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Effect of paclobutrazol on *in vitro* formation of potato microtubers and their sprouting after storage

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Abstract

Paclobutrazol, the gibberellin biosynthesis inhibitor, accelerated *in vitro* tuber initiation of potato cv. Rema and increased the uniformity of tuberization period. However, the high concentrations (10 - 1000 mg l^{-1}) of this retardant, strongly decreased mass and/or number of microtubers. The microtubers were harvested and stored in darkness (22 ± 2 °C) for 250 d. After this period both sprouting and growth of sprouts were affected by previous paclobutrazol treatment.

Introduction

Microtuberization *in vitro* is the method currently used for rapid potato multiplication. Much of the published work on the induction of potato microtubers *in vitro* has focussed on the effects of plant growth regulators. In particular, emphasis has been placed on the role of cytokinins, auxins, ethylene, abscisic acid, and antigibberellin compounds (Garcia-Tores and Gomez-Campo 1973, Wang and Hu 1982, Hussey and Stacey 1983, Mangat *et al.* 1984).

Compounds that inhibit vegetative growth frequently stimulate tuberization. Thus, many kinds of growth retardants and growth inhibitors have been used for *in vitro* tuberization studies. These compounds include abscisic acid (ABA), chlorocholine chloride (CCC), maleic hydrazide (MH), *Alar* and *Amo-1618* (for review see Wang and Hu 1985). The application of growth inhibitors typically leads to a reduction in the level of endogenous gibberellins (GA), which otherwise strongly depress tuber initiation (Koda and Ojazawa 1983).

One of the most active inhibitors of gibberellin biosynthesis is paclobutrazol (*PP 333*) (Davis *et al.* 1988). Paclobutrazol is known to increase potato tuberization *in vitro* under a wide range of photoperiod conditions from total darkness to 16 h light per day (Harvey *et al.* 1991, Šimko 1991, 1993).

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Besides microtuber number and size, important factors for *in vitro* microtuberization are uniformity of tuberization and of microtuber sprouting after the dormancy period. The control of uniformity at the time of *in vitro* tuberization is probably the most important economic variable. The uniform tuberization period is critical for the harvest of microtubers of high physiological uniformity and the uniform early growth of plants derived from microtubers (McCown and Joyce 1991).

The experiment reported here focussed on the effect of a wide range of concentrations of paclobutrazol on *in vitro* potato tuberization, uniformity of tuber formation and sprouting of microtubers after storage.

Materials and methods

The medium early potato cultivar Rema (*Solanum tuberosum* L.) was used in the experiment. The stock material was maintained in 200 ml jars containing five nodal explants on 25 ml of medium. The medium contained MS salts and organic supplements (Murashige and Skoog 1962), 2 % (m/v) sucrose and 0.8 % *Difco Bacto* agar. The pH was adjusted to 5.7 before autoclaving for 15 min at 121 °C and 100 kPa. The cultures were kept in a growth-room at 20 °C and 100 μmol(photon) m^{-2} s^{-1} fluorescent light with a 16 h photoperiod. The same conditions were used for cultivation of plants in the first four weeks of the experiment. After four weeks, 25 ml of the inductive solution was added to each of the jars to give a total of 50 ml as a double layer. The inductive solution contained 8 % m/v sucrose and various concentrations of paclobutrazol (0, 0.001, 0.01, 0.1, 1, 10, 100 or 1000 mg l^{-1}). Paclobutrazol (M_r = 293.8) was added to the inductive solution in the form of the commercial plant growth regulator *Cultar* (250 g(paclobutrazol) l^{-1}). After the inductive solution was added the photoperiod was reduced to 8 h. Cultures were evaluated three times a week until the end of tuberization, which was considered to be when the plants had not formed additional tubers in any treatment for two weeks. At that time (9 weeks) the microtubers were harvested and analyzed. The uniformity of tuberization in this experiment is expressed as the time between the appearance of 25 and 75 % of all microtubers in each jar. The experiment was performed three times with ten, five and six replications in each treatment. Extreme storage conditions (in temperature and length) were used in the test of paclobutrazol effect on microtuber storage and sprouting. After 250 d of storage in darkness at 22 ± 2 °C the growth of microtuber sprouts was measured.

A one-way *ANOVA* programme was used for statistical analyses, with percentage data transformed to arcsin values prior to analysis although results are presented in a non-transformed format. The logarithmic regression ($y = A + B \times \ln x$) was used for making a regression analysis between paclobutrazol concentration and time of tuber initiation (for control 0.0001 concentration was used instead of 0).

Results and discussion

Increasing concentrations of paclobutrazol decreased the time to the beginning of tuberization, but tuber formation also stopped earlier in comparison with the control (Fig. 1). The plants treated with 100 - 1000 mg l^{-1} paclobutrazol formed sessile tubers, which were situated on the upper part of the stem. Concentrations of paclobutrazol from 0.01 to 10 mg l^{-1} also stimulated the formation of sessile microtubers which were, however, situated on the bottom part of the plant stem. Rarely, the tubers were formed on the end of short stolons. In the treatments both without paclobutrazol and with very low paclobutrazol concentration (0.001 mg l^{-1}) all microtubers were formed on long stolons, which grew into the medium in most cases (Fig. 2).

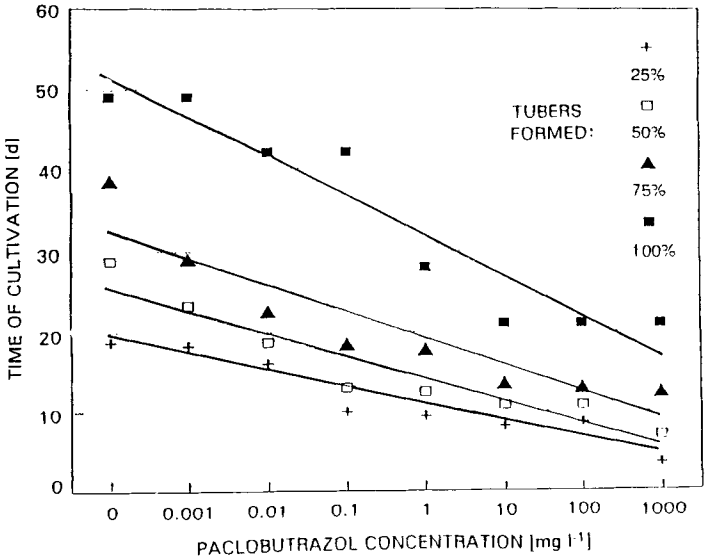

Fig. 1. Effect of paclobutrazol treatments on microtuber initiation (% of all formed tubers) during the tuberization period (4 weeks old potato plantlets cultured *in vitro* at 20 °C, 8 h photoperiod and irradiance of 100 μmol m^{-2} s^{-1}).

Logarithmic regression:

25%: $y = 10.4786 - 0.91977 \times \ln x$ $r^2 = 0.912^{**}$

50%: $y = 14.1774 - 1.23567 \times \ln x$ $r^2 = 0.897^{**}$

75%: $y = 18.8083 - 1.50194 \times \ln x$ $r^2 = 0.863^{**}$

100%: $y = 31.6667 - 2.13528 \times \ln x$ $r^2 = 0.906^{**}$

**statistically significant regression

High concentrations of paclobutrazol (10 - 1000 mg l^{-1}) in the inductive solution led to a decrease in the number of microtubers per jar compared with control (Table 1). Likewise the mean fresh mass of the tuber was lower when the same paclobutrazol concentrations (10 - 1000 mg l^{-1}) were used. Among lower concentrations no significant differences were found. This result is in agreement with previous reports, where paclobutrazol in concentration from 0.125 mg l^{-1} (Šimko

1991) to 2.938 mg l^{-1} (Hervey *et al.* 1991) did not decrease the number and/or mass of *in vitro* formed microtubers.

The lowest uniformity of tuberization was observed on control plants, where 19.7 d were needed for initiation of 50 % of all microtubers (from 25 % to 75 %) (Table 1). Paclobutrazol treatment (from 0.01 mg l^{-1}) significantly increased the uniformity of tuberization in comparison with the control. It seems that the time of tuberization and also its uniformity is a complex function of the type of cultured tissue and the tuberization stimuli (McCown and Joyce 1991), which paclobutrazol can affect *via* gibberellin biosynthesis.

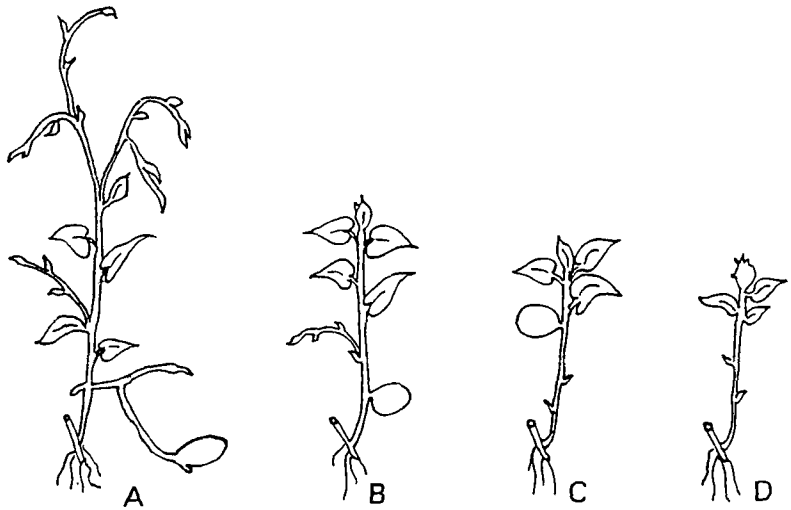

Fig. 2. Response of plant morphology to paclobutrazol concentration (*A* = 0 - 0.001; *B, C* = 0.01 - 10; *D* = 100 - 1000 mg l^{-1}) in inductive solution. Four weeks old plantlets were cultivated after paclobutrazol addition at 20 °C, 8 h photoperiod and 100 μmol m^{-2} s^{-1} irradiance during next 9 weeks.

During the nine weeks of tuberization, the stem elongation was 58 mm on the treatment without paclobutrazol. The lowest concentration of inhibitor (0.001 mg l^{-1}) had no effect on the elongation, whereas concentrations from 0.01 to 0.1 mg l^{-1} inhibited elongation by about 52 - 57 %, and in the higher concentrations (1 - 1000 mg l^{-1}) up to 90 - 95% (Table 1). These results are in agreement with previous experiments, where paclobutrazol strongly inhibited *in vitro* shoot growth of potato plants (Belcher and Abbott 1987, Šimko 1991, 1993).

The growth habit of the plants was also dependent upon the amount of added paclobutrazol. Plants at high concentrations (100 - 1000 mg l^{-1}) showed almost no growth, and early leaf senescence was observed. In the treatments with 0.01 to 10 mg l^{-1} paclobutrazol new, dark green leaves were formed and the length of internodes was shorter (especially in the treatments with 1 - 10 mg l^{-1} of inhibitor) as compared to control. The plants treated with 0.001 mg l^{-1} or no paclobutrazol grew steadily and formed a lot of new leaves. A multitude of stolons filled the space of the culture jar and grew into the medium.

From these results it follows that paclobutrazol concentrations suitable for use in *in vitro* microtuberization of potato plants are from 0.01 to 1 mg l^{-1}. These concentrations of inhibitor both stimulated an earlier start to tuber initiation as compared to control and did not decrease the mean fresh mass of the microtubers (Harvey *et al.* 1991, Šimko 1991). It seems that the inhibition of biosynthesis of GA, which arose after adding the paclobutrazol to the plant (Davis *et al.* 1988), led not only to acceleration but also to more uniform microtuberization in *in vitro* conditions. Similar results were found with another plant growth inhibitor - CCC (Hussey and Stacey 1983) where it was reported that all tubers were formed rapidly on the ends of short stolons above the agar. Swelling was initially rapid, but the rates of swelling declined sharply after four weeks, and therefore small tubers were produced quickly within a few weeks if CCC was added to the medium. These authors, however, used 500 - 1000 mg l^{-1} CCC, whereas effective concentrations of paclobutrazol are here much lower (0.01 - 1 mg l^{-1}).

Table 1. Effect of paclobutrazol on potato microtuberization at 9 weeks (20 °C, 8 h photoperiod, irradiance 100 μmol m^{-2} s^{-1}) and microtuber sprouting after 250 d of storage in darkness at 22 °C. Data are the combined from three experiments. Uniformity of tuberization is expressed as the time between the appearance of the 25 % and 75 % of all microtubers in each jar.

Paclobutrazol concentration [mg l^{-1}]	Numbers of. microtubers per jar (5 plants)	Mean fresh mass per microtuber [mg]	Uniformity of tuberization [d]	Stem elongation during tuberization [mm]	Microtubers sprouting after storage [%]	Mean length of sprout [mm]
0 (control)	7.1^{a}	230^{a}	19.7^{a}	58^{a}	72^{ab}	31^{a}
0.001	6.3^{ab}	242^{a}	10.8^{ab}	58^{a}	73^{ab}	35^{a}
0.01	5.7^{ab}	260^{a}	6.5^{b}	28^{b}	75^{ab}	20^{b}
0.1	6.0^{ab}	236^{a}	8.4^{b}	25^{b}	85^{a}	25^{b}
1	5.9^{ab}	230^{a}	8.2^{b}	6^{c}	80^{a}	24^{b}
10	5.4^{b}	119^{b}	5.3^{b}	5^{c}	60^{b}	5^{c}
100	2.3^{c}	52^{c}	4.3^{b}	3^{c}	0^{c}	-
1000	0.8^{d}	55^{c}	8.8^{b}	3^{c}	0^{c}	-

The same letter indicates no significant difference within column by Tukey's Test at the 5 % level.

After 250 d of storage the percentage of sprouted microtubers and both the number and length of sprouts was measured. The number of sprouts per sprouting microtuber (1.3 - 1.8) was independent on paclobutrazol treatment (data not shown). There were no significant differences among the percentages of sprouting of five paclobutrazol treatments (from 0 to 1 mg l^{-1}) (Table 1). Paclobutrazol at a concentration 10 mg l^{-1} decreased the percentage of microtuber sprouting compared to 0.1 - 1 mg l^{-1}, and the two highest concentrations (from 100 to 1000 mg l^{-1}) totally inhibited the sprouting of microtubers after storage. This inhibition was probably caused by the high level of residues from paclobutrazol and/or to the very small size of microtubers (as little as 75 mg fresh mass) which may become dry during the storage period. In general, it seems that the paclobutrazol also decreased the height of potato sprouts derived from

microtubers (Table 1). However, sprouts formed from paclobutrazol-induced microtubers at 0.01 - 1 mg l^{-1} concentration looked more vigorous than the control sprouts. These sprouts from treated tubers had the same number of internodes as the control sprouts, but they were shorter, thicker and less wilted (data not shown).

Paclobutrazol concentrations from 0.01 to 1 mg l^{-1} were optimal in the present experiment for potato cv. Rema for *in vitro* tuberization and sprouting after storage. However, significant interactions between cultivar and paclobutrazol treatment have been found (Harvey *et al.* 1991), and so these concentrations cannot be generally recommended for all potato cultivars. Even though the effect of paclobutrazol on the potato plant can be explained by such its antigibberellin properties (Balamani and Poovaiah 1985), there is evidence that paclobutrazol may influence the amounts of other phytohormones as ABA, ethylene, cytokinin and auxin (for review see Davis and Curry 1991).

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