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IMPROVING ONION SEED GERMINATION USING PRIMING TREATMENTS

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Summary

The effects of osmopriming 'Wolska' onion seeds on the percentage of normal and abnormal seedlings as well as the mean germination time (MGT) were studied. The priming was carried out with polyethylene glycol (PEG 6000 and PEG 8000) at -1.0 MPa and -1.5 MPa solutions. The seeds were treated in a column bioreactor for 6, 24, 48, 72 and 96 hours at 15 and 20ºC. Compared to the control (untreated seeds), both PEG 6000 and PEG 8000 osmotics similarly increased the percentage of normal seedlings, reduced the percentage of abnormal seedlings and shortened MGT. Seed priming at -1.0 MPa promoted a higher percentage of normal seedlings and shorter MGT than treatment at -1.5 MPa, but both osmotic potentials enhanced those germination features relative to the untreated seeds. Compared to the control, both priming temperatures improved the tested germination traits, but treatment at 20ºC gave better results than at 15ºC. Seeds primed for 48 hours produced the highest percentage of normal seedlings and the lowest number of abnormal seedlings, but treatment for 72 hours reduced MGT the most. The best ratios of normal to abnormal seedlings were obtained after priming at 20ºC for 24 hours at -1.0 MPa and after treating for 48 hours at -1.5 MPa. The seeds primed at 20ºC for 72 hours at -1.0 MPa germinated two times faster than the control. The study showed that some of the applied priming treatments notably improved the germination characteristics of the tested 'Wolska' onion seed lot.

Key words: *Allium cepa* L., germination capacity, MGT, osmopriming, seedlings

INTRODUCTION

Poland, producing about 551,000 tons of onion (*Allium cepa* L.) bulbs a year on a cultivation area of 20,100 ha, is a European leader in terms of the growing area and the amount of production, as well the level of consumption of this vegetable (CSO 2014). Modern agriculture requires seeds of the highest quality that are needed in order to be able to perform precision sowing. Furthermore, sowing of high quality seeds is necessary to get rapid and uniform seedling emergence, which has a major impact on the final vegetable yield and its quality. Unfortunately, onion seeds usually have a low quality, resulting in slow and asynchronous germination as well as seeds producing a high number of abnormal seedlings, especially during field stress conditions after planting in the early spring (Borowski and Michałek 2006).

So far, several priming techniques have been found to be beneficial pre-sowing vegetable seed treatments that increase the speed of germination and seedling emergence, as well as improve the tolerance of seeds to field stress conditions such as lack of water or adverse temperatures. Proper standardization of the pre-sowing seed treatment method and methodology for individual crops and cultivars is the most important determinant of the success of seed priming. Thus, the priming conditions should be determined by trials and errors for each lot (Khan 1992). Osmopriming is one of the most popular pre-sowing seeds treatments. Because salt solutions can be toxic to seeds (Haigh and Barlow 1987), most researchers have suggested using a polyethylene glycol (PEG) solution for *Allium* seed osmopriming (Bujalski *et al*. 1989, Bujalski *et al*. 1991, Bujalski and Nienow 1991, Petch *et al*. 1991, Murray *et al*. 1992, Bujalski *et al*. 1993, Dabrowska and Kolasińska 1998, Dorna and Marcinek 2002, Ya-hong *et al*. 2003, Borowski and Michałek 2006, Ya-hong and Dorna 2006). The effect of osmopriming is usually dependent on the duration and temperature of treatment, the osmotic potential and the type of priming solution (Khan 1992, Bujalski *et al*. 1994). However, the most efficient method for onion seed priming treatment, which would not only improve the speed of germination but also increase germination capacity and reduced the amount of abnormal seedlings, has still yet to be determined (Caseiro *et al*. 2004, Tajbakhshet *et al*. 2004, Selvarani and Umarani 2011).

The aim of this experiment was to compare the effect of several osmopriming factors such as priming solutions, osmotic potential, priming temperature and priming duration on the percentage of normal and abnormal seedlings as well as the mean germination time using ‘Wolska’ onion seeds with low germination capacity.

MATERIAL AND METHODS

The commercial seed lot of the onion (*Allium cepa* L.) of the 'Wolska' cultivar was used. The germination capacity of seeds was very low (63%), so the seeds were not commercially acceptable. The initial moisture of the tested seeds had a content of 7%. The experiment was carried out in the Seed Science Laboratory of the Unit of Genetics, Plant Breeding and Seed Science at the University of Agriculture in Krakow (Poland).

Seeds were subjected to a pre-germination treatment using osmopriming. Seed samples (70 g) were primed in a column bioreactor with 700 ml solutions of two polyethylene glycols (PEG 6000 and PEG 8000) at two osmotic potentials: -1.0 MPa and -1.5 MPa (Michel and Kaufmann 1973, Michel 1983) for 6, 24, 48, 72 and 96 hours in the dark, in an incubator at 15 and 20ºC. In order to stop microorganism progress during treatment, 0.1% thiram was added into the priming solutions. Seeds treated in bioreactor columns with priming solutions were mixed and aerated using a pump. After conditioning, samples of 400 seeds were removed from the priming solutions and rinsed three times in demineralized distilled water. Next, the seed samples were dried for 24 hours at room temperature in thin layers using an air flow at about 40% RH. Untreated seeds were used as the control.

Following priming, germination tests were performed according to international standards for seed testing recommendations (ISTA 2012). A completely randomised design was used with four replications, each consisting of 100 clusters taken at random. The seeds were uniformly placed in 150 mm Petri dishes on three layers of wet filter paper (Filtrak, 3w, 65 g m^{-2}) moistened by demineralised distilled water. These Petri dishes were placed in the incubator with forced air at 15ºC circulation in the darkness. The measurements of the percentage of normal seedlings (germination capacity) and the percentage of abnormal seedlings were performed using the ISTA handbook for seedling evaluation guidelines (Don 2003) twelve days after planting. Seedlings were classified for normal and abnormal germinants. In order to evaluate the mean germination time (MGT), seedlings that had started germination (with a protruded radicle of 2 mm long) were counted daily, at the same time, from the moment of planting until the final count made 12 days after planting. MGT (days) was calculated according to the following equation: $MGT = \Sigma (D \times G) / \Sigma G$, where D is the number of days counted from the beginning of germination, and G is the number of seeds which were germinated on day D.

Statistical analysis was conducted using the STATISTICA software (ver. 9). The data from the experiment were subjected to a general analysis of variance (ANOVA). The comparison of means for the germination capacity, number of

abnormal seedlings and MGT were calculated using the Duncan least significant difference test at $P = 0.05$.

RESULTS AND DISCUSSION

The main objective of the experiment was to evaluate the influence of different osmopriming factors on the percentage of normal and abnormal seedlings as well as mean germination time (MGT) using 'Wolska' onion seeds with a low germination capacity (63%) and high percentage of abnormal seedlings (30%). The seeds were treated for 6, 24, 48, 72 and 96 hours at 15 and 20ºC in solutions of PEG 6000 and PEG 8000 used at two osmotic potentials: -1.0 MPa and -1.5 MPa.

In regard to the tested priming solutions, PEG 6000 and PEG 8000, both had a similar effect on the percentage of normal and abnormal seedlings as well as the MGT (Table 1). In comparison with the control (untreated seeds), both solutions increased the percentage of normal seedlings, up to 12% by using PEG 8000, reduced the number of abnormal seedlings by 7%, and shortened the MGT almost 1.5 days. The seeds of particular species differ in their responses to individual osmotic solutions (Haigh and Barlow 1987, Selvarani and Umarani 2011). Reports on the osmotica used for osmopriming onion seeds have been conflicting; most of them recommend the use of high-molecular weight polyethylene glycol such as PEG 6000 (Dearman *et al*. 1986, Bujalski and Nienow 1991, Dabrowska and Kolasinska 1998) or PEG 8000 (Hill *et al*. 1989, Murray *et al*. 1992, Dorna and Marcinek 2002, Ya-hong *et al*. 2003, Caseiro *et al*. 2004, Borowski and Michałek 2006, Ya-hong and Dorna 2006) as the most beneficial for germination. However, Furutani *et al*. (1986) reported that onion seed osmopriming using NaCl, $KNO_3 + K_3PO_4$ or mannitol significantly reduced the time of germination and resulted in a higher percentage of germination than priming in PEG 6000 solution. On the other hand, Haigh and Barlow (1987) found that onion seed priming using K_2HPO_4, $K_2HPO_4 + KNO_3$, KNO_3, K_3PO_4 or $K_3PO_4 + KNO_3$ was less beneficial than PEG 6000.

In the present research, seed priming at -1.0 MPa solution promoted higher germination capacity and shorter MGT than treatment at -1.5 MPa (Table 1). However, both used osmotic potentials have enhanced those germination traits compared to untreated seeds. Haigh and Barlow (1987) suggest that the final results of priming depend more on the type of solution used than its osmotic potential, nevertheless they recommended -1.5 or -1.7 MPa for onion seeds. Thus, osmotic potential at -1.5 MPa is generally used during onion seed priming (Hill *et al*. 1989, Bujalski and Nienow 1991, Murray *et al*. 1992, Ya-hong *et al*. 2003, Ya-hong and Dorna 2006) or at around -1.0 MPa solutions (Furutani *et al*.

1986, Dabrowska and Kolasinska 1998, Dorna and Marcinek 2002, Caseiro *et al*. 2004, Borowski and Michałek 2006, Selvarani and Umarani 2011).

Table 1. Effect of 'Wolska' onion seed osmopriming factors on the percentage of normal and abnormal seedlings as well as mean germination time (MGT)

Treatment		Normal seedlings (%)	Abnormal seedlings (%)	MGT (days)
Priming solution	PEG 6000	74 a*	23 a	5.11 a
	PEG 8000	75 a	23 a	5.05 a
	control	63 b	30 b	6.50 b
Osmotic potential	-1.0 MPa	76 a	22 a	4.80 a
	-1.5 MPa	73 b	24 ab	5.35 b
	control	63 c	30 b	6.50 c
Priming temperature	15ºC	72 b	25 b	5.26 b
	20ºC	77 a	21 a	4.90 a
	control	63 c	30 c	6.50 c
Priming duration	6 h	67 d	31 c	6.40 d
	24 h	82 b	16 a	5.65 c
	48 h	85 a	13 a	4.72 b
	72 h	76 c	22 b	3.84 a
	96 h	64 e	34 d	4.78 b
	control	63 e	30 c	6.50 d

*Means followed by the same letter are not significantly different at $P < 0.05$

Regardless of the priming temperature used in the current study, all germination traits were improved compared to the control. However, the pre-sowing conditioning performed at 20ºC was more advantageous than at 15ºC (Table 1). On the contrary, Bujalski and Nienow (1991), when testing temperature effects on the osmotic priming of leek seeds, noted the best results in terms of reducing germination time when seeds were treated at temperatures lower than 20ºC. Consequently, most scientists perform onion seed priming at 10ºC (Murray *et al*. 1992) or 15ºC (Dearman *et al*. 1986, Haigh and Barlow 1987, Hill *et al*. 1989, Bujalski and Nienow 1991, Dabrowska and Kolasinska 1998, Dorna and Marcinek 2002, Ya-hong *et al*. 2003, Caseiro *et al*. 2004, Borowski and Michałek 2006, Ya-hong and Dorna 2006), although some of them have treated onion seeds at room temperature (Selvarani and Umarani 2011). Furutani *et al*. (1986), when comparing onion seed priming at 10 and 24ºC, noted significantly slower germination in the case of the higher temperature.

Table 2. Effect of the interaction of select priming treatments of 'Wolska' onion seed on the percentage of normal and abnormal seedlings as well as mean germination time (MGT)

Treatment		Normal seedlings (%)	Abnormal seedlings (%)	MGT (days)
Osmotic potential	Priming duration			
-1.0 MPa	6 h	70 d*	28 d	NS**
	24 h	89 a	9 a	NS
	48 h	82 b	16 b	NS
	72 h	76 c	22 c	NS
	96 h	65 e	32 e	NS
-1.5 MPa	6 h	64 ef	33 e	NS
	24 h	75 c	22 c	NS
	48 h	88 a	10 a	NS
	72 h	75 c	22 c	NS
	96 h	62 g	35 f	NS
Control		63 fg	30 d	NS
Osmotic potential	Priming temperature			
-1.0 MPa	15ºC	NS	NS	5.16 b
	20ºC	NS	NS	4.46 a
-1.5 MPa	15ºC	NS	NS	5.37 b
	20ºC	NS	NS	5.34 b
Control		NS	NS	6.50 c

*Means followed by the same letter are not significantly different at $P < 0.05$
**Not significant at $P < 0.05$

Seed priming continued in the present experiment for 48 hours produced the highest percentage of normal seedlings and the lowest number of abnormal seedlings (Table 1). In turn, the seed treatment for 72 hours reduced the MGT the most. Compared to the control, a priming period of 96 hours did not increase the percentage of normal seedlings and did not reduce the percentage of abnormal seedlings, while priming for 6 hours did not change the percentage of abnormal seedlings as well as the MGT. It can be seen that irrespective of the temperature and osmotic potential of the priming solution, continued treatment for 24-72 hours resulted in an improvement of all of the tested germination features as compared to the control. Thus it was found that the priming carried out for 6 and 96 hours was the least efficient from the point of view of improving the observed germination characteristics. Furutani *et al*. (1986), Bujalski and Nienow (1991)

and Selvarani and Umarani (2011) reported that the delayed conditioning period of onion seeds resulted in an acceleration of their germination. Onion seed priming at 15ºC using PEG 6000 or PEG 8000 solutions at a range from -1.0 to -1.5 MPa was usually continued for 6-7 days (Dorna and Marcinek 2002, Ya-hong *et al*. 2003, Borowski and Michałek 2006, Ya-hong and Dorna 2006) or 14 days (Dearman *et al*. 1986, Bujalski and Nienow 1991, Dabrowska and Kolasinska 1998), and sometimes 2 days (Caseiro *et al*. 2004).

Significant interactions of the priming factors were found only between osmotic potential and priming duration for the percentage of normal seedlings, which was the highest, and in the case of the percentage of abnormal seedlings, which was the lowest when the seeds were primed for 24 hours at -1.0 MPa and for 48 hours at -1.5 MPa, as compared to the other treatments (Table 2). In addition, a clear interaction was noted between osmotic potential and priming temperature for MGT that was greatly reduced when the seeds were treated at 20ºC at -1.0 MPa, as compared to the other treatments.

It was noted that some of the applied combinations of priming factors notably improved the MGT as well as the ratio of normal and abnormal seedlings (Table 3). The best results in terms of the percentage of normal and abnormal seedlings were obtained after priming at 20ºC for 24 hours at -1.0 MPa solution (91% and 7%, respectively) and after treating for 48 hours at -1.5 MPa solution (90% and 8%, respectively). Furthermore, the MGT of these seeds was significantly better, by about 1.5 days, compared to the control. The shortest MGT was found in the case of seeds primed at 20ºC for 72 hours at -1.0 MPa solution. Additionally, these seeds germinated two times faster than the control, but the other tested germination traits were average. The speedy germination of primed onion seeds is well known and often reported by researchers. Against, research has rarely noted an improvement of the germination capacity of primed onion seeds, nevertheless it has been reported by Bujalski *et al*. (1989), Bujalski and Nienow (1991), Dabrowska and Kolasinska (1998), Dorna and Marcinek (2002), Ya-hong *et al*. (2003), Tajbakhsh *et al*. (2004) and Selvarani and Umarani (2011). Most of the authors have noted a lack of the influence of pre-sowing *Allium* seed treatment on the percentage of abnormal seedlings (Bujalski *et al*. 1989, Bujalski and Nienow 1991, Bujalski *et al.* 1991, Petch *et al*. 1991, Bujalski *et al*. 1993). Tajbakhsh *et al*. (2004) was the first to document the reduction of abnormal onion germinants using a priming treatment. In contrast, the others have obtained a significant development of abnormal leek seedlings (Nienow *et al*. 1991, Bujalski *et al*. 1994, Maude *et al*. 1994).

Table 3. Effect of 'Wolska' onion seed osmopriming in polyethylene glycol on the percentage of normal and abnormal seedlings as well as mean germination time (MGT)

Treatment			Normal seedlings (%)	Abnormal seedlings (%)	MGT (days)
Priming temperature	Osmotic potential	Priming duration			
15ºC	-1.0 MPa	6 hours	67 e*	31 f	6.48 f
		24 hours	87 b	11 b	5.71 e
		48 hours	80 c	18 d	4.80 c
		72 hours	74 d	24 e	3.93 b
		96 hours	63 fg	35 g	4.88 cd
	-1.5 MPa	6 hours	62 fg	36 g	6.70 f
		24 hours	73 d	25 e	5.93 e
		48 hours	86 b	12 bc	5.00 cd
		72 hours	73 d	24 e	4.13 b
		96 hours	60 g	36 g	5.08 d
20ºC	-1.0 MPa	6 hours	72 d	26 e	5.78 e
		24 hours	91 a	7 a	5.03 cd
		48 hours	84 b	14 c	4.10 b
		72 hours	78 c	20 d	3.23 a
		96 hours	67 e	30 f	4.15 b
	-1.5 MPa	6 hours	67 e	30 f	6.66 f
		24 hours	78 c	20 d	5.93 e
		48 hours	90 a	8 a	4.98 cd
		72 hours	77 c	20 d	4.09 b
		96 hours	64 f	34 g	5.05 cd
Control			63 fg	30 f	6.50 f

*Means in columns followed by the same letter are not significantly different at $P < 0.05$

The obtained results of the experiment present the possibility to develop an advantageous priming method that significantly improves onion seed germination parameters such as the shortening of germination time, increase of germination capacity and reduction of the percentage of abnormal seedlings. However, it should be noted that the details of this pre-sowing treatment must be selected on the basis of trial and error to a specific seed lot, especially when the germination capacity of the seeds is low.

CONCLUSION

1. The two osmotic solutions, PEG 6000 and PEG 8000, promoted a similar, as well comparable to the control, effect on the percentage of normal and abnormal seedlings as well as the MGT of 'Wolska' onion seeds.
2. Seed priming at -1.0 MPa promoted a higher germination capacity and shorter MGT than treatment at -1.5 MPa. Compared to the control, both of the osmotic potentials used improved those germination traits.
3. Compared to the control, both of the priming temperatures used improved the tested germination traits, but seed treatment at 20ºC gave better results than at 15ºC.
4. Priming for 48 hours produced the highest percentage of normal seedlings and the lowest number of abnormal seedlings, but treatment for 72 hours reduced the MGT the most. Compared to the control, priming for 96 hours did not increase the percentage of normal seedlings and did not reduce the percentage of abnormal seedlings and priming for 6 hours did not change the percentage of abnormal seedlings as well as the MGT.
5. The most beneficial ratio of normal and abnormal seedlings was obtained after priming at 20ºC for 24 hours at -1.0 MPa and for 48 hours at -1.5 MPa. The seeds treated at 20ºC for 72 hours at -1.0 MPa germinated two times faster than the control.

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