

## Brassinolide effect on growth of apical meristems, ethylene production, and abscisic acid content in potato tubers

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### Abstract

Treatment of intact potato (*Solanum tuberosum* L., cv. Nevskii) tubers with 24-epibrassinolide (EB) resulted in prolonged deep dormancy, increased production of ethylene and higher contents of free and bound abscisic acid (ABA) in buds. EB at the most efficient concentration  $0.01 \text{ mg dm}^{-3}$ , applied immediately after tuber harvest, inhibited sprouting by 36 - 38 d, increased ethylene formation after 1 and 7 d of storage by almost 300 and 150 %, respectively, and increased the content of both free and bound ABA during the whole period of storage (on average by about 80 %). Electron microscopic and morphometric studies showed that EB brings about a decrease in cell volume in tunica and all types of meristems and an increase in the number of vacuoles, accompanied by a decrease in their volume.

*Additional key words:* *Solanum tuberosum* L., tuber dormancy.

### Introduction

The transition of plants from one physiological state to another, including induction, duration and the end of dormancy, is related to the function of endogenous growth regulators. Studies on potato tubers showed that all known phytohormones, gibberellic acid (GA), abscisic acid (ABA), ethylene, cytokinins, and auxins are involved in regulation of tuber dormancy. Auxins activate sprout growth after the breaking of dormancy (Shih and Rappaport 1970, Engelbrecht and Bielinska-Czarnecka 1972, Van Staden and Dimala 1978, Wareing and Phillips 1983, Hemberg 1985, Turnbull and Hanke 1985a,b, Korableva *et al.* 1980, Súkhova *et al.* 1987, 1993). Ethylene plays an important part in induction and maintenance of tuber dormancy, the ratio of ABA to GA determines the physiological state of potato tubers (Poapst *et al.* 1968, Rylski *et al.* 1974, Korableva *et al.* 1989, 1997, Cvíkrová *et al.* 1994). A rise in ethylene

production in tubers is usually accompanied by an increased synthesis of ABA and induction or prolongation of deep dormancy (Korableva *et al.* 1989, 1997, Cvíkrová *et al.* 1994). Ethylene production is rather low in intact tubers, but it may be enhanced under the action of exogenous ethylene or other stimulators (Korableva and Platonova 1995). Brassinosteroids were shown to be stimulators of ethylene production in different plants (Mandava and Thompson 1984, Arteca *et al.* 1988, Eun *et al.* 1989, Clouse *et al.* 1992) and also stimulators of elongation growth (together with auxins) (Sasse 1999). However, their role in tuber dormancy has never been studied.

The aim of this work was to study the effect of 24-epibrassinolide (EB) on deep dormancy of potato tubers, growth of apical meristems, ethylene production and ABA content in potato tuber apical meristems (buds).

### Materials and methods

**Plant material and EB treatment:** Potato plants (*Solanum tuberosum* L., cv. Nevskii) were used in the

experiments. The harvested tubers were immersed in EB solution of different concentrations ( $0.001 - 0.01 \text{ mg dm}^{-3}$ ),

*Received 20 March 2001, accepted 1 June 2001.*

*Abbreviations:* ABA - abscisic acid; EB - 24-epibrassinolide.

*Acknowledgement:* The authors thank to Dr. Ivana Macháčková of the Institute of Experimental Botany, Academy of Sciences of the Czech Republic for critical reading of the manuscript.

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dried and stored at 4 °C and 85 % humidity. The duration of deep dormancy was determined as the number of days needed for appearance of 1 - 5 mm sprouts in 90 - 95 % of the tubers used. For this purpose, the treated potato tubers were placed under conditions optimal for sprouting (temperature of 25 °C, relative humidity of 85 - 90 %).

**Ethylene determination:** The content of ethylene was determined by gas chromatography (*Tsvet, Gazochrom*, Moscow, Russia, equipped with a *Porapak N* column,  $3000 \times 2$  mm, carrier gas He  $23 \text{ cm}^3 \text{ min}^{-1}$ , oven, injector and detector temperature 80, 100 and 150 °C, respectively, flame ionization detector) as described in Sukhova *et al.* (1987).

**ABA determination:** ABA was extracted and determined as described earlier (Korableva *et al.* 1980, Sukhova *et al.* 1987). ABA content was analyzed in purified methanol extract by the above mentioned gas chromatograph (with an electron capture detector, *OV-1* column, carrier gas  $\text{N}_2$   $30 \text{ cm}^3 \text{ min}^{-1}$ , oven, injector and detector temperatures 210, 250, and 270 °C, respectively). Bound ABA was determined after hydrolysis of the extract with 10 % NaOH for 1 h at 60 °C.

**Morphometric analysis:** Tuber apices of virus-free potato plants grown in a greenhouse were used in the experiments. In the period of deep dormancy, intact

tubers were immersed in EB solution of growth-inhibiting concentrations (0.01; 0.001 mg  $\text{dm}^{-3}$ ) (Platonova and Korableva 1994). Tubers treated with distilled water were used as control. After storage for 2 - 3 months at 4 °C, when water-treated tubers started sprouting, we excised the apices from both EB- and water-treated tubers under a binocular microscope. Then, the apices were fixed with 2.5 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.0 - 7.2, postfixed with 1 %  $\text{OsO}_4$ , and embedded in *EPON-812* epoxide resin (Luft 1962). The cells of the tunica, the central and rib meristems and the lateral meristems at the base of leaf primodium were compared morphometrically (Avetisova and Stefanov 1979). Apical regions were determined under a light microscope, using semithin sections. Ultrathin sections obtained with an *LKB-3* ultramicrotome (*LKB*, Uppsala, Sweden) were stained with lead citrate as described in Reynolds (1963) and examined under a *JEM-100 C* electron microscope (*Jeol*, Tokyo, Japan). We measured the total volumes of cells and vacuoles. In triplicates, we used 25 - 30 electron micrographs of cells in each meristem region studied with a final magnification of 30 000 $\times$ . To perform the measurements, we laid a morphometric grid with meshes of  $10 \times 10$  mm over the cell micrograph and counted the total number of the points of grid line intersections inside the outlines of the entire cell and all vacuoles. The data presented are the means with their standard errors.

## Results

**Dormancy and sprouting:** The treatment of harvested potato tubers with EB at concentrations of 0.001 - 0.01 mg  $\text{dm}^{-3}$  before storage inhibits sprout growth (Fig. 1A). Deep dormancy of water-treated potato tubers lasted 90 d. After 7-month storage, 97 - 98 % of water-treated tubers started sprouting. The length of deep dormancy markedly increased in EB-treated tubers, being the longest (126 - 128 d) in those treated with 0.01 mg  $\text{dm}^{-3}$  EB. A delay in sprouting, as compared to water-treated tubers, was observed in EB-treated tubers irrespective of the duration of storage. The delay depended on EB concentration and with 0.01 mg  $\text{dm}^{-3}$  was 38, 36, 17 and 7 d after storage for 2, 4, 5 and 7 months, respectively (Fig. 1A).

**Ethylene production:** The formation of ethylene increased in EB-treated potato tubers (Fig. 1B). The rate of ethylene synthesis varied depending on the degree of dormancy (deep dormancy, break of dormancy, sprouting) of potato tubers and EB concentration. Water-treated tubers evolved the least amount of ethylene during deep dormancy and the highest amount in the course of sprouting. The highest production of ethylene is observed in potato tubers treated with 0.01 mg  $\text{dm}^{-3}$  EB on days 1 and 7 after treatment. 30 and 60 d after the treatment, the

stimulating effect of EB disappeared and treated tubers produced the same amount of ethylene as non-treated ones. Thus, EB showed the highest stimulating effect on ethylene synthesis during the first 7 d after the treatment of potato tubers with 0.01 mg  $\text{dm}^{-3}$  EB in the course of deep dormancy.

**ABA content:** The amounts of both ABA and ethylene depended on the time of analysis and EB concentration (Table 1). In control tubers, the highest content of free ABA was detected in apices during deep dormancy, *i.e.* on the 30<sup>th</sup> and 60<sup>th</sup> days of storage. On completion of deep dormancy, *i.e.* on the 120<sup>th</sup> and 150<sup>th</sup> days of storage, the content of free ABA in apices decreased about 3- and 5-fold, respectively. Changes in the content of bound ABA were less dramatic, its amount in apices of water-treated potato tubers decreasing about 2-fold on sprouting. Treatment of potato tubers with 0.001 and 0.01 mg  $\text{dm}^{-3}$  EB increased the content of free ABA that remained enhanced not only during deep dormancy, but also after its completion and on sprouting (Table 1). The content of bound ABA slightly increased under the effect of EB and the level remained increased until sprouting: after storage for 120 and 150 d, the amount of bound

ABA was about two fold higher in EB-treated tubers than in water-treated ones. Hence, treatment of potato tubers with EB before storage increases ABA level in tuber apices and changes the ratio of free to bound ABA.

**Morphometric analysis:** Electron microscopic study and morphometry allowed us to determine the total volumes of cells and vacuoles on sections from different apical regions of potato tubers. Treatment with EB (0.001 - 0.01 mg dm<sup>-3</sup>) caused a decrease in the total volumes of cells and vacuoles (Table 2). The total cell volume was

diminished 1.3 and 1.7 fold in cells of the tunica and central meristem, respectively, and 2.2 and 1.2 fold in cells of the rib and lateral meristems, respectively. The total vacuole volume was reduced 1.6 and 3.8 fold in cells of the central and rib meristems, respectively, and 1.2-fold in cells of the tunica and lateral meristems. The decrease in the total vacuole volume was accompanied with an increase in the number of vacuoles (Table 2) that was especially noticeable in cells of the rib meristem (2.1 fold). However, the number of vacuoles did not change in cells of the lateral meristem.

Table 1. Effect of epibrassinolide (EB) on the content of free and bound abscisic acid [ $\mu\text{g g}^{-1}$  (f.m.)] in apical regions of potato tubers during their storage lasting for 30, 60, 120 and 150 d. Means  $\pm$  SE,  $n = 3$ . Initial content: free ABA 8.4, bound ABA 1.8 mg g<sup>-1</sup> (f.m.).

EB [mg dm <sup>-3</sup> ]	30 d free ABA	30 d bound ABA	60 d free ABA	60 d bound ABA	120 d free ABA	120 d bound ABA	150 d free ABA	150 d bound ABA
0	6.1 $\pm$ 0.8	1.6 $\pm$ 0.1	5.2 $\pm$ 0.5	1.7 $\pm$ 0.1	1.9 $\pm$ 0.1	0.8 $\pm$ 0.1	1.1 $\pm$ 0.1	0.8 $\pm$ 0.1
0.0001	6.8 $\pm$ 0.7	2.2 $\pm$ 0.2	5.4 $\pm$ 0.6	2.1 $\pm$ 0.1	2.2 $\pm$ 0.2	1.2 $\pm$ 0.2	1.3 $\pm$ 0.1	0.9 $\pm$ 0.2
0.0010	8.4 $\pm$ 0.9	2.6 $\pm$ 0.2	6.2 $\pm$ 0.4	2.7 $\pm$ 0.3	3.8 $\pm$ 0.5	2.4 $\pm$ 0.3	2.3 $\pm$ 0.3	1.6 $\pm$ 0.2
0.0100	12.5 $\pm$ 1.0	2.7 $\pm$ 0.3	9.8 $\pm$ 1.1	2.6 $\pm$ 0.2	7.6 $\pm$ 0.9	2.5 $\pm$ 0.3	4.2 $\pm$ 0.8	2.4 $\pm$ 0.3

Table 2. Effect of EB on the volumes of cells and vacuoles in cells of different regions of potato tuber apices [arbitrary morphometric units].

Tuber regions	EB [mg dm <sup>-3</sup> ]	Cell volume	Number of vacuoles in a section	Total volume of vacuoles	Volume of one vacuole
Tunica	0	304 $\pm$ 13	5.8 $\pm$ 0.3	20 $\pm$ 0.7	3.4 $\pm$ 0.2
	0.001	228 $\pm$ 12	7.8 $\pm$ 0.2	16 $\pm$ 0.6	2.0 $\pm$ 0.2
	0.010	250 $\pm$ 12	8.0 $\pm$ 0.2	17 $\pm$ 0.6	2.1 $\pm$ 0.2
Central meristem	0	664 $\pm$ 28	6.2 $\pm$ 0.4	87 $\pm$ 7.0	15.0 $\pm$ 0.9
	0.001	402 $\pm$ 18	8.6 $\pm$ 0.5	52 $\pm$ 4.0	11.5 $\pm$ 1.5
	0.010	420 $\pm$ 18	9.0 $\pm$ 0.5	57 $\pm$ 5.0	10.4 $\pm$ 0.8
Rib meristem	0	930 $\pm$ 39	2.9 $\pm$ 0.2	350 $\pm$ 18.0	129.0 $\pm$ 9.0
	0.001	400 $\pm$ 18	6.0 $\pm$ 0.3	86 $\pm$ 10.0	14.0 $\pm$ 0.5
	0.010	410 $\pm$ 18	6.5 $\pm$ 0.2	96 $\pm$ 9.0	15.0 $\pm$ 1.5
Lateral meristem	0	512 $\pm$ 22	6.7 $\pm$ 0.6	30 $\pm$ 4.5	4.6 $\pm$ 0.4
	0.001	480 $\pm$ 16	7.2 $\pm$ 0.4	26 $\pm$ 3.5	3.6 $\pm$ 0.3
	0.010	490 $\pm$ 16	7.7 $\pm$ 0.4	24 $\pm$ 3.5	3.1 $\pm$ 0.3

## Discussion

It was shown earlier that inhibition of sprouting during deep dormancy of potato tubers is connected with the effect of ABA, while the breakdown of dormancy is accompanied with a decrease in the content of ABA and an increase in the content of GA in apical meristems (Hemberg 1985, Korableva *et al.* 1980). Potato tubers were shown to produce less ethylene during deep dormancy than on sprouting (Korableva *et al.* 1997), what does not suggest any relation between the duration

of deep dormancy and the ability of tuber tissues to produce ethylene. However, presented data show that this is not the case. We showed earlier that treatment of potato tubers with the ethylene producer, 2-chloroethylphosphonic acid stimulated ABA biosynthesis in apices and prolonged the deep dormancy period (Korableva *et al.* 1989, Cvíkrová *et al.* 1994). The results of our experiments (Korableva and Platonova 1995) allowed us to suggest that stimulation of endogenous

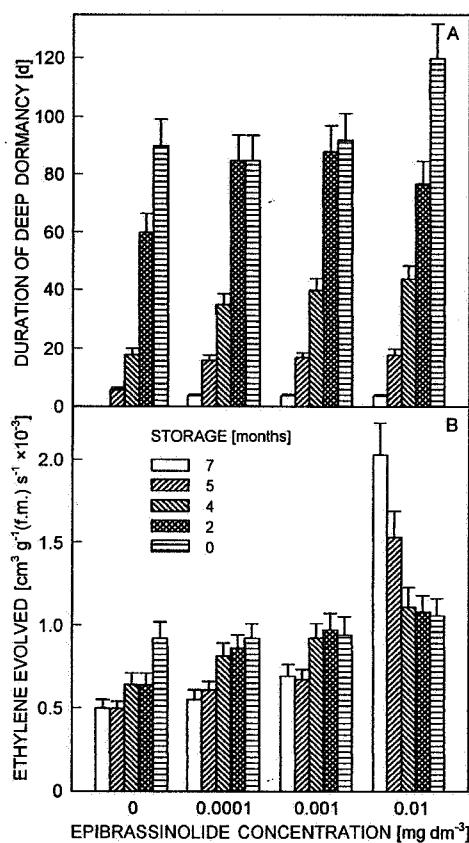


Fig. 14. Effect of epibrassinolide on duration of deep dormancy of potato tubers. Tubers were kept at optimal temperature (25 °C) and humidity (85 %) after 0, 2, 4, 5 and 7 months of storage at 4 °C and 85 % humidity, respectively.

Fig. 1B. Effect of epibrassinolide on ethylene evolution by intact potato tubers. The columns represent 1, 7, 30, 60 and 150 d of storage at 4 °C and 85 % humidity.

ethylene synthesis may lead to increased synthesis of ABA and prolonged deep dormancy of potato tubers. The data obtained in this work show that brassinosteroids (BS) are suitable for this purpose. Some authors (Mandava and Thompson 1984, Artega *et al.* 1988, Eun *et al.* 1989) reported the enhanced ethylene production under the effect of EB in different plants. Our experiments showed that EB stimulated ethylene formation in intact potato tubers and ABA synthesis in cells of tuber apices. The EB-induced changes correlate with the duration of deep dormancy that also increases. The experiments with metabolic inhibitors,  $\text{Co}^{2+}$  and aminoacetic acid revealed that EB increased the activities of both ACC-synthase and ACC-oxidase (Korableva *et al.* 1997). Ultramorphometric analysis of functionally different regions of potato tuber apices demonstrated that inhibition of cell stretching is one of the EB effects on the cell level. The most sensitive to EB was the rib meristem. The treatment with sprouting-inhibiting concentrations of EB suppressed cell stretching 2-fold. This effect is of special importance as the rib meristem plays a leading part at the initial stages of growth of potato tuber apices (Platonova and Korableva 1992). Cells of the rib meristem of potato tubers are assumed to be target cells responding to the EB effect through changes in the ratios of hormones.

We are going to continue this work in order to elucidate if there is a relation between stimulation of ethylene synthesis and enhanced ethylene evolution by potato tubers. Besides, further studies on intracellular changes in different regions of apical meristems may be of special interest in the context of regulation of potato tuber dormancy by means of EB.

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