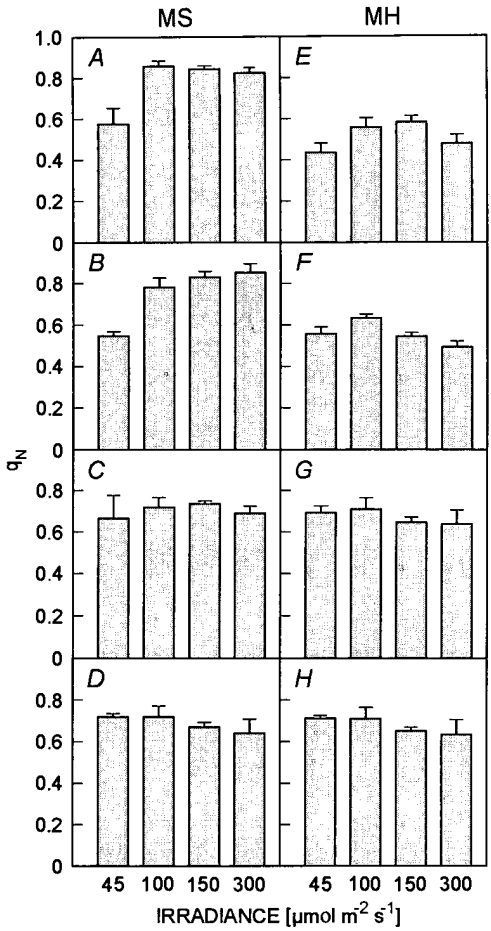

Fig. 3. Variation of the nonphotochemical quenching (q_N) under different irradiances (45, 100, 150, and 300 µmol m^{-2} s^{-1}) for plantlets cultivated under MS conditions (*A* to *D*) or MH conditions (*E* to *H*). *A, E*: 7-d-old plantlets in rooting phase; *B, F*: 14-d-old plantlets in rooting phase; *C, G*: 35-d-old plantlets in acclimation; *D, H*: 56-d-old plantlets in acclimation.

Discussion

The MH conditions (PPFD of 100 µmol m^{-2} s^{-1}) induced an increase in F_v/F_m 7 d after rooting. In MS conditions (45 µmol m^{-2} s^{-1} during the rooting phase and 100 µmol m^{-2} s^{-1} during the acclimation), the 7- and 14-d-old plantlets showed a lower F_v/F_m. The decline of F_v/F_m is linearly correlated with the quantum yield of light-limited oxygen evolution (Björkman and Demming 1987) and the number of functional PS2 centres (Öquist *et al.* 1992). It is also an indication that changes in climatic conditions during the transfer from the multiplication stage to rooting in

hydroponic medium (from 45 to 100 μmol m^{-2} s^{-1}) did not create stress and photoinhibition 7 d after this transfer. On the contrary, in these conditions the increase in irradiance induced an increase in the number of functional PS2 centres. This effect could be attributed to the suppression of sugar in the medium. Several authors have reported a repressive effect of sucrose on the *in vitro* photosynthetic activity of isolated cells (Neumann *et al.* 1989) or plantlets (Van Huylenbroeck *et al.* 1995, Synková 1997) but it seems also that sugar in the medium limits the differentiation of chloroplasts and the development of the structural system implied in the primary reaction of photosynthesis.

The decline in F_v/F_m during the first weeks after the transfer from *in vitro* to *ex vitro* conditions indicates that climatic conditions create stress in MS-plantlets but not in the MH-plantlets. The resistance against high irradiance increased as the plants rooted and new leaves were formed, and we observed that the MH-plantlets had more leaves than the MS-plantlets (values not shown). Similar results were observed on *Spathiphyllum* (Van Huylenbroeck *et al.* 1995). The decrease of F_v/F_m and the increase of q_N are directly related to damage and degradation of the D1 protein in the reaction centre. So the decrease in F_v/F_m when plantlets were transferred to the greenhouse suggested damages of photosystems of MS-plantlets. The resistance against high irradiance increased when new leaves were formed which was reflected by an increased F_v/F_m after weeks of acclimation. Similar results were observed on *Eucalyptus* plantlets (Kirdmanee *et al.* 1995).

For all plantlets, the increase in q_N after transferring to the greenhouse suggested that a part of the energy was used to protect photosystems. This dissipation process involves the xanthophyll cycle with the formation of zeaxanthin and indicates photoprotective non-radiative dissipation of excitation energy (Demmig-Adams and Adams 1992). But in MS conditions, the photoprotective process that could be estimated by q_N was not sufficient to suppress the decrease in F_v/F_m during the first weeks of acclimation. At the same time, P_N and J_f of 14-d-old MH-plantlets showed a significant increase. The stage of growth during the transfer to the greenhouse could explain the greater resistance of plantlets in hydroponic conditions. Otherwise, after 6 weeks of acclimation, no difference was found between MS- and MH-plantlets.

The study on photoinhibition (exposure 5 h under PPFD of 100, 150, or 300 μmol m^{-2} s^{-1}) allowed to state that all plantlets were sensitive to higher PPFD. But the decrease in F_v/F_m appeared more rapidly and was more marked for MS-plantlets. These high irradiances had an effect on plantlets cultivated under MS conditions during rooting and after 21 d of acclimation. After six weeks of this stage, only a PPFD of 300 μmol m^{-2} s^{-1} induced an F_v/F_m decrease. The sudden increase of irradiance induced an increase of q_N. This increase was higher as the F_v/F_m decreased, but the growth conditions were not sufficient to inhibit it. On the contrary, the F_v/F_m of MH-plantlets during rooting was strongly decreased only under a PPFD of 300 μmol m^{-2} s^{-1}. The difference of sensitivity between MS- and MH-plantlets may be explained by lower irradiance and sucrose medium. These differences involved the poor differentiation of chloroplasts in *in vitro* formed leaves (Lee *et al.* 1985) but they also indicated that the suppression of sugar and increased irradiation

had not the same effect on plantlet aging. Our study indicates that during the two weeks of rooting under a higher PPFD and without sugar, the primary reactions of photosynthesis were strongly modified. This effect was maintained during the following three weeks of acclimation.

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