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Influence of Paclobutrazol on in Vitro Growth of Sweet Cherry Shoots

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Abstract. **Shoot tips from in vitro-established shoot tip cultures of *Prunus avium* L. cv. Hedelfingen and cv. Starkrimson were cultured on sweet cherry propagation medium to which several concentrations of paclobutrazol (PP333) were added and gibberellic acid (GA_3) omitted, in order to reduce the elongation of the shoots. A reduction in both shoot length and bud number was achieved even with the lowest concentration of paclobutrazol (0.2 mg·liter^{-1}). Shoot tips from the inhibited cultures when replanted on regular propagation medium overcame the inhibition at the first transfer. In order to use this system for cold preservation experiments, the cultures were examined under dark and low temperature conditions (0.5° ± 0.5°C). There was a significant difference between the PP333 system and the control after certain periods of cold preservation. The survival ratio of the PP333-inhibited culture was higher than the control after removing from the cold preservation conditions and recultured. Chemical name used: β-[(4- chlorophenyl)methyl]-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol.**

One of the methods for preserving germplasm of fruit trees using tissue culture systems is to maintain the cultures at low temperatures to slow the growth process; however, the plant material should be kept alive and ready to resume active growth when removed from the preservation conditions. Cold conditions may not be sufficient to inhibit growth, so other factors in the medium (minerals, hormones, sugars, etc.) may be evaluated.

Growth retardants also have been used, and Westcott (8) used abscisic acid (ABA) with some success. The plant bioregulator paclobutrazol (PP333) has been a promising growth retardant for use in orchards. It retards plant growth by interfering with gibberellic acid biosynthesis (9). Because paclobutrazol is effective with most members of the Rosaceae family (1, 9) including cherry (3, 7), and because it has no long-term effect, it appeared promising for in vitro preservation of sweet cherry.

Shoot tip cultures of sweet cherries ('Hedelfingen' and 'Starkrimson') were used. These cultures were prepared from budwood collected in January from 1-year-old trees. The budwood was kept moist in plastic bags at 6°C for at least 3 months before dissecting buds and establishing cultures (4).

As basic medium, the ordinary proliferation medium of Snir (4) was used, with these modifications: omission of GA_3 and addition of paclobutrazol (0.2, 0.4, and 0.8 mg·liter^{-1}). Paclobutrazol is heat-stable and is unchanged by autoclaving. For rooting, a medium including 2-(1-naphthyl)acetic acid (NAA) was used (4).

The cultures were kept in a growth chamber at 25° ± 2°C with 16-hr of light per day and 50 μmol·s^{-1}·m^{-2} photosynthetic photon flux (PPF). The cold room was maintained at 0.5° ± 0.5° with complete darkness. The test tubes for the preservation experiments were sealed with Parafilm to prevent external fungal contamination.

Each treatment was applied to 20 and 30 culture tubes (replicates). The mean values, along with their SEs, are presented in Tables 1–4. PP333 was provided from ICI Americas. The stock solution of PP333 contained 50 mg·liter^{-1}.

Paclobutrazol reduced the elongation of the sweet cherry shoots and affected the number of developing buds (Table 1). The weight of the 'Hedelfingen' culture was not correlated with the length. At 0.2 mg·liter^{-1}, there was no reduction and, at 0.4 mg·liter^{-1}, even an addition (in fresh weight). The reason for this phenomenon is that only the upper part of the shoot (above the agar) is influenced by the growth retardant. At the base of the treated shoots, massive calluses were formed (such calluses were not formed in the control shoots). These calluses resembled those that appeared at the base of rooted cuttings; they had no autonomous capacity to grow and they failed to develop when transferred to fresh medium without the shoots.

When considering paclobutrazol as a GA_3 synthesis inhibitor, it can be seen that it acts antagonistically with GA_3, which is used to inhibit root formation on cuttings. Following paclobutrazol treatment, the color of the treated leaves is darker and the leaves themselves are smaller and more curled than the leaves in the control cultures. When tips from these cultures were transferred to regular propagation medium containing GA_3, a full recovery from the temporary influence of paclobutrazol was obtained (Table 2). Several subcultures were prepared from these treatments with no observed differences between the growth of treated cultures and control cultures (length of the shoots, shape of the leaves, and growth rate).

Use of paclobutrazol in combination with cold preservation was tested by comparing cultures maintained at 0.5° ± 0.5°C with and without paclobutrazol. There was no significant difference (Table 3) in growth inhibition at levels >0.2 mg·liter^{-1} of paclobutrazol, but there was a clear difference between the control and the growth retardant treatments in both cultivars. This difference persisted especially after 13 to 15 months due to the inhibition effect of paclobutrazol on the elongation of the etiolated shoot (Table 3).

Dark conditions had an etiolation effect that could be minimized by low temperature, although it was still observed at 0.5° ± 0.5°C. The long, white shoots that grew under these conditions were tender and suspectible to brown spots and necrosis.

The 'Hedelfingen' preservation experiment was terminated after 13 months to check the viability of the cultures. The test tubes were removed from the cold room, and the upper part of each shoot (≈10 mm) was

Table 1. Effect of paclobutrazol on the length of sweet cherry shoots.[z]

Paclobutrazol treatment (mg·liter^{-1})	Length (mm ± SE)	No. buds (± SE)	Culture wt (g ± SE)
		Starkrimson	
Control	23.29 ± 1.19	4.64 ± 0.57	---[y]
0.2	15.05 ± 0.63	5.07 ± 0.54	---
0.4	11.67 ± 0.59	2.61 ± 0.36	---
0.8	9.61 ± 0.68	1.40 ± 0.22	---
Significance[x]	***	***	
		Hedelfingen[w]	
Control	11.51 ± 0.66	---	0.649 ± 0.074
0.2	9.94 ± 0.40	---	0.805 ± 0.072
0.4	7.29 ± 0.36	---	1.021 ± 0.090
Significance	***		**

[z]The number of buds that developed and their weight. Measurements were done 43 days after treatment.
[y]Not determined.
[w]The mean initial length of the shoot tips at zero time was 3.45 ± 0.18 mm.
[x]Significance by F test at 1% (**) and at 0.1% (***).

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Table 2. Removal of paclobutrazol inhibition by transfer of sweet cherry shoot tips to regular propagation medium (including GA_3)[z].

Paclobutrazol pretreatment (mg·liter^{-1})	Length (mm ± SE)
Starkrimson	
Control	13.48 ± 1.50
0.2	13.09 ± 0.46
0.4	14.86 ± 1.35
0.8	14.31 ± 1.10
Significance	NS[y]
Hedelfingen	
Control	12.34 ± 0.44
0.2	12.41 ± 0.71
0.4	13.77 ± 0.68
Significance	NS

[z]The measurements were taken 21 days after the transfer.
[NS]Not significant, F test.

planted on fresh medium and cultured for 21 days at ordinary growth chamber conditions (25.0° + 0.5°C). The number of newly established cultures from the original preserved cultures represented the survival ratio. Survival ratio increased with 0.2 mg·liter^{-1} paclobutrazol (Table 4). After 21 days, the cultures were subcultured onto fresh medium. Shoots from the second subculture were checked to determine if the rooting capacity of these shoots was influenced by the former cold preservation treatment. The rate of rooting (within 16 days) and the rooting percentage were almost the same in all treatments (Table 4).

Table 4. The effect of paclobutrazol on the survival and rooting of 'Hedelfingen' shoots after 13 months in cold storage.

PP333 treatment (mg·liter^{-1})	Survival (%)	Rooting (%)
Control	50.0	83.3
0.2	91.6	83.8
0.4	76.0	87.5
Significance	---	NS

[NS]Not significant.

Wang (6) found that paclobutrazol significantly postponed symptoms of chilling injury of cucumber seedlings at 5°C. Holubowicz (2) reported frost resistance of cherry trees induced by paclobutrazol applications. It seems that, in the in vitro cold-preservation system of sweet cherry, paclobutrazol might act in two ways, e.g., as an inhibitor of shoot elongation and as an inhibitor of the chilling injuries.

Upadhyana et al. (5) suggested that paclobutrazol delays dark-induced senescence in attached soybean leaves. If this mechanism is working also in cherry shoots, it can be another reason to use this chemical in the cold preservation system.

It seems promising to use paclobutrazol in the in vitro cold preservation system, since it reduced significantly the undesirable elongation of cherry shoots and maintained culture viability.

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Table 3. The effect of paclobutrazol on the length of sweet cherry shoots in cold preservation[z].

Paclobutrazol treatment mg·liter^{-1}	Length (mm ± SE)				
	Starkrimson			Hedelfinger	
	5 months	12 months	15 months	8 months	15 months
Control	8.94 ± 0.61	32.25 ± 3.95	48.50 ± 4.09	18.46 ± 1.07	39.30 ± 5.56
0.2	6.75 ± 0.50	15.12 ± 1.11	17.14 ± 1.01	12.70 ± 0.69	17.57 ± 0.92
0.4	6.88 ± 0.66	14.40 ± 1.30	16.07 ± 1.07	13.35 ± 0.66	17.46 ± 0.72
0.8	6.63 ± 0.46	13.80 ± 0.97	14.64 ± 0.99	---	---
Significance[y]	*	***	***	***	***

[z]Seven-day-old cultures were exposed to 0.5 ± 0.5°C in the dark.
[y]Significance by F test + 0.1% (***), 1% (**), and 5% (*).