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Influence of growth room and vessel humidity on the *in vitro* development of rose plants

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Abstract

An increase of the vapor pressure deficit (VPD) of the growth room atmosphere (from 600 Pa up to 2000 Pa) induced a variation in the air VPD inside the vessels used for rose micropropagation.

During the photoperiod, the *in vitro* plants lost water by evaporation. During the night period, depending upon the VPD of the growth room, plants could take water from the vessel atmosphere.

According to the intensity of the transpiration, large changes in the growth and morphology were observed: decrease in multiplication rate, modification of leaf colour and area, reduction of the elongation and changes of the level of axillary buds which grew.

Introduction

Plants cultivated *in vitro* exhibit malfunctioning stomata, which could explain poor water regulation (Brainerd & Fuchigami 1982; Conner & Conner 1984). Under *in vitro* conditions, growth and morphogenesis of the plantlets were influenced by the air volume above the organs (Bateson et al. 1987) and by the water potential of the agar medium (Debergh et al. 1981; Kosai et al. 1986). Inside the vessels, the vapor pressure deficit depended on how long the culture lasted, on the temperature and on the plant development (Sallanon & Coudret 1990).

During the mass propagation of the rose cultivar Madame Georges Delbard (R) deladel, the *in vitro* plants exhibited morphological changes in winter even though most growth conditions were constant. The only parameter that changed throughout the year of production was the vapor pressure deficit (VPD) of the growth room atmosphere, because during winter, the air temperature was low and when this air was heated to the growth room temperature, its humidity was reduced.

The purpose of this work was to verify if and how the VPD of the growth chambers can modify the VPD inside the containers and consequently affect the plants development.

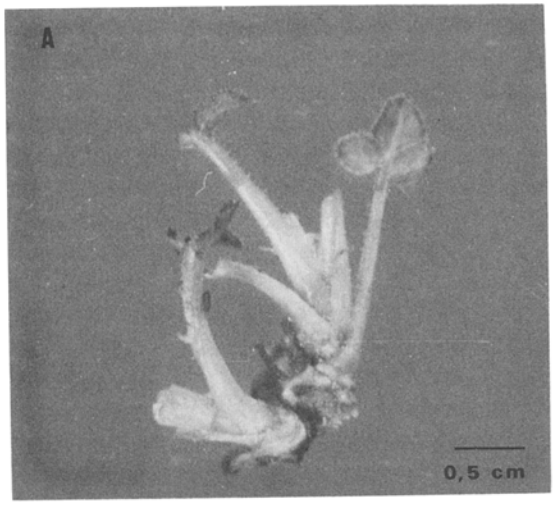
Materials and methods

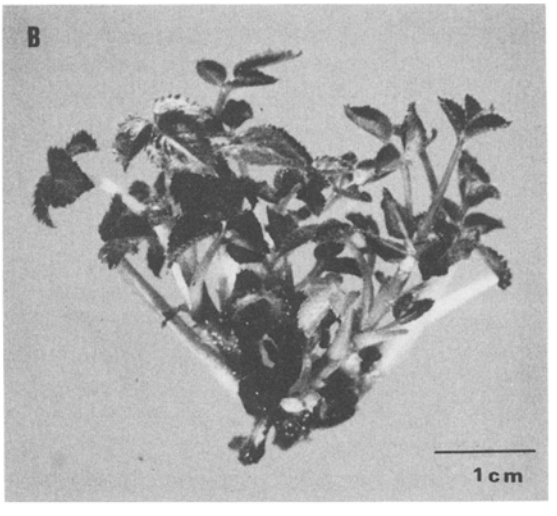
Plant material and growth conditions

The greenhouse rose cultivar Madame G. Delbard R Deladel was cultivated *in vitro*. The axillary budding medium consisted of Murashige and Skoog (1962) salts, supplemented with benzyl amino purine ($6.6\,\mu M\,l^{-1}$) and sucrose ($87.7\,mM\,l^{-1}$) and solidified with Bacto agar ($7\,g\,l^{-1}$). After adjustment to pH 5.5, 120 ml of medium were poured in 850 ml glass containers (verreries Champenoises) closed with polycarbonate lids that allows gas exchange and water loss (3 to 6 g during a micropropagation run of 21

days). After autoclaving (120°C, 30 min) 20 rose explants were inoculated per vessel.

The multiplication explant consisted of a small clump of two trimmed shoots of 8 mm long (Fig. 1 A). Within 21 days in the growth rooms, four to six new shoots developed from the axillary buds and elongated up to 2 to 2.5 cm (Fig. 1 B and C). The vessels were kept in a growth room adjusted to provide day/night temperature of 24°C/22°C and a 16 h light period with a quantum flux density of 50 μmol m^{-2} s^{-1} incident on the plants (Mazda, daylight). For two multiplication runs, 6 batches of vessels were used in two chambers with the air vapor pressure of 550 Pa at 22°C and 600 Pa at 24°C (relative humidity: 80%) for the first and 1910 Pa at 22°C and 2100 Pa at 24°C (relative humidity 30%) for the second. To set the air VPD, water saturated air





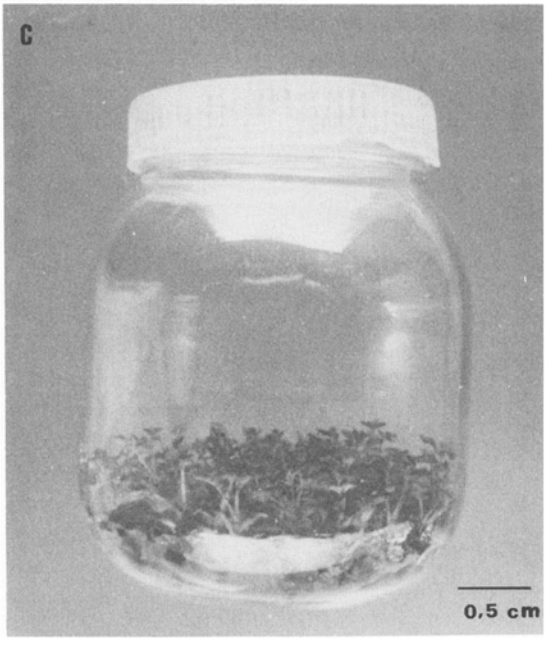


Fig. 1. (*A*) *in vitro* plant: one day old, (*B*) *in vitro* plant: 21 day old, (*C*) growth vessel inoculated with 20 plantlets (21 day old).

was maintained at the dew point temperature with four cryostats working together with the photoperiod. The air was then heated to 22°C or 24°C. For each chamber, three jars contained only agar medium while the others were inoculated as previously mentioned.

Measurements

On day 21 of the multiplication cycle, the air VPD inside the containers was evaluated night and day, every 30 min, in the middle of the container and above the agar level or the plants with a Wescor HP 115 psychrometer. With the same apparatus, plant and medium water potentials were measured. The atmosphere humidity was expressed as water potential and as VPD, in order to compare atmosphere and medium or plant water retention capacities. The mathematic relationship between water potential and VPD is as following:

$$\psi = (RT/V)\,\mathrm{Ln}\,(e/e_s)$$

where $VPD = e_s - e$; ψ: water potential, VPD: vapor pressure deficit, e = water vapor partial pressure; e_s: saturation partial pressure of water vapour; R: universal gas constant; V: partial molar volume of water; T: temperature.

Morphology and growth

The multiplication rate was evaluated according to production criteria which take in account only shoots taller than 1 cm which are suitable for rooting. Shoot and leaf colour, swollen axillary buds level of 40 plants, have been compared at the end of multiplication for both growth conditions.

Results

The air VPD inside the vessels was higher during the photoperiod (T = 24°C) than during the night period (T = 22°C) (Tables 1 and 2). The air VPD in a vessel containing only medium was weaker than when the vessel contained plants (Tables 1 and 2). Inside the vessel, air VPD was generally greater in the middle than in the lower part (Tables 1 and 2). Whatever the temperature and the vessel content were, air VPD inside the vessels was greater under high evaporating rate (room air VPD = 1910 and 2100 Pa) than under low evaporating rate (room air VPD = 550 and 600 Pa) (Tables 1 and 2).

There were no or slight ψ gradients between medium and plants (Table 2), while significant differences were recorded between ψ air and ψ plants or ψ medium (Tables 1 and 2). Without plants, ψ air was always weaker than ψ medium

Table 1. Vapor pressure deficit (VPD) and water potential (ψ) of the atmosphere and the medium inside vessels that contained only a medium kept under high and low evaporation conditions. (Mann and Whitney's test, $*\alpha < 1\%$; $**\alpha < 5\%$; only not evident significant differences were indicated).

Vapor pressure deficit (Pa) and water potential (MPa)	Evaporation condition			
	High		Low	
	VPD (Pa)	ψ (MPa)	VPD (Pa)	ψ (MPa)
Photoperiod (T = 24°C)				
air inside the vessels				
middle part	62 ± 4	−2.9 ± 0.2	35 ± 2	−1.6 ± 0.1
base	*48 ± 3	**−2.2 ± 0.15	*17 ± 2	*−0.8 ± 0.1
medium		**−0.53 ± 0.03	ns	*−0.44 ± 0.04
Night period (T = 22°C)				
air inside the vessels				
middle part	30 ± 2	−1.5 ± 0.1	12 ± 3	−0.6 ± 0.15
base	*19 ± 2	*−0.97 ± 0.1	*7.5 ± 1	*−0.38 ± 0.05
medium		*−0.5 ± 0.05	ns	ns−0.47 ± 0.05

Table 2. Vapor pressure deficit (VPD) and water potential (ψ) of the atmosphere, the medium and the plants inside vessels inoculated with plants and kept under high and low evaporation conditions (Mann and Whitney's test, $*\alpha < 1\ \%$; $**\alpha < 5\ \%$; only not evident significant differences were indicated).

Vapor pressure deficit (Pa) and water potential (MPa)	Evaporation condition			
	High		Low	
	VPD (Pa)	ψ (MPa)	VPD (Pa)	ψ (MPa)
Photoperiod (T = 24°C)				
air inside the vessels				
middle part	37 ± 2	−1.7 ± 0.1	24 ± 4	−1.1 ± 0.2
base	*30 ± 4	*−1.4 ± 0.2	*10 ± 1	*−0.46 ± 0.1
medium		*−0.65 ± 0.15	ns	ns−0.49 ± 0.1
plants		ns−0.47 ± 0.05	ns	ns−0.41 ± 0.07
Night period (T = 22°C)				
air inside the vessels				
middle part	13 ± 15	−0.66 ± 0.07	9 ± 1	−0.45 ± 0.05
base	ns12 ± 1	ns−0.6 ± 0.05	*5 ± 1	*−0.25 ± 0.05
medium		ns−0.51 ± 0.04	ns	*−0.45 ± 0.06
plants		ns−0.47 ± 0.05	ns	ns−0.41 ± 0.07

during photoperiod. The results were the same during night period, but only under high evaporating rate (Table 1). With plants, under low evaporating rate, ψ air and ψ plants were similar during photoperiod, while during night period ψ plants was weaker than ψ air (Table 2). Under high evaporating rate, ψ air was lower than ψ medium during day and it was equal to that during night (Table 2).

Under high evaporating conditions, the multiplication rate was 1.2, leaves were large and dark green colored, shoots were short (<1 cm) and they began to lignify. The upper axillary buds emerged but their growth stopped after preformed leaves had developed. In contrast, under low evaporating conditions, the multiplication rate was significantly higher (1.5), leaves were small, light green coloured and slightly epinastic, shoots were long (1.5 ± 0.2 cm) and pink-green coloured, evidence of anthocyanin pigments, which are characteristic of juvenility in this plant material (Table 3).

Table 3. Morphological characteristics of plants grown under high and low evaporation conditions. (Mann and Whitney's test, $*\alpha < 1\%$).

	Evaporation condition		
	High		Low
Multiplication rate	1,2 ± 0,1	**	1,5 ± 0,1
Leaf area per vessel (cm^2)	11 ± 2	*	14 ± 1,5
Shoot length (cm)	1 ± 0,2	**	1,8 ± 0,3
Average number of axillary buds per explant starting from			
the base	1,3 ± 0,1	**	2,2 ± 0,2
the high part	2 ± 0,3	**	0

Discussion

Inside the vessels, air VPD was dependent on the ambiant temperature, on the vessel content and on the growth room humidity. Daily variations of air VPD inside the vessels were correlated to the temperature changes. The presence of *in vitro* plants induced a smaller inner VPD, in response to an increased evaporation; due to the *in vitro* plants transpiration. The air VPD inside the vessels decreased with the growth room air VPD, the latter controlled the water vapour loss throughout the lids of the containers. Atmospheric VPD was dependent on the evaporation intensity in the vessel (influence of the vessel content) and on the water vapor loss outside the vessels (influence of the growth room humidity).

Within the range of the VPD values, inside the containers, the relationship between VPD and water potential was roughly linear. The possible water movements that occur according to de-

creasing water potential gradients, can be estimated by comparing the medium, plant and air water potentials. Inside the jars without plants, there was a water flux from medium to the atmosphere only during the photoperiod under low evaporating conditions. Under high evaporating conditions, this water flux was throughout the day cycle long. When the jars contained plants, these transpired during the light cycle under low evaporating conditions; during the night period water potential gradients allowed water to come from the atmosphere to the plants, keeping in mind that the top and the bottom of the jars were wetter than the middle (Sallanon & Coudret 1990). Under high evaporating conditions, there was not only plant transpiration during the photoperiod, but also during the night period, and the possibility of a water flux from the air to the plant was very little.

The increase of transpiration intensity (under high evaporating conditions) did not induce significant plant water potential decreases but it heavily modified plant growth and morphology. Two hypotheses could explain these variations in growth and morphology. Since the stomata are opened and do not regulate water exchanges, under high evaporation, transpiration would be too high to allow optimal growth during light period and the water losses would not be counterbalanced by an uptake of water from the atmosphere during night period. The second hypothesis involves a decrease of water availability into the agar medium at the base of explants, due to excessive evaporation. The plantlets were water stressed and grew less vigourously.

Whatever the kind of water stress, these two statements may explain the altered morphology that has been recorded. A decrease in the terminal bud activity, because of water deficit, would limit the apical dominance, and axillary buds are released. The other morphologic features (large leaf surface, dark green color, partial lignification, etc. . .) were evidences of less juvenile plants that were not actively grown.

These data show that the reduction of explants water loss during the multiplication stage leads to higher multiplication rates. The control of the growth rooms humidity and the intensity of water losses throughout the multiplication phase seems to be therefore as important as media composition, light and temperature parameters to manage the plants.

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