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EFFECT OF ETHANOL AND PLANT GROWTH REGULATORS ON TERMINATION OF POTATO MICROTUBER DORMANCY

ABSTRACT

The duration of dormancy varies significantly among cultivars, but even short dormancy can limit usage of potato microtubers for seed production. The aim of the research was to test efficacy of dormancy breaking by treatment with an aqueous solution of ethyl alcohol, saccharose, gibberellic acid and kinetin (ethanol treatment) in comparison to treatment with aqueous solution of thiourea, daminozide and gibberellic acid (standard treatment). Prolonging the period of microtuber production at the *in vitro* stage significantly favored the shortening of the dormancy and facilitated its breaking. While the standard treatment had the strongest effect, the ethanol treatment was slightly less efficient. The statistically significant differences were only observed during the first 13 days after the microtuber treatment. After that time, efficacy of ethanol and standard treatments was similar to control treatment with water. The investigated treatments had no effect on the natural decrease of ABA level in microtubers.

Key words: abscisic acid, ethanol, gibberellic acid, kinetin, sucrose, dormancy, sprouting, microtubers

INTRODUCTION

Dormancy duration is often long and varies significantly among cultivars. Thus, it has negative impact on application of potato microtubers in seed production (Coleman and Coleman 2000). Shortening the duration of dormancy has practical significance as it enables prompt multiplication of the seed material (Jansky and Hamernik 2015). The breaking of tuber dormancy was mostly investigated for conventional seed tubers. However, microtubers are similar to

conventional seed potatoes in terms of structure, metabolic processes, enzymes activity, biochemical components, effects of hormonal activities and physiological changes due to environmental factors (Leclerc *et al.* 1995, Donnelly *et al.* 2003). Thus, methods developed for conventional seed tubers may be also effective for microtubers. Despite extensive investigation of dormancy termination, only few practical protocols were developed so far (Kim *et al.* 1999, Coleman and Coleman 2000, Salimi *et al.* 2010).

Similarly to conventional seed tubers, the duration of minitubers dormancy depends mainly on potato genotype and microtuber size (Leclerc *et al.* 1995, Tabori *et al.* 1999). It can be significantly affected by environmental conditions both during the *in vitro* growth of plants and during subsequent *ex vitro* storage of harvested minitubers (Tabori *et al.* 1999, Andrenelli *et al.* 2005, Coleman and Coleman 2000). According to Coleman and Coleman (2000), application of 8 h photoperiod instead of continuous darkness during microtuber induction and development, combined with increased sucrose concentration (from 4 to 16%) in induction medium, positively affected the yield of microtubers and shortened the duration of dormancy in cultivar-dependent manner by 46-86 days. Subsequent *ex vitro* 24 h treatment with ethyl bromide vapor followed by a 72 h storage in the controlled atmosphere resulted in a rapid dormancy release of freshly harvested minitubers (Coleman and Coleman 2000). Effective termination of microtuber dormancy was also achieved by rindite (Kim *et al.* 1999) or carbon disulphide (Salimi *et al.* 2010) treatments. Currently these methods are the most efficient in termination of potato tuber dormancy, but their application is limited due to high cost and/or hazardous nature of chemicals used in these treatments.

The application of growth regulators either before (van Ittersum *et al.* 1993, Caldiz 1996, Alexopoulos *et al.* 2006, Gregorius *et al.* 2008, Gregorius *et al.* 2008) or after harvest (Suttle 2008, Alexopoulou *et al.* 2008, Kullen *et al.* 2011) was reported to effectively shorten or terminate dormancy duration. According to Suttle (2004) endogenous cytokinins are main plant hormones leading to the loss of tuber dormancy while gibberelins are responsible for subsequent sprout growth (Suttle 2004, Hartmann *et al.* 2011). In practice, exogenous treatment with cytokinins was effective only in the later stages of dormancy (Suttle 2008), while treatment with gibberellic acid (GA) could break dormancy of freshly harvested tubers (Jansky and Hamernik 2015). However, GA effect was cultivar dependent and some cultivars hardly responded to the treatment (Wróbel 2008, Wróbel and Robak 2011, 2014).

Recently, it was reported that 0.5% ethanol in combination with 1% sucrose rapidly breaks dormancy of *in vitro*-cultured growing and maturing microtubers (Claassens *et al.* 2005). However, the *ex vitro* effect of ethanol on dormancy duration of microtubers was not investigated.

In our preliminary studies we investigated the effect of ethanol on termination of dormancy of in eye-plugs cut of field grown seed tubers. We found that ethanol alone was ineffective but in combination with GA and kinetin effi-

ciently terminated the dormancy and induced sprout growth at similar level to the control treatment, composed of GA, thiourea and daminozide (unpublished results).

The present study examined the effect of optimized ethanol treatment on breaking the dormancy of microtubers and subsequent sprout and plant growth in comparison to treatment with GA, thiourea and daminozide using treatment with water as a control treatment.

MATERIALS AND METHODS

Research materials

The studied cultivars are described in Table 1. Single-nodal pieces of *in vitro* plantlets were planted in sterile plastic containers with Murashige & Skoog medium (Murashige and Skoog 1962). The tuberisation was induced by the same medium supplemented with saccharose ($8 \text{ g} \cdot \text{L}^{-1}$) and 6-benzylaminopurine ($2 \text{ mg} \cdot \text{L}^{-1}$). Subsequently the containers were incubated for 3 – 4 weeks at 20°C and 16h/8h day/night cycle under cold white light (3000 lux). Next, the containers with well-rooted *in vitro* plantlets were incubated 9-21 weeks in darkness at $18\text{-}20^\circ\text{C}$. After this period, microtubers were harvested, cleaned of the remains of the medium and placed in a cold room at 4°C .

In total, seven series, separated over time, were produced, using potato microtubers of varying age (from 113 to 147 days old). The age of microtubers was defined as a period from the planting of the *in vitro* propagated plantlets until harvesting microtubers. Dormancy breaking treatments were made from 6 to 22 days after harvest.

Table 1

Potato cultivars used in the experiment

Cultivar	Maturity	Breeder	Microtubers [average value]	
			weight [mg]	diameter [mm]
Etiuda	mid early	HZ Zamarte, Poland	230	7.2
Gwiazda	early	HZ Zamarte, Poland	287	7.7
Hubal	early	HZ Zamarte, Poland	163	6.5
Jubilat	mid early	PMHZ Strzekęcin, Poland	195	6.8

Chemicals

GA and kinetin were purchased from Alfa Aesar GmbH, organic solvents for extraction and chromatographic analysis were supplied by Sigma-Aldrich Co. B-Nine 85 SG is a commercial product containing $850 \text{ g} \cdot \text{kg}^{-1}$ of daminozide. For

the preparation of solutions and tuber rinsing, deionized water with a conductivity of $0.06 \mu\text{S} \times \text{cm}^{-1}$ was used.

Treatments

Microtubers, were either immersed for 30 min in the solution containing 4% ethanol, $1 \text{ mg} \times \text{L}^{-1}$ GA and $1 \text{ mg} \times \text{L}^{-1}$ kinetin (ethanol treatment) or for 15 min in the solution containing $1 \text{ mg} \cdot \text{L}^{-1}$ GA, $10 \text{ g} \times \text{L}^{-1}$ thiourea and $3 \text{ g} \times \text{L}^{-1}$ B Nine 85 SG (standard treatment). Control microtubers were soaked for 15 min in water (control treatment). Standard treatment is used for many years in the Department of Potato Protection and Seed Science in Bonin to terminate the dormancy of field-grown tubers.

Table 2

Investigated treatments. The concentrations of GA and kinetin were $1 \text{ mg} \times \text{L}^{-1}$.

No	Composition ($\text{g} \times \text{L}^{-1}$)	Soaking time [min]
1.	Control (deionized water)	15
2.	Standard (GA, 10 g thiourea, 3 g B-Nine 85 SG) ¹	15
3.	Ethanol (4.0% ethanol, 1% sucrose, GA, kinetin)	30

¹ commonly used method for breaking dormancy employed by the Department of Potato Protection and Seed Science in Bonin; B-Nine 85 SG (active ingredient – daminozide – $85 \text{ g} \cdot \text{kg}^{-1}$)

Method

From 6 to 22 days after harvest, three replicates per treatment were treated with ethanol, standard or water solution (control). Due to the size variation, 10 microtubers of similar size were selected for each repetition. Following treatments, minitubers were placed on wet, fine sand in glass Petrie dishes. The dishes were stored in a phytotrone, in darkness, at 21°C and 85-90% RH.

Observations

The number of sprouted microtubers and sprout length were measured starting from 7th day after treatment every 3-4 days for 28 days. Dormancy was considered to be broken once a sprout of minimum length 2 mm had formed. To determine the effect of treatments on plant emergence, once sprout growth assay was terminated (28 dat.), microtubers were planted into pots filled with peat substrate and placed in a greenhouse. Emergence and growth of the offspring plants were recorded every 3-4 days for 28 days.

ABA level analysis

For ABA determination two samples of 6 microtubers per treatment were selected. One sample was frozen at -80°C directly after each treatment and second – 28 days later. Both samples were stored at -80°C until the assay. To determine ABA concentration GC-MS was applied. Assay included the deuterated internal standard, according to the method described by Wilmowicz *et al.* (2008). Each sample of 6 frozen microtubers was homogenized in liquid nitrogen with 20 ml 80% (v/v) methanol including 100 ng $[6\text{-}^2\text{H}_3]$ ABA. Homogenate was transferred to an Erlenmeyer flask and extracted twice for 12 hours at room temperature in 20 ml 80% (v/v) of methanol. The combined methanol fractions were filtered through a Büchner's funnel and the obtained filtrate was concentrated at 40°C until its volume was about 3 ml, using a rotary vacuum evaporator.

The samples were acidified using 2N HCl to pH 2.0, and then centrifuged 15 minutes at $10000 \times g$. Then, the supernatant was extracted three times with 5 ml of ethyl acetate. The organic fraction was evaporated until dry using a rotary vacuum evaporator, and the dry sample was dissolved in 3 ml 80% (v/v) methanol and cleaned using the liquid-solid extraction method (SPE) in small columns containing distributed silica Eluate containing ABA was evaporated until dry and the solid samples were dissolved in 200 μl of 20% (v/v) methanol and separated by High Performance Liquid Chromatography (HPLC) on a SUPELCOSIL ABZ PLUS column using 1% acetic acid solution in water and methanol gradient from 20 to 80% (v/v). At the correct elution time points the fractions were collected, evaporated until dry in a rotary vacuum evaporator, and the remains were transferred to little glass bottles to which ether solution of diazomethane was added for transformation into volatile derivatives. After 1-2 hours ether and the remains of diazomethane were removed by blowing with Nitrogen, and the samples were dissolved in 100 μl of methanol and analyzed in an Auto-System XL gas chromatograph paired with a PerkinElmer Turbomass detector using column MDN-5 (30 m $\times$ 0.25 mm, 0.25 μm ; Supelco). The separation was carried out over a temperature program ranging from 120 to 250°C (initially 120°C for 1 min, and then temperature was increased up to 250°C at speed $10^{\circ}\text{C}\cdot\text{min}^{-1}$, flow $1.5 \text{ mL} \times \text{min}^{-1}$, feeder temperature was 280°C). GC/MS –SIM analysis was carried out by monitoring of ions m/z 162 and 190 for endogenous ABA and 166 and 194 for $[6\text{-}^2\text{H}_3]$ ABA.

Abscisic acid content in the samples was assessed by means of comparison of the ion peaks profile that is characteristic for endogenous ABA (with m/z 162, 190), with the profile for peaks of corresponding ions characteristic for added internal standard $[6\text{-}^2\text{H}_3]$ ABA (with m/z 166, 194), taking into consideration adequate correction coefficients.

Results analysis

The results (percentage values) for normalization underwent Bliss's transformation according to the formula (Wójcik *et al.* 1976):

$$y = \arcsin\sqrt{x}$$

where

y – value after transformation

x – percentage values

Next, the obtained values were analyzed using ANOVA variance analysis. For assessment of significant differences between the researched combinations, mean values were tested using Tukey's test. To significantly increase the results credibility coefficient, the assumed significance level was $\alpha=0.01$. Pearson correlation coefficients were calculated between sprouting of microtubers and their later growth in the soil, as well as between the length of growth period of microtubers at the in vitro stage and duration of dormancy breaking following the harvest (research series) and the number of sprouted microtubers. Statistical calculations were carried out using Statistica 10.0 program (StatSoft, Inc. 2011). After the analysis, the obtained values were retransformed into percentage values and are presented in this form in the paper.

RESULTS

Table 3

Impact of duration of microtuber growth at the in vitro stage and time of dormancy breaking treatment on their sprouting and later growth

Series	Share [%] of sprouted microtubers after 28 days	Share [%] of plants after 28 days since microtubers planting in soil	DMPC	PMHADB	DMPC+PMHADB
1	76.3 b ¹	79.6 ab ¹	135	21	156
2	62.1 c	76.7 ab	115	15	130
3	95.2 a	84.3 a	136	22	158
4	94.5 a	80.8 ab	147	17	164
5	68.7 bc	84.0 a	147	7	154
6	55.3 c	74.4 ab	113	6	119
7	77.7 b	69.1 b	119	10	129
Correlation coefficient for the share of sprouted microtubers after 28 days			0.62	0.68	0.76
Correlation coefficient for share of plants after 28 days since planting the sprouted microtubers			0.79	0.42	0.80

DMPC – duration of microtuber production cycle (days)

PMHADB – period from microtuber harvest until dormancy breaking treatment (days)

¹ mean values marked with the same letters do not differ significantly from each other (Tukey $p=0.01$)

A high correlation coefficient (0.76) was observed between the duration of microtuber production cycle expressed in days (DCPM) and the period from microtuber harvest until dormancy breaking treatment (PMHADB) as well as the number of sprouted microtubers (Table 3). Both, increasing the length of production period (approximately to 147 days) and postponing dormancy breaking treatment to 3 weeks after harvest, significantly influenced the sprouting of microtubers as well as later plant growth (Table 3).

Analysis of averages from all treatments (standard, ethanol and control) showed, that after dormancy termination, cultivars differed significantly in sprouting rates (Fig. 1). The highest rate was observed for cv. Gwiazda, showing that depth of dormancy in this cultivar was the lowest. Cultivars Hubal and Jubilat had the slowest rates of sprouting, what indicated that their dormancy was deeper comparing to other investigated cultivars (Fig. 1).

Fig. 1. Sprouting of 4 cultivars of potato microtubers (mean values from all treatments for 7 series – production cycle) mean values for each observation day between cultivars are marked with the same letters mean values marked with the same letters do not differ significantly from each other (Tukey $p=0.01$)

A statistically significant difference in breaking the dormancy between combinations was observed only during the first 13 days after treatments (Table 4). Later these differences were statistically insignificant, what indicates that the share of sprouted microtubers was at a similar level for all combinations. Standard treatment was found to be the best method, although ethanol treatment had

similar efficacy. After 28 days of observations, on average from 76% (control) to 79% (standard) of microtubers have developed 2 mm sprouts.

Table 4

Impact of the studied treatments on breaking microtuber dormancy (average numbers for 4 potato cultivars – 7 series).

Combinations	Share of sprouted microtubers in subsequent days of observation [%]						
	7	10	13	16 ¹	20	24	28
Control	2.5 b	15.7 b	27.0 b	36.8 b	55.0 a	64.9 a	75.8 a
Standard	7.4 a	29.1 a	39.8 a	48.2 a	62.4 a	71.0 a	78.8 a
Ethanol	3.8 b	19.8 b	33.1 ab	44.7 ab	59.4 a	67.8 a	78.3 a

Mean values for each day of the observation marked with the same letters do not differ significantly from one another (Tukey $p=0.01$)

¹ only significant differences for $p=0.05$

After 4-weeks of sprouting, microtubers were planted into soil for further observation of their growth. The percentage of plant emergence (Table 5) was largely consistent with earlier observations from the microtuber sprouting stage (compare Table 5 vs. Table 4).

Table 5

Impact of the studied treatments on plant emergence after 28 days of microtuber sprouting (mean values for 4 potato cultivars – 7 series).

Treatments	Share of emergent plants in subsequent days of observation [%]						
	7	10	13	16	20	24	28
Control	12.8 b	28.7 b	42.4 b	56.6 a	63.9 a	69.2 a	77.4 a
Standard	22.3 a	40.5 a	53.2 a	62.5 a	68.3 a	74.5 a	79.8 a
Ethanol	17.4 ab	35.4 ab	51.0 ab	59.9 a	65.9 a	72.1 a	78.7 a

Mean values of each day of the observation are marked with the same letters and do not differ significantly from each other (Tukey $p=0.01$)

The assessment of ABA contents in microtubers is presented in Table 6. Directly after treatments, microtubers of investigated cultivars, differed significantly in ABA concentration. As expected, significant differences in ABA concentrations between treatments for given cultivar were lacking. The highest ABA concentration was found in microtubers of cv. Gwiazda, where average from all treatments equaled to 307.3 ng/g FW. For cultivars Hubal and Etiuda ABA concentration equaled respectively to 227 and 190 ng/g FW. The lowest ABA concentration (152.7 ng/g FW) was recorded for cv. Jubilat.

Comparing to ABA assayed directly after treatments, the ABA concentrations were significantly lower 28 day after treatments for all investigated cultivars. Almost 4-fold and 3-fold decrease of ABA concentrations was recorded respectively for cultivars Gwiazda and Jubilat and 2-fold decrease for cultivars Etiuda and Hubal.

Table 6
ABA content in microtubers Values are means in ng·g FW⁻¹ (mean values for 7 series, ±SD)

Treatments	Etiuda		Gwiazda		Hubal		Jubilat	
	After soaking	28 days later	After soaking	28 days later	After soaking	28 days later	After soaking	28 days later
Control	195 ±58	94 ±9	331 ±82	71 ±24	232 ±22	111 ±27	186 ±50	61 ±21
Standard	186 ±34	99 ±11	310 ±33	108 ±25	220 ±37	112 ±32	130 ±52	59 ±14
Ethanol	189 ±56	94 ±22	281 ±38	67 ±25	229 ±43	99 ±23	142 ±39	53 ±15

SD – Standard deviation

DISCUSSION

Recently, it was reported that ethanol can rapidly terminate dormancy in *in vitro*-cultured growing and maturing tubers (Claassens *et al.* 2005). In their work a medium containing 0.5% ethyl alcohol and 1% sucrose induced termination of dormancy of 5 – 8 weeks old microtubers. Over 80% of microtubers sprouted as little as 6 days after placing on ethanol containing medium. Efficacy of ethanol was similar to control treatment with 5 mM GA (Claassens *et al.* 2005, Vreugdenhil *et al.* 2006). In that work, microtubers were not harvested from nodes but, cultured *in vitro* through entire experiment. Thus the *ex vitro* effect of ethanol was not known. In our preliminary work, performed on field-grown seed tubers, ethanol alone was not effective, but after supplementing with GA and kinetin had similar effect to standard treatment (unpublished results). Thus in this work we compared the effect of optimized ethanol treatment (Table 2) on dormancy termination in *ex vitro* microtubers. The standard treatment (Table 2) was the most efficient (Table 4), but the ethanol treatment, was only slightly less effective (Table 4). The statistically significant difference in efficacy of dormancy breaking between treatments was observed only during the first 13 days following the microtuber treatment (Table 4). In work described by Claassens *et al.* (2005), microtubers were uptaking the ethanol from media through entire experiment duration. In our work, microtubers were submerged in ethanol treatment only for limited time (30 min). Thus it is possible that longer exposition to the treatment or increased ethanol concentration could have more profound effect on microtuber dormancy.

Rylski *et al.* (1974) reported dual action of ethylene, which significantly shortened the dormancy of potato tubers but inhibited elongation of the sprouts during extended treatment. In this work, ethylene chlorohydrin had even more profound effect on dormancy termination (Rylski *et al.* 1974). Ethylene ability to terminate dormancy was also reported by Coleman (1998), who additionally observed that the ethylene treatment lowered ABA content in treated tubers. It

is known that physical stress (wounding, low oxygen, short cycles of high and low temperatures) may induce both ethylene biosynthesis and dormancy termination (Struik and Wiersema 1999). Thus, one of possible explanations of ethanol effect on dormancy may result from “stress” ethylene production due to reaction to alcohol.

Prolonging the duration of microtuber production and postponing dormancy breaking treatment after their harvest had a significant impact on termination of microtuber dormancy and subsequent growth of sprouts (Table 3). Andrenelli *et al.* (2005) explain this by claiming that a higher total temperature during microtuberisation accelerates metabolic processes responsible for dormancy shortening and onset of sprouting. This is also confirmed by earlier research by Ranalli *et al.* (1994) who, assessing microtubers of 13 potato cultivars, stated that the duration of dormancy tends to be linear and inversely proportional to the duration of storage. Additionally, they observed that smaller microtubers (less than 250 mg) had a longer dormancy than larger microtubers because the concentration of inhibitors responsible for sprouting was higher than in larger tubers. This is confirmed in research by Lommen (1994), who claimed that in the case of minitubers, the duration of dormancy is also inversely related to the size of tuber. Moreover, it is substantially longer than in the case of traditional field-grown seed potatoes.

Leclerc *et al.* (1995) suggest that longer dormancy duration in small microtubers can be connected with the difference in age of tubers at the time of harvest. Different results with respect to the size of microtubers were obtained by Andrenelli *et al.* (2005), who stated that smaller microtubers (less than 6 mm) had a significantly shorter dormancy period than those whose size was over 6 mm.

The observed significant differences between cultivars in rates of microtuber sprouting (Fig. 1) after termination of dormancy were consistent with earlier reports (Leclerc *et al.* 1995, Tabori *et al.* 1999). Wróbel (2008) and Wróbel and Robak (2011, 2014), reported significant differences in the difficulty level of dormancy breaking on the basis of investigating response of 96 potato cultivars to dormancy terminating treatment. Most cultivars sprouted directly after harvest but some required 5 or 10 weeks storage before treatment was effective. In these studies, dormancy was terminated efficiently using water solution of thiourea, diaminozide (B-Nine 85 SG) and gibberellin (Wróbel 2008, Wróbel and Robak 2011, 2014), which is the same as standard treatment in this work (Table 2). The result showed that the time of effective treatment did not correlate with the natural dormancy duration or maturity group of the investigated genotypes (Wróbel and Robak 2014). Also in this work standard treatment had the highest impact on microtuber dormancy termination. This may be explained by the application of additional ingredients such as daminozide and thiourea. The former is a growth regulator responsible for shortening of internodes, i.e. the plant's height, the latter stimulates dormancy shortening. It is known that nitrogen compounds shorten the dormancy of e.g. seeds (Hilhorst and Karssen

1988, Bethke *et al.* 2004, Alboresi *et al.* 2005, Oracz *et al.* 2008, Arc *et al.* 2013), whereas the application of nitrates at the plant vegetation stage decrease ABA content in mature dry seeds (Matakiadis *et al.* 2009). In the case of potato tubers, thiourea has similar properties. According to Hosseini *et al.* (2011), with the increase of thiourea concentration, the duration of minituber dormancy clearly shortened, whereas the number and length of sprouts increased. Similar results were obtained by Germchi *et al.* (2011), who observed also that an hour-long soaking of minitubers in thiourea solution significantly influenced faster plant emergence, number of stalks, their height, number of tubers and tuber yield, but had a negative impact on the weight of individual tubers. Mani *et al.* (2013b) also reported positive effects of thiourea on potato tuber yield. In another work, Mani *et al.* (2013a), assessing the variation of thiourea dose (19-76 g·l⁻¹), found that a lower dose was more favorable as it enabled faster and more even tuber sprouting, whereas with a larger thiourea concentration, the dormancy duration was prolonged and the number of sprouted tubers decreased.

On the basis of the author's own research (unpublished data) it was observed that too high concentration of nitrates can cause extensive rotting of tuber eye-plugs at an early stage of sprouting. According to Bajji *et al.* (2007), thiourea inhibits catalase and leads to an increase in peroxidase activity. Thus intracellular excess of hydrogen peroxide stimulates superoxide dismutase, which in turn stimulates sprouting. Moreover, the inhibition of catalase by thiourea facilitates longer availability of intracellular hydrogen peroxide for ascorbate and glutathione peroxidases than for catalase, thus these enzymes perform the role of providing a hydrogen peroxide metabolism what results in dormancy breaking (Bhate and Ramasarma 2009).

Selection of the right cultivar and the duration of the treatment play an important role in this type of research. Postponing the treatment after tuber harvest significantly facilitated dormancy breaking, possibly due to the natural decrease of ABA level in the tubers during storage (Wróbel 2008; Wróbel and Robak 2011, 2014).

Clear variation in the ABA content in microtubers of particular cultivars was observed (Table 6), but this not correlated with subsequent cultivar-dependent variation in sprouting rates. The cv. Gwiazda had the greatest initial content of ABA and was also the fastest to sprout. However, for this cultivar, the greatest reduction (almost 4-fold) in endogenous ABA after 28 days was observed. These results are consistent with the previously reported observation that albeit ABA positively regulates dormancy, there is no defined concentration threshold, which triggers bud break (Suttle 2007). Therefore, the main source of variation in ABA content in cultivars is potato genotype.

No differences in ABA content in microtubers were observed between treatments (Table 6). However, a clear drop in content was observed 28 days after the treatment for dormancy breaking. It is likely that this would be clearly visible as soon as a week and/or after two weeks. Such a conclusion might be sug-

gested by the results obtained on the basis of microtuber sprouting observations (Table 4). Suttle (2008) in his research observed a natural drop in apical eyes of endogenous ABA by over 60% during the 15 days following transfer of the tuber to normal room temperature. Moreover, treating minitubers with synthetic cytokinins: CP (N-(2-chloro-4pyridyl)-N'-phenylurea) or NG (1-(α -ethylbenzyl)-3-nitroguanidine) did not affect ABA lowering, whereas treating them with gibberellin limited the decrease of ABA content. Suttle and Hultstrand (1994) observed that as ABA content grows, dormancy in tubers increases and microtubers do not sprout, thus endogenous ABA is essential for induction and maintenance of potato microtubers in dormancy. Leclerc *et al.* (1995) later proved that the ABA level in cells and the duration of microtuber dormancy were positively correlated. This was subsequently confirmed by Suttle (2004). He claimed that ABA and ethylene are the major factors responsible for tuber dormancy, though only ABA is responsible for maintaining the dormancy. According to Claassens *et al.* (2005), ethanol treatment had no impact on ABA concentration, what was consistent with our result (Table 6).

CONCLUSIONS

Prolonging the time of microtuber production favored dormancy shortening and facilitated its breaking.

The most effective dormancy breaking was achieved using standard treatment, but significant differences in its efficacy were confirmed only for the first 13 days following the treatment of the microtubers. During this period efficacy of ethanol treatment was lower, but the difference was not high.

The applied research treatments did not affect the lowering of ABA level in microtubers – its content was reduced in time for all combinations through a natural process.

In spite of the positive effects of ethyl alcohol application at the *in vitro* stage in shortening potato microtuber dormancy, as discussed by Claassens *et al.* (2005) in their research, the application of ethyl alcohol for the purpose of dormancy breaking following the harvest did not produce the expected results. Thus, from a practical point of view, when dealing with cultivars which have a deep and difficult to break dormancy, the investigated treatments may be inefficient and further research is needed to develop treatment which will efficiently break potato microtuber dormancy and will be health and environment friendly.

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