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Research Note

Rates of net photosynthesis and dark respiration of *in vitro* vine plants during acclimatization

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K e y w o r d s : grapevine, gas exchange, light, temperature, in vitro culture.

Introduction: In breeding programs, *in vitro* propagation can be successfully used as an alternative for rapid multiplication. To improve this technology it is important to increase the limiting factors of *in vitro* plant photosynthesis, low CO_2 concentration and low light intensities. (SCHOCH *et al.* 1989; FALQUE *et al.* 1991; DÜRING and HARST 1996). There is a lack of knowledge concerning the biological behaviour of plantlets during acclimatization (BRENDEL 1986; GRIBAUDO and FRONDA 1993) when slowly growing plants are extremely sensitive to environmental conditions, especially to water stress. Experiments on gas exchange are of particular interest (SLAVTCHEVA and DIMITROVA 1997).

In this paper, rates of net photosynthesis and dark respiration of *in vitro* plants during their acclimatization were determined. Special emphasis is given to the effects of light and temperature and to the mode of *in vitro* culture.

Materials and Methods: P l a n t m a t e r i a l : Investigations were carried out with clones of Dimiat (4/24, 4/38) and Italian Riesling (3/47). Two groups of plantlets were used: initially *in vitro*-propagated plants (IP) obtained from one-node cuttings of field-grown vines; micropropagated *in vitro* plants (MP) of the 17th (Dimiat 4/24), 15th (Dimiat 4/38) and 15th (Riesling 3/47) subculture, which were obtained from one-node cuttings (single leaves attached) of *in vitro* plantlets. The explants were cultured on modified MS medium with half-strength macrosalts, full-strength microsalts and indole-3-acetic acid (1mg·l^{-1}) (SLAVTCHEVA and DIMITROVA 1997). The explants were kept at 26-28 °C, 60-100 µmol quanta·m^{-2}·s^{-1} and a photoperiod of 16h. After *in vitro* culture for one month, the plantlets were transferred to non-sterile conditions for adaptation and acclimatized to 100 µmol quanta·m^{-2}·s^{-1} and 26-28 °C. On days 18 and 32 of the adaptation period plant growth characteristics were determined.

G a s e x c h a n g e : Two series of measurements of photosynthesis (Pn) and dark respiration (Rd) were carried out. Each series had a duration of 4 d: from day 14 to day 17 after transfer from *in vitro* culture and from day 28 to day 31 respectively. The vinelets (5 per variant) were placed in exiccators connected to an open measuring circuit (details: SLAVTCHEVA and DIMITROVA 1997). Pn and Rd were determined by means of an infra-red gas analyser (URAS-2, Germany) connected to a 12-point-recorder (Polycomp). Air humidity in the laboratory was 80-85 % and CO_2 was 350 ppm. As light sources, high pressure mercury vapour lamps (LRF-400 W, Poland) were used. Four light intensities were tested: 20, 100, 200 and 400 µmol·m^{-2}·s^{-1}. The measurements were carried out at 21 °C (on days 14, 15, 28 and 29 after transfer from *in vitro* culture) and 26 °C (on days 16, 17, 30 and 31).

S t a t i s t i c a l a n a l y s i s : The data were statistically processed by analysis of variance (BAROV and NAYDENOVA 1969).

Results and Discussion: P l a n t g r o w t h : The plantlets developed well after transfer from *in vitro* culture (Table). Root and shoot growth of MP plants was improved probably due to the better adaptability to cultural conditions. According to ROUSSEVA and PANDELIEV (pers. comm.) shoot and root development of *in vitro* plants is more intensive and requires less time if single leaves are present on the microcuttings. In this trial, shoots as well as roots were longer (7-64 % and 10-50 %, respectively) compared to IP ones. Leaf area of MP plants was also larger (13-38 %). The most considerable differences in plant growth were found with Dimiat 4/24.

P h o t o s y n t h e s i s : Net photosynthetic rates increased significantly with light intensity (Figure), especially from 20 to 200 µmol·m^{-2}·s^{-1}. However, in no case light saturation of Pn was observed. The values were similar to *in vivo*-cultured potted plants (STOEV and SLAVTCHEVA 1982).

T a b l e

In vitro plant growth during the acclimatization period (mean values of two measurements)

	Shoot		Root		Leaf
	length mm	number of nodes	length mm	number	area dm^2
D1 IP	73.5	8.6	266.2	5.0	0.38
D1 MP	97.2	10.4	293.4	5.7	0.43
D2 IP	61.6	8.3	344.0	6.9	0.40
D2 MP	100.8	11.7	550.5	9.4	0.55
R IP	76.5	8.4	288.2	5.8	0.40
R MP	81.5	9.7	357.9	5.2	0.45

IP: Initially *in vitro*-propagated from field-grown vines.
MP: Micropropagated of 15th, 17th, and 15th subcultures, resp.
D1: Dimiat 4/38; D2: Dimiat 4/24; R: Italian Riesling 3/47.

At low light intensities (20 or 100 µmol·m^{-2}·s^{-1}), an increase of temperature was related to a decline of Pn in the first series of measurements (Figure, A and C) which is due to the more rapid increase of Rd compared to Pn with temperature. However, the temperature enhancement affected Pn augmentation at 400 µmol quanta·m^{-2}·s^{-1} and partly at 200 µmol quanta·m^{-2}·s^{-1}. It has been demonstrated (STOEV and SLAVTCHEVA 1982), that the temperature optimum of Pn of *in vivo* plants shifts to higher temperatures with increasing light intensity. The temperature effect on Pn was found in the second measuring series as well (Figure, B and D). In

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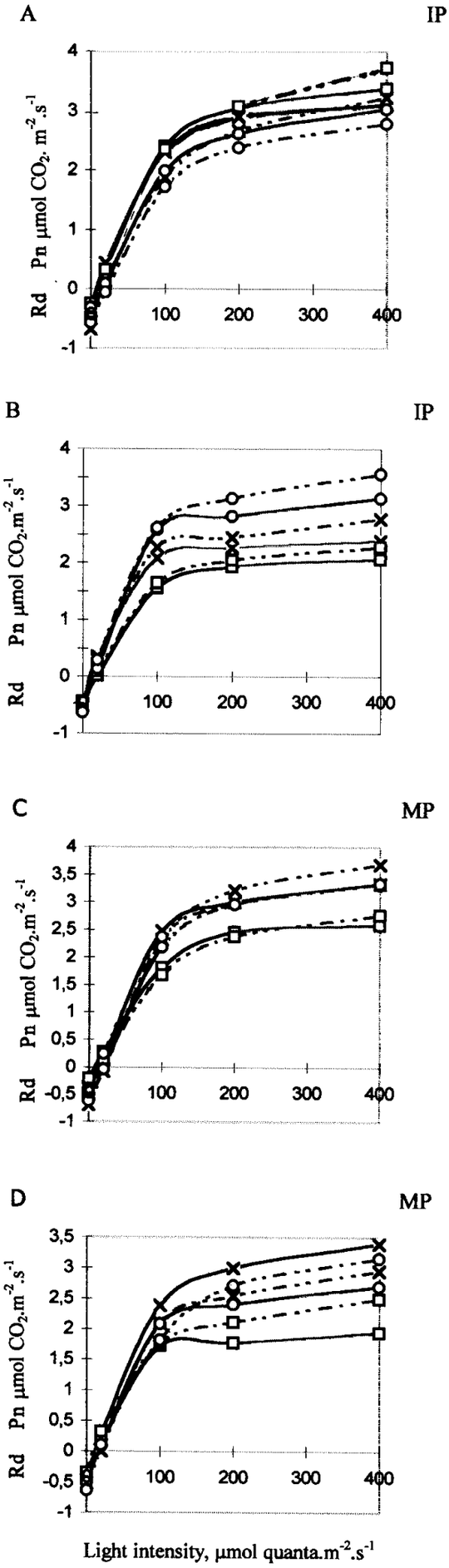

contrast to the first series, higher rates of Pn occurred at 26 °C at all light intensities. Differences among the variants were shown to be not significant at 20 and 100 µmol·m^{-2}·s^{-1}, but significant at 200 and especially at 400 µmol·m^{-2}·s^{-1}.

Under the experimental conditions, Pn was not affected by subculturing. However, higher photosynthetic rates were measured most frequently at 200 and 400 µmol quanta·m^{-2}·s^{-1} with MP plantlets.

Differences between vine varieties were related to the light and temperature conditions and to the mode of *in vitro* culture. The lowest photosynthetic rates in this trial were obtained with Dimiat 4/24. Variety-specific differences of *in vivo* plants occurred generally with respect to the compensation point, light saturation, and temperature optimum (STOEV and SLAVTCHEVA 1982).

Dark respiration: An increase of ambient temperature by 5 °C led to a significant Rd increment (1.6-2.3 times) in the first series of measurements (Figure, A and C), but to small changes (1.0-1.3 times) in the second series (Figure, B and D), which can be explained with the adaptation of plantlets to the environmental conditions. The values of Rd (from -0.7 to -0.3 µmol CO_2·m^{-2}·s^{-1}) were in the range of those found on *in vivo*-cultured pot plants (STOEV and SLAVTCHEVA 1982), or a little bit higher. In the first series of measurements, the light compensation point was 12-18 µmol·m^{-2}·s^{-1} at 21 °C and 16-22 µmol·m^{-2}·s^{-1} at 26 °C; in the second series it was 14-22 µmol·m^{-2}·s^{-1} at the two temperatures.

No significant differences were observed between the two groups of plants with respect to Rd. Taking into consideration whole plantlets, MP plants had a higher Rd which corresponded to their better vegetative growth. Irrespective of the plantlet group, significant differences occurred with regard to Dimiat 4/24, but not between Dimiat 4/38 and Italian Riesling 3/47.

BAROV, V.; NAYDENOVA, P.; 1969: Statistical Methods for Field and Vegetation Experiments. Zemizdat, Sofia.

BRENDEL, G.; 1986: Einsatz der *in vitro*-Kultur zur Produktion von Vorstufenpflanzgut in der Züchtung. Wein-Wiss. **41**, 264-269.

DÜRING, H.; HARST, M.; 1996: Stomatal behaviour, photosynthesis and photorespiration of *in vitro*-grown grapevines: Effects of light and CO_2. Vitis **35**, 163-167.

FALQUE, M.; COMPAN, D.; GALZY, R.; 1991: A method for the study of CO_2 exchange in *in vitro* cultured *Vitis rupestris* plantlets. Plant Cell Tiss. Org. Cult. **27**, 175-181.

GRIBAUDO, I.; FRONDA, A.; 1993: L'ambientamento delle piante frutticole micropropagate. Riv. Fruttic. **55**, 75-80.

SCHOCH, P. G.; LEFEVRE, B.; TEISSON, C.; GANRY, Y.; 1989: Photosynthese et respiration de bananier *in vitro*. Photosynthetica **23**, 113-118.

SLAVTCHEVA, T.; DIMITROVA, V.; 1997: Gas exchange with in vitro cultured vine plants during their acclimatization. Riv. Vitic. Enol. **50(2)**, 3-9.

STOEV, K.; SLAVTCHEVA, T.; 1982: La photosynthese nette chez la vigne (*V. vinifera* L.) et les facteurs ecologiques. Connaiss. Vigne Vin **16**, 171-185.

Figure: Rates of net photosynthesis (Pn) and dark respiration (Rd) (mean values) of initially propagated (IP) and micropropagated (MP) further subcultured *in vitro* plants measured at 21 °C (–) and 26 °C (---) during acclimatization. **A** and **C**: from day 14 to day 17; **B** and **D**: from day 28 to day 31. D1, D2, R: see Table.