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The effects of hydro- and osmopriming on the germination, vigour and hydrolytic enzymes activity of common zinnia (*Zinnia elegans* Jacq.) seeds

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ABSTRACT

Priming is one of the most common methods of improving seed quality. The aim of this experiment was to study the effects of hydro- and osmopriming on the germination, vigour and hydrolytic enzymes (*α*-amylase, *β*-glucosidase, exopeptidase and lipases) activity of zinnia (*Zinnia elegans* Jacq.) seeds. Seeds of three cultivars: Jowita, Kirke and Orys, and *Z. elegans* fl.pl., a mixture of cultivars (Mix), were tested. The seeds were hydroprimed in a restricted volume of water (200 µl H_2O g^{-1} seed, 24 h at 15°C) and osmoprimed in a polyethylene glycol solution (-1.0 MPa PEG 8000, 5 days at 20°C). Untreated seeds served as the control. The cultivars differed significantly in terms of seed quality as well as response to priming. On average, 'Jowita' seeds were characterised by the highest quality, expressed by the total number of germinating seeds (G_{max}) and germination at the 1st and 2nd counts. Mix seeds showed the lowest quality, expressed by germination at the 1st and 2nd counts and vigour. Generally, an increase in *α*-amylase activity and a decrease in lipase activity was found in hydroprimed and osmoprimed seeds, and *α*-amylase activity was significantly higher in 'Jowita' seeds than Mix seeds. The applied treatments did not affect *β*-glucosidase and exopeptidase activity in the tested seeds. Osmopriming accelerated seed germination and influenced *α*-amylase and lipase activity to a greater extent than hydropriming. Among the assayed enzymes, only the activity of *α*-amylase may be potentially useful for the seed industry as a physiological marker of zinnia seed vigour and the effectiveness of osmopriming.

Key words: *α*-amylase, *β*-glucosidase, exopeptidase, lipases, polyethylene glycol

INTRODUCTION

Priming is one of the most common and universally used methods for improving seed quality. The process permits partial seed hydration so that pregerminative metabolic activity proceeds but germination is prevented. Properly conducted priming causes an increase in germination percentage and germination rate, enables seeds to germinate under a broader range of environmental conditions and improves seedling vigour and growth (McDonald 2000). The positive effects of priming are attributed to the induction of the biochemical mechanisms of cell repair, activation

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of the antioxidant defence system and induction of enzymes catalysing the decomposition and mobilisation of storage compounds (Di Girolamo and Barbanti 2012). Seeds generally contain starch, proteins and triacylglycerols, in proportions depending on the plant species, as sources of matter and energy. During germination, these three major nutrient reserves are hydrolysed specifically by amylases, proteases and lipases, respectively (Barros et al. 2010, Di Girolamo and Barbanti 2012). α-Amylase hydrolyses starch into the simple sugar glucose (Kaneko et al. 2002). β-Glucosidases have been implicated in the degradation of β-glucans produced by the endo-type β-glucanases of cell walls (Akiyama et al. 1998). Aminopeptidases participate in the final stages of protein degradation and hydrolyse peptide bonds, yielding amino acids from N-terminal peptides and proteins (Abdala et al. 1999). Lipases catalyse the hydrolysis of long-chain triacylglycerols into glycerol and free fatty acids, although their substrates can also be esters of medium- or short-chain fatty acids (Cavalcanti et al. 2007). An increase in α-amylase activity in primed seeds has been reported by many authors (Sung and Chang 1993, Lee and Kim 1999, Singh et al. 1999, Lee and Kim 2000, Mukasa et al. 2003, Basra et al. 2005, Sathish and Sundareswaran 2010, Dehghanpour Farashah et al. 2011). However, there are not many reports on the activity of other hydrolytic enzymes after seed priming.

Zinnia (*Zinnia elegans* Jacq.), cultivated usually on flowerbeds and for cut flowers, belongs to the worldwide most popular group of annual ornamental plants. The results of previous experiments have shown that hydropriming as well as osmopriming may significantly accelerate the germination of zinnia seeds (Szopińska and Tylkowska 2009, Szopińska and Wojtaszek 2011, Szopińska 2011). However, there are no reports about the enzyme activity in these seeds. The activity of some enzymes may be potentially used in the seed industry as physiological markers of seed germination and priming efficacy. Hence, the aim of the conducted study was to determine the effect of two priming techniques, hydro- and osmopriming, on the germination and vigour of zinnia seeds in relation to the activity of hydrolytic enzymes.

MATERIAL AND METHODS

Four samples of *Zinnia elegans* Jacq. seeds produced by seed company TORSEED in Toruń (Poland) were used in the study: 'Jowita', 'Kirke', 'Orys' and *Z. elegans* fl.pl., a mixture of cultivars (Mix). The optimal conditions for the hydro- and osmopriming of zinnia seeds were established during a series of preliminary experiments.

Hydropriming

For hydropriming, seeds were placed in 100 cm^3 flasks and 200 µl of distilled water per 1 g of seeds was added. Then the flasks were sealed with parafilm and aluminium foil and incubated in darkness at 15°C for 24 h. After the treatment, seeds were dried in semi-open Petri dishes at 20°C and 45% relative humidity for 24 h to an equilibrium moisture content.

Osmopriming

For osmopriming, 50 seeds were placed in each 9 cm Ø Petri dish on four layers of blotters moistened with 5 cm^3 of polyethylene glycol (PEG 8000, Sigma-Aldrich Co.) solution of osmotic potential of -1.0 MPa and incubated for five days in darkness at 20°C. To obtain this solution, 284 g of PEG was dissolved in 1 litr of sterile water (Michel and Kaufman 1973). The Petri dishes were sealed with parafilm. After priming, the seeds were washed under tap water for 3 min. and rinsed three times in sterile distilled water to remove PEG. Then the seeds were dried with filter paper and next in semi-open Petri dishes at 20°C and 45% relative humidity for 24 h to an equilibrium moisture content.

Seed germination and vigour

For the germination test, eight replications of 50 seeds from each treatment were tested. The seeds were placed in 9 cm Ø Petri dishes (25 seeds per dish) containing six layers of moistened blotters and incubated in darkness, at 20°C. Percentages of normal seedlings (germination at the 1st and 2nd count) and abnormal diseased seedlings were calculated after four and 10 days according to ISTA rules (ISTA 2012). Additionally, the total number of germinating seeds (G_{max}) was determined on the base of the vigour test.

To characterise seed vigour, eight replications of 50 seeds were incubated under the same conditions as in the germination test. Radicle protrusion was scored daily for 10 days. The germination rates, characterising seed vigour, i.e.: T_{10} – time to 10% of the total number of germinating seeds (G_{max}) and MGT – mean germination time were evaluated using GERMINATOR software (Joosen et al. 2010).

Enzymes assays

To obtain the embryos for all enzymatic analyses, the seeds were placed in 9 cm Ø Petri dishes on

four layers of blotters soaked with distilled water and incubated at 20 ± 2°C for 24 h in darkness. Each enzymatic analysis was repeated four times.

The activity of α-amylase (1,4-α-D-glucanohydrolase; EC 3.2.1.1) was determined by the Bernfeld (1955) method consisting of measuring the coloured product of the reduction of 3,5-dinitrosalicylic acid by the reducing group of sugars developed due to starch hydrolysis. The 0.1 g samples of embryos were ground in a cooled mortar with 2.5 cm^3 of sodium phosphate buffer of pH 6.9 with sodium chloride in a concentration of 0.6 mM. Subsequently, the samples were centrifuged at 15,000 × *g* for 20 min. and the obtained supernatant was an enzymatic extract. The reaction mixture, containing 0.5 cm^3 of enzymatic extract and 0.5 cm^3 of 1% soluble starch solution dissolved in an extraction buffer, was incubated for 3 min. at 25°C. Then, 1 cm^3 of 3,5-dinitrosalicylic acid was added and the samples were heated for 5 min. in a boiling water bath to terminate enzymatic reaction. After cooling down, 5 cm^3 of distilled water was added to the samples. Absorbance was measured spectrophotometrically at a wavelength of 540 nm.

The activity of β-glucosidase (β-D-glucoside glucohydrolase; EC 3.2.1.21) was determined by the method of Nicols et al. (1980). The 0.1 g of embryos were ground in cooled mortar and extracted with 5 cm^3 of 0.1 M potassium phosphate buffer of pH 7.0, containing 0.5% polyethylene glycol (PEG 6000) and 50 mg of Polyclar AT. The samples were centrifuged at 10,000 × *g* for 15 min. The reaction mixture containing equal volumes of the 10-fold dissolved enzyme extract and 4-nitrophenyl-β-D-glucopyranoside (2 mg cm^{-3}) was incubated for 60 min. at 35°C. The reaction was terminated by an addition of 0.2 M Na_2CO_3 (1.5 ml cm^{-3}). Formed *p*-nitrophenol was determined spectrophotometrically at 400 nm.

The activity of exopeptidase (leucyl aminopeptidase; EC 3.4.11.1) was determined by Worthington's method (1993). The 0.1 g samples of embryos were ground in a cooled mortar with 10 cm^3 of 0.02 M TRIS-maleic buffer of pH 6.0. Then, the samples were centrifuged at 12,000 × *g* for 40 min. The obtained supernatant was an enzymatic extract. The reaction mixture contained 0.4 cm^3 of enzymatic extract, 1 cm^3 of 0.02 M TRIS-maleic buffer of pH 6.0 and 1 cm^3 of substrate (0.6% L-leucine-β-naphtylamide hydrochloride). The samples were incubated at 30°C for 60 min. and then transferred to a water bath and kept at 70°C for 10 min. to stop the enzymatic reaction. After cooling down, the samples were centrifuged at 3,000 × *g* for 10 min. and then 5 cm^3 of 0.02% Fast Garnet GBC dye was added to a precipitate of each sample to bind naphthylamine and to obtain a coloured complex. Absorbance was measured spectrophotometrically at a wavelength of 500 nm.

Lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) activity was determined by the method of Achakzai et al. (2003). Acetonic powder was obtained by homogenizing the embryos of zinnia seeds in acetone and separating acetone from the solid part by filtration and evaporation at room temperature (Cavalcanti et al. 2007). Next, 0.1 g of the powder was extracted with 2 cm^3 of 0.05 mM phosphate buffer of pH 8.0 for 45 min. at 26°C. Subsequently, the extract was centrifuged at 11,000 × *g* for 30 min. 3.75 cm^3 of 48% ammonium sulphate was added to 1.5 cm^3 of supernatant and was placed for 30 min. at 4°C. Next, samples were centrifuged at 5,000 × *g* for 30 min., the supernatant was discarded and the precipitate was dissolved in 1.5 cm^3 of 0.05 mM phosphate buffer of pH 8.0. The obtained solution was an enzymatic extract. The reaction mixture contained 0.1 cm^3 of enzymatic extract, 2.3 cm^3 of 0.1 M TRIS buffer of pH 9.0 and 0.6 cm^3 of 4-nitrophenyl laurate (the emulsion was obtained by a solution of 1 cm^3 of 20 mM laurate solution in acetone with 8 cm^3 of 0.1 M acetate buffer of pH 3.8, 4 cm^3 of 1% polyvinyl alcohol and was supplemented to 20 cm^3 with 0.1 M acetate buffer of pH 3.8). Samples were incubated for 1 h at 30°C. Absorbance was measured spectrophotometrically at a wavelength of 405 nm.

Statistical analysis

Data related to germination, vigour and enzymes activity were processed by two-way ANOVA (the first factor was the treatment, the second was the cultivar) using STAT software. The significance of differences was evaluated by Duncan's multiple range test at $p = 0.05$.

RESULTS

The examined cultivars differed significantly in terms of seed germination and vigour as well as response to priming (Tabs 1 and 2). Before priming, a very high total number of geminating seeds was observed for 'Jowita' and 'Kirke' (93.8 and 95.8%, respectively), a lower one for 'Orys' (85.5%) and the lowest for Mix (74.8%). After hydro- and osmopriming, a significant decrease in this parameter was observed only for

Table 1. The effect of hydro- and osmopriming on the germination of zinnia seeds (%)

Item	Treatment	Jowita	Kirke	Orys	Mix	Mean
Total number of germinating seeds (G_{max})	Control	93.8 de*	95.8 e	85.5 bc	74.8 a	87.4 B
	Hydropriming	92.5 d	85.3 bc	87.0 c	79.5 ab	86.1 B
	Osmopriming	93.3 de	79.3 ab	82.8 bc	74.3 a	82.4 A
	Mean	93.2 C	86.8 B	85.1 B	76.2 A	
Germination at 1st count	Control	60.3 e-h	42.3 bc	53.0 de	35.8 ab	47.8 A
	Hydropriming	66.3 gh	46.3 cd	58.0 e-g	29.8 a	50.1 A
	Osmopriming	67.8 h	61.0 e-h	63.0 f-h	54.5 d-f	61.1 B
	Mean	64.8 D	49.8 B	58.0 C	40.0 A	
Germination at 2nd count	Control	88.0 e	82.3 de	59.8 c	45.8 b	68.9 B
	Hydropriming	84.3 de	47.8 b	67.5 c	34.0 a	58.4 A
	Osmopriming	80.0 d	63.8 c	67.0 c	58.5 c	67.3 B
	Mean	84.1 C	64.6 B	64.8 B	45.9 A	
Abnormal diseased seedlings	Control	6.5 b	10.5 bc	24.8 de	32.3 ef	18.5 B
	Hydropriming	2.5 a	38.5 f	16.8 cd	41.5 f	24.8 C
	Osmopriming	0.8 a	14.5 c	13.8 c	14.8 c	10.9 A
	Mean	3.3 A	21.2 B	18.4 B	29.5 C	

*Values marked with the same letter, for each parameter separately, do not differ significantly at p = 0.05, according to Duncan's test

Table 2. The effect of hydro- and osmopriming on zinnia seed vigour (h)

Item	Treatment	Jowita	Kirke	Orys	Mix	Mean
Time to 10% germination (T_{10})	Control	32.3 bc*	28.7 b	27.1 b	38.6 c	31.7 C
	Hydropriming	25.2 ab	29.5 b	19.3 a	34.2 bc	27.1 B
	Osmopriming	19.0 a	19.6 a	19.6 a	20.1 a	19.6 A
	Mean	25.5 AB	25.9 AB	22.0 A	30.9 B	
Mean germination time	Control	38.5 cd	39.1 d	40.1 d	46.0 e	40.9 C
	Hydropriming	39.9 d	34.1 c	23.7 b	41.8 de	34.9 B
	Osmopriming	19.4 ab	18.6 a	19.5 ab	20.0 ab	19.4 A
	Mean	32.6 B	30.6 B	27.8 A	35.9 C	

*Explanations: see Table 1

'Kirke'. The total number of germinating seeds for other cultivars was not affected by the applied treatments. On average, the deterioration of this parameter was noted for osmoprimed seeds (Tab. 1). However, the mean seed germination at 1st count was higher for osmoprimed seeds than for untreated and hydroprimed ones. Osmopriming significantly improved this parameter for 'Kirke', 'Orys' and Mix, while hydropriming did not affect it for any cultivar as compared to the control (Tab. 1). Hydropriming significantly decreased seed germination at the 2nd count for 'Kirke' and Mix. On the other hand, osmopriming resulted in a deterioration of this parameter for 'Jowita' and 'Kirke' and an improvement for Mix (Tab. 1). On average, 'Jowita' seeds were characterised by the highest germination at the 1st and 2nd counts, whereas the lowest values of these parameters were observed in the case of Mix seeds (Tab. 1). The reduction of seed germination at the 2nd count for 'Kirke' after hydropriming was connected with a significant increase in the percentage of abnormal diseased seedlings. On the other hand, the percentage of these seedlings decreased significantly for 'Jowita' after hydropriming and for 'Jowita', 'Orys' and Mix after osmopriming. Hydropriming generally deteriorated this parameter as compared to the control, while osmopriming improved it. On average, the highest percentage of abnormal diseased seedlings was observed for Mix and the lowest for 'Jowita' (Tab. 1).

In general, the values of the T_{10} parameter for seeds of all samples did not differ significantly (Tab. 2). 'Orys' seeds were characterised by a lower MGT value than the other cultivars (Tab. 2). On the other hand, the highest values of both parameters

Table 3. Enzymes activity in embryos of germinating hydro- and osmoprimed zinnia seeds

Item	Treatment	Jowita	Kirke	Orys	Mix	Mean
α-Amylase (µmol maltose 100 mg^{-1} h^{-1})	Control	36.4 ab*	36.3 ab	37.7 ab	37.6 ab	37.0 A
	Hydropriming	46.2 bc	44.8 abc	51.5 c	34.2 a	44.2 B
	Osmopriming	54.7 c	50.5 c	49.2 c	45.2 bc	49.9 C
	Mean	45.8 B	43.8 AB	46.1 B	38.0 A	
Lipases (ΔA 100 mg^{-1} acetone powder h^{-1})	Control	0.52 g	0.35 bc	0.41 de	0.42 e	0.42 C
	Hydropriming	0.46 f	0.34 b	0.38 cd	0.36 bc	0.38 B
	Osmopriming	0.40 de	0.20 a	0.41 de	0.33 b	0.34 A
	Mean	0.46 D	0.30 A	0.40 C	0.37 B	

*Explanations: see Table 1

were found for Mix seeds. On average, both applied treatments improved the vigour of zinnia seeds, but osmopriming did so to a larger extent than hydropriming. After osmopriming there were not significant differences in the values of T_{10} and MGT between the tested cultivars, while hydropriming improved T_{10} only for 'Orys' and MGT for 'Kirke' and 'Orys' (Tab. 2).

The enzyme activity depended on the cultivar and the method of seed priming applied (Tab. 3). On average, an increase in α-amylase activity was found in osmo- and hydroprimed seeds. However, hydropriming increased the activity of this enzyme only in 'Orys' seeds, while osmopriming increased it in 'Jowita', 'Kirke' and 'Orys' seeds (Tab. 3). The applied treatments did not affect β-glucosidase and exopeptidase activity in the seeds of the tested cultivars. The observed values ranged from 4.00-5.54 µmol *p*-nitrophenol mg^{-1} h^{-1} and 0.36-0.49 ΔA 100 mg^{-1} h^{-1} for β-glucosidase and exopeptidase activity, respectively (data not shown). On the other hand, a significant decrease in lipase activity in hydroprimed and osmoprimed 'Jowita' and Mix seeds as well as in osmoprimed 'Kirke' seeds was found. However, none of the treatments affected the activity of these enzymes in 'Orys' seeds. Generally, the activity of lipases in osmoprimed seeds was lower than in hydroprimed seeds (Tab. 3).

DISCUSSION

In the present experiment, the seeds of the tested cultivars differed in their response to priming. McDonald (2000) reported, on the basis of many findings, that initial seed quality may significantly influence priming success. Moreover, individual species and even seed lots may vary in response to various priming techniques. The results of previous experiments have shown that hydropriming as well as osmopriming significantly accelerated zinnia seed germination (Szopińska and Tylkowska 2009, Szopińska and Wojtaszek 2011, Szopińska 2011). However, in the present work, the positive effects of osmopriming were more conspicuous than those of hydropriming, although the level of seed moisture content achieved by using both priming methods was comparable, and ranged from 48.8 to 50.3% in individual samples. In 'Kirke' and Mix, a decrease in seed germination at the 2nd count after hydropriming was associated with an increase in the percentage of abnormal diseased seedlings and dead seeds (data not shown). The deterioration of seed health after priming has been reported by many authors (Biniek 1994, Tylkowska and Biniek 1996, Nascimento and West 1998, Dorna et al. 2001, Tylkowska and Van den Bulk 2001, Dorna et al. 2005), also in relation to hydro- and osmoprimed zinnia seeds (Szopińska and Tylkowska 2009, Szopińska and Wojtaszek 2011). High humidity and incubation temperature during priming, especially hydropriming, favour the growth of a variety of microorganisms. On the other hand, the low osmotic potential of PEG solution could be a factor limiting the growth of fungi during osmopriming (Szopińska 2001). Nevertheless, a good solution may be combining zinnia seed priming with a chemical or biological treatment (Tylkowska and Biniek 1996, Dorna et al. 2001, Dorna et al. 2005, Szopińska 2011).

An increase in α-amylase activity after priming has been found in the seeds of many species, e.g.: maize (Sung and Chang 1993, Sathish and Sundareswaran 2010), marigold (Afzal et al. 2011), milk thistle (Sedghi et al. 2010), muskmelon (Singh et al. 1999), oregano (Dehghanpour Farashah et al. 2011), rice (Lee and Kim 1999, Lee and Kim 2000, Basra et al. 2005) and sugar beet (Mukasa et al. 2003). Sedghi et al. (2010) reported that α-amylase activity in milk thistle seeds primed in GA_3 solution

for 24 h was higher after five days of germination than in unprimed seeds. Singh et al. (1999) observed an increase in α-amylase activity in muskmelon seeds primed in PEG and KNO_3 solutions for three days. Mukasa et al. (2003) reported that the levels of amylase activity in primed sugar beet seeds were 1.9 to 11.5 times higher than those of the control seeds. An increase of α-amylase activity was also found in *Tagetes erecta* L. and *T. patula* L. seeds primed in mannitol solution (Afzal et al. 2011), in hydroprimed and haloprimed *T. patula* seeds (Mukhtar et al. 2013) and in oregano seeds after priming in a PEG solution (Farashah et al. 2011). According to Basra et al. (2005), an increase in α-amylase activity in hydroprimed and haloprimed rice seeds caused an increase in soluble sugar content, resulting in faster and more uniform plant emergence. This phenomenon was also observed in our research in primed zinnia seeds, especially after osmopriming. The lowest activity of this enzyme was detected in Mix seeds, which showed the lowest germination at the 1st and 2nd counts and the highest values of T_{10} and MGT parameters. Galani et al. (2011) and Lee and Kim (2000) observed a positive correlation between α-amylase activity and germination percentage and the germination rate of rice seeds. In rice seeds of reduced viability and vigour, amylase activity has been shown to be lower than in seeds characterised with high germination and vigour (Nandi et al. 1995, Galani et al. 2011). Khan et al. (2013) reported that in recent harvest pea seeds, α- and β-amylase activity was higher compared to stored seeds. The storage period significantly affected the activity of these enzymes and a decreasing trend was observed as the storage period was prolonged and seed vigour deteriorated. In the present experiment, the untreated seeds of all of the cultivars showed the same level of α-amylase activity despite the fact that they varied in seed germination and vigour. Nevertheless, the values of the T_{10} parameter and MGT in all cultivars decreased after osmopriming and did not differ significantly, while the α-amylase activity increased and also reached the same level. The improvement of seed vigour associated with an increase of α-amylase activity after hydropriming was observed only in one of the tested cultivars (Orys). These results show that α-amylase activity may be a good physiological marker in the case of osmopriming zinnia seeds but it's not fully useful as a marker of hydropriming.

In the present experiment, we did not find differences in β-glucosidase and exopeptidase activity in unprimed and primed zinnia seeds. However, Akiyama et al. (1998) observed that the activity of β-glucosidase increased more than eight-fold within five days of rice seed germination. The authors suggested that the enzyme is probably involved not only in hydrolysis but also in the modification of oligosaccharides in the cell walls of germinating rice seeds. On the other hand, Mukasa et al. (2003) reported that there was little change in β-glucosidase activity in sugar beet seeds after priming. The different priming protocols and specific seed physiology of individual species might be reasons for these varying results. Yoshiara et al. (2012) found the highest level of β-glucosidase activity in soybean seedlings only after 144 h of germination. Simos et al. (1994) observed that the β-glucosidase of barley seeds did not increase during germination, even in the presence of exogenously added gibberellic acid. However, the germination process affected the physical properties of β-glucosidase in terms of charge and apparent molecular weight. According to Gallardo et al. (2002), β-glucosidase is activated in the final stage of germination and plays a part in loosening the cell walls of the embryo, which allows cell elongation and root growth.

Isola and Franzoni (1996) found that initially high aminopeptidase activity in resting peanut seeds continuously declined after imbibition. The authors assumed that these enzymes may be involved in protein turnover as well as in the activation of certain apoenzymes. Kirmizi and Güleryüz (2006) observed that at the beginning of broad bean germination the activity of exopeptidase in cotyledons was higher than after five days of germination. Similarly, Politycka and Gmerek (2008) found the highest activity of this enzyme in cucumber and pea seeds after 24 h of germination and the lowest after 72 h. This correlation was not observed during the germination of zinnia seeds. β-glucosidase and aminopeptidase are most likely activated in further stages of seed germination in this species.

In the present work, a higher level of lipase activity on average was found in untreated zinnia seeds than in hydro- and osmoprimed ones. In cucumber seeds, the highest level of lipase activity was observed after 24 h of germination and after 48 and 72 h this activity decreased systematically (Politycka and Gmerek 2008). Most likely, the lipolytic activity in primed zinnia seeds took place earlier than in unprimed seeds. Kubala et al. (2015) identified several genes encoding enzymes

playing a role in lipid catabolism in oilseed rape seeds and found that some of them were strongly up-regulated already during osmopriming. During germination, triacylglycerols stored in oil bodies are quickly utilized in energy production for the synthesis of the structural compounds required for the growth of the embryo (Barros et al. 2010). The free fatty acids, products of triacylglycerols hydrolysis, are transported to peroxisome, where *β*-oxidation is initiated. The acetyl-CoA produced by *β*-oxidation enters the glyoxylate cycle and takes a part in glyconeogenesis to produce sugars used by the embryo as an energy source during germination (Quettier and Eastmond 2009).

CONCLUSIONS

1. The obtained results demonstrate that, regardless of the initial quality of the treated seeds, osmopriming is a more effective method of improving the germination and vigour of zinnia seeds than hydropriming.
2. Among the assayed enzymes, only the activity of α-amylase may be potentially useful for the seed industry as physiological marker of zinnia seed vigour and the effectiveness of osmopriming.

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AUTHOR CONTRIBUTIONS

Both authors (D.S. and B.P.) equally contributed to designing the experiments, performing analytical measurements and manuscript writing. D.S. performed the statistical analysis.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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