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THE EFFECT OF PRIMING ON GERMINATION AND VIGOUR OF PANSY (*Viola × wittrockiana* Gams.) SEEDS

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Abstract. Three seed priming techniques: hydropriming, halopriming and osmopriming, were compared for their effects on germination and vigour of pansy (*Viola × wittrockiana* Gams.) seeds at 20°C, 30°C and 35°C. Seeds were hydroprimed in the restricted volumes of water (600 and 700 µl $H_2O \cdot g\ seed^{-1}$, 2, 3 or 4 days), haloprimed in KNO_3 solution (-1.5 MPa, 5 days), and osmoprimed in polyethylene glycol solutions (-1.0, -1.25 or -1.5 MPa PEG 8000, 7 days) at 15°C or 20°C. Seed germination and vigour tests were performed for untreated and primed seeds. Generally, hydropriming negatively affected the speed of germination, the percentage of germinating seeds and germination capacity. Halopriming accelerated seed germination at 20°C, 30°C and 35°C but did not influence the percentage of germinating seeds and germination capacity. Osmopriming of seeds in PEG solution of osmotic potential –1.0 MPa at 20°C not only improved germination rates at 20°C, 30°C, and 35°C to the highest extent, but also increased percentage of germinating seeds at 30°C and 35°C most effectively and positively affected seed germination capacity at 20°C and 30°C.

Key words: pansy seed, priming techniques, germination capacity, vigour

INTRODUCTION

Priming is a pre-sowing, controlled-hydration treatment in which seeds are exposed to an external water potential sufficiently low to prevent radicle protrusion but stimulating physiological and biochemical activities [Bradford 1986, Khan 1992]. This process may improve speed and uniformity of germination and germination percentage, especially under adverse conditions such as low and high temperature, salinity and matric stress [Wurr and Fellows 1984, Pill and Finch-Savage 1988, Frett and Pill 1989, Pill et al. 1991, Dursun and Ekinci 2010, Nawaz et al. 2013, Di Girolamo and Barbanti 2012, Manonmani et al. 2014]. Various priming methods are used to enhance seed quality. In

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hydropriming seeds are submerged in water, whereas osmotic priming is the process that involves the use of osmotic solutions with a low water potential to control water uptake eg. polyethylene glycol. Halopriming refers to soaking seeds in solutions of inorganic salts i.e. NaCl, KNO_3, $CaCl_2$ etc. Some salt solutions may exert direct or indirect nutritional effects [Nawaz et al. 2013, Di Girolamo and Barbanti 2012, Manonmani et al. 2014].

Temperature stress is one of the most important factors affecting growth and development of many plants. At supra-optimal temperatures seeds may enter into the state of thermoinhibition or thermodormancy [Horowitz and Taylorson 1983]. Thermoinhibited seeds fail to germinate at high temperatures, but are able to do so when the temperature will be decreased. However, the dormant state must be released by some form of dormancy-breaking treatment, even if the seeds are placed at their optimum germination temperature [Vidaver and Hsiao 1975, Black et al. 2006]. Moreover, in both thermoinhibited and thermodormant seeds, too high temperature maintained for an extended period could result in thermal death [Horowitz and Taylorson 1983]. The most often produced bedding plant is pansy (*Viola* × *wittrockiana* Gams.). Production of this plant starts usually during summer months when conditions are often unfavorable for plant development, because high temperatures during pansy seed imbibition can delay or completely inhibit germination [Carpenter and Boucher 1991, Hamrick 2005].

There are many reports showed that thermodormancy could be overcome by seed priming [Cantliffe et al. 1981, Valdes et al. 1985, Cantliffe 1991, Carpenter and Boucher 1991, Parera and Cantliffe 1992, Weges et al. 1991, Yoon et al. 1997]. The best-known example is that of lettuce [Cantliffe et al. 1984, Szopińska and Tylkowska 2004, Schwember and Bradford 2005], leek [Parera and Cantliffe 1992] and tomato [Odell and Cantliffe 1986], where the upper temperature limit for germination was increased by priming treatment. Similar, though less obvious, phenomenon had been found in pansy seeds subjected to hydropriming [Łabenska 2007, Matczyński 2008], halopriming [Yoon et al. 1997] and osmopriming [Carpenter and Boucher 1991, Yoon et al. 1997, Łabenska 2007, Matczyński 2008]. However, any of these studies compared effectiveness of all of these methods.

The aim of the experiment was to determine and compare the effects of various priming methods i.e. hydropriming, halopriming and osmopriming on germination and vigour of pansy seeds at optimal and supra-optimal temperatures.

MATERIALS AND METHODS

Commercially produced seeds of pansy (*Viola* × *wittrockiana* Gams.) cv. 'Pirna Sachsenland' obtained from CNOS-PNOS Seed Company in Poznań were used in the study. Germination and vigour tests were performed for untreated seeds (control) and seeds subjected to hydropriming, halopriming and osmopriming.

Hydropriming. For hydropriming 2.5 g of seeds were placed in conical 100 ml flasks and imbibed in 1500 µl (600 µl $H_2O \cdot g\ seed^{-1}$) or 1750 µl (700 µl $H_2O \cdot g\ seed^{-1}$) of distilled water. Next the flasks were sealed with parafilm and aluminum foil and incu-

bated for 2, 3 or 4 days at 15°C or 20°C in darkness. After priming the seeds were dried for 48 h in semi-open Petri dishes at 20°C and 45% relative humidity.

Halopriming. For halopriming 50 seeds were placed in 9 cm diameter Petri dishes on 4 layers of blotters moistened with 5 ml of KNO_3 solution of osmotic potential -1.5 MPa. The plates were sealed with parafilm and kept for 5 days at 15°C or 20°C in darkness. After priming the seeds were washed under tap water for 5 min and then rinsed three times with distilled water. For drying the seeds were placed in semi-open Petri dishes at 20°C and 45% relative humidity for 48 h.

Osmopriming. Polyethylene glycol 8000 (PEG 8000, Sigma-Aldrich) was used for osmopriming of pansy seeds. Fifty seeds were placed in 9 cm diameter Petri dishes on 4 layers of blotters moistened with 5 ml of PEG solutions of osmotic potential of -1.0, -1.25 and -1.5 MPa. The Petri dishes sealed with parafilm were placed for 7 days at 15°C or 20°C in darkness. After osmopriming the seeds were washed and dried according to the procedure described for halopriming.

Seed germination test. In germination test percentage of normal seedlings (germination capacity at final count), abnormal seedlings and ungerminated seeds were determined after 7 and 21 days according to the ISTA rules [International Rules for Seed Testing 2012]. Six replicates of 50 seeds from each treatment (300 seeds) were placed in 9 cm diameter Petri dishes containing six layers of moistened blotters and incubated at 20°C, 30°C and 35°C in darkness. Additionally, the percentage of germinating seeds (G_{max}) was determined during seed vigour evaluation on the base of a number of seeds with visible radicle counted daily.

Seed vigour test. Six replicates of 50 seeds from each treatment (300 seeds) were incubated in the same way like in germination test. Radicle protrusion was scored daily for 21 days. The parameters: T_{10} – time to 10% of G_{max}, T_{50} – time to 50% of G_{max}, and $U_{75\text{-}25}$ – time between 25 and 75% of G_{max}, were determined.

Moisture content determination. The moisture content determination was carried out for untreated and primed seeds on two replicates of 0.5 g from each treatment. The high constant temperature oven method was applied [International Rules for Seed Testing 2012]. In case of primed seeds moisture content was evaluated before and after drying.

Statistical analysis. SeedCalculator version 2.1 software [Jalink and Van der Schoor 1999] was used to analyze G_{max} and vigour data. All results were compared by means of variance analysis followed by the Duncan's test.

RESULTS

Seed germination. The increase of incubation temperature during germination resulted in decrease of the percentage of germinating seeds (G_{max}) in control from 80.3% at 20°C to 67.7% at 30°C and 26.0% at 35°C (tab. 1). None of the applied priming methods improved this parameter at 20°C. Some priming treatments even decreased G_{max} compared with untreated seeds. Osmopriming seeds in PEG solutions of -1.25 and -1.5 MPa osmotic potential at 15°C and in PEG solution of -1.0 MPa osmotic potential at 20°C increased the percentage of germinating seeds significantly at 30°C, while other

treatments did not affect this parameter. Osmopriming seeds, in all combinations used, improved the percentage of germinating seeds significantly at 35°C. The best result was observed if seeds were primed in PEG solution of -1.0 MPa osmotic potential at 20°C. Meanwhile, halopriming and hydropriming did not affect G_{max} at the highest temperature.

Table 1. The effect of priming on the percentage of germinating seeds (G_{max}) at 20, 30, and 35°C

	Seed treatment				Temperature, °C					
	duration of priming, days	temperature of priming, °C	amount of water, µl $H_2O \cdot g\ seed^{-1}$	osmotic potential, MPa	20		30		35	
Untreated seeds					80.3	a-c	67.7	d–h	26.0	f–i
Hydropriming	2	15	600	–	84.0	ab	60.7	g–i	11.3	ij
			700	–	83.0	ab	53.3	i	6.67	j
		20	600	–	82.3	a–c	64.0	e–i	25.7	f–i
			700	–	81.3	a–c	68.0	c–g	21.0	g–j
	3	15	600	–	79.0	a–d	66.0	d–h	21.3	g–j
			700	–	77.0	b–e	65.7	d–h	29.7	f–h
		20	600	–	79.3	a–c	69.3	b–g	12.7	ij
			700	–	79.7	a–c	65.7	d–h	19.3	g–j
	4	15	600	–	72.3	de	72.7	a–g	18.7	g–j
			700	–	81.3	a–c	62.0	f–i	25.0	f–i
		20	600	–	79.0	a–d	68.3	b–g	32.7	e–g
			700	–	71.0	e	55.0	hi	16.3	h–j
Halopriming	5	15	–	-1.5	75.3	c–e	70.7	a–g	30.7	f–h
		20	–	-1.5	70.7	e	63.3	e–i	39.3	d–f
Osmopriming	7	15	–	-1.0	84.0	ab	73.7	a–f	45.7	c–e
			–	-1.25	80.0	a–c	80.0	ab	49.3	b–d
			–	-1.5	83.0	ab	79.3	a–c	55.0	bc
		20	–	-1.0	81.7	a–c	81.7	a	69.0	a
			–	-1.25	84.3	ab	76.7	a–d	63.3	ab
			–	-1.5	85.0	a	75.0	a–e	52.3	b–d

Means in columns followed by the same letter do not differ significantly at $\alpha = 0.05$ according to Duncan's test

Only osmopriming at 20°C in PEG solutions of -1.0 and -1.25 MPa osmotic potential increased significantly germination capacity at 20°C (tab. 2). At higher temperature hydropriming seeds in volume of water 600 µl $H_2O \cdot g\ seed^{-1}$ for 3 days at 15°C and osmopriming at 20°C positively affected this parameter. Generally, normal seedlings (germination capacity) were not observed in control as well as in primed seeds at 35°C. Only after osmopriming seeds in PEG solution of -1.0 MPa osmotic potential at 15°C this parameter reached 0.3%.

None of the applied methods of priming decreased the percentage of abnormal seedlings regardless of temperature (tab. 3). Some of the treatments at lower temperatures, and almost all of them at 35°C increased the number of these seedlings.

Table 2. The effect of priming on the germination capacity at the final count at 20, 30, and 35°C (%)

	Seed treatment				Temperature, °C					
	duration of priming, days	temperature of priming, °C	amount of water, µl $H_20 \cdot g\ seed^{-1}$	osmotic potential, MPa	20		30		35	
Untreated seeds					66.0	b–d	42.7	e–i	0	b
Hydropriming	2	15	600	–	72.3	ab	43.3	e–i	0	b
			700	–	72.0	ab	40.7	g–i	0	b
		20	600	–	72.7	ab	42.7	e–i	0	b
			700	–	69.7	bc	45.0	b–h	0	b
	3	15	600	–	66.0	b–d	52.0	a–d	0	b
			700	–	66.0	b–d	37.3	h–j	0	b
		20	600	–	67.3	bc	44.3	c–i	0	b
			700	–	68.0	bc	48.3	a–g	0	b
	4	15	600	–	63.7	b–d	46.3	a–g	0	b
			700	–	64.7	b–d	40.3	g–i	0	b
		20	600	–	64.7	b–d	49.7	a–f	0	b
			700	–	60.7	b–d	41.3	f–i	0	b
Halopriming	5	15	–	-1.5	64.0	b–d	36.3	ij	0	b
		20	–	-1.5	58.0	d	30.0	j	0	b
Osmopriming	7	15	–	-1.0	64.7	b–d	49.3	a–f	0.3	a
			–	-1.25	69.3	bc	44.0	d–i	0	b
			–	-1.5	70.7	b	51.0	a–e	0	b
		20	–	-1.0	80.0	a	54.7	a	0	b
			–	-1.25	80.0	a	53.0	ab	0	b
			–	-1.5	68.7	bc	52.7	a–c	0	b

For explanations see table 1

Osmopriming seeds in PEG solutions of -1.0 and -1.25 MPa osmotic potential at 20°C reduced significantly the percentage of ungerminated seeds compared with control at 20°C (tab. 4). At higher temperature all osmopriming treatments at 20°C and osmopriming in PEG solutions of -1.25 and -1.5 MPa osmotic potential at 15°C decreased significantly this parameter. Very high percentage of ungerminated seeds was found in control at 35°C (99.0%). Most of the hydropriming treatments reduced the number of these seeds significantly. After osmopriming seeds at 15°C and after osmopriming in PEG solution of -1.0 MPa osmotic potential at 20°C the percentage of ungerminated seeds was lower than in control. Halopriming seeds at 20°C also reduced the number of these seeds.

Table 3. The effect of priming on the percentage of abnormal seedlings at 20, 30, and 35°C

	Seed treatment				Temperature, °C					
	duration of priming, days	temperature of priming, °C	amount of water, µl $H_2O \cdot g\ seed^{-1}$	osmotic potential, MPa	20		30		35	
Untreated seeds					6.0	f–h	13.0	f–h	1.0	f
Hydropriming	2	15	600	–	5.7	gh	15.0	c–h	2.0	ef
			700	–	7.7	c–h	16.0	b–h	2.0	ef
		20	600	–	5.7	gh	19.3	a–c	3.0	c–f
			700	–	5.7	gh	12.0	gh	5.0	b–d
	3	15	600	–	11.3	ab	11.0	h	4.3	b–e
			700	–	12.3	a	15.0	c–h	4.0	b–e
		20	600	–	10.7	a–c	13.0	f–h	3.7	b–e
			700	–	9.3	a–f	14.0	d–h	4.3	b–e
	4	15	600	–	8.7	b–g	16.7	b–g	4.0	b–e
			700	–	9.7	a–e	16.7	b–g	3.7	b–e
		20	600	–	10.3	a–d	16.7	b–g	5.7	b
			700	–	8.0	b–h	18.3	b–e	3.3	b–f
Halopriming	5	15	–	-1.5	6.7	e–h	14.7	c–h	2.7	d–f
		20	–	-1.5	7.7	c–h	13.3	e–h	4.3	b–e
Osmopriming	7	15	–	-1.0	6.7	e–h	14.3	c–h	8.7	a
			–	-1.25	7.0	d–h	23.3	a	4.3	b–e
			–	-1.5	9.0	a–f	15.7	b–h	5.3	bc
		20	–	-1.0	7.0	d–h	18.7	b–d	4.0	b–e
			–	-1.25	5.0	h	17.7	b–f	2.0	ef
			–	-1.5	9.7	a–e	20.3	ab	2.7	d–f

For explanations see table 1

Seed vigour. Osmopriming followed by halopriming improved the speed of germination at 20°C to the largest extent (tabs 5 and 6). Hydropriming in restricted volumes of water gave diverse results. It accelerated seed germination generally at an initial stage, decreasing significantly T_{10} parameter (tab. 5). Only seeds hydroprimed in 600 µl $H_2O \cdot g\ seed^{-1}$ for 3 and 4 days at 20°C showed significantly lower T_{50} (tab. 6). On the other hand, hydropriming in 600 µl $H_2O \cdot g\ seed^{-1}$ for 2 days at 20°C negatively affected this parameter. Osmoprimed and haloprimed seeds germinated faster than untreated and hydroprimed seeds also at 30°C and 35°C.

Hydropriming negatively affected uniformity of germination at 20°C. This deleterious effect was also observed at 30°C if seeds were hydroprimed for 2 days at 15°C, regardless of the amount of water and if seeds were primed at 20°C in higher volume of water. Moreover, prolongation of time between 25 and 75% total germination was noted at 35°C, when seeds were hydroprimed for 2 and 3 days at 20°C, and after priming at 15°C in 600 µl $H_2O \cdot g\ seed^{-1}$ for 3 days and in 700 µl $H_2O \cdot g\ seed^{-1}$ for 4 days. Both halopriming at 20°C and osmopriming, regardless of temperature and osmotic potential of PEG, improved significantly the uniformity of germination at 30°C compared with untreated seeds. At the lower as well as at the higher temperature this effect was not observed (tab. 7).

Table 4. The effect of priming on the percentage of ungerminated seeds at 20, 30, and 35°C

	Seed treatment				Temperature, °C					
	duration of priming, days	temperature of priming, °C	amount of water, µl $H_2O \cdot g\ seed^{-1}$	osmotic potential, MPa	20		30		35	
Untreated seeds					27.7	a–d	44.3	b–d	99.0	a
Hydropriming	2	15	600	–	22.0	c–e	41.7	b–d	98.0	ab
			700	–	20.3	d–f	43.3	b–d	98.0	ab
		20	600	–	21.7	c–e	38.3	d–f	97.0	a–d
			700	–	24.7	b–d	43.0	b–d	95.0	c–e
	3	15	600	–	22.7	c–e	37.0	d–g	95.7	b–e
			700	–	21.7	c–e	47.7	bc	96.0	b–e
		20	600	–	22.0	c–e	41.0	b–f	96.3	b–e
			700	–	22.7	c–e	37.7	d–f	95.7	b–e
	4	15	600	–	27.7	a–d	37.0	d–g	96.0	b–e
			700	–	25.7	b–d	40.3	c–f	96.3	b–e
		20	600	–	28.3	a–d	33.7	d–f	94.3	e
			700	–	31.3	ab	40.3	c–f	96.7	a–e
Halopriming	5	15	–	-1.5	29.3	a–c	49.0	b	97.3	a–c
		20	–	-1.5	34.3	a	56.7	a	95.7	b–e
Osmopriming	7	15	–	-1.0	28.7	a–d	36.3	d–h	91.3	f
			–	-1.25	23.7	b–d	32.7	f–i	95.7	b–e
			–	-1.5	20.3	d–f	33.3	e–i	94.7	de
		20	–	-1.0	13.0	f	26.7	i	96.0	b–e
			–	-1.25	15.0	ef	29.0	g–i	98.0	ab
			–	-1.5	24.7	b–d	27.0	i	97.3	a–c

For explanations see table 1

Table 5. The effect of priming on the speed of germination expressed by T_{10} parameter (time to 10% of total seed germination), at 20, 30, and 35°C (days)

	Seed treatment				Temperature, °C					
	duration of priming, days	temperature of priming, °C	amount of water, µl $H_20 \cdot g\ seed^{-1}$	osmotic potential, MPa	20		30		35	
Untreated seeds					2.81	a	2.23	a	3.37	bc
Hydropriming	2	15	600	–	1.94	d–f	1.66	bc	2.38	cd
			700	–	1.81	ef	0.88	d	2.04	d
		20	600	–	2.37	bc	2.06	ab	2.66	b–d
			700	–	2.50	b	2.04	ab	2.83	b–d
	3	15	600	–	2.24	b–d	1.72	bc	2.31	cd
			700	–	2.33	bc	1.73	bc	2.92	b–d
		20	600	–	2.10	c–e	1.44	c	2.89	b–d
			700	–	2.17	cd	2.01	ab	2.83	b–d
	4	15	600	–	1.66	f	1.33	c	3.53	b
			700	–	1.95	d–f	1.53	c	4.77	a
		20	600	–	1.33	g	1.43	c	2.97	b–d
			700	–	1.68	f	1.66	bc	3.69	b
Halopriming	5	15	–	-1.5	0.31	ij	0.21	f	0.39	e
		20	–	-1.5	0.06	j	0.05	f	0.27	e
Osmopriming	7	15	–	-1.0	0.83	h	0.63	de	0.69	e
			–	-1.25	0.73	h	0.18	f	0.54	e
			–	-1.5	0.59	hi	0.42	ef	0.37	e
		20	–	-1.0	0.03	j	0.00	f	0.00	e
			–	-1.25	0.08	j	0.00	f	0.17	e
			–	-1.5	0.31	ij	0.05	f	0.22	e

For explanations see table 1

Moisture content. The moisture content of untreated seeds was 8.2%. After priming this parameter ranged from 42.3% to 45.9%. The highest moisture content was observed for haloprimed seeds. Drying seeds after priming resulted in a decrease in moisture content to 8.4%.

Table 6. The effect of priming on the speed of germination expressed by T_{50} parameter (time to 50% of total seed germination), at 20, 30, and 35°C (days)

	Seed treatment				Temperature, °C					
	Duration of priming, days	Temperature of priming, °C	Amount of water, µl $H_20 \cdot g\ seed^{-1}$	Osmotic potential, MPa	20		30		35	
Untreated seeds					3.88	b–d	3.49	cd	5.15	d
Hydropriming	2	15	600	–	3.65	c–e	4.23	a–c	3.63	e
			700	–	3.77	b–e	5.04	a	3.28	ef
		20	600	–	5.72	a	4.47	ab	7.74	ab
			700	–	3.61	c–e	5.02	a	7.04	ab
	3	15	600	–	4.05	bc	3.98	bc	6.27	b–d
			700	–	4.16	b	4.55	ab	6.48	b–d
		20	600	–	3.33	e	3.60	cd	6.09	cd
			700	–	3.39	de	4.46	ab	6.92	bc
	4	15	600	–	3.63	c–e	3.99	bc	6.96	bc
			700	–	3.67	b–e	4.26	a–c	8.98	a
		20	600	–	2.81	f	2.99	d	5.93	cd
			700	–	3.29	e	4.02	bc	6.83	bc
Halopriming	5	15	–	-1.5	1.56	g	1.15	ef	1.10	gh
		20	–	-1.5	0.85	hi	0.46	e–g	0.84	gh
Osmopriming	7	15	–	-1.0	1.23	gh	1.18	e	1.32	gh
			–	-1.25	1.39	g	10.8	ef	1.52	gh
			–	-1.5	1.38	g	1.17	e	1.98	fg
		20	–	-1.0	0.44	i	0.17	g	0.21	h
			–	-1.25	0.74	i	0.33	e–g	1.15	gh
			–	-1.5	1.23	gh	0.68	e–g	1.46	gh

For explanations see table 1

DISCUSSION

In the present study the effects of three priming techniques: hydro-, halo- and osmopriming on germination and vigour of pansy seeds were determined. The moisture content levels, which reached seeds after priming (42.3–45.9%), were sufficient to initiate germination processes [McDonald 2000].

Table 7. The effect of priming on the uniformity of germination expressed by U_{75-25} parameter (time between 25% and 75% of total seed germination), at 20, 30, and 35°C (days)

Seed treatment					Temperature, °C					
	duration of priming, days	tempera-ture of priming, °C	amount of water, µl $H_2O \cdot g\ seed^{-1}$	osmotic potential, MPa	20		30		35	
Untreated seeds					1.59	e–g	3.49	c	2.99	cd
Hydropriming	2	15	600	–	3.08	ab	5.37	b	1.68	d
			700	–	3.58	a	7.57	a	2.18	d
		20	600	–	2.95	a–c	4.56	bc	7.05	a
			700	–	2.39	b–d	5.33	b	7.28	a
	3	15	600	–	2.92	a–c	4.74	bc	5.86	ab
			700	–	2.78	a–c	4.79	bc	4.82	bc
		20	600	–	2.24	c–e	3.82	c	7.05	a
			700	–	2.61	b–d	4.28	bc	7.28	a
	4	15	600	–	3.12	ab	4.68	bc	4.66	bc
			700	–	3.00	a–c	4.45	bc	5.79	ab
		20	600	–	2.48	b–d	3.60	c	4.64	bc
			700	–	2.66	b–d	3.67	c	4.49	bc
Halopriming	5	15	–	-1.5	2.30	b–e	2.30	b–e	1.12	d
		20	–	-1.5	1.91	d–f	1.91	d–f	0.87	d
Osmopriming	7	15	–	-1.0	0.98	g	1.15	d	1.11	d
			–	-1.25	1.50	e–g	1.72	d	1.92	d
			–	-1.5	1.56	e–g	1.51	d	3.00	cd
		20	–	-1.0	1.17	fg	0.80	d	1.13	d
			–	-1.25	1.54	e–g	1.24	d	2.00	d
			–	-1.5	1.56	e–g	1.67	d	2.32	d

For explanations see table 1

Almost all of the hydropriming treatments, used in the experiment, had an adverse effect on the percentage of germinating seeds, germination capacity, as well as speed and uniformity of germination at 20°, 30° and 35°C. Some acceleration was only observed at an initial stage of germination. On the contrary, Matczyński [2008] found that in most cases hydropriming pansy seeds, at volumes of 400–700 µl $H_2O \cdot g\ seed^{-1}$ for 48–72 h, enhanced their germination rates at 20°C and 25°C, but not at 35°C. The author also observed an improvement of the percentage of germinating seeds after some hydropriming treatments. However, none of these treatments increased germination

capacity. Rajabalipour et al. [2013] hydroprimed pansy seeds in distilled water for 6, 12, 18 and 24 h. They did not observed any improvement in the percentage of germinating seeds at alternating temperatures 25/18°C, and 24 h treatment showed even an adverse effect on this parameter. According to Soon et al. [2000], the earlier and synchronized germination might be connected with increased metabolic activities in hydroprimed seeds. On the other hand, the rate of hydration in this method of priming is difficult to control, and some types of seeds may actually incur imbibitional damages if the rate of hydration is too rapid.

In the present study priming in KNO_3 solutions accelerated seed germination at 20°C, 30°C and 35°C, but did not improve the percentage of germinating seeds and germination capacity. Lara et al. [2014] found that potassium nitrate priming improved tomato seed germination time and germination rate to a larger extent than PEG priming. The authors suggested that the observed benefits were related to the activity of the enzyme nitrate reductase in the production of nitrite/nitric oxide, which promoted a faster germination. The nitrate (NO_3) could be absorbed and used in the metabolism of the embryo, through the enzyme nitrate reductase. Priming with KNO_3 increased also to the largest degree the activity of some antioxidant enzymes: superoxide dismutase and catalase in tomato seeds.

Yoon et al. [1997] tested various priming methods, including treating seeds in numerous salt solutions and polyethylene glycol (PEG 15000) at osmotic potential -1.0 and -2.0 MPa, for 3, 6 and 9 days at 23°C, on pansy seed germination at high temperature. The authors observed that seeds primed with $CaCl_2$ at -1.0 MPa for 3 days had significantly higher germination at 35°C than other examined treatments and effectively increased seedling emergence and reduced emergence time in summer greenhouse studies. Moreover, the authors found that salts solutions had been easier to handle and to remove from seeds than polyethylene glycol. Rajabalipour et al. [2013] found that priming pansy seeds in 0.3, 0.5 and 0.7% KNO_3 solutions for 6–24 h in most cases reduced their germination percentage, especially at the highest concentration. Cushman et al. [1990] tested germination of *Viola tricolor* seeds at 18 and 30°C after 2, 4, 6 and 8 days priming in aerated solutions of 300 and 400 mM NaCl. The authors found that priming increased germination at the higher temperature to the level similar to the optimum temperature. However, the cultivars differed in the response to priming solution. Bradford [1995] reported that in some cases, because of low molecular size of the salts, they may be absorbed by the seeds, resulting in toxic effects. Therefore, PEG is a preferred osmoticum by many in research and seed industry, because the large size of PEG molecules prevented them from entering the living cells of the seeds.

The present study showed that the best method of priming pansy seeds with respect to the speed of germination as well as the percentage of germinating seeds and germination capacity was osmopriming in PEG solution of osmotic potential -1.0 MPa at 20°C for 7 days. This treatment not only enhanced significantly the speed of germination at 20°C, 30°C and 35°C, but also increased to the largest extent the percentage of germinating seeds at 30°C and 35°C and seed germination capacity at 20°C and 30°C. Carpenter and Boucher [1991] also found that the optimum method for pansy seeds priming was soaking seeds in aerated osmotic solutions of PEG 8000 at -1.0 MPa for 7 days, however the authors primed seeds at lower temperature (15°C). After priming the seeds

had five times higher germination at 35°C of non-primed seeds, germinated faster and more uniformly. Moreover, the authors observed that prolongation of priming of more than 7 days resulted in an increase of the number of abnormal seedlings. Rajabalipour et al. [2013] primed pansy seeds in PEG solutions of the osmotic potential of -0.5, -1.0, -1.5 and -2.0 MPa for 6, 12, 18 and 24 h. The higest germination rate and percentage at alternating temperatures 25/18°C showed the seeds primed in -0.5 MPa solution for 12 h. The positive effects of priming on seed germination of many species are attributed to the induction of biochemical mechanisms of cell repair. The resumption of the metabolic activity can restore cellular integrity, through the synthesis of nucleic acids (DNA and RNA), proteins and the improvement of the antioxidant defence system [Di Girolamo and Barbanti 2012].

The results of present experiment showed that the improvement of the percentage of germinating seeds at the higher temperatures, especially 35°C, was not related with improvement of germination capacity or even an increase of the number of abnormal seedlings. The same phenomenon was also observed by Łabenska [2007], Matczyński [2008], and Song [2011]. The researchers observed significant differences between total percentage of geminating seeds, evaluated on the base of seed vigour test, and the percentage of germinating seeds, i.e. normal and abnormal seedlings, in standard germination test, at 35°C. We assumed that during standard germination test the slowly germinating seeds were very fast overgrown at the higher temperature by saprophytic fungi, which caused early death of the seedlings, and resulted in classification of these seedlings to the death seeds category. That problem did not appear during vigour test because the seedlings were removed from the plates directly after radicle appearance, before the fungi started to grow. This phenomenon may also partially explain results obtained by Carpenter and Boucher [1991]. The authors did not determine standard germination but made only daily germination counts, corresponding to our vigour test. Although they observed higher percentage of germination at constant 35°C after priming, radicle lengths were reduced and emergent seedlings lacked their vigour necessary for commercial production. Szopińska and Tylkowska [2004] also reported that after incubation at 35°C germinated seedlings of lettuce showed characteristic abnormalities, i.e. thickened and shortened radicle. Origins of those abnormalities have physiological and biochemical character and may be connected with high temperature [Bekendam and Grob 1979].

The obtained results allow to conclude, that regardless of priming method pansy seed germination at a temperature above 30°C may be unsatisfactory, and the study on thermodormancy-breaking treatment of pansy seeds should be continued.

CONCLUSIONS

1. Generally hydropriming did not influence significantly or adversary affected the percentage of germinating seeds, germination capacity and speed of germination.

2. Halopriming accelerated seed germination at 20, 30 and 35°C but did not affect total germination and germination capacity.

3. Osmopriming seeds in PEG solution of -1.0 MPa osmotic potential at 20°C for 7 days improved significantly germination rates at 20, 30 and 35°C, and increased the percentage of germinating seeds at 30 and 35°C and germination capacity at 20 and 30°C.

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WPŁYW KONDYCJONOWANIA NA KIEŁKOWANIE I WIGOR NASION BRATKA OGRODOWEGO (*Viola* × *wittrockiana* Gams.)

Streszczenie. Porównywano wpływ trzech metod kondycjonowania: hydrokondycjonowania, kondycjonowania w solach mineralnych oraz osmokondycjonowania na kiełkowanie i wigor nasion fiołka ogrodowego w temperaturze 20, 30 i 35°C. Nasiona hydrokondycjonowano w ograniczonej ilości wody (600 i 700 µl $H_2O \cdot g$ nasion^{-1}, 2, 3 lub 4 dni), kondycjonowano w roztworze KNO_3 (-1.5 MPa, 5 dni) oraz osmokondycjonowano w roztworach glikolu polietylenowego (-1,0, -1,25 lub -1,5 MPa PEG 8000, 7 dni) w temperaturze 15 lub 20°C. Oceniano kiełkowanie i wigor nasion niekondycjonowanych i kondycjonowanych. Na ogół hydrokondycjonowanie negatywnie wpływało na szybkość kiełkowania, procent kiełkujących nasion oraz zdolność kiełkowania. Kondycjonowanie w roztworze KNO_3 przyśpieszyło kiełkowanie nasion w temperaturze 20, 30 i 35°C, ale nie miało wpływu na procent nasion kiełkujących i zdolność kiełkowania. Osmokondycjonowanie nasion w roztworze PEG o potencjale osmotycznym -1.0 MPa w temperaturze 20°C nie tylko znacząco poprawiało wskaźniki wigoru w temperaturze 20, 30 i 35°C, ale również najkorzystniej z zastosowanych metod wpływało na procent nasion kiełkujących w temperaturze 30 i 35°C i istotnie zwiększyło zdolność kiełkowania nasion w temperaturze 20 i 30°C.

Słowa kluczowe: nasiona fiołka ogrodowego, metody kondycjonowania, zdolność kiełkowania, wigor

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