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Changes in the levels of free IAA and cytokinins in potato tubers during dormancy and sprouting

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Abstract

Changes in the levels of free indol-3-ylacetic acid (IAA) and free cytokinins were determined in the course of dormancy and sprouting period in potato tubers (*Solanum tuberosum* L., cv. Nevskii) stored at 4 °C. The same analyses were performed in potato tubers after Ethrel application, which prolongs dormancy. No significant changes were found in free IAA level during dormancy followed by a rapid decrease during sprouting. After Ethrel application a significant lower IAA level was found 3 weeks after application, but further on no changes in free IAA level between treated and non-treated tubers were detected. Cytokinin level was relatively low and constant till sprouting and increases then by about 55 %, mainly due to an increase in the level of zeatin riboside and isopentenyladenosine. Ethrel application decreased cytokinin level during dormancy slightly, but postpones the increase coupled with sprouting by about 1 month. Thus, IAA does not seem to have a significant effect on tuber dormancy, while cytokinins are probably necessary for sprouting initiation.

Introduction

Tuber dormancy and sprouting is known to be controlled by phytohormones, the most important of which are abscisic acid (ABA) and gibberellins. Application of abscisic acid induces and/or prolongs dormancy of potato tubers (Korableva *et al.* 1980, de Bottini *et al.* 1982, Wareing and Phillips 1983), while gibberellins are usually able to break the dormancy (Rappaport and Wolf 1968, Shih and Rappaport 1970). Another phytohormone with the capability to influence dormancy is ethylene. Potato tubers produce low amounts of ethylene (Poapst *et al.* 1968, Rylski *et al.* 1974, Sukhova *et al.* 1989) and exogenous application of ethylene-releasing compound (Ethrel) prolonged significantly dormancy duration. It was shown that Ethrel application brings about an increase in ABA level (Metlitskii *et al.* 1982,

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Sukhova *et al.* 1987). Moreover, it had an impact on its own formation - both the level of 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene formation were increased after Ethrel application (Korableva *et al.* 1989). The role of auxins and cytokinins in the regulation of tuber dormancy was so far not sufficiently documented. Applied cytokinins were shown to be able to induce sprouting in some cases (Hemberg 1985). Engelbrecht and Bielinska-Czarnecka (1972) reported an increase in cytokinin level in tuber buds and surrounding tissue during dormancy. Turnbull and Hanke (1985 a,b) showed that dormancy control by cytokinins involves both changes in the level of and in the sensitivity to cytokinins. Applied cytokinins induced bud sprouting in the second half of dormancy (Turnbull and Hanke 1985a). Studies of changes in endogenous cytokinin levels showed their dramatic decrease, namely in zeatin riboside, immediately after tuber harvest, a very slow rise in the bud meristems during and a more rapid one in the end of dormancy (Turnbull and Hanke 1985 b). There are almost no data on IAA levels and on its role in the dormancy control. Thus, the aim of the present work was to show the changes in free IAA and cytokinin levels in potato tubers during dormancy and after its prolongation due to Ethrel application and to try to assess their role in the control of tuber dormancy.

Materials and methods

Potato tubers (*Solanum tuberosum* L., cv. Nevskii) were used. Plants were sprayed with 0.2 % aqueous solution of Ethrel in the field 2 weeks before the harvest, a treatment shown to prolong dormancy (Korableva *et al.* 1989). After the harvest, potato tubers from both control and sprayed plants were kept in a special cold storage room with high relative humidity (*ca.* 90 %) at 4 °C in darkness. For hormone analyses the apical buds were cut off the tubers after different periods of storage, frozen immediately in liquid nitrogen and freeze-dried. Free IAA level was determined by HPLC with fluorometric detection after thorough extract purification (Eder *et al.* 1988). ^{3}H-IAA (*Amersham*, specific activity 2.04 GBq mmol^{-1} and actual activity 3 kBq) was used for recovery control. Cytokinins were determined by ELISA using polyclonal antibodies against isopentenyladenosine (iPA) and zeatin riboside (ZR), cytokinin conjugates with alkaline phosphatase and *p*-nitrophenylphosphate as substrate. ELISA was preceeded by thorough purification of the extract (water residue after methanol evaporation) on a P-cellulose column in diluted acetic acid pH 3.0, from which the cytokinins were eluted by 0.3 N ammonia and after adjusting pH to 6.5 purified further on a DEAE-cellulose column coupled with a *Sep-pak* cartridge and by HPLC separation to zeatin (Z), ZR, isopentenyladenine (iP) and iPA fractions (Strnad *et al.* 1989, Macháčková *et al.* 1993). ^{3}H-*m*-hydroxybenzyladenine (synthesized in the Institute of Nuclear Biology and Radioisotopes, Academy of Sciences of the Czech Republic, Prague, specific activity 1.6 TBq mol^{-1}, actual activity 3 kBq) was used to control recovery. Both experiments were repeated twice with similar results. Results of one experiment in the case of IAA and of both experiments in the case of cytokinins are given. S.E. gives the error within one set of data (repeated determinations in the frame of one experiment).

Results and discussion

The levels of free cytokinins (this pool contains also nucleotides - cleavage by acid phosphatase is included in the purification protocol) at the beginning, in the middle and at the end of the dormancy period is given in Table 1. It is obvious that cytokinin levels kept constant from the beginning of dormancy over several months (August - January). It increased in the period of sprouting, namely the level of both nucleosides, ZR and iPA. This is in good correlation with the results of van Staden and Dimala (1978) and Turnbull and Hanke (1985) and is in agreement with the envisaged role of cytokinins in promoting cell division.

Table 1. Levels of free cytokinins (mean ± S.E.) in apical buds of tubers of control and Ethrel-treated potato plants in the beginning, in the middle and in the end of dormancy period (tubers were stored at 4 °C).

Sample	Sample	Cytokinin level [ng g^{-1}(f.m.)]				
		Z	ZR	iP	iPA	total
Control	24 Aug. 1990	11.5 ± 1.6	11.0 ± 1.7	17.9 ± 2.0	12.5 ± 1.8	52.9
Ethrel	24 Aug. 1990	8.3 ± 1.0	6.4 ± 0.8	16.9 ± 1.9	14.9 ± 1.3	46.5
Control	15 Jan. 1991	10.8 ± 1.2	8.7 ± 0.9	14.2 ± 1.5	13.6 ± 1.5	47.3
Ethrel	15 Jan. 1991	7.1 ± 0.6	10.4 ± 1.2	14.1 ± 1.6	13.1 ± 1.4	44.7
Control	10 Apr. 1991	11.6 ± 1.1	22.5 ± 2.4	16.8 ± 1.9	22.4 ± 2.3	73.3
Ethrel	10 Apr. 1991	5.1 ± 0.6	5.8 ± 0.6	15.0 ± 1.7	10.2 ± 1.1	36.1
Control	5 Oct. 1991	15.6 ± 2.1	14.8 ± 1.8	18.3 ± 2.3	16.7 ± 2.1	65.4
Ethrel	5 Oct. 1991	13.1 ± 1.7	12.4 ± 1.4	16.5 ± 1.8	15.6 ± 1.7	57.6
Control	10 Jan. 1992	14.3 ± 1.2	13.1 ± 1.3	16.9 ± 1.8	15.8 ± 1.4	60.1
Ethrel	10 Jan. 1992	12.5 ± 1.3	11.9 ± 1.2	14.5 ± 1.6	14.7 ± 1.6	53.6
Control	1 Mar. 1992	13.9 ± 1.4	13.0 ± 1.3	16.1 ± 1.7	16.3 ± 1.6	59.3
Ethrel	1 Mar. 1992	12.6 ± 1.3	12.1 ± 1.1	14.7 ± 1.5	15.2 ± 1.7	54.6
Control	5 Apr. 1992	16.2 ± 1.7	26.4 ± 3.1	19.8 ± 2.1	27.5 ± 2.9	89.9
Ethrel	5 Apr. 1992	11.9 ± 1.3	12.5 ± 1.4	15.2 ± 1.6	14.3 ± 1.5	53.9
Ethrel	3 May 1992	14.3 ± 1.8	27.8 ± 3.1	17.6 ± 1.9	25.1 ± 2.8	84.8

Free IAA level was undetectable during the first week after Ethrel application (*i.e.* 1 week before the harvest). Three weeks later in control tubers IAA level already attained the value which was then kept constant during dormancy period and sharply decreased at the time of sprouting (Table 2). It is a surprising finding, as one would expect sprouting initiation being coupled with enhanced level of IAA, which is thought to promote both cell division and cell elongation. The analyses, however, were made when sprouts were already visible and we cannot exclude that their real initiation is coupled with temporarily increased IAA level.

Ethrel (2-chloroethylphosphonic acid) was shown to prolong tuber dormancy (Metlitskii *et al.* 1982, Korableva *et al.* 1989). The effect of its application 2 weeks before harvest (0.2 %) on free cytokinin and IAA levels in the tubers during dormancy (Tables 1 and 2). Ethrel had no effect on free cytokinin level in the first two samplings, but significantly decreased cytokinin level in the last sampling in the period of sprouting of control tubers. This finding is in good agreement with the above-mentioned role of cytokinins in sprouting induction and the delay of sprouting by Ethrel application. Free IAA level was not changed significantly after Ethrel application (Table 2). The only difference was observed 3 weeks after Ethrel application when treated tubers had much lower IAA level than the control ones.

Table 2. Free IAA level (mean ± S.E.) in apical buds of tubers of control and Ethrel-treated potato plants during dormancy (tubers were stored at 4 °C).

Date of sampling	Free IAA level [ng g^{-1}(f.m.)] control	Ethrel
10 Aug. 1990	traces	traces
13 Aug. 1990	traces	traces
5 Oct. 1990	28.6 ± 2.9	3.6 ± 0.4
15 Nov. 1990	29.9 ± 3.3	32.1 ± 3.0
1 Dec. 1990	27.7 ± 3.1	30.0 ± 2.9
22 Dec. 1990	28.8 ± 3.6	29.7 ± 2.6
24 Jan. 1991	32.6 ± 3.7	32.4 ± 3.3
23 Feb. 1991	34.3 ± 3.1	30.5 ± 3.2
24 Mar. 1991	34.8 ± 3.2	28.9 ± 3.1
10 May 1991	10.4 ± 1.3	9.9 ± 1.2

It may be concluded that cytokinins play an important role in the control of dormancy duration, in other words stimulation of sprouting. On the basis of the presented data it is difficult to ascribe any role to auxin in dormancy and sprouting regulation. It is interesting to note that treatment with Ethrel had no effect on IAA level throughout dormancy period. It was recently shown (Korableva *et al.* 1989) that IAA application to isolated tuber buds increased significantly ethylene formation. The system of isolated tuber buds can be thus used for deeper study of interrelationship between IAA and ethylene.

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