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Effect of Benzylaminopurine and Thidiazuron on *in vitro* Shoot Proliferation of *Tilia cordata* Mill., *Sorbus aucuparia* L. and *Robinia pseudoacacia* L.*

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Abstract. Benzylaminopurine and thidiazuron stimulated shoot proliferation of *Tilia*, *Sorbus* and *Robinia*. Low concentration of BAP (0.2—1.0 mg l^{-1}) promoted axillary bud formation and shoot elongation. Thidiazuron displayed high cytokinin activity at very low concentrations (0.002—0.05 mg l^{-1}). Shoot number induced on media containing thidiazuron was large. Numerous shoots were produced on the media containing BAP together with thidiazuron. Shoots produced on media containing thidiazuron or BAP together with thidiazuron rooted after transfer to medium supplemented with low concentration of auxin (IBA or NAA).

The progress in the field of *in vitro* propagation of forest trees is closely connected with a better understanding of the role of plant hormones. Good knowledge of the effect of plant growth regulators is an important factor in the achievement of rapid proliferation of forest tree cultures. *In vitro* propagation of superior genotypes that grow faster and are resistant to diseases is a perspective way to the acceleration of forest tree improvement programs.

In our experiments the effects of cytokinins and auxins on stimulation of shoot proliferation of broadleaved forest trees were investigated. In this report the effects of two cytokinins, benzylaminopurine and thidiazuron, on shoot proliferation of *Tilia cordata*, *Sorbus aucuparia* and *Robinia pseudoacacia* are discussed.

MATERIAL AND METHODS

Shoots excised from proliferating cultures were used for experiments. Shoots were grown either on a modified MS medium (Murashige and Skoog 1962), or on BTM or WPM (composition of the media used was described earlier — Chalupa 1981, 1983 a,b, 1984). In order to study the effects of growth regulators on shoot proliferation, each medium was supplemented with different concentrations of 6-benzylaminopurine-BAP (N-(phenylmethyl)-1H-purin-6-amine) or with thidiazuron (N-phenyl-N'-1,2,3-thi-

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diazol-5-ylurea), either alone or in combination with indol-3-ylbutyric acid (IBA) or naphthyl-acetic acid (NAA) and gibberellic acid (GA_3). BAP was tested in concentrations 0.1, 0.2, 0.4, 0.6, 1.0, 2.0 and 4.0 mg l^{-1}, thidiazuron in concentrations 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.5 and 1.0 mg l^{-1}. IBA or NAA was added in concentration 0.1 or 0.2 mg l^{-1} and GA_3 in concentration 0.2 or 0.5 mg l^{-1}. Difco Bacto agar (6 g l^{-1}) was added to solidify the medium. Sucrose (20 or 30 g l^{-1}) was used as a carbon source. The medium was autoclaved for 20 min at 121 °C. Cultures were grown under controlled environment at 25 °C (day) and 20 °C (night), under 16 h photoperiod of cool white fluorescent light with illuminance of 5—8 klx.

For each treatment 24 shoot tips (10—15 mm long) were used and each experiment was repeated at least twice. Shoot tips were transferred after 4 weeks to a fresh medium of the same composition, and the number and length of new shoots were evaluated after 8 weeks. Shoot cultures of linden (*Tilia cordata* MILL.), mountain-ash (*Sorbus aucuparia* L.) and robinia (*Robinia pseudoacacia* L.) were used for experiments.

RESULTS

Effect of BAP and Thidiazuron on Shoot Proliferation of *Tilia cordata* MILL.

Of the media tested (MS, BTM, WPM), MS medium stimulated best the growth and proliferation of shoots. MS medium was used for the evaluation of the effect of BAP and thidiazuron on shoot proliferation.

Low concentration of BAP (0.2—0.6 mg l^{-1}) stimulated growth and elongation of shoots (Table 1, Fig. 1 B). The number of shoots produced on media supplemented with a low concentration of BAP was significantly larger than on the medium lacking cytokinin. With increasing concentration of BAP (0.1—1.0 mg l^{-1}) the shoot number increased. The high concentration of BAP (4.0 mg l^{-1}) resulted in the formation of shorter shoots.

Thidiazuron effected the growth and proliferation of shoots very significantly. Even a very low concentration of thidiazuron (0.002—0.01 mg l^{-1}) stimulated formation of new axillary shoots. Shoots elongated slowly on media containing higher concentrations of thidiazuron and were significantly shorter than those produced on media containing BAP. Even leaves were smaller on media containing thidiazuron. Thidiazuron in low concentrations stimulated formation of axillary shoots, and in high concentrations it inhibit-

TABLE 1

The effect of BAP and thidiazuron on shoot number and length of shoots of *Tilia cordata* MILL. (cultured on MS medium supplemented with 0.1 mg l^{-1} IBA)

BAP [mg l^{-1}]	Number of news hoots	Mean shoot length [cm]	Thidiazuron [mg l^{-1}]	Number of new shoots	Mean shoot length [cm]
0.0	1.3 ± 0.8	1.7 ± 0.4	0.0	1.3 ± 0.8	1.7 ± 0.4
0.2	4.7 ± 2.2	2.3 ± 0.5	0.005	9.5 ± 3.2	1.4 ± 0.4
0.6	6.1 ± 2.4	2.5 ± 0.6	0.01	15.2 ± 6.4	1.2 ± 0.4
1.0	7.3 ± 2.5	2.2 ± 0.5	0.02	16.9 ± 7.3	1.1 ± 0.3
4.0	5.4 ± 2.3	1.6 ± 0.4	0.05	13.3 ± 5.6	0.7 ± 0.3

ed shoot elongation. The number of shoots produced on media with a low concentration of thidiazuron was significantly larger than on media supplemented with BAP (Table 1), but shoots were shorter (Fig. 1 C, D).

To stimulate axillary bud formation and shoot growth, the shoot tips were grown on the medium containing BAP together with thidiazuron, and IBA or NAA. If the nutrient media were supplemented with a low concentration of growth regulators, the proliferation and elongation of shoots were stimulated. MS medium supplemented with a low concentration of BAP (0.2—0.6 mg l^{-1}), thidiazuron (0.005—0.02 mg l^{-1}) and IBA or NAA (0.1—0.2 mg l^{-1}) stimulated development of axillary buds and proliferation of shoots (Fig. 1 A).

Effect of BAP and Thidiazuron on Shoot Proliferation of *Sorbus aucuparia* L.

Of the nutrient media tested, MS medium promoted best the growth and proliferation of shoots. MS medium supplemented with a low concentration of BAP (0.2—0.4 mg l^{-1}) stimulated the formation and elongation of shoots (Table 2). Shoots grown on these media attained a considerable length. Increased concentration of BAP (0.6—1.0 mg l^{-1}) promoted formation of a larger number of shoots (Fig. 2 B). MS media containing a higher concentration of BAP (2.0—4.0 mg l^{-1}) stimulated formation of numerous short shoots.

TABLE 2

The effect of BAP and thidiazuron on shoot number and length of shoots of *Sorbus aucuparia* L. (cultured on MS medium supplemented with 0.1 mg l^{-1} IBA)

BAP [mg l^{-1}]	Number of new shoots	Mean shoot length [cm]	Thidiazuron [mg l^{-1}]	Number of new shoots	Mean shoot length [cm]
0.0	1.4 ± 0.7	2.2 ± 0.5	0.0	1.4 ± 0.7	2.2 ± 0.5
0.2	5.3 ± 2.3	2.4 ± 0.6	0.005	7.2 ± 3.1	1.5 ± 0.5
0.6	12.7 ± 4.8	2.1 ± 0.6	0.01	10.1 ± 4.3	1.4 ± 0.4
1.0	15.4 ± 5.6	1.9 ± 0.5	0.02	15.2 ± 7.6	1.2 ± 0.4
4.0	18.3 ± 6.2	0.4 ± 0.2	0.05	14.1 ± 6.9	0.5 ± 0.3

Thidiazuron stimulated formation of numerous axillary shoots. The tips grown on MS medium supplemented with a low concentration of thidiazuron (0.005—0.02 mg l^{-1}) produced numerous axillary shoots (Fig. 2 D). Shoots produced on media containing higher concentrations of thidiazuron were short (Fig. 2 C). A high concentration of thidiazuron (0.5 mg l^{-1}) inhibited elongation of shoots from induced buds.

Shoot tips grown on MS medium containing BAP together with thidiazuron and auxin in low concentration produced numerous elongated shoots. MS media supplemented with BAP (0.2—0.6 mg l^{-1}), thidiazuron (0.005—0.02 mg l^{-1}) and IBA (0.1—0.2 mg l^{-1}) stimulated formation of multiple elongated shoots (Fig. 2 A).

Effect of BAP and Thidiazuron on Shoot Proliferation of *Robinia pseudoacacia* L.

MS medium stimulated best the shoot proliferation of robinia. MS medium containing a low concentration of BAP (0.2—0.6 mg l^{-1}) stimulated formation of axillary shoots and promoted proliferation of shoots (Fig.

TABLE 3

The effect of BAP and thidiazuron on shoot number and length of shoots of *Robinia pseudoacacia* L. (cultured on MS medium supplemented with 0.1 mg l^{-1} IBA)

BAP [mg l^{-1}]	Number of new shoots	Mean shoot length [cm]	Thidiazuron [mg l^{-1}]	Number of new shoots	Mean shoot length [cm]
0.0	3.2 ± 1.8	2.4 ± 0.6	0.0	3.2 ± 1.8	2.4 ± 0.6
0.2	5.8 ± 2.2	2.5 ± 0.6	0.005	5.1 ± 2.3	2.2 ± 0.6
0.6	6.3 ± 2.3	2.3 ± 0.5	0.01	6.8 ± 2.6	2.0 ± 0.5
1.0	7.4 ± 2.6	2.1 ± 0.5	0.02	7.1 ± 3.0	1.7 ± 0.5
4.0	6.8 ± 2.5	1.7 ± 0.4	0.05	7.8 ± 2.8	1.4 ± 0.4

B). Shoot tips grown on MS medium supplemented with a high concentration of BAP (4 mg l^{-1}) produced abundant callus and shorter shoots (Table 3).

Thidiazuron stimulated formation of axillary shoots. Low concentrations of thidiazuron promoted axillary bud development and shoot proliferation (Fig. 3 C, D). High concentrations of thidiazuron caused thickening of stem and induced callus formation on the base of shoot tips.

Shoot tips grown on MS media containing BAP together with thidiazuron and auxin in low concentration produced numerous elongated shoots. MS media supplemented with BAP (0.2—0.6 mg l^{-1}), thidiazuron (0.005—0.02 mg l^{-1}) and IBA (0.1—0.2 mg l^{-1}) stimulated proliferation of shoots (Fig. 3 A).

DISCUSSION

Recently discovered new compounds show high cytokinin activity comparable to that of the most active adenine-type cytokinins. Cytokinin activity of thidiazuron was detected in the *Phaseolus lunaris* callus bioassay. Thidiazuron displayed high cytokinin activity which was similar to that of the highly active cytokinins (MOK *et al.* 1980, 1982).

In our experiments the effects of thidiazuron and adenine-type cytokinin (BAP) on shoot proliferation of some broadleaved forest trees were compared. Thidiazuron showed high cytokinin activity. Low concentrations stimulated formation of axillary shoots of forest tree species tested. Thidiazuron also stimulated formation of callus tissue and effected elongation of shoots. Thidiazuron displayed high cytokinin activity at lower concentration than BAP. On account of the high cytokinin activity, thidiazuron appears to be one of the new cytokinins which might play an important role in micropropagation of hardwood trees. Thidiazuron can be used either alone or together with adenine-type cytokinin. Shoots of *Tilia cordata*, *Sorbus aucuparia* and *Robinia pseudoacacia* produced on media containing thidiazuron or BAP together with thidiazuron rooted after transfer to medium supplemented with low concentration of auxin (IBA or NAA).

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Figures at the end of the issue.

BOOK REVIEW

MILLER, J. R., MILLER, T. A. (ed.): INSECT-PLANT INTERACTIONS. — Springer Series in Experimental Entomology, Springer Verlag, New York—Berlin—Heidelberg—London—Paris—Tokyo 1986. 342 pp., 65 figs. DM 158,—.

This new volume of the Springer Series in Experimental Entomology is a unique section through the spectrum of methods appropriate to current research in insect-plant interactions. The publication is divided into 10 chapters, which are devoted to different aspects of insect-plant interaction. Many contributors have managed to offer an authoritative overview of their respective areas as well as address themselves to their primary charge, *i.e.* to cover the approaches and methods at a level useful to both non-expert and expert researchers.

The introductory chapter reports on a number of techniques and methodologies of direct observation of insect behaviour relevant for the study of insect-plant relationships. The second one is devoted to the assessment of host-plant finding by insect, including the types of movement of insect during host-plant finding, mechanism of finding an odour source, observation of insect responses to plant in the field, and different techniques and devices used in studying these problems. The definitions of oviposition preference, performance, some plant characters, such as acceptability and suitability, measurement of preference and its mean in insect-plant relationships are given in Chapter 3. The next chapter presents survey of assays of insect feeding. Some illustrations of equipment used in testing the insect choice of food are added. The bioassay procedures outlined in Chapter 5 can be adapted to tests for physiological activities of plant substances on insects irrespective of their mode of action, first of all to evaluation of effects of ingestion of antimetabolites, proteinase inhibitors and acute toxicants. Chapter 6 is focused on measurement of intake and utilization of food by insect as a basis for defining diet quality and also deals with mathematical processing of these data. The next chapter reviews biochemical aspects of plant nutrients (such as proteins and sugars) and allelochemics (such as gossypol, saponins and tannins) affecting insect digestion and food intake. In contrast to other contributions in this volume, which have largely the character of a review, Chapter 8 introduces new chemical methods of isolating and identifying phytochemicals (phytoecdysteroids) biologically active in insects. The development and deployment of crop cultivars defended against or tolerant of insect attack is a major tactics in pest management. In this context, Chapter 9 is of great importance since it summarizes techniques for evaluating plant resistance to insects which are appropriate to the appraisal and development of insect resistant cultivars. It also analyse plant traits that contribute to plant resistance. The last chapter deals with current techniques used to recording and analysing the response of individual sensory cells associated with various types of insect sensilla.

Most chapters are supplemented by a number of instructive graphs, figures and photos, and all contributions have a comprehensive bibliography. Due to its high scientific level, this book can be recomended to all those dealing with problems of insect-plant interactions, as well as to all specialists whose cooperation is needed in further progress in this field.

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