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BRIEF COMMUNICATION

Water relations in grapevine micro-cuttings grown *in vitro*

J. GUERRIER-JULIEN, A. DELOIRE* and A. COUDRET**

Manufacture de Pneumatiques Michelin, F-63040 Clermont-Ferrand Cédex, France.
Biologie Cellulaire et Ampélologie, Université de Reims, UR4R, UFR Sciences,
*B.P. 1039, F-51867 Reims Cédex 2, France**
Physiologie et Biotechnologies Végétales, Université Clermont Ferrand,
*4 Rue Ledru, F-63000 Clermont-Ferrand, France***

Abstract

Dry mater, water content, water, osmotic and pressure potentials, content of saccharides and potassium were measured during *in vitro* cultivation of *Vitis* rootstocks. Three cases were compared: *a*) the micro-cuttings with normal growth; *b*) micro-cuttings which stop their growth after 15 d of culture, and *c*) micro-cuttings reactivated by 6 d of continuous darkness.

Major differences were observed in water content and osmotic potential. The stopping of growth was not a specific property of buds, but was probably due to restriction of translocation of saccharides and water in the shoot.

Additional key words: *in vitro* propagation, osmotic potential, pressure potential, *Vitis*, water potential.

The problem of the *Vitis* rootstock we study, is that 50 % of the micro-cuttings stop their development after the budbreak: it means after having 1 or 2 leaves. These micro-cuttings (called inactive plants) have no visible root primordia even after 15 d of culture and stay at this stage during whole culture cycle (50 d). Nevertheless if these plants have been submitted to 6 d of continuous darkness, normal growth appeared (called reactived plants). With the aim to understand the cause of the above mentioned differences in micro-cuttings development, we have measured intracellular pH and water relation of active, inactive and reactived plants, at different stages of their development.

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*To whom correspondence should be addressed.

The grapevine rootstocks 41 B (*Vitis vinifera* cv. Chasselas × *Vitis berlandieri*) were growned on Murashige and Shoog (1962) medium modified by the company SA Moët et Chandon (Deloire, unpublished communication). The micro-cuttings were grown without hormones, under a photoperiod of 16 h, irradiance 50 µmol m^{-2} s^{-1} and day/night temperature 25/20 °C (for details see Jacob *et al.* 1989). During micropropagation of *Vitis* rootstocks, preformed leaves are developed and roots primordia appear on micro-cuttings already after 8 d. After 45 to 50 d of culture a miniplant is obtained with well developed roots and 5 to 6 leaves with new axillary buds.

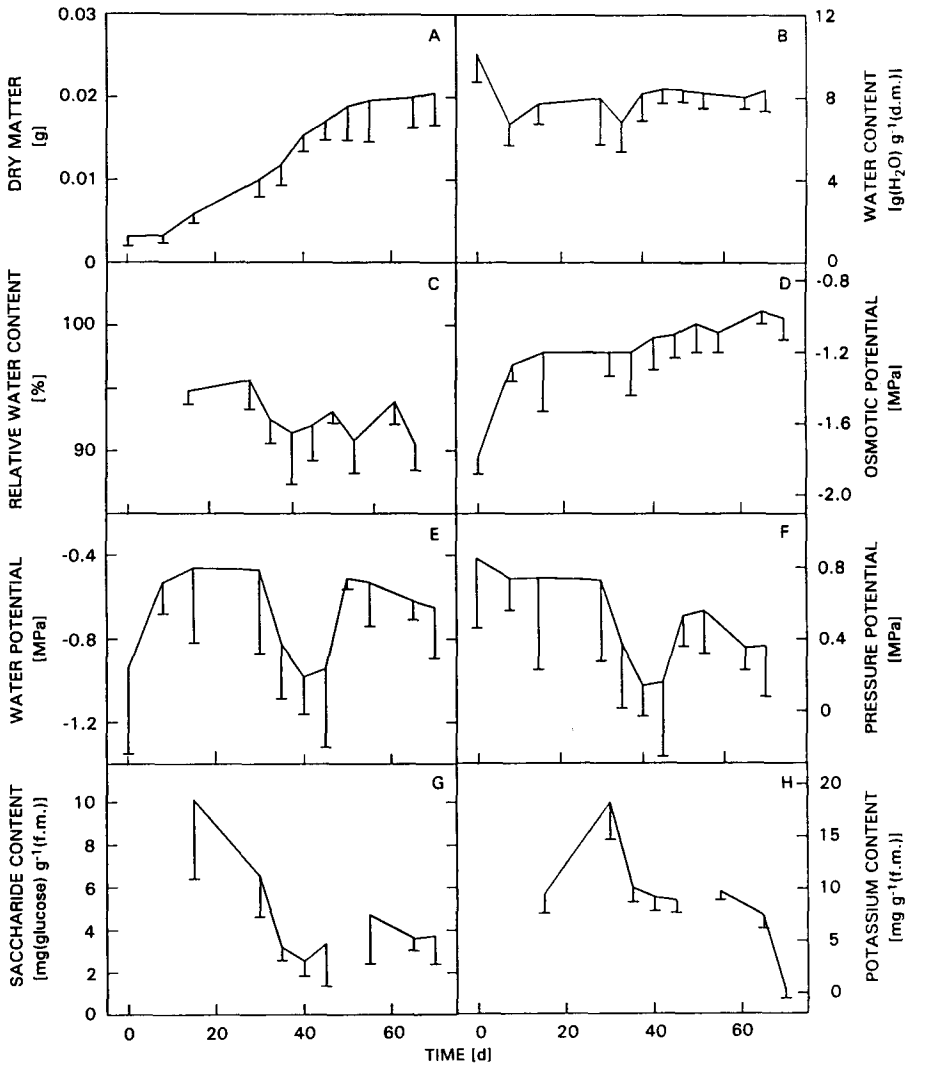

Fig. 1. Evolution of different parameters during a subculture of active plants, in function of time after beginning of the subculture.

The water content per unit of dry matter and the relative water content were determined gravimetrically. The osmotic potential was measured with a freezing point osmometer in cell sap extracted from entire plant, according to Houchi (1986). The water potential was measured with a *WESCOR* dew point hygrometer (Sallanon and Coudret 1990).

Total soluble saccharides were extracted by boiling the entire plant in methanol (40 %, v/v) and after incubation per 24 h at 4 °C; they were measured by Nelson technique; results were expressed in mg glucose per g of fresh matter. The concentration of K^+ was measured by flame photometry.

During the first 8 d of culture, dry matter predictably did not change (Fig. 1A); only 2 or 3 leaves began to grow. An increase of fresh matter took place and micro-cuttings had a high pressure potential (0.7 MPa) (Fig. 1F). At the 35th day, pressure potential was lower, dry matter increased, the plants have developed roots which were able to absorb water and nutrients (Fig. 1H). In the same time, water and saccharides concentration decreased (Fig. 1B,C,G). Beyond the 50th day of culture, dry matter was stabilized and pressure potential and relative water content increased: growth slow down and stopped when the plants reached the top of jar. From the 35th day of culture, micro-cuttings were at the optimal phase of growth and this was the period when new micro-cuttings sliced up from these plants were the most suitable for the following subcultures.

Table 1. Comparsion of water content [g g^{-1}(d.m.)], osmotic potential [MPa], pressure potential [MPa], soluble saccharide content [mg(glucose) g^{-1}(f.m.)] and potassium content [mg g^{-1}(f.m.)] (mean ± SE) in active, inactive and reactived *in vitro* micro-cuttings of grapevine.

Age [d]		Water content	Osmotic potential	Pressure potential	Saccharide content	Potassium content
13	active plant	6.56 ± 41	- 1.37 ± 1.7	0.72 ± 2.5	7.03 ± 2.00	8.62 ± 1.5
	inactive plant	5.51 ± 40	- 1.69 ± 3.4	0.92 ± 4.0	9.12 ± 2.00	10.30 ± 2.0
	reactivated plant	5.51 ± 40	- 1.69 ± 3.4	0.92 ± 4.0	9.12 ± 2.00	10.30 ± 2.0
19	active plant	5.52 ± 86	- 1.27 ± 0.6	0.43 ± 1.9	7.79 ± 1.49	7.61 ± 2.5
	inactive plant	5.12 ± 32	- 1.46 ± 2.7	0.91 ± 2.8	8.94 ± 1.30	7.76 ± 1.5
	reactivated plant	6.77 ± 32	- 1.31 ± 1.2	0.74 ± 3.0	6.59 ± 1.40	7.70 ± 1.7
30	active plant	7.35 ± 39	- 1.20 ± 1.0	0.38 ± 1.2	9.48 ± 0.40	7.66 ± 0.9
	inactive plant	6.47 ± 61	- 1.67 ± 2.4	0.38 ± 3.2	9.47 ± 5.00	7.24 ± 0.7
	reactivated plant	6.31 ± 96	- 1.34 ± 0.7	0.59 ± 1.1	4.28 ± 1.00	8.03 ± 1.0

When we compared active, inactive and reactivated plants, major difference seemed to be the water content and osmotic potential (Table 1). The water content of non active plants was 5.51 g g^{-1} (d.m.) while water content of active plants was 7.20 g g^{-1} (d.m.); osmotic potential of active plants was 0.3 MPa higher than that of inactive one. The water deficit in inactive plants resulted in a lower osmotic potential, confirmed by higher concentrations of soluble saccharides and potassium in the plant. So, stopping of growth was not due to a carbon or nutrition deficit of the entire plant.

After treatment by darkness, all measured parameters in the reactivated plants had similar values as those of active 13 d-old plants. Particularly, this treatment produced a water influx in the micro-cuttings (120 % increase of water content) which increased osmotic potential. After darkness, roots are developed by micro-cuttings which allow necessary water absorption.

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