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IMPROVEMENT OF RED BEET MONOGERM CLUSTERS GERMINATION USING RUBBING AND WATER TREATMENTS

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Abstract

The aim of the research was to improve the germination parameters of red beet monogerm clusters using pre-sowing treatments. Red beet (*Beta vulgaris* L.) clusters of two monogerm breeding lines, AR79A and W411A, differed in the initial germination capacity, were studied. Five seed treatments including rubbing, leaching for 4, 8 and 24 hours, and soaking for 24 hours, were performed. Based on the germination test carried out at 15 and 20ºC, the germination traits, such as the mean germination time (MGT), coefficient of uniformity of germination (CUG), coefficient of velocity (COV), germination capacity (GC) and germination rate (GR), were measured. Compared to the control, treatments used in the study improved the most of measured germination features for both tested lines. The leaching of clusters for 24 hours yielded the best enhancement in most of the germination traits. The rubbing was the least effective technique in terms of improvement in the germination parameters. The germination of treated clusters at 15 and 20°C presented comparable, and better than the control, values of germination features. The interaction between treatment method and germination temperature showed that leaching of clusters for 24 hours and germination at 20°C was the technique that successfully influenced the majority of the germination characteristics. Finally, it was noted that each pre-sowing technique significantly improve germination parameters, however the line

AR79A with lower initial GC (65%) presented better improvement of MGT, CUG and GC than the line W411A with higher initial GC (84%).

Keywords: *Beta vulgaris* L., breeding line, leaching, rubbing, soaking

INTRODUCTION

Red beet (*Beta vulgaris* L.) is a member of Amaranthaceae family grown in Europe, North America, Middle East and Asia. The red beet is cultivated mainly as the root vegetable, but also leaves are consumed. The nutritional value of the red beet is highly beneficial to the human diet. Moreover, the betalains obtained from the roots is used as the natural dye for food products (Goldman and Navazio 2008, Neelwarne and Halagur 2013). The harvest of red beet swollen storage roots in Poland in the year 2015 was 297,000 tons. The cultivated area of red beet in our country is over 6.2% of the total cultivation area of vegetables (CSO 2016).

The modern agriculture requires new red beet cultivars valuable in terms of production and quality. For the producers of this vegetable is also important high quality and monogermity of clusters. Unluckily, most of red beet cultivars listed in the *Polish National List of Vegetable Plant Varieties* (PNLVPV 2016) are either traditional – multigerm or open-pollinated. Among the 27 red beet cultivars only one – 'Patryk' – is monogerm, and just 5 cultivars are hybrid cultivars. Hence, today the breeding programme of red beet strongly focuses on the creation of hybrid cultivars that produce high and good quality yield. Monogermity, a significant red beet breeding strategy enables precision seed sowing and allows avoiding the thinning of young plants (Goldman and Navazio 2008).

The multigerm clusters of red beet are botanically a fructification that can have several embryos. Monogermity of clusters, conditioned by the recessive alleles *mm*, is the effect of mutation. Usually, the monogerm clusters are the single fruits – seed boll. However, the pericarp of monogerm seed bolls is very thick and rich in germination inhibitors that causes slow and asynchronous seed germination (Taylor *et al.* 2003, Rochalska and Orzeszko-Rywka 2008). Therefore, the seed industry is still developing new technologies such as rubbing (decoating), washing, leaching, soaking or priming and others that improve the clusters quality.

The rubbing removes the surface layer of clusters pericarp that facilitates the penetration of water into the seed and eliminates some of the germination inhibiting substances. The rubbing of sugar beet monogerm clusters increased the vigour of seeds (Orzeszko-Rywka and Podlaski 2003), improved the mean time of germination (Rochalska and Orzeszko-Rywka 2008) and influenced faster and more regular seedling emergence (Orzeszko-Rywka and Podlaski 2010).

In the case of multigerm clusters of red beet, the rubbing significantly improved the germination energy (GE) and GC (Domoradzki *et al.* 2007 a, b). Katzman *et al.* (2001) observed also high uniformity of germination for decoated seeds of spinach, which is derived from the same family as beets.

The water treatments of beet clusters, such as leaching, soaking, washing or priming, remove the majority of germination inhibitors from the pericarp. The leaching of red beet multigerm clusters caused the increase in GE and GC (Domoradzki *et al.* 2003, Domoradzki *et al.* 2007 b). The washing of sugar beet clusters increased the GR (Khazaei 2001) and the vigour (Orzeszko-Rywka and Podlaski 2003) as well as the field emergence (Orzeszko-Rywka and Podlaski 2010). The study published by Katzman *et al.* (2001) presented the rise of GR of leached spinach seeds. The soaking of clusters improved the field emergence both in the red beet (Taylor *et al.* 1985) and sugar beet (Murray *et al.* 1993). Habib (2010) reported positive influence of soaking on GC, MGT, GR and time necessary to reach 10% of final germination percentage of the sugar beet clusters. Also hydropriming of the beet clusters is recommended by other researches as the beneficial pre-sowing water treatment. Costa and Villela (2006), and Nirmala and Umarani (2008) reported significant improvement of germination parameters of red beet clusters that were primed with the water. The advancement of germination characteristics in the case of hydroprimed sugar beet clusters was observed by Capron *et al.* (2000), Mukasa *et al.* (2003), Rochalska and Orzeszko-Rywka (2008), Dias *et al.* (2009). Sacała *et al.* (2016) noted also the positive effect of sugar beet clusters hydropriming during the entire growing season.

The purpose of the research was to improve the quality of red beet monogerm clusters using five pre-sowing seed treatments including rubbing and water treatments, such as leaching at three different durations of 4, 8 and 24 hours, and soaking for 24 hours. The germination traits, such as the MGT, CUG, COV, GC, GR of two breeding lines (AR79A and W411A) that differed in terms of the initial germination capacity, were measured.

MATERIAL AND METHODS

The experiment was conducted in the Institute of Plant Biology and Biotechnology (IPBB) at the University of Agriculture in Kraków (Poland). Red beet (*Beta vulgaris* L.) clusters of two monogerm cytoplasmatic male sterile breeding lines, AR79A and W411A, were subjected to five pre-sowing treatments including rubbing and water treatments, such as leaching at three different duration of 4, 8 and 24 hours, and soaking for 24 hours. As the control untreated raw clusters were used. AR79A is a new breeding line selected in IPBB and the line W411A was bred in the University of Wisconsin – Madison (USA). The clusters of both studied lines were collected in September 2013 at the experimental field of IPBB. The experiment was carried out in the Seed Science Laboratory of IPBB in May 2014. The seeds of tested lines differed in the initial GC that was 65% for AR79A and 84% for W411A.

Each of the pre-sowing treatments: rubbing, leaching for 4, 8 and 24 hours and soaking for 24 hours, were performed using 50 g clusters samples of each of the breeding line. The rubbing of clusters was provided by hand using emery paper. The aim of that treatment was to remove the external layer of clusters pericarp. The leaching of clusters was carried out for 4, 8 and 24 hours, at 25°C, in demineralized slowly flowing distilled water that washed the clusters. The soaking of clusters was performed for 24 hours in 1.5 litres of demineralized distilled water at 25°C in plastic columns located in the incubator. Water and seeds were mixed and aerated using a pump. After the leaching and soaking treatments, the clusters were dried for a week in thin layer using an air stream at 25°C.

After the rubbing, leaching and soaking, germination test of seeds was performed according to *International Rules for Seed Testing* recommendations (ISTA 2012) at two different temperatures, 15°C and 20°C. Based on the germination test the mean germination time (MGT), coefficient of uniformity of germination (CUG), coefficient of velocity (COV), germination capacity (GC) and germination rate (GR), were assessed. The completely randomized design with four replications, each consisting of 100 seeds taken at random, was used. The clusters were uniformly placed in plastic boxes (120 × 210 mm) on wet pleated filter paper (MUNKTELL) with 50 bellows (110 × 20 mm, 120 g m^{-2}) moistened by demineralized distilled water up to 55% of the total water capacity. The germination was performed in the incubator with forced air at 15°C and 20ºC circulations, in darkness. For the evaluation of the MGT, CUG, COV and GR the seedlings that initiated 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 14 days after planting. The MGT, CUG, COV and GR were calculated using the following formulas: $MGT = \Sigma (D \times N) / \Sigma N$; $CUG = \Sigma N / \Sigma (MGT - D)^2 \times N$; $COV = \Sigma N / \Sigma (D / N) \times 100$, and $GR = \Sigma N / \Sigma (D \times N)$, where N is the number of clusters which germinated on day D, and D is the number of days counted from the beginning of germination. The germination capacity was measured 14 days after planting. The assessment of seedlings was performed on the basis of recommendations of *ISTA Handbook for Seedling Evaluation Guidelines* (Don 2009).

The data obtained in the experiment were statistically analysed using the package STATISTICA (version 12). The clusters of tested breeding lines differed in the initial germination capacity, therefore the results were subjected to general analysis of variance (ANOVA, $P < 0.01$) separately for each of the genotype. The study was carried out as two-factorial experiment. As the first factor of the research – five pre-sowing techniques were considered and as the second factor of the study – two temperatures of seed germination were applied. To estimate the significant differences between the means for the MGT, CUG, COV, GC and GR, Duncans test at $P = 0.05$ was adopted.

RESULTS AND DISSCUSION

The germination traits observed in the experiment, such as the MGT, CUG, COV, GC and GR, were significantly ($P < 0.01$) affected by all tested factors, including pre-sowing treatments and temperatures of germination. The interaction of pre-sowing treatments × temperatures of germination also significantly ($P < 0.01$) influenced studied germination characteristics.

The effect of the pre-sowing methods on the germination features, presented as the mean for two applied temperatures of germination, is presented in Table 1. The techniques used in the study, including rubbing and water treatments, improved the majority of the germination parameters in both tested monogerm red beet breeding lines compared to the untreated control clusters. Among the water methods used in the research, the leaching of clusters for 24 hours was the most profitable technique. The leaching of clusters for 24 hours yielded the best values of MGT, COV and GR in the case of the line AR79A and showed the highest improvement of MGT, CUG, COV and GR in the line W411A. Significantly high increase in the value of CUG of the line AR79A was noted after the leaching of clusters for 8 and 24 hours as well as after the soaking. Each of the leaching treatments raised GC in both tested lines to the same high level. As the leaching duration increased, the values of MGT, COV and GR of the line AR79A, and MGT, CUG, COV and GR of the line W411A, were improved. Also Khazaei (2001) and Habib (2010) noted the improvement of germination parameters along with the increase in the duration of the sugar beet clusters water treatments. The soaking of clusters for 24 hours was recommended by Murray *et al.* (1993) as the beneficial treatment for the sugar beet clusters germination. In the present study, the soaking of clusters better influenced MGT and COV of the line AR79A as well as MGT, COV and GR of the line W411A, than leaching for 4 hours. The soaking and the leaching of clusters for 4 and 8 hours resulted comparable level of GR of the line AR79A and similar values of CUG and GC of the line W411A. In the case of the line AR79A, the soaking and the leaching treatments yielded comparable results of CUG. In contrast, GC of the line AR79A was much higher after the leaching treatments than after the soaking of clusters. The rubbing was the least effective pre-sowing technique applied in the present experiment. Although, in comparison with the control, the rubbing positively influenced all studied germination characteristics of the line W411A and improved most of the germination traits of the line AR79A, except CUG that for the control and rubbed clusters was comparable. The minor improvement in the germination of rubbed sugar beet clusters, in comparison with water treatment, was reported also by Orzeszko-Rywka and Podlaski (2003) as well by Rochalska and Orzeszko-Rywka (2008).

Table 1. Effect of the pre-sowing treatments of clusters (P < 0.01) on the germination traits of monogerm red beet breeding lines presented as the mean for two applied temperatures of germination

Pre-sowing treatments	MGT* (days)		CUG		COV		GC (%)		GR	
	AR79A	W411A	AR79A	W411A	AR79A	W411A	AR79A	W411A	AR79A	W411A
Rubbing	3.88 d**	3.52 e	0.39 c	0.61 c	25.8 d	28.4 e	75.0 c	91.5 c	19.3 d	25.9 e
Leaching for 4 h	2.58 c	3.18 d	0.69 b	0.71 bc	38.9 c	31.5 d	85.0 a	93.5 ab	33.1 c	29.4 d
Leaching for 8 h	2.38 b	2.23 b	0.87 a	0.76 b	42.0 b	44.9 b	85.5 a	93.5 ab	35.8 b	41.9 b
Leaching for 24 h	2.14 a	1.96 a	0.82 ab	1.09 a	46.9 a	51.2 a	85.5 a	94.5 a	40.1 a	48.4 a
Soaking for 24 h	2.36 b	2.51 c	0.80 ab	0.82 b	42.6 b	39.9 c	80.0 b	92.5 bc	34.1 bc	36.8 c
Control	4.60 e	3.91 f	0.42 c	0.38 d	21.8 e	25.6 f	65.0 d	84.0 d	14.1 e	21.5 f
Improvement***	2.46	1.95	-0.45	-0.71	-25.1	-25.6	-20.5	-10.5	-26.0	-26.9

*MGT – mean germination time, CUG – coefficient of uniformity of germination, COV – coefficient of velocity, GC – germination capacity, GR – germination rate
**Means in columns followed by the same letter are not significantly different at $P < 0.05$
***The difference between value of the control and the most beneficial value of the treatment in the column

Table 2. Effect of the germination temperature of treated clusters (P < 0.01) on the germination traits of monogerm red beet breeding lines, presented as the mean for studied pre-sowing techniques

Temperatures of germination	MGT* (days)		CUG		COV		GC (%)		GR	
	AR79A	W411A	AR79A	W411A	AR79A	W411A	AR79A	W411A	AR79A	W411A
15ºC	2.76 a**	2.74 a	0.65 ab	0.86 a	37.8 a	38.3 a	81.5 a	92.5 a	31.1 a	35.6 a
20ºC	2.58 a	2.62 a	0.78 a	0.74 a	40.7 a	40.1 a	82.5 a	93.0 a	33.8 a	37.4 a
Control at 15ºC	4.76 b	4.02 b	0.36 c	0.33 b	21.0 b	24.9 b	64.0 b	83.5 b	13.5 b	20.8 b
Control at 20ºC	4.44 b	3.79 b	0.47 bc	0.43 b	22.5 b	26.4 b	65.5 b	84.5 b	14.7 b	22.3 b

*MGT – mean germination time, CUG – coefficient of uniformity of germination, COV – coefficient of velocity, GC – germination capacity, GR – germination rate
**Means in columns followed by the same letter are not significantly different at P < 0.05

The clusters of studied genotypes differed in term of the initial GC that was 65% for the line AR79A and 84% for the line W411A (Table 1). The means of treatments, presented as the average for two applied temperatures of germination, showed that the line AR79A presented better improvement of MGT, CUG and GC than the line W411A, compared to the control. In the case of COV and GR the improvement was similar for both tested genotypes of red beet.

After pre-sowing treatments the clusters were germinated at two different temperatures, 15°C and 20°C that similarly influenced the values of all evaluated germination traits in both examined lines (Table 2). Also for both studied genotypes, the treated clusters germinated at 15°C or at 20°C showed significantly better results of the germination, than the control germinated at the same temperatures. On the other hand, Katzman *et al.* (2001) testing the rubbing and the water treatments of spinach seeds, reported that different temperatures of germination can influence the final effect of germination.

Table 3. Effect of interaction of pre-sowing treatments × temperatures of germination of the clusters ($P < 0.01$) on the germination traits of monogerm red beet breeding line AR79A

Pre-sowing treatments	Temperatures of germination	MGT* (days)	CUG	COV	GC (%)	GR
Rubbing	15ºC	3.96 h**	0.46 ef	25.3 g	74.0 c	18.7 f
	20ºC	3.81 g	0.31 f	26.3 g	75.5 c	19.8 f
Leaching for 4 h	15ºC	2.67 f	0.58 de	37.5 f	84.0 a	31.5 e
	20ºC	2.48 de	0.79 bc	40.3 de	86.0 a	34.7 d
Leaching for 8 h	15ºC	2.39 de	0.86 ab	41.9 cd	85.0 a	35.6 cd
	20ºC	2.37 cd	0.89 ab	42.2 c	85.5 a	36.0 cd
Leaching for 24 h	15ºC	2.25 bc	0.63 cde	44.4 b	85.5 a	37.9 b
	20ºC	2.03 a	1.01 a	49.3 a	85.5 a	42.2 a
Soaking for 24 h	15ºC	2.51 e	0.72 bcd	39.9 e	79.5 b	31.7 e
	20ºC	2.21 b	0.88 ab	45.3 b	80.5 b	36.4 c
Control	15ºC	4.76 j	0.36 f	21.0 h	64.0 d	13.5 g
	20ºC	4.44 i	0.47 ef	22.5 h	65.5 d	14.7 g

*MGT – mean germination time, CUG – coefficient of uniformity of germination, COV – coefficient of velocity, GC – germination capacity, GR – germination rate
**Means in columns followed by the same letter are not significantly different at $P < 0.05$

The interaction of pre-sowing treatments × temperatures of germination of clusters showed that in the case of both tested genotypes the germination performed at 20°C positively influenced almost all studied germination traits of the clusters that were leached for 24 hours (Tables 3 and 4). However, the exceptions was GC that values were similar in both studied temperatures and, additionally, CUG measured for the clusters of the line W411A that germinated better at 15°C than at 20°C. The clusters of both genotypes that were leached for 4 hours, next germinated at 20°C, revealed better results of MGT, COV and GR, as well as in the case of the line AR79A also CUG, than clusters that after leaching for 4 hours were germinated at 15°C. The clusters of the line W411A after leaching for 4 and 8 hours presented higher CUG when the germination test was performed at 15°C, than at 20°C. The soaking for 24 hours and then the germination of treated clusters performed at 20°C, yielded favourable effects of MGT, COV and GR, but only in the line AR79A. Furthermore, the clusters of the line AR79A that after rubbing were germinated at 20°C presented better value of MGT, comparing with the clusters that after rubbing were germinated at 15°C.

Table 4. Effect of interaction of pre-sowing treatments × temperatures of germination of the clusters (P < 0.01) on the germination traits of monogerm red beet breeding line W411A

Pre-sowing treatments	Temperatures of germination	MGT* (days)	CUG	COV	GC (%)	GR
Rubbing	15ºC	3.58 g**	0.58 e	27.9 fg	91.0 c	25.4 g
	20ºC	3.45 fg	0.64 e	28.9 f	91.5 bc	26.5 fg
Leaching for 4 h	15ºC	3.31 f	0.78 d	30.2 f	93.0 abc	28.1 f
	20ºC	3.05 e	0.65 e	32.8 e	93.5 abc	30.7 e
Leaching for 8 h	15ºC	2.21 bc	0.94 bc	45.2 c	93.5 abc	42.2 c
	20ºC	2.25 c	0.58 e	44.6 c	93.5 abc	41.6 c
Leaching for 24 h	15ºC	2.04 ab	1.15 a	49.1 b	94.0 ab	46.2 b
	20ºC	1.88 a	1.01 b	53.2 a	95.0 a	50.6 a
Soaking for 24 h	15ºC	2.57 d	0.83 cd	38.9 d	92.0 bc	35.9 d
	20ºC	2.45 d	0.81 d	40.8 d	92.5 abc	37.7 d
Control	15ºC	4.02 i	0.33 f	24.9 h	83.5 d	20.8 h
	20ºC	3.79 h	0.43 f	26.4 gh	84.5 d	22.3 h

*MGT – mean germination time, CUG – coefficient of uniformity of germination, COV – coefficient of velocity, GC – germination capacity, GR – germination rate
**Means in columns followed by the same letter are not significantly different at *P* < 0.05

The current research presenting some of the pre-sowing techniques, such as rubbing and water treatments that may be useful in the germination improvement of monogerm clusters of two red beet breeding lines. The rubbing and the leaching of red beet multigerm clusters already were tested by Taylor *et al.* (1985), Domoradzki *et al.* (2003) and Domoradzki and Korpal (2007 a, b), which reported the usefulness of these methods in improving of the germination parameters. In the present experiment each of the used treatments successfully influenced the germination characteristics for both studied monogerm breeding lines. However, the most effective technique was leaching of clusters for 24 hours and the least efficient method was rubbing. It was noted, that in the case of clusters with lower initial GC, equal 65%, the improvement of germination traits was much higher than in terms of the clusters with better initial GC, equal 84%. It was also found that both applied germination temperatures similarly influenced the values of measured features. Although, the germination performed at 20°C, in the case of clusters leached for 24 hours, was more profitable than the germination at 15°C. Finally, the results of the study showed the possibility of selecting the most effective and suitable pre-sowing treatment that improve the quality of clusters of monogerm red beet breeding lines.

CONCLUSIONS

1. The pre-sowing treatments applied in the study improved the most of the germination characteristics of the monogerm clusters of both tested red beet breeding lines.
2. The leaching for 24 hours of clusters of both genotypes was the most profitable technique and the rubbing was the least effective method in terms of enhancement germination parameters, across two germination temperatures.
3. The clusters of both lines germinated at 15°C and at 20°C presented similar values of germination traits that were calculated as the mean for all applied methods.
4. The interaction of pre-sowing treatments × temperatures of germination showed that leaching of clusters for 24 hours and then germination at 20°C, was the most promising way to improve the majority of the germination features in the case of both tested genotypes.
5. The line AR79A with the initial GC 64% presented better improvement of the MGT, CUG and GR than the line W411A with the initial GC 84%.

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