HortScience 27(2):111-113. 1992.

Paclobutrazol and Reduced Humidity Improve Resistance to Wilting of Micropropagated Grapevine

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Additional index words. in vitro culture, stomata, *vitis vinifera*

Abstract. **Plantlets of *Vitis vinifera* L. 'Moscato Bianco' were grown in vitro in cellulose plugs (Sorbarods) saturated with a modified Murashige and Skoog rooting medium. Both the inclusion of 0.5-l mg paclobutrazol/liter in the rooting medium and the use of culture vessels that reduce the relative humidity from 100% to 94% improved resistance of plantlets to wilting after transplanting. Maximum benefit was obtained with a combination of paclobutrazol and reduced humidity; it resulted in smaller stomatal apertures, shorter stems, reduced leaf area, and more and thicker roots. Chemical names used: (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-01 (paclobutrazol).**

Grapevine can be rapidly propagated in vitro from shoot tips and axillary buds (Harris and Stevenson, 1982), but micropropagated plantlets are susceptible to rapid desiccation after transfer to soil and require acclimatization. Our aim was to produce hardier plants by modification of Stage III procedures and thus avoid acclimatization with its associated costs of labor and equipment. Three Stage III procedures ameliorated a similar, though less acute, problem in chrysanthemum *[Dendrathema grandiflorum* (Ramat) Kitamura]. These three were 1) protection of roots by cellulose plugs (Sorbarods) (Roberts and Smith, 1990), 2) inclusion of a triazole growth retardant (paclobutrazol) in the culture medium (Smith et al., 1990a), and 3) the use of culture vessels with reduced humidity (Smith et al., 1990b). In the present invesigation, we rooted plantlets of *V. vinifera* 'Moscato Bianco' in vitro in Sorbarods and studied the effects of paclobutrazol and reduced humidity on their morphology and resistance to wilting.

Stage II culture was on solid medium in glass jars (300 ml) with translucent polypropylene screwcaps (MELI, Brussels, Belgium). The medium consisted of half-strength MS (Murashige and Skoog, 1962) salts, MS vitamins, 30 g sucrose/liter and 8 g Difco bacto agar/liter. The pH was adjusted to 5.6 with KOH before addition of agar. Stage III culture vessels were of clear polystyrene with a rectangular base (155 × 110 mm, 450 ml) and contained 60 Sorbarods (Fig. 1). The lid (800 ml) had five holes (20-mm diameter) drilled along both flanks. These were overlaid with a strip of Tyvek Europeel T (Du Pont de Nemours International SA, Geneva) that acted as a tough bacteriological barrier through which water vapor could diffuse. The vessel, with Sorbarods included, was supplied by the manufacturer (Baumgartner Papiers SA, Lausanne, Switzerland) after sterilization by gamma irradiation. Liquid medium (300 ml/vessel) was identical to Stage II medium except for the exclusion of agar and the addition, in some treatments, of 0.5-1.0 mg paclobutrazol/liter (analytical grade; ICI, Bracknell, U.K.). The vessels were maintained at 94% relative humidity (RH), but in some treatments, the membrane was covered by adhesive tape to establish 100% RH. The relative humidity in the culture vessels was measured with an HL 240 D humidity meter (Lee-Dickens, Kettering, U.K.).

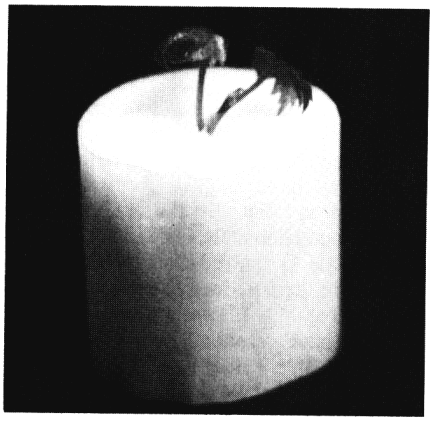

Fig. 1. Sorbarod, consisting of a cylinder (20 mm long × 18 mm diameter) of cold-crimped cellulose wrapped in cellulose paper, with shoot tip inserted.

The barrel of probe was sealed into a circular hole cut in the lid and held in a central position. All cultures were maintained at 23C in a 16-h photoperiod provided by cool-white fluorescent lights (Thorn, London) giving 57 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the plant surface. Shoot tips (5 mm) were inserted into the Sorbarods (Fig. 1) to initiate Stage III culture. Plantlets were assigned numbers according to their position in the culture vessels and taken randomly for wilting tests and measurements after 4 weeks.

Plantlets (25 per treatment) were transferred, rooted in Sorbarods, to water-saturated potting compost in plant pots (250 ml) and assessed for wilting. They were maintained at 100% RH for 0.5 h, then exposed, in a randomized matrix, to 62% ± 2% RH at 30 ± 1C under continuous lighting supplied by warm-white fluorescent tubes giving 318 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the plant level. Wilting was scored individually on a scale of 0 (turgid) to 4 (completely wilted) at intervals for 6 h and mean wilting scores were calculated for each treatment.

Stomata1 apertures were assessed after exposure for 18 h to stimuli that induce closure in vivo; that is, a dry atmosphere (20% RH at 23C) and darkness. Stomata1 impressions were then made by applying nail varnish to the abaxial surface of leaves. The apertures of 20 stomata on the second youngest expanded leaf on each of five plantlets per treatment were measured with a microscope equipped with a micrometer eyepiece.

Plantlets cultured without paclobutrazol at 100% RH wilted severely when transferred to 62.5% RH for 6 h and were subsequently unable to regain turgor. Plantlets treated with 0.5-1.0 mg paclobutrazol/liter at 100% RH and plantlets cultured without paclobutrazol at 94% RH wilted less and subsequently regained full turgor when re-

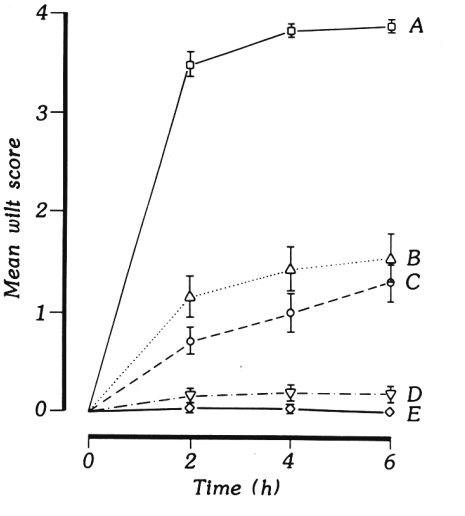

Fig. 2. Mean wilt scores in relation to time following exposure to 62% ± 2% RH at 30 ± 1C. Bar linesindicate SE. Treatments : 0 mg paclobutrazol/liter, 100% RH **(A);** 0.5 mg paclobutrazol/liter, 100% RH **(B);** 1 mg paclobutrazol/liter, 100% RH **(C);** 0 mg paclobutrazol/liter, 94% RH **(D);** 1 mg paclobutrazol/liter, 94% RH **(E).**

Received for publication 19 Feb. 1991. Accepted for publication 10 Sept. 1991. We thank Baumgartner Papiers SA,. Lausanne, Switzerland, for the gift of Sorbarods and culture vessels and Imperial Chemical Industries Plc, Jealott's Hill Research Station, Bracknell, UK, for the gift of paclobutrazol. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Table 1. The effect of paclobutrazol and relative humidity (RH) on various morphological characters of 'Moscato Bianco' plantlets after 4 weeks in Stage III culture. The means are based on 2.5 plantlets per treatment.

	Relative humidity (%)						
	100	100	94	94			
	Paclobutrazol (mg·liter^{-1})				Two-way analysis of variance[z]		
Plant organ	0	1	0	1	Factor	F value	Probability
Stems							
Length (mm)	34	11	21	10	RH	18.74	<0.01
					Paclobutrazol	201.66	<0.01
					Interaction	17.29	<0.01
Expanded leaves							
No./plant	3.6	3.1	2.8	2.0	RH	60.53	<0.01
					Paclobutrazol	29.98	<0.01
					Interaction	2.30	>0.05
Area/leaf (mm^2)	104	62	176	85	RH	12.98	>0.01
					Paclobutrazol	28.29	<0.01
					Interaction	0.86	>0.05
Area/plant (mm^2)	380	192	519	175	RH	1.56	>0.05
					Paclobutrazol	30.07	<0.01
					Interaction	2.57	>0.05
Roots							
No./plant	4.6	8.2	4.5	5.9	RH	4.48	<0.01
					Paclobutrazol	22.37	<0.01
					Interaction	3.57	>0.05
Avg diam (mm)	0.7	1.0	0.7	1.1	RH	18.35	<0.01
					Paclobutrazol	278.00	<0.01
					Interaction	0.248	>0.05

[z]Transformations of data to achieve normality and homoscedasticity: log for stem length and leaf area; square-root for leaf number and root number.

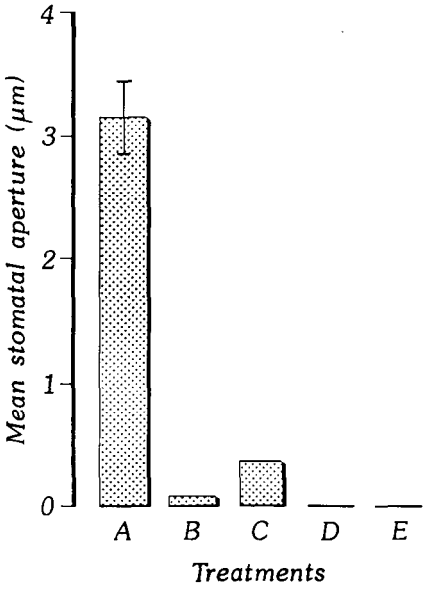

Fig. 3. Mean stomata1 apertures of the second youngest leaf per shoot following exposure to 20% RH at 23C for 18 h in darkness. Means are based on 20 stomata on each of five leaves. Bar line indicates SE. Treatments : 0 mg paclobutrazol/liter, 100% RH **(A);** 0.5 m.g paclobutrazol/liter, 100% RH (B); 1 mg paclobutrazol/liter, 100% RH **(C);** 0 mg paclobutrazol/liter, 94% RH **(D);** 1 mg paclobutrazol/liter, 94% RH **(E).**

turned to a humid atmosphere. Plantlets treated with 1 mg paclobutrazol/liter at 94% RH showed negligible wilting (Fig. 2). Smaller stomata1 apertures in water-stressed plantlets treated with paclobutrazol and reduced humidity, separately and in combination (Fig. 3), apparently minimized water loss. Reductions in leaf area per plant in response to paclobutrazol probably reduced transpiration (Table 1). Thicker roots and shorter stems induced by paclobutrazol and reduced humidity, and the greater number of roots induced by paclobutrazol (Table 1) may have facilitated replacement of the water lost by the leaves.

Increased resistance to wilting associated with improved stomata1 responses, reduction in stem length, leaf number and leaf area, and increases in root diameter have also been observed in response to paclobutrazol in chrysanthemum cultured in vitro (Smith et al., 1990a). Rooting was also promoted by paclobutrazol in *Plectranthus australtis* R. Br. and *Phaseolus vulgaris* L. grown in vivo (Davis et al., 1985) and by another growth retardant, ancymidol, in *Asparagus officinalis* L. cultured in vitro (Chin, 1982). Improved stomata1 responses in plantlets grown in vitro at reduced humidity have also been reported in chrysanthemum (Short et al., 1987; Smith et al., 1990b; Wardle et al., 1983) and carnation *(Dianthus calyophyllus* L.) (Ziv et al., 1987). We postulate that improved stomata1 behavior under reduced humidity may have resulted from increased solute concentrations in the epidermis following evaporation at the leaf surface. Shorter stems (Table 1) and red discolorations were observed in the first-formed leaves of plantlets grown at 94% RH without paclobutrazol and may have resulted from water stress. There was little evidence of such stress in plantlets grown at 94% RH with 1 mg paclobutrazol/liter, which indicates that paclobutrazol ameliorated this problem. This view is supported by the highly significant interaction between relative humidity and paclobutrazol on stem length (Table 1). It seems, therefore, that maximum benefit is to be gained by exploiting the complementary effects of relative humidity and paclobutrazol rather than by increasing the concentration of paclobutrazol beyond 1 mg·liter^{-1} or further reducing humidity.

After transfer to soil in temperate greenhouse conditions, without acclimatization, plantlets of grapevine that are rooted in Sorbarods and treated with paclobutrazol at 1 mg·liter^{-1} at 94% RH rapidly regain any lost turgor. Leaves that were formed in vitro persist for a further 3 to 4 weeks.

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