PHOTOSYNTHETICA 39 (1): 29-33, 2001

Gas exchange of *in vitro* and *ex vitro* grown grapevine plants

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Abstract

Net photosynthetic rate (P_N) and dark respiration rate (R_D) were measured in *Vitis vinifera* L. cvs. Dimiat 4/24 (23rd subculture), Dimiat 4/38 (22nd subculture), and Italian Riesling 3/47 (22nd subculture) on days 3, 2, and 1 (1st series) before transfer from the *in vitro* culture and on days 14, 15, 16 (2nd series) and 28, 29, 30 (3rd series) after the transfer. P_N of *in vitro* and *ex vitro* plants was strongly affected by irradiance. P_N and R_D of *in vitro* plantlets were lower and transpiration rate (E) was higher compared to those of *ex vitro* plantlets. P_N, R_D, and E changed in the course of acclimation.

Additional key words: acclimation; dark respiration rate; fresh mass; leaf area; net photosynthetic rate; shoot and root lengths; shoot node number; transpiration rate; *Vitis vinifera L.*

Introduction

Clonal propagation of herbaceous and woody plants has become a well-established practice. Micro-propagation offers rapid multiplication of virus-free plants continuously during the year. To improve this technology, the effects of limiting factors during *in vitro* cultivation of the plantlets must be minimised. The nutrient medium improvement was investigated with *in vitro* grapevine plants (Galzy *et al.* 1990, Dimitrova 1998). Plantlets are generally supplied by saccharides (mainly sucrose) as sources of carbon and energy (Genoud *et al.* 1999). Nevertheless, *in vitro* plantlets develop photosynthetic apparatus enabling photoautotrophy under suitable environmental conditions. The limiting factors for *in vitro* plant photosynthesis are mainly low irradiance and low CO_2 concentration in culture vessels (Lee *et al.* 1985, Pospíšilová *et al.* 1997). Their increase led to an improved photosynthesis (Schoch *et al.* 1989, Solárová *et al.* 1989). Corresponding investigations with grapevine plants are rare (Falque *et al.* 1991, Lima da Silva *et al.* 1996).

A further complication to commercial production has been poor acclimation (Gribaudo and Fronda 1993). Slowly growing plants are extremely sensitive to environmental conditions, especially to water stress (Lakso *et al.* 1986, Lewandowski 1991). The study of environmental effects on photosynthesis, respiration, and transpiration during acclimation help to improve plant survival and growth (Slavtcheva and Dimitrova 1997, 1999). Finally, appropriate methods and precise measuring techniques are needed for a study of gas exchange of *in vitro* or *ex vitro* plants (Falque *et al.* 1991, Lima da Silva *et al.* 1996, Slavtcheva and Dimitrova 1997).

In this paper, a comparison between gas exchanges of *in vitro* and *ex vitro* grapevine plants is demonstrated. Special emphasis is given to the effects of irradiance on P_N of both groups of plants.

Materials and methods

Plants: *Vitis vinifera* L. cvs. Dimiat 4/24 (23rd subculture) Dimiat 4/38 (22nd subculture), and Italian Riesling 3/47 (22nd subculture) were grown in vessels (fruit-jars) as one node micro-cuttings (single leaves attached) on MS medium with half-strength macro-salts, full-strength micro-salts, and 1 g m^{-3} indole-3-acetic acid (Slavtcheva and Dimitrova 1997, Dimitrova 1998). The *ex vitro* plants were cultured at temperature of 26-28 °C, irradiance of 60-100 μmol m^{-2} s^{-1}, and a photoperiod of 16 h. Following *in vitro* culture for one month, plantlets were transferred to non-sterile conditions for adaptation before their transplanting into greenhouse or field. Roots of plantlets were cut to 1-cm length. As substrate, perlite was placed in small (55×35×65 mm) plastic vessels. Plantlet acclimation occurred at irradiance of 100 μmol m^{-2} s^{-1}, temperature of 24-26 °C, and air humidity of 90-

Received 27 July 2000, *accepted* 20 November 2000.
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70 %. Main plant growth characteristics were determined during *in vitro* cultivation and acclimation.

Gas exchange measurements: Three series of measurements of P_N and R_D were carried out: (*a*) on days 3, 2, 1 (1st series) before transfer from *in vitro* culture; and (*b*) on days 14, 15, 16 (2nd series) and 28, 29, 30 (3rd series) after transfer from *in vitro* culture. Two vessels per variant, *i.e.*, 10 plantlets, were placed in exsiccators connected to an open measuring circuit. Large blower prevented from CO_2-reduction in the air within the exsiccators. Air flow rate through the circuit was 22.2-27.8 $cm^3 s^{-1}$. P_N and R_D were determined by means of infrared gas analyser *URAS-2* (Frankfurt/Main, Germany) connected to a 12-point-recorder *Polycomp* (Frankfurt/Main, Germany). During measurements, air temperature and relative humidity in the laboratory were regulated with an air conditioner *KT-2* (Schkeuditz, Germany). The ambient temperature was 21-23 °C, air humidity 80-85 %, and CO_2 concentration 800 mg m^{-3}. High-pressure mercury vapour lamps *LRF-400 W* (Warsaw, Poland) gave irradiances of 20, 100, 200, and 400 μmol $m^{-2} s^{-1}$.

E was determined gravimetrically on day 1 before transfer from *in vitro* culture and on days 17 and 31 after the transfer. Fresh mass of whole plantlets was determined on an analytical balance *Sartorius* (Göttingen, Germany). *E* was determined at low irradiance of 20 μmol $m^{-2} s^{-1}$, 21 °C, and 70 % air humidity for 6 min.

Leaf area was determined gravimetrically by means of leaf copies.

Statistical analysis: Analysis of variance and regression was made following Barov and Naidenova (1969).

Results and discussion

Plant growth characteristics:

***In vitro* plants**: The plantlets developed well under the experimental conditions (Table 1). Considerable cultivar-specific differences were found in root growth. In this trial, the longest root (173.7 mm) was found in Italian Riesling. However, shoot lengths and node numbers were smaller in this cultivar.

***Ex vitro* plants**: Plantlets developed rapidly (within 31 d) a good root system (254-274 mm). More considerable differences (Table 1) in plant growth characteristics occurred during third and fourth weeks of adaptation period compared to the first and second weeks. The findings corresponded to the results of Pospíšilová *et al.* (1999) with tobacco plantlets.

Table 1. Main growth characteristics of *in vitro* and *ex vitro* plants. * Roots of plantlets were cut to 1 cm. Means of 9-11 replications ± standart error of mean.

	Variant	Shoot length [mm]	node number	Total root length [mm]	Leaf area [cm^2 $plant^{-1}$]	Fresh mass [g $plant^{-1}$]
In vitro plants	Dimiat 4/38	54.2 ± 4.8	4.4 ± 0.2	112.4 ± 13.8	12.3 ± 0.2	0.280 ± 0.030
	Dimiat 4/24	57.3 ± 3.0	4.4 ± 0.2	122.9 ± 12.4	17.0 ± 0.9	0.342 ± 0.032
	Italian Riesling 3/47	38.8 ± 3.5	3.0 ± 0.2	173.7 ± 19.1	12.9 ± 0.3	0.389 ± 0.041
Ex vitro plants, 17 d	Dimiat 4/38	64.7 ± 4.5	5.7 ± 0.3	96.5* ± 9.1	19.0 ± 0.4	0.540 ± 0.047
	Dimiat 4/24	62.6 ± 4.5	5.7 ± 0.3	120.4 ± 9.7	21.6 ± 0.2	0.581 ± 0.016
	Italian Riesling 3/47	65.3 ± 3.8	6.0 ± 0.3	123.3 ± 12.1	15.5 ± 0.2	0.438 ± 0.039
Ex vitro plants, 31 d	Dimiat 4/38	80.2 ± 3.7	6.3 ± 0.3	253.7 ± 24.1	24.4 ± 0.6	1.152 ± 0.078
	Dimiat 4/24	83.8 ± 3.9	6.1 ± 0.2	245.1 ± 29.3	30.8 ± 2.3	1.445 ± 0.011
	Italian Riesling 3/47	70.3 ± 1.7	6.7 ± 0.3	274.3 ± 23.8	29.6 ± 1.9	1.278 ± 0.031

Photosynthetic rate:

***In vitro* plants**: P_N increased with increasing irradiance (Table 2), larger changes being found from 20 to 200 μmol $m^{-2} s^{-1}$ (by 83-90 %) than from 200 to 400 μmol $m^{-2} s^{-1}$. However, saturation irradiance was not reached with any cultivar at even at the highest irradiance used. The effect of irradiance on P_N was significant ($F_{exp} = 279.80 > F_{0.001} = 7.55$). P_N increased from the first to the third measuring day before transfer. The differences, although small, were significant ($F_{exp} = 26.76 > F_{0.001} = 9.61$). Further, there were clones in the trial significantly different ($F_{exp} = 8.21 > F_{0.01} = 5.85$) among themselves. The plantlets of Italian Riesling 3/47 and Dimiat 4/24 had higher P_N compared to Dimiat 4/38 ($F_{exp} > F_{0.001}$or $F_{0.05}$, respectively).

Table 2. Rates of dark respiration (R_D) and net photosynthesis (P_N) [μg(CO_2) m^{-2} s^{-1}] of *in vitro* and *ex vitro* grapevine plants measured on days 3, 2, and 1 before transfer (marked by *minus* sign), and on days 14, 15, 16, 28, 29, and 30 of acclimation, respectively. P_N was determined at irradiances of 20-400 μmol m^{-2} s^{-1}. Means ± SE, $n = 10$.

Cultivar	Day	R_D	P_N 20	100	200	400
Dimiat 4/38	−3	-9.4 ± 1.9	11.1 ± 3.1	33.3 ± 3.3	62.2 ± 3.3	64.4 ± 2.5
	−2	-9.2 ± 1.9	10.8 ± 3.1	48.9 ± 1.7	60.8 ± 3.9	73.6 ± 3.9
	−1	-12.5 ± 1.9	15.6 ± 3.3	57.2 ± 3.1	70.0 ± 3.6	77.8 ± 4.7
	14	-20.6 ± 2.0	-2.5 ± 3.9	85.0 ± 3.9	112.2 ± 5.6	118.1 ± 4.2
	15	-30.0 ± 0.8	10.3 ± 2.8	85.6 ± 4.4	106.4 ± 3.3	114.4 ± 6.7
	16	-26.4 ± 0.8	2.2 ± 2.8	83.6 ± 3.3	110.3 ± 5.3	115.8 ± 6.1
	28	-36.1 ± 2.2	-5.6 ± 4.7	84.7 ± 1.7	105.3 ± 3.6	129.4 ± 6.9
	29	-45.6 ± 3.3	-7.8 ± 2.8	92.2 ± 1.9	108.3 ± 2.5	143.9 ± 7.8
	30	-40.6 ± 2.8	+3.1 ± 4.2	111.4 ± 2.8	123.6 ± 2.5	167.2 ± 8.3
Dimiat 4/24	−3	-8.3 ± 1.1	16.1 ± 3.3	48.1 ± 3.9	60.3 ± 3.6	70.0 ± 2.8
	−2	-8.9 ± 1.9	11.1 ± 3.6	54.4 ± 2.8	65.6 ± 2.8	73.9 ± 4.6
	−1	-8.3 ± 1.9	21.7 ± 3.6	63.1 ± 3.3	73.6 ± 4.0	81.1 ± 2.5
	14	-17.8 ± 1.9	-6.4 ± 3.6	70.3 ± 1.7	99.2 ± 3.3	110.0 ± 2.8
	15	-25.6 ± 0.8	13.1 ± 2.5	74.7 ± 2.2	93.1 ± 2.2	101.7 ± 4.7
	16	-23.6 ± 0.8	9.7 ± 2.5	71.1 ± 1.7	102.2 ± 3.3	109.2 ± 2.5
	28	-37.8 ± 1.4	1.4 ± 1.1	75.0 ± 1.7	93.9 ± 3.6	116.7 ± 6.1
	29	-41.9 ± 2.5	-5.8 ± 3.3	83.1 ± 2.2	94.4 ± 2.5	128.3 ± 6.1
	30	-37.2 ± 1.4	0.3 ± 3.6	98.1 ± 2.8	105.8 ± 3.9	148.1 ± 5.0
Riesling Italian 3/47	−3	-13.9 ± 1.1	10.3 ± 3.3	40.6 ± 2.2	66.9 ± 3.3	80.6 ± 4.2
	−2	-14.2 ± 1.7	11.7 ± 3.6	55.3 ± 1.9	74.2 ± 3.1	84.4 ± 4.7
	−1	-15.6 ± 1.1	14.2 ± 3.1	63.6 ±3.5	73.3 ± 3.3	94.2 ± 4.2
	14	-16.1 ± 1.1	-1.4 ± 3.9	86.4 ± 4.7	126.4 ± 6.1	146.7 ± 8.1
	15	-37.2 ± 0.8	15.8 ± 2.5	99.4 ± 4.7	118.6 ± 7.2	133.1 ± 8.6
	16	-32.8 ± 1.7	18.1 ± 3.3	97.5 ± 5.0	122.5 ± 5.6	137.8 ± 7.2
	28	-33.3 ± 2.8	-0.6 ± 2.2	96.4 ± 1.7	113.3 ± 5.0	154.7 ± 6.7
	29	-44.4 ± 2.5	-0.8 ± 5.0	125.5 ± 3.1	126.9 ± 3.6	172.8 ± 6.7
	30	-41.9 ± 3.3	9.2 ± 4.4	125.0 ± 2.5	148.1 ± 4.2	199.4 ± 8.1

***Ex vitro* plants:** P_N was more strongly affected by irradiance (Table 2). The values were in the range of those found with small pot-plants cultured *in vivo* (Stoev and Slavtcheva 1982). The differences were significant: (F_{exp} = 394.58 > $F_{0.001}$ in the 2nd series; F_{exp} = 411.59 > $F_{0.001}$ in the 3rd series). P_N changed from the first to the third measuring day not significantly in the 2nd series (F_{exp} < $F_{0.05}$), but the differences were significant (F_{exp} > $F_{0.001}$) in the 3rd series, probably due to their already better acclimation in this time. Also cultivar-specific differences were found (F_{exp} > $F_{0.001}$). The lowest values of P_N were established with Dimiat 4/24, the highest ones with Italian Riesling 3/47. The changes in the course of time during acclimation were not significant at low irradiance (20-100 μmol m^{-2} s^{-1}), but significant at irradiance of 400 μmol m^{-2} s^{-1}.

The changes in P_N at different irradiance with time were described by linear regressions:
at 20 μmol m^{-2} s^{-1}, y = 15.3 − 0.6 x, t_{exp} = 1.331 < $t_{0.05}$;
at 100 μmol m^{-2} s^{-1}, y = 66.1 + 1.1 x, t_{exp} = 2.163 < $t_{0.05}$;
at 200 μmol m^{-2} s^{-1}, y = 105.6 + 0.3 x, t_{exp} = 0.597 < $t_{0.05}$;
at 400 μmol m^{-2} s^{-1}, y = 87.5 + 2.2 x, t_{exp} = 2.945 > $t_{0.05}$,
where y was P_N [μg(CO_2) m^{-2} s^{-1}] and x the time in days [d] after transfer from *in vitro* culture.

Finally, P_N of *ex vitro* plants at 100-400 μmol m^{-2} s^{-1} was higher than that of the *in vitro* ones: 1.3-1.8 times in the 2nd series, and 1.5-2.1 times in the 3rd series. We found that the photosynthetic apparatus of *in vitro* grown grapevine plants was functional and there was positive CO_2 balance. The plantlets were capable for simultaneous saccharide uptake from the culture medium and CO_2 assimilation. P_N might vary according to cultivar and especially to environment. The acclimation procedure used in our laboratory was suitable and the plantlets survived satisfactorily this critical step. P_N was high enough to support plant growth after transfer from *in vitro* culture.

Dark respiration rate:

***In vitro* plants**: The mean values found with *in vitro* plantlets (Table 2) were from –14.6 up to –8.5 μg(CO_2) m^{-2} s^{-1}. Compensation irradiance was 6 μmol m^{-2} s^{-1} (Dimiat 4/24), 10 μmol m^{-2} s^{-1} (Dimiat 4/38), or 12 μmol m^{-2} s^{-1} (Italian Riesling 3/47). The values were lower or similar to those in *in vivo* plants (Geisler 1963, Stoev and Slavtcheva 1982). Thus the *in vitro* vine plants could utilise low doses of photons better than the *in vivo* grown plants. Cultivar-specific differences in R_D were also found ($F_{exp} > F_{0.01}$). Nevertheless, the Dimiat clones had lower R_D and differed significantly from the Italian Riesling clone, but not between themselves. Non-significant differences were observed among the R_D values on the different measuring days.

***Ex vitro* plants**: R_D of *ex vitro* plantlets were (Table 2) from –28.3 up to –22.3 μg(CO_2) m^{-2} s^{-1} in the 2nd series, and from –40.8 up to –39.0 μg(CO_2) m^{-2} s^{-1} in the 3rd series, *i.e.*, 1.9-4.7 times higher than in the *in vitro* plants. In the 2nd series, compensation irradiance was 16 μmol m^{-2} s^{-1} (Dimiat 4/24), 20 μmol m^{-2} s^{-1} (Dimiat 4/38), and 14 μmol m^{-2} s^{-1} (Italian Riesling 3/47). In the 3rd series, the compensation irradiance was higher (20, 22, 22 μmol m^{-2} s^{-1}, respectively). The values were in the range found with *in vivo* pot plants (Geisler 1963, Stoev and Slavtcheva 1982). The findings correspond to our previous results with *in vitro* grown plants during acclimation (Slavtcheva and Dimitrova 1997, 1999). Cultivar-specific differences were not found during acclimation. Significant differences existed among R_D on different measuring days ($F_{exp} = 8.46 > F_{0.05}$; $F_{exp} = 10.66 > F_{0.05}$) of the two series. R_D on the 2nd and 3rd day differed significantly from that on the 1st day.

R_D changed during acclimation. The effect of timing was significant. The relationship was described by linear regression:

$$y = -8.3 - 1.1\,x, \text{ with } t_{exp} = 3.844 > F_{0.05},$$

where y is R_D [μg(CO_2) m^{-2} s^{-1}] and x is the time [d] after transfer under *ex vitro* conditions. In fact, R_D (absolute value) increased during acclimation.

Table 3. Rates of transpiration [g(H_2O) kg^{-1}(plant fresh mass) s^{-1}] of *in vitro* and *ex vitro* grapevine plants. Means ± SE, $n = 10$.

Time [d]	Dimiat 4/38	Dimiat 4/24	Italian Riesling 3/47
1 before transfer	0.283 ± 0.013	0.295 ± 0.015	0.271 ± 0.015
17 after transfer	0.116 ± 0.004	0.154 ± 0.006	0.171 ± 0.007
31 after transfer	0.084 ± 0.004	0.088 ± 0.004	0.121 ± 0.006

Transpiration rate:

***In vitro* plants**: *E* of whole plantlets was measured before their transfer to non-sterile conditions (Table 3). *E* was between 0.271 (Italian Riesling 3/47) and 0.295 (Dimiat 4/24) g(H_2O) kg^{-1}(plant fresh mass) s^{-1}: these values were in the range usual for *in vivo* cultured plants (Vignes *et al.* 1974). However, *E* for *in vitro* plants was higher than for *ex vitro* plants of grapevine (Slavtchevaand Dimitrova 1997). Thus the roots must actively replace water loss, absorbing water from the medium and thus maintaining open stomata.

***Ex vitro* plants**: *E* (Table 3) was 1.6 to 3.4 times lower than in *in vitro* plants. It was from 0.116 (Dimiat 4/38) up to 0.171 (Italian Riesling 3/47) g(H_2O) kg^{-1}(plant fresh mass) s^{-1} two weeks after transfer from *in vitro* culture, from 0.084 (Dimiat 4/38) up to 0.121 (Riesling 3/47) four weeks after transfer. Highest *E* was found in Riesling 3/47. According to Iacono *et al.* (1992), water control by genotype exists with vine plants grown *in vitro* or *in vivo*: *Vitis riparia* showed higher *E* than *Vitis rupestris* in reducing water use efficiency (WUE). In *V. vinifera*, Bravdo *et al.* (1972) observed cultivar-specific differences in physiological behaviour of *in vivo* cultured grapevine plants. Queen of the Vineyards and Sultanina had significantly higher *E* than Muscat Hamburg. The differences in *E* among our grapevine cultivars were significant ($F_{exp} = 19.18 > F_{0.01}$). The result corresponds to our previous findings with *in vitro* grown plants during acclimation (Slavtcheva and Dimitrova 1997). *E* measured with *ex vitro* plants was in the range of values established with *in vivo* cultured plants under non-optimal conditions (Vignes *et al.* 1974).

E decrease with time during acclimation could be described by a linear regression:

$$y = 0.207 - 0.0035\,x, \text{ with } t_{exp} = 7.313 > t_{0.05},$$

where y is *E* [g(H_2O) kg^{-1}(plant fresh mass) s^{-1}] and x the time [d] after transfer under *ex vitro* conditions.

These findings correspond with those of Pospíšilová *et al.* (1999) obtained with tobacco plants where *E* gradually decreased because stomatal regulation of water loss became more effective and cuticle and epicuticular waxes developed. However, as the fresh mass of our experimental plants increased (from 0.520 to 1.292 g) during the adaptation period, *E* per plant increased with time.

References

Barov, V., Naidenova, P.: [Statistical Methods for Field and Vegetative Experiments.] – Zemizdat, Sofia 1969. [In Bulg.]

Bravdo, B., Lavee, S., Samish, R.M.: Analysis of water consumption of various grapevine cultivars – Vitis **10**: 279-291, 1972.

Dimitrova, V.: Clonal micropropagation of cv. Pamid (*Vitis vinifera* L., ssp. *sativa*) and nutrient medium composition optimization. – Wein-Wissenschaft **53**: 168-171, 1998.

Falque, M., Compan, D., Galzy, R.: A method for the study of CO_2 exchange in *in vitro* cultured *Vitis rupestris* plantlets. – Plant Cell Tissue Organ Cult. **27**: 175-181, 1991.

Galzy, R., Compan, D.: Remarks on mixotrophic and autotrophic carbon nutrition of *Vitis* plantlets cultured *in vitro*. – Plant Cell Tissue Organ Cult. **31**: 239-244, 1992.

Galzy, R., Haffner, V., Compan, D.: Influence of three factors on the growth and nutrition of grapevine microcuttings. – J. exp. Bot. **41**: 295-301, 1990.

Geisler, G.: Art- und sortenspezifische CO_2-Assimilationsraten von Reben unter Berücksichtigung wechselnder Beleuchtungsstärken. – Mitt. Klosterneuburg **A 13**: 301-305, 1963.

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

Gribaudo, I., Fronda, A.: [Acclimation of micropropagated fruit plants.] – Riv. Frutt. **55**: 75-80, 1993. [In Ital.]

Iacono, F., De Micheli, L., Campostrini, F., Falcetti, M., Martinelli, L.: Comparison between photosynthesis and transpiration of *in vitro* cultured plantlets and *in vivo* cultured plants of genus *Vitis*. Implications of transplanting. – In: Gay, G., Gribaudo, I., Novello, V., Schubert, A. (ed.): Proc. 4th Int. Symp. Grapevine Physiol. Pp. 507–511. Giovanni Dalmasso Found., Turin 1993.

Lakso, A.N., Reisch, B.I., Mortensen, J., Roberts, M.H.: Carbon dioxide enrichment for stimulation of growth of *in vitro*-propagated grapevines after transfer from culture. – J. amer. Soc. hort. Sci. **111**: 634-638, 1986.

Lee, N., Wetzstein, H.Y., Sommer, H.E.: Effects of quantum lux density on photosynthesis and chloroplast ultrastructure in tissue-cultured plantlets and seedlings of *Liquidambar styraciflua* L. towards improved acclimatization and field survival. – Plant Physiol. **78**: 637-641, 1985.

Lewandowski, V.T.: Rooting and acclimatization of micropropagated *Vitis labruska* "Dalaware". – Hort. Sci. **26**: 586-589, 1991.

Lima da Silva, A., Hariscain, P., Ollat, N., Doazan, J.P.: Estimation de la capacité photoautotrophique de vitroplants de porte-grefte de vigne (var. Gravesac): mise au point d'un systeme de mesure de la photosynthèse nette de vitroplants. – Vitis **35**: 73-78, 1996.

Pospíšilová, J., Čatský, J., Šesták, Z.: Photosynthesis in plants cultivated *in vitro*. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 525-540. M. Dekker, New York – Basel – Hong Kong 1997.

Pospíšilová, J., Tichá, I., Kadleček, P., Haisel, D., Plzáková, S.: Acclimatization of micropropagated plants to *ex vitro* conditions. – Biol. Plant. **42**: 481-497, 1999.

Schoch, P.-G., Lefèvre, B., Teisson, C., Ganry, J.: Photosynthèse et respiration de bananier *in vitro*. – Photosynthetica **23**: 113-118, 1989.

Slavtcheva, T., Dimitrova, V.: Gas exchange with *in vitro* cultured vine plants during their acclimatization. – Riv. Vitic. Enol. **50**: 3-9, 1997.

Slavtcheva, T., Dimitrova, V.: Rates of net photosynthesis and dark respiration of *in vitro* vine plants during acclimatization. – Vitis **38**: 183-184, 1999.

Solárová, J., Pospíšilová, J., Čatský, J., Šantrůček, J.: Photosynthesis and growth of tobacco plantlets in dependence on carbon supply. – Photosynthetica **23**: 629-637, 1989.

Stoev, K., Slavtcheva, T.: La photosynthèse nette chez la Vigne (*V. vinifera* L.) et les facteurs ecologiques. – Connais. Vigne Vin **16**: 171-185, 1982.

Vignes, D., Monange, Y., Carles, J.: Morphologie foliare et échanges gazeux chez la Vigne vierge. – Compt. rend. Acad. Sci. Paris **D 278**: 1365-1368, 1974.