

the excess sprouting of shoots by rejuvenation, therefore, brought about a reduction in cut flower quality. The micropropagated Madam Violet used for this study sprouted many leader shoots without the excess sprouting, a known characteristic of this cultivar. The cut flower production of 'Madame Violet' was of a higher and better quality when compared with that of 'Carl Red'.

We expect to investigate cultivar differences in relation to the productivity of micropropagated plants and their sprouting ability.

LITERATURE CITED

- Bjarnason, E.N., B.C. Hanger, J.R. Moran, and J.A. Cooper.** 1985. Production of *Prunus* necrotic ringspot virus-free roses by heat treatment and tissue culture. *N. Z. J. Agr. Res.* 28:151-156.
- Curir, P., C. Damiano, and T. Cosmi.** 1986. In vitro propagation of some rose cultivars. *Acta Hort.* 189:221-224.
- Davies, D.R.** 1980. Rapid propagation of roses in vitro. *Sci. Hort.* 13:385-389.
- Dubois, A.M. and D.P. Vries.** 1992. Vitrocultuur geeft onderstam eugdige groeikracht terug. *Valblad voor de Bloemisterij* 15:42-43.
- Francllet, A., M. Boulay, F. Bekkaoui, Y. Fouret, B. Verschoore-Martouzet, and N. Walker.** 1987. Rejuvenation. p. 232-248. In: J.M. Bonga and D.J. Durzan (eds.). *Cell and Tissue Culture in Forestry*. Vol. 1. Martinus Nijhoff Publishers, Dordrecht.
- Jones, O.P.** 1994. Physiological change and apparent rejuvenation of temperate fruit trees from micropropagation. p.323-331. In: P.J. Lumsden, J.R. Nicholas, and W.J. Davies (eds.). *Physiology, Growth and Development of Plants in Culture*. Kluwer Academic Publishers, Netherlands.
- Kitamura, H., T. Kawai, S. Yamada, K. Watanabe, and K. Hasegawa.** 1992. Methods of shoot apex culture and performance test of roses. *J. Japan. Soc. Hort. Sci.* 61 (Suppl. 2):470-471.
- Valles, M.** 1987. Micropropagation of several *Rosa hybrida* L. cultivars. *Acta Hort.* 212:611-617.

Photoautotrophic micropropagation of *Cymbidium*: Effects of CO₂ Concentration, Photosynthetic Photon Flux Density and Sucrose Concentration on Plantlet Growth

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Cymbidium plantlets are produced in vitro normally under heterotrophic or mixotrophic conditions in the presence of sucrose. Leafy nodes of other species have been grown successfully to plantlets through photoautotrophic culture (i.e. without sucrose) under CO₂ enrichment. Therefore, this study was undertaken to determine if cymbidiums in leaf can also be micropropagated without sucrose, and if so, to determine the optimum culture conditions.

Cymbidium PLBs with two or three leaves were cultured in vitro on half strength Murashige and Skoog (1962) medium under varying concentrations of sucrose, CO₂ and photosynthetic photon flux density (PPFD) for 42 days; the treatments were

Table 1. Description of experimental conditions.

Plant material	<i>Cymbidium</i> cv. Marilyn Monroe
Medium	
Basal medium	Half strength Murashige & Skoog (1962)
Supporting material	Agar (7 g liter ⁻¹)
Growth regulator	None
pH	5.6 before autoclaving
Amount	70 ml/vessel
Vessel	
Type	Polycarbonate box (370 ml)
Number of air-exchange	5.4 h ⁻¹
Culture room	
Photoperiod	16 h d ⁻¹
Air temperature	Light period 19-22C Dark period 24-26C
Culture period	42 days

Table 2. Treatment descriptions.

Treatment code	CO ₂ conc. ¹ (μmol mol ⁻¹)	PPFD ² (μmol m ⁻² s ⁻¹)	Sucrose conc. ³ (g liter ⁻¹)
AL00	500	50	0 - 0
AL03	500	50	0 - 30
AL30	500	50	30 - 0
AL33	500	50	30 - 30
AH00	500	100	0 - 0
AH03	500	100	0 - 30
AH30	500	100	30 - 0
AH33	500	100	30 - 30
BL00	1000	50	0 - 0
BL03	1000	50	0 - 30
BL30	1000	50	30 - 0
BL33	1000	50	30 - 30
BH00	1000	100	0 - 0
BH03	1000	100	0 - 30
BH30	1000	100	30 - 0
BH33	1000	100	30 - 30

¹ Concentration in the culture room.² Photosynthetic photon flux density on the empty shelf.³ Concentration in the medium during the first and second culture period.

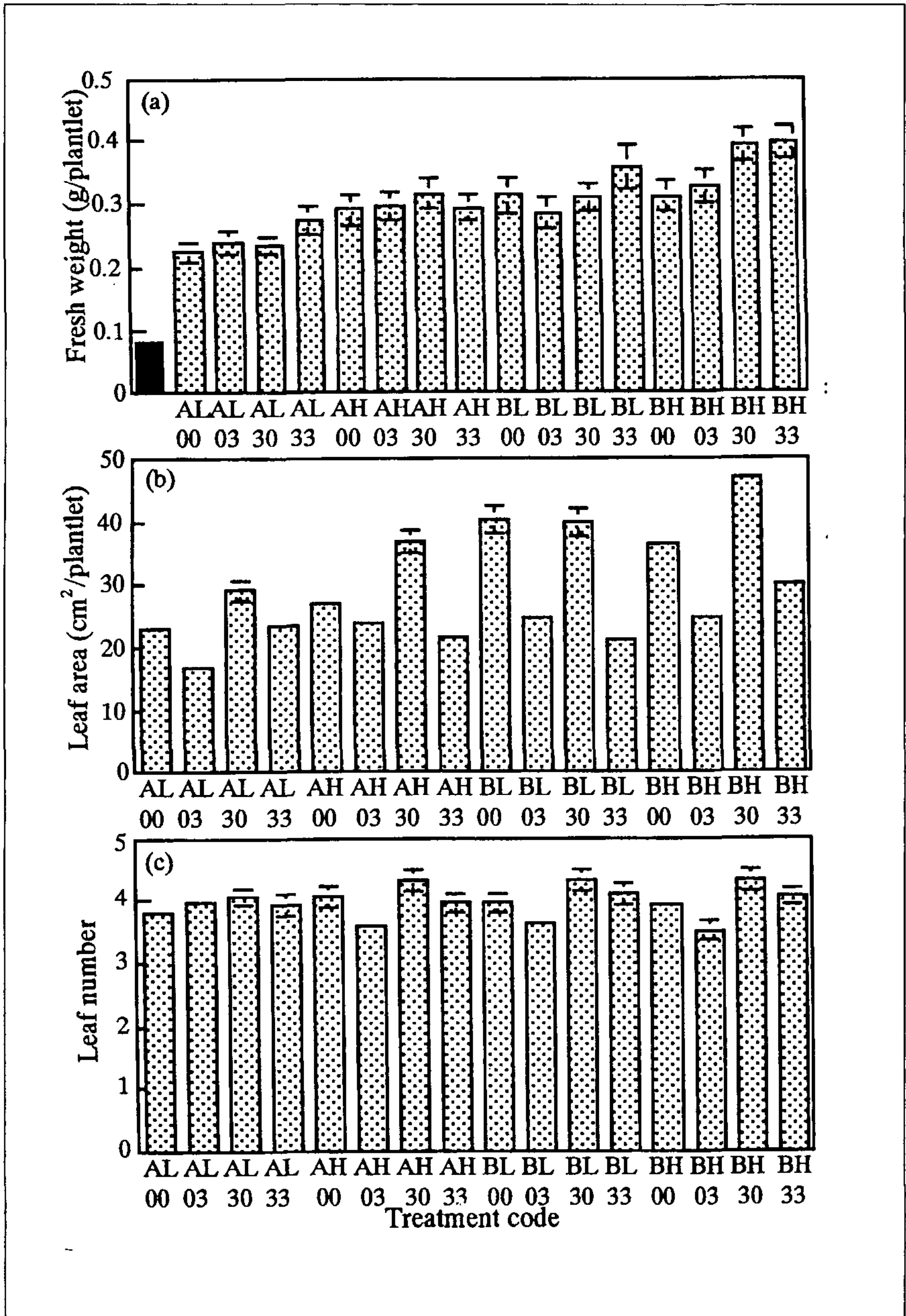


Figure 1. Total fresh weight (a), leaf area (b), and leaf number of (c) per plantlet on days 42 of *Cymbidium* plantlets (mean \pm SE); ■ represents total fresh weight on day 0.

divided into two periods: 0 to 14 days and 15 to 42 days (Tables 1 and 2). During each period sucrose concentration was maintained at 0 or 30 g liter⁻¹, CO₂ at 500 or 1000 μmol mol⁻¹, and PPFD at 50 or 100 μmol m⁻² s⁻¹. PLBs were on one culture for the first period and changed to the new culture for the second period. At the end of each period, total fresh weight, leaf area, and leaf number per plantlet were measured.

At the end of the first period, leaf area was greater under the combination of no sucrose, 1000 μmol mol⁻¹ CO₂, 50 μmol m⁻² s⁻¹ PPFD. The effect of the culture conditions on the total fresh weight and leaf number was not significant ($P \leq 0.01$) during this period. At the end of the second period, total fresh weight and leaf number were greater without sucrose under 1000 μmol mol⁻¹ CO₂ and 100 μmol m⁻² s⁻¹ PPFD than under other treatments. When both periods are considered together, all the three parameters were greater under 30 g liter⁻¹ sucrose, 1000 μmol mol⁻¹ CO₂, and 100 μmol m⁻² s⁻¹ PPFD (first period), and 0 g liter⁻¹ sucrose, 1000 μmol mol⁻¹ CO₂, and 100 μmol m⁻² s⁻¹ PPFD (second period) (Table 3 and Figure 1).

The above results suggest that *Cymbidium* plantlets can be produced photoautotrophically under conditions of high PPFD and CO₂ enrichment. This could help reduce the micropropagation cost for cymbidiums.

Table 3. Statistical summary on treatment effects of *Cymbidium* plantlets.

Variable	Growth criterion		
	FW	Leaf area	Leaf number
Day 14			
CO ₂ conc.	NS ²	NS	NS
PPFD	NS	NS	NS
Sucrose conc. ¹	NS	**	NS
Day 42			
CO ₂ conc.	**	**	**
PPFD	**	**	NS
Sucrose conc. ³ (g liter ⁻¹)	NS	**	**
0 - 0	a ⁴	ab	b
0 - 30	a	b	c
30 - 0	a	a	a
30 - 30	a	b	b

¹ Medium sucrose concentration in the first culture period (0 or 30 g liter⁻¹).

² NS and ** indicate nonsignificant or significant at the P 0.01, respectively (analysis of variance).

³ Combination of sucrose concentration in the first period and second period.

⁴ Means within a column followed by different letters are significantly different at the P 0.05.