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Activity of thidiazuron in *in vitro* shoot cultures of *Prunus* sp. and *Morus alba*

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Abstract

Thidiazuron incorporated into MS medium stimulated rosettes formation only in some treatments. This effect was more pronounced in cultures of *Morus alba* than *Prunus* sp. Mulberry cultures responded to the optimal concentration of thidiazuron (0.2 mg l^{-1}) not only with shoot formation but also with growth of large leaves and poor development of callus tissue. In cultures of both investigated genera the shoot elongation was inhibited. Shoots of mulberry cultures growing on proliferation medium supplemented with thidiazuron formed roots, in many cases.

Introduction

Thidiazuron has been shown to possess in several culture systems, the higher cytokinin activity than or comparable to that of BA (Nieuwkerk and Zimmerman 1986, Chalupa 1987, Mok 1987). Some *Prunus* species and *Morus alba* propagated *in vitro* in our laboratory, show low proliferation rate on medium containing BA. The purpose of our work was to induce higher proliferation rate of difficult to multiplication cultures replacing BA by thidiazuron, in MS medium.

Material and methods

Shoots from existing *in vitro* cultures of *Prunus cerasus* L. - cvs. Schattenmorelle and North Star, *Prunus avium* L. - seedlings, *Prunus mahaleb* L. - seedlings and selections 3/9 and Heineman 10 also *Morus alba* L. - "large leaf" selection for silk worms, were cultivated on MS medium. Medium containing 1 mg l^{-1} BA, 0.01 mg l^{-1} NAA and 0.1 mg l^{-1} GA_3 was designed as a standard for *Prunus* sp. and containing 2 mg l^{-1} BA, 0.1 mg l^{-1} NAA and 0.1 mg l^{-1} GA_3 for *Morus alba*. The growth of cultures on this medium was compared with cultures growing on medium

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supplemented with thidiazuron, added instead of BA, at concentrations 0.02, 0.1, 0.2, 0.4, 1.0 mg l^{-1}. To determine possible interaction of cytokinins and auxins in mulberry cultures, thidiazuron was added along with BA at concentrations 0.25, 0.5, 1.0, 2.0 mg l^{-1} and NAA at concentrations 0.01, 0.1, 0.2, 0.5 mg l^{-1}. Medium pH was adjusted to 5.2 for cherries and 5.8 for mulberry.

The cultures of *Prunus* sp. were maintained at 25 °C and *Morus alba* 27 °C, under fluorescent light (irradiance 4 W m^{-2}) with 16/8 h day/night light regime.

Cultures were examined according to our earlier findings (Borkowska 1986). The effect of thidiazuron was assessed after 5 to 6 weeks of culture period by determination the number of rosettes (shoots shorter than 10 mm), number and length of long shoots (shoots longer than 10 mm), total mass of shoot clusters and content of dry matter (expressed in percent). For mulberry cultures also callus tissue was weighed.

Each treatment in every series consisted of 3 replicates with 10 single shoot cultures as one replicate. Data were treated by a standard analysis of variance, with Duncan's multiple range test for the determination of significant differences at P=0.05. For some treatments the results were presented as percent of control (standard) treatment.

Results and discussion

***Prunus* sp.** Among the thidiazuron treatments, higher number of rosettes in comparison to standard treatment was obtained only for *P. avium* (in all investigated doses) and in a less degree for *P. mahaleb* seedlings (1 mg l^{-1}). In cultures of cv. Schattenmorelle proliferation rate was half lower than for standard (Table 1). Also in experiments described by Chalupa (1987), with shoot cultures of forest tree species the effect of thidiazuron was stronger than BA only for *Tilia cordata* Mill.

Table 1. Number of new rosettes of *Prunus sp.* cultures after 6 weeks on proliferation medium. Means followed by the same letter do not differ statistically (separately for each vertical column).

Cytokinin [mg l^{-1}]		*P. cerasus*		*P. mahaleb*			*P.avium*
		Schatt.	N. Star	Hein.	3/9	seedling	seedling
BA	1.0	8.1c	19.1c	5.2d	3.9c	6.2bc	10.5a
Thid	0.1	2.0a	8,8a	2.2a	2.5ab	3.5a	13.8b
	0.5	4.6b	13.3b	4.0bc	3.4bc	5.3b	14.0b
	1.0	3.9b	19.4c	3.7b	2.0a	7.9c	13.2b
	2.0	4.4b	20.9c	5.0cd	1.9a	6.1bc	13.9b

On media containing thidiazuron, the number of long shoots was evidently lower than those produced on media containing BA. The inhibition of extension growth has been described by authors (Mok 1987, Singha and Bhatia 1988).

Dry matter content of cherries cultures cultivated on medium with thidiazuron at lower concentrations was higher than in presence of BA. However, higher content of dry matter was coupled with the lowest rate of proliferation.

Morus alba. The highest number of rosettes was formed at concentration of thidiazuron 0.2 mg l^{-1}. In majority of passages this value was higher than on BA medium. Elongation of shoots was inhibited (Table 2).

Table 2. Number of new rosettes and long shoots and mass of *Morus alba* shoot clusters after 5 weeks on proliferation medium (data from 2 series).

Cytokinin [mg l^{-1}]		Rosettes		Long shoot		Mass [mg]	
		Series 1	Series 2	Series 1	Series 2	Series 1	Series 2
BA	2.0	3.4b	4.3b	4.6c	3.2c	253a	278a
Thid	0.02	1.0a	0.7a	2.3b	2.2b	204a	223a
	0.1	3.1b	4.2b	4.7c	2.3bc	370b	307b
	0.2	7.2d	5.5b	2.4b	1.5ab	476c	4-5c
	0.4	5.7c	5.3b	1.3a	1.0a	396b	344bc
	1.0	4.1b	4.1b	1.1a	0.9a	355b	291b

The leaves formed in presence of thidiazuron, especially at concentration 0.2 mg l^{-1} were larger than on medium containing BA and as result, the weight of the shoot clusters was higher than in remained treatments (Table 2).

At the base of mulberry shoots cultured on medium containing BA, abundant callus is normally formed. In treatments with thidiazuron callus formation was highly inhibited. This result was repeated in several passages (Table 3). However, even when callus formation was suppressed, as average, it was growing accidentally, similarly to BA containing medium. The effect of callus inhibition on medium supplemented with thidiazuron is contrary to that described by Chalupa (1987), for cultures of forest tree, and by Capelle *et al.* (1983), for *Phaselous lunatus* L. cultures.

Table 3. The effect of thidiazuron and BA on callus formation in cultures of *Morus alba* in two series.

Cytokinin [mg l^{-1}]		Callus mass [mg]	
		1	2
BA	2.0	282c	150b
Thid	0.02	45a	37a
	0.1	37a	25a
	0.2	97a	29a
	0.4	91a	48a
	1.0	199b	44a

The possible explanation of this discrepancy could be different sensitivity of plant genera to investigated cytokinin and its concentration.

Desired effects of thidiazuron found in mulberry cultures we tried to enhance by simultaneously application of BA. Presence of BA below standard concentration (0.25 mg l^{-1}) along with thidiazuron (0.2 mg l^{-1}) affected shoot proliferation with weak callus formation (Table 4).

Table 4. Effect of thidiazuron applied along with BA on shoot and callus growth in *Morus alba* cultures, expressed as percent of control

Cytokinin [mg l^{-1}] Thid.	BA	Rosettes	Long shoots	Callus
0	2.00	100	100	100
0.1	0.00	81	63	62
0.1	0.25	55	57	52
0.1	0.50	88	76	152
0.2	0.00	111	36	43
0.2	0.25	137	46	55
0.2	0.50	90	60	110

Changing the ratio of thidiazuron to NAA had not pronounced influence on shoot proliferation within concentrations assessed but the leaves were well developed what was expressed in high weight of shoot clusters (Table 5). Number of shoots proliferated on thidiazuron containing medium formed roots.

Table 5. Effect of thidiazuron applied along with NAA on number of shoot and callus growth in *Morus alba* cultures.

Cytokinins [mg l^{-1}] Thid.	BA	NAA	Mass [mg]	Rosettes	Long shoots	Callus [mg]
0	2.0	0.1	165a	2.4a	2.9b	258b
0.2	0.0	0.01	227ab	2.3a	1.1a	40a
0.2	0.0	0.1	326bc	2.6a	1.3a	198b
0.2	0.0	0.2	384c	2.7a	1.5a	362b
0.2	0.0	0.5	183ab	2.4a	1.0a	271b

In conclusion, in cultures of *Prunus* sp. replacement of BA by thidiazuron has no expected effect. However, in cultures of *Morus alba* thidiazuron enhances the efficiency cultured material by increase the number of shoots, better development of leaves and supression of callus formation

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