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Differences in Traits of Seeds and Seedlings of Perennial Ryegrass Cultivars after Nine Months Storage at Different Temperatures

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Abstract

This study shows results of laboratory analyses of the traits of seeds (germination and germination energy) and seedlings (root and hypocotyl length, total seedling length) of the two perennial ryegrass cultivars: diploid 'Bartwingo'and tetraploid 'Calibra' before and after 9 months of storage at the temperatures of 10°C, -20°C and -80°C in tightly sealed containers. Seed traits were significantly different at different temperatures (p=0.01). For both cultivars germination and germination energy gained the best results at -80°C in comparison to the values before storage. Obtained values of the seedling traits tested were significantly lower after 9 months storage (p=0.01). For the 'Bartwingo' cultivar, the slightest difference in the values of seedling traits before storage was gained at 10°C, while the 'Calibra' cultivar gained the same at -20°C. After 9 months storage at different temperatures the 'Bartwingo' diploid cultivar showed higher values of germination and germination energy, while the 'Calibra' tetraploid cultivar developed longer root and hypocotyl length, and total seedling length. Such investigations provide an opportunity for preserving perennial ryegrass seeds at lower temperatures in long term storage.

Keywords: perennial ryegrass, seeds, storage, germination, temperature

Introduction

Seed quality is a complex trait that depeds on a number of various factors. According to Marcos-Filho and McDonald (1998) seed quality is determined by its genetic, physical, physiological and health condition properties that are influenced by agroecological conditions in the course of vegetation (McDonald, 1998), as well as seed processing, (Schaffer and Vanderlip, 1999), storage conditions and storage period (Saxena *et al.*, 1987; Vieira *et al.*, 2001). Seed quality in the period of storage is primarily influenced by seed moisture, temperature, and relative humidity in warehouse. Optimum storage conditions also depend on the crop species of the seeds stored: grass seeds, in general, can be better preserved than maize seeds, while maize seeds can be better preserved than soybean seeds (Elias *et al.*, 2002). Under favourable conditions seeds of crops provided for roughage (grass and small-grained fodder legumes) can preserve quality or viability over a longer period. Dormancy as a pronounced trait that can be the result of physical and physiological mechanisms in the seed is a contributory factor (Hampton and Hill, 2002). Studies of Marsall and Lewis (2004) showed that seeds of small-grained legumes and grass could be successfully stored for 12 years with control of relative humidity and temperature in a warehouse (2°C and relative humidity between 10 and 20%) as opposite to the conditions with mere temperature control (4°C). Under previously mentioned controlled storage conditions, seeds of small-grained legumes and grass had slightly lower moisture content after 10 years storage when compared to the seeds introduced into the warehouse (Lewis *et al.*, 1998). Research findings on the influence of storage conditions on seed traits make significant contribution to seed quality preservation over the storage period. The objective of the research was to monitor and determine the influence of storage temperature at 10°C, -20°C and -80°C on traits of seeds and seedlings of two different perennial ryegrass cultivars during long term storage period. This study shows results obtained after 9 months storage.

Materials and methods

In this research we tested seeds of two perennial ryegrass cultivars 'Bartwingo' and 'Calibra' whose origin, 1000-seed mass, seed moisture, and ploidity can be seen in Tab. 1. The seeds were stored in tightly sealed glass jars at the temperature of 10°C, -20°C and -80°C and relative humidity of 35% during a 9 months period. Seed sprouting was done in a clime chamber by the method of rolled filter paper. Traits of the seeds (germination energy and seed germination) and seedlings (root and hypocotyl length and total seedling length) were determined before and 9 months after storage according the described methods of ISTA, 1999. For each treatment 100 seeds were sown on wet filter paper in 4 replications. The rolled filter paper with seeds was placed in PVC bags, and deposited in clime chamber at the temperature of 7°C (with prior cool-

Tab. 1. The origin, 1000-seed mass, the seed moisture and the ploidity of perennial ryegrass cultivars

Cultivar	Origin	1000-seed mass (g)	Seed moisture (%)	Ploidity
'Bartwingo'	Netherlands, Barenburg, expiry date 31.12.2010	1.92	8.4	diploid
'Calibra'	Denmark, DLF Trifolium-Denmark, expiry date 31.12.2010	3.06	10.4	tetraploid

ing) for 10 days, and then at the temperature of 20°C for 8 days. The fifth day after temperature increase the germination energy was recorded; seed germination was recorded three days later. Finally, root and hypocotyl length and total seedling length of 25 randomly chosen seedlings were measured. Results were statistically processed by analysis of variance (ANOVA) and LSD-test using VVSTAT software (Vukadinovic, 1985).

Results and discussion

Obtained values for tested traits of seeds and seedlings of perennial ryegrass cultivars before and after 9 months storage at different temperatures are given in Tab. 2.

Mean values of germination energy for both cultivars were significantly different at different storage temperatures (p=0.01). Significantly lower values of germination energy for both cultivars were obtained at the temperature of 10°C if compared to the values before storage at temperatures of -20°C and -80°C. The highest germination energy of the 'Bartwingo' cultivar was recorded before storage. 9 months later germination energy decreased, as follows, by 6% at 10°C, by 2.7% at -20°C, and by 1.2% at -80°C. The lowest germination energy of the 'Calibra' cultivar was recorded at 10°C. By preserving seeds at low temperatures after 9 months storage this cultivar exhibited an increase in germination energy by 1.5% at -20°C, relative to the significant 9.5% at -80°C in comparison to the values before storage. On average, at all the storage temperatures the 'Bartwingo' cultivar showed significantly higher values of germination energy than the 'Calibra' cultivar (p=0.01).

Mean seed germination values for both cultivars were significantly different at different storage temperatures (p=0.01). By preserving seeds of the 'Bartwingo' cultivar at 10°C a significant decrease of 5.5% in germination values was recorded relative to the seed germination before storage; 2.8% at -20°C; and 2.3% at -80°C 9 months after storage. On the contrary, the germination of the 'Calibra' cultivar was increased at all the temperatures, as follows, by 1.3% at 10°C; by 4.3% at -20°C; and by a significant 15.8% at -80°C relative to the germination before storage. On average, at all the storage temperatures the 'Bartwingo' cultivar exhibited significantly higher germination values than the 'Calibra' cultivar (p=0.01).

Differences between cultivars can be presumed to be the result of differences in seed size. Smaller seeds of the 'Bartwingo' diploid cultivar to activate enzymes in degrading nutrients and initiating the germination process require less water supplies and a shorter time in comparison to the 'Calibra' tetraploid cultivar. Namely, early growth and persistence of perennial ryegrass is greater in diploids (Hall, 1992) due to the possible endosperm imbalance for ploidity, or to mother-father genome interaction in their endosperm (Burton and Husband, 2000). Moreover, apart from storage temperature seed viability is significantly influenced by seed moisture: in the same conditions of temperature and relative humidity in warehouse, seeds of the 'Calibra' cultivar having higher moisture content exhibited significantly lower germination energy and germination (p=0.01) relative to the 'Bartwingo' cultivar with lower moisture content. According to Ritz (1988) critical seed moisture for perennial ryegrass storage is 14%. Studies of Welty *et al.* (1987) showed that loss in seed germination of perennial ryegrass occurred after 2-3 months at the seed moisture level of 15% and storage temperature of 20oC or 30oC. Furthermore, Cattani (2007) reported that perennial ryegrass seed viability could be sustained for 14.5 years at a storage temperature of 5°C and seed moisture of 10.5%, or, for 7 years with the same seed moisture and temperature of 10°C.

Mean values of seedling root length for both cultivars were significantly different at different storage temperatures (p=0.01). Seedling root length of the 'Bartwingo' cultivar before storage (5.281 cm) was slightly decreased, as follows, at a storage temperature of -20°C by 0.126 cm and at -80°C by 0.211 cm. At 10°C a slight increase of 0.012 cm was recorded. On the contrary, values of seedling root length for the 'Calibra' cultivar significantly depended on changes in temperatures. The lowest seedling root length value (5.598 cm) was recorded at 10°C. At temperatures before storage, and at -20°C and -80°C root length values were higher, as follows, by 0.924 cm, 0.985 cm, and 0.677 cm. In general, mean values of seedling root length for both cultivars were decreased relative to the values before storage. Moreover, the 'Calibra' cultivar had on average significantly longer seedling roots by 1.045 cm, relative to the 'Bartwingo' cultivar which can be a result of seed size, or ploidity. In addition, as reported by Smith *et al.* (2003) seedlings of tetraploid cultivars were longer relative to the diploid ones.

Mean values of seedling hypocotyl length for both cultivars significantly decreased after 9 months storage relative to the values before storage (p=0.01). The 'Bartwingo' cultivar had hypocotyl length of 8.109 cm before storage, and after 9 months it decreased by 0.953 cm at 10°C, and, as follows, even by 1.19 cm and 1.189 cm at -20°C and -80°C. Hypocotyl length of the 'Calibra' cultivar before storage (9.33 cm) significantly decreased after 9 months

Tab. 2. Germination energy, seed germination (%), root and hypocotyl length and total seedling length (cm) of seedlings of perennial ryegrass cultivars before and after 9 months storage at different temperatures

Cultivar (C)	Values before storage	Storage temperature (°C)			Mean (C)
		10	-20	-80	
Germination energy (%)					
'Bartwingo'	82.5	76.5	79.8	81.2	80.0[a]
'Calibra'	68.5	67.0	70.0	78.0	70.8[b]
Mean (T)	75.5[b]	71.8[c]	74.9[b]	79.6[a]	75.4
LSD	T	C		TxC	
p= 0.05	1.6701	0.8965		2.0832	
p= 0.01	2.3996	1.2569		2.9536	
Germination (%)					
'Bartwingo'	85.8	80.8	83.0	83.5	83.[3a]
'Calibra'	68.5	69.8	72.8	84.3	73.8[b]
Mean (T)	77.1[b]	75.3b[c]	77.9[b]	83.9[a]	78.5
LSD	T	C		TxC	
p= 0.05	1.6338	1.0461		2.1897	
p= 0.01	2.3474	1.4666		3.0982	
Seedling root length (cm)					
'Bartwingo'	5.281	5.293	5.155	5.070	5.200[b]
'Calibra'	6.522	5.598	6.583	6.275	6.245[a]
Mean (T)	5.902[a]	5.445[c]	5.869[a]	5.673[b]	5.722
LSD	T	C		TxC	
p= 0.05	0.0833	0.0669		0.1253	
p= 0.01	0.1197	0.0938		0.1769	
Seedling hypocotyl length (cm)					
'Bartwingo'	8.109	7.156	6.919	6.920	7.276[b]
'Calibra'	9.330	7.272	8.128	8.199	8.232[a]
Mean (T)	8.720[a]	7.214b[c]	7.523[b]	7.560$_{b}$	7.754
LSD	T	C		TxC	
p= 0.05	0.3276	0.3338		ns	
p= 0.01	0.4704	0.4680		ns	
Total seedling length (%)					
'Bartwingo'	13.390	12.449	12.074	11.990	12.476[b]
'Calibra'	15.825	12.870	14.711	14.474	14.477[a]
Mean (T)	14.621[a]	12.660[c]	13.392[ab]	13.232[ab]	13.476
LSD	T	C		TxC	
p= 0.05	0.8934	0.6815		ns	
p= 0.01	1.2836	0.9555		ns	

ns - non significant, T - temperature, C- cultivar

storage by 1.202 cm and 1.101 cm at -20°C and -80°C, and even by 2.058 cm at 10°C. On average, the 'Calibra' cultivar had 0.956 cm longer hypocotyls than the 'Bartwingo' cultivar, which can also be a result of seed ploidity.

Total seedling length significantly decreased after 9 months storage at different temperatures (p=0.01). Total length of the 'Bartwingo' cultivar was 13.390 cm before storage, and it decreased by 0.941 cm at 10°C, and by 1.316 cm and 1.4 cm at -20°C and -80°C. Total length of the 'Calibra' cultivar was 15.825 cm before storage, and it decreased even by 2.955 cm at 10°C, and by 1.114 cm and 1.351 cm at -20°C and -80°C. On average, the 'Calibra' cultivar had 2.001cm higher total seedling length values than the 'Bartwingo' cultivar.

If we compare obtained results of seed (germination energy and germination), and seedling traits (root and hypocotyl length, total seedling length) between the 'Bartwingo' and 'Calibra' cultivars it can be seen that after 9 months storage at different temperatures the 'Bartwingo' cultivar had more viable seeds, while the 'Calibra' cultivar developed more viable seedlings. This means that tetraploid cultivars exhibited significantly higher competitive abilities and lower tolerance to cold and low temperatures relative to diploid cultivars. In other words, according to Sugiyama (1998) having two sets of chromosomes means higher influence on competitive ability of tolerance and resistance to cold due to the genetic difference between populations of different ploidity degrees. These differences in seedling traits of tested cultivars can be ascribed to dif-

ferences in physiological dormancy (embryonic dormancy) which is apparently more pronounced in 'Bartwingo' diploid cultivar relative to the tetraploid 'Calibra'.

Conclusions

The results of our investigations have proved possibilities of preserving viable traits of seeds and seedlings of perennial ryegrass diploid and tetraploid cultivars at low temperatures over a long term storage period.

For both cultivars' seed traits (germination energy and germination) most favourable temperature was -80°C at which values of germination energy and germination for the 'Bartwingo' cultivar slightly decreased relative to values before storage, while the same values for the 'Calibra' cultivar significantly increased relative to the values before storage.

Considering seedling traits (root and hypocotyl length and total seedling length) the most favourable temperature for the 'Bartwingo' cultivar was 10°C when the lowest difference was recorded in traits relative to the values before storage, while 'Calibra' responded similarly at -20°C.

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