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BIOMASS OF PERENNIAL RYEGRASS CULTIVARS SOWED AFTER 5 YEAR SEED STORAGE PERIOD AT DIFFERENT TEMPERATURES

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SUMMARY

Seeds of two perennial ryegrass (*Lolium perenne* L.) cultivars (diploid 'Bartwingo' and tetraploid 'Calibra') were stored in hermetically sealed glass jars at four different temperatures (-80, -20, 10 and 20°C) for five years. After the storage period the seeds were sowed in containers filled with commercial substrate. Initial growth and development occurred under natural sunlight and manual watering to maintain optimum substrate moisture. After 60 days of vegetation plants were taken from the substrate, developed plants were counted, their roots were washed and whole plants were measured for shoot and root dry-weight and total biomass. Stems and leaves per plant were counted too. The research has revealed significant effects ($p<0.01$) of storage temperature, cultivar and their interaction to all of the investigated traits. When averaged over cultivars the highest values were observed upon storage temperature of -20°C for all the traits except root dry-weight which did not differ between -20 and -80°C. The lowest values of all investigated traits were observed upon storage at 20°C. When averaged over storage temperatures, diploid cultivar had greater number of stems and leaves and the tetraploid one had greater root dry-weight, shoot dry-weight and total biomass.

Key words: perennial ryegrass, seed age, temperature, biomass dry-weight

INTRODUCTION

Perennial ryegrass is one of the most prevailing pasture species (Hannawey, 1999). In nature it occurs as a diploid, but tetraploid cultivars have become available due to colchicine application. Tetraploids are recognized for their greater seed weight (Sugiyama, 1998), larger cells (Wilkins and Sabanci, 1990) and larger leaves and stems (Smith et al., 2001). Quality of seed for perennial ryegrass establishment is of crucial importance due to the persistence of the stand. Favorable conditions along with the use of high quality seed will allow for optimum yields and persistence in pasture mix. Seed quality is a complex trait comprised of genetic, physical, physiological and health traits (Marcos-Filho and McDonald, 1998). Agroecological conditions during the seed crop vegetation (Shlepetys, 1995; McDonald, 1998), seed processing (Schaffer and Vanderlip, 1999) and seed storage conditions and storage period (Saxena et al., 1987) altogether affect the seed quality. At improper storage conditions and upon seed aging seed quality can be considerably impaired (Vieiera et al., 2001; Karman et al., 2014).

Optimum storage conditions are specific to the species being stored. Critical factors in the storage according to Elias et al. (2002) are: kind of seed, seed moisture content, initial viability of the seed, storage temperature and relative humidity, duration of storage and protection from storage fungi and insects.Unlike majority of field crops (i.e. maize, soybean, Elias et al., 2002; Šimić et al., 2005) seeds of small-seeded forages can maintain viability for long time if stored in optimal conditions (Griffiths and Pegler, 1964; Lewis et al., 1998; Rincker, 1981; Bukvić et al., 2009; Rozman et al., 2010; Stanisavljević et al., 2010; Stanisavljević et al., 2001; Bukvić et al., 2015). Seeds of perennial ryegrass stored with greater moisture (20%) and at higher temperature (25°C) lose viability sooner than seeds stored with lower seed moisture (10.5%) and at lower storage temperature (5°C) (Cattani, 2007). Ching and Calhoun (1968) and Ching and Schoolcraft (1968) investigated the causes of seed viability and vigor loss in crimson clover and perennial ryegrass during a 10-year storage period with different seed moisture (6, 8, 12, 16 and 20%) and at different temperatures (3, 22 and 38°C). Upon their results they concluded thatloss of viability and vigor was not due to depletion of food but appeared to be related to the activity of proteases, phytase, and phosphatases since an increase of permeability, amino acids, and inorganic phosphate was observed in the aged material. The magnitude of these increases was related to species, seed moisture and to a lesser degree storage temperature. After seed moisture, the seed storage temperature is the second most important element determining the seed longevity and germination (Justice and Bass, 1978; Vertucci and Roos, 1990; Vertucci and Roos, 1993). Seed germination is the most common indicator of seed quality. Though, germination testing is conducted by standard germination method in ideal moisture and temperature conditions (Siddique and Wright, 2004) the observed values often surpass field emergence (Hamman et al., 2002). Therefore the aim of this research was to investigate the seed quality of two perennial ryegrass cultivars after five years of storage at different temperatures by means of growing plants in containers filled with substrate, exposed to natural sunlight and with maintained moisture of substrate by watering.

MATERIAL AND METHODS

Seeds of two perennial ryegrass cultivars ('Bartwingo' and 'Calibra') were stored for five years at different temperatures (20, 10, -20 and -80°C) in hermetically sealed jars. Initial seed and seedling traits were measured before the storage treatment (Tables 1 and 2).

Table 1 Origin, thousandweight, seed moisture and ploidy of investigated perennial ryegrass cultivars
Tablica 1. Podrijetlo, masa 1000 sjemenki, vlaga sjemena i ploidnost kultivara engleskoga ljulja

Cultivar *Kultivar*	Origin *Podrijetlo*	Thousand weight(g) *Masa 1000 sjemenki (g)*	Seed moisture (%) *Vlaga sjemena (%)*	Ploidy *Ploidnost*
Bartwingo	Netherlads	1.92	8.4	diployd
Calibra	Denmark	3.06	10.4	tetraployd

Table 2 Seed and seedling traits
Tablica 2. Svojstva sjemena i klijanaca

Cultivar *Kultivar*	Germination (%) *Klijavost (%)*	Seedlings root length (cm) *Dužina korijena klijanaca (cm)*	Seedling stem length (cm) *Dužina stabljike klijanaca (cm)*
Bartwingo	85.8	5.28	8.11
Calibra	68.5	6.52	9.33

After seed storage treatments, the seeds were sown in polystyrene containers. Seed of perennial ryegrass was sown in 32 polystyrene containers. Each container contains 26 sowing places. Containers were filled with commercial substrate, and in each sowing place a single seed was sown with of perennial ryegrass. Seeds of each storage temperature treatment and of both cultivars were sowed in 4 replications (i.e., containers). After sowing, containers were placed outdoor and protected with a metal net. Containers were additionally covered with PVC folia in the case of rain to prevent ablation of the substrate. Rainfall was collected and used for watering containers in order to maintain the optimum substrate moisture. After 60 days of vegetation, the developed plants were taken from containers for counting. Shoots were separated from roots. Stems and leaves per plantwere counted. After drying (105°C), shoots and roots per replication (container) were weighed. Total dry biomass was a sum of root and shoot dry mass. ANOVA and LSD tests were performed using SAS Software 9.1.3 (2002-2003).

Table 3 Seed storage temperature and cultivar effects on number of developed plants, number of stems and leaves per plant and dry weight of shoots, roots and total plant mass

Tablica 3. Utjecaj temperature skladištenja i kultivara na broj razvijenih biljaka, broj stabljika i listova po biljci te suhu masu korijena, izdanaka i ukupnu biljnu masu

Cultivar (C) *Kultivar*	Storage temperature (T) / *Temperatura skladištenja* (T)				Average *Prosjek*
	-80ºC	-20ºC	10ºC	20 ºC	
	Number of plants / *Broj biljaka*				
Bartwingo	22.5	23.0	23.5	8.2	19.3[b]
Calibra	23.7	25.0	22.0	10.0	20.2[a]
Average/*Prosjek*	23.1[b]	24.0[a]	22.7[bc]	9.1[c]	19.7
LSD	T**	C**	C x T**		
0.05	0.377	0.334	0.600		
0.01	0.542	0.468	0.847		
	Number of stems per plant / *Broj stabljika po biljci*				
Bartwingo	2.7	3.0	3.0	1.8	2.6[a]
Calibra	2.6	2.7	2.7	1.3	2.3[b]
Average/*Prosjek*	2.6[b]	2.9[a]	2.8[a]	1.6[c]	2.5
LSD	T**	C**	C x T**		
0.05	0.078	0.055	0.1095		
0.01	0.112	0.077	0.155		
	Number of leaves per plant / *Broj listova po biljci*				
Bartwingo	8.3	9.2	8.2	5.1	7.7[a]
Calibra	7.6	7.7	7.7	4.0	6.8[b]
Average/*Prosjek*	8.0[b]	8.5[a]	8.0[b]	4.5[c]	7.2
LSD	T**	C**	C x T**		
0.05	0.115	0.099	0.181		
0.01	0.165	0.139	0.255		
	Root dry weight (g) / *Suha masa korijena (g)*				
Bartwingo	2.972	2.692	2.475	0.428	2.142[b]
Calibra	3.485	3.770	2.778	0.672	2.676[a]
Average/*Prosjek*	3.229[a]	3.231[a]	2.626[b]	0.550[c]	2.409
LSD	T**	C**	C x T**		
0.05	0.217	0.122	0.275		
0.01	0.312	0.171	0.390		
	Shoot dry weight (g) / *Suha masa izdanaka (g)*				
Bartwingo	3.585	4.335	3.880	0.910	3.178[b]
Calibra	4.320	4.815	4.285	0.885	3.576[a]
Average/*Prosjek*	3.952[b]	4.575[a]	4.082[b]	0.897[c]	3.377
LSD	T**	C**	C x T**		
0.05	0.101	0.133	0.214		
0.01	0.146	0.187	0.300		
	Total plant dry weight (g) / *Ukupna suha masa (g)*				
Bartwingo	6.555	7.035	6.360	1.340	5.323[b]
Calibra	7.810	8.590	7.060	1.560	6.255[a]
Average/*Prosjek*	7.183[b]	7.813[a]	6.710[c]	1.450[d]	5.789
LSD	T**	C**	C x T**		
0.05	0.235	0.138	0.304		
0.01	0.338	0.194	0.430		

RESULTS AND DISCUSSION

Number of developedplantlets

After five years of perennial ryegrass seed storage of the same cultivars, Bukvić et al. (2018) have found that storage period has more or less decreased germination of stored seeds. In this research, average number of developed plants after 5-year seed storage varied between 8.2 and 25 of total 26 sowed seeds (Table 3). Storage temperature treatments, cultivars and their interactions significantly ($p<0.01$) affected the number of plants. When averaged over cultivars, the highest number was observed at -20°C storage temperature whilst the lowest was at 20°C. Tetraploid cultivar ‘Calibra’ had greater average number of plants than diploid ‘Bartwingo’. Cultivar ‘Bartwingo’ had the highest number of developed plantsat -20 and 10 °C and ‘Calibra’at -20°C. Both cultivars had the lowest number of plants upon the storage at 20°C.

Number of stems per plant

Number of stems per plant significantly depended ($p<0.01$) on seed storage temperature, cultivar and their interaction (Table 3). According to Kursvuran (2011) perennial ryegrass in field conditions can develop 523 to 656 stems per plant, depending on nitrogen fertilization. In this research average number of stems per plant varied from 1.3 to 3.0 and was similar to findings of Smith et al. (2003). In their trial with 45 perennial ryegrass cultivars they found 2.3 to 3.2 stems per plant after 33 to 37 days of initial growth in controlled condition. Neuteboom et al. (1988) found 3.7 and 4.7 stems per plant of two perennial ryegrass cultivars grown for five weeks at the lowest nitrogen fertilization and 8.1 and 11.5 at highest nitrogen fertilization. In this research the highest number of stems per plant was observed upon storage at -20 and 10°C, and the lowest upon 20°C (Table 3). Diploid cultivar ‘Bartwingo’ had greater number of stems per plant than tetraploid ‘Calibra’. Greater number of stems per plant at diploid cultivars than in tetraploid ones was reported by previous researchers. Neutboom et al. (1988) have found greater number of stems per plant in diploid perennial ryegrass cultivar ‘Wendy’ (4.4 to 11.5) than in tetraploid cultivar ‘Condesa’ (3.7 to 8.1) when grown in controlled conditions. In field conditions Solomon et al. (2017) have found greater stem density in diploid cultivar ‘Marsall’ (1383 stems per m^2) than in tetraploid ‘Maximus’ (1191 stems per m^2). Smith et al. (2001) have found greater number of stems per plant in diploid than in tetraploid cultivars. Contrary to that, mass of stems per plant was greater in tetraploids than in diploids. Inconsistently to a.m. findings, Smith et al. (2003) have found slightly greater number of stems in tetraploids than in diploids in their research on 18 diploid and 27 tetraploid cultivars of perennial ryegrass.

Number of leaves per plant

Number of leaves per plant varied between 4.0 and 9.2 (Table 3). Similar number of leaves per plant was found by Smith et al. (2003) in 45 perennial ryegrass cultivars after 33 to 37 days of initial growth: 3.9 to 4. The greatest count of leaves in this research was observed upon storage at -20°C, and the lowest at 20°C, whilst there were no significant differences at temperatures -80 and 10°C. Considering the interaction, cultivar 'Bartwingo' had greatest leaf number upon storage at -20°C and 'Calibra' at -20 and 10°C. When averaged over storage temperatures, diploid cultivar 'Bartwingo' had significantly greater number of leaves per plant than tetraploid 'Calibra'. These findings are in accordance with research ofNeutboom et al. (1988) who observed greater number of leaves per plant in diploid cultivar 'Wendy' (8.49) than in tetraploid 'Condesa' (7.95).

Root dry weight

Average root dry weight varied from 0.428 to 3.770 g (Table 3). Crush et al. (2009) found root dry weight between 0.259 to 1.574 g in wild types and between 0.371 and 1.469 g for inbred-lines of perennial ryegrass after 110 days of initial growth in a glasshouse. In this research root dry weight was greater at -80 and -20°C than at 10 and 20°C ($p<0.01$)when averaged over cultivars. The lowest root dry weight was found upon storage at 20°C. Tetraploid cultivar 'Calibra' had greater average root dry weight than diploid 'Bartwingo' ($p<0.01$). Oppositely, Deru et al. (2012) have found greater root dry weight in diploid than in tetraploid cultivars in their field experiment with 8 perennial ryegrass cultivars.

In this research the different levels of storage temperature differently affected root dry weight with significant interaction of storage temperature and cultivar ($p<0.01$). Incultivar 'Bartwingo' the greatest root dry weight was found upon storage at -80°C and it was gradually decreased with the increase of storage temperature. In cultivar 'Calibra' the greatest root dry weight was found at -20°C, followed by -80°C, then 10°C, and the lowest was upon the storage at 20°C.

Shoot dry weight

Average shoot dry weight varied between 0.885 and 4.81 g (Table 3). Crush et al. (2009) have found shoot dry weight between 0.310 and 1.223 g for wild types and between 0.712 and 1.870 g for inbred lines of perennial ryegrass plants grown for 110 days in tubes filled with sand.In this research the greatest shoot dry weight was found upon the storage at -20°C, significantly lower was upon -80 and 10°C, and the lowest upon 20°C, when averaged over cultivars. Significant interactions of temperature and cultivars were observed and reflected to absence of significant difference between -80 and 10°C for cultivar 'Calibra' and to lower shoot dry weight at -80 than at 10°C for cultivar 'Bartwingo'. When averaged over storage temperatures, greater shoot dry weight was found in tetraploid 'Calibra' than in diploid 'Bartwingo' ($p<0.01$). Smith et al. (2001) have also found a greater shoot dry weight in tetraploids than in diploids in their research on three diploid and three tetraploid cultivars of perennial ryegrass. Though, in the Smith et al. (2003) research on ploidy level and seed mass effects to the initial growth of perennial ryegrass, authors didnot find significant effect of ploidy to shoot weight. However, tetraploid cultivars tended to have a longer and wider leaves.The authors noted that longer and wider leaves of tetraploid cultivars were result of the “Gigas” effect, and part of this variation between and within diploid and tetraploid perennial cultivars may be explained by a genetic effect on auxin concentrations, cell wall extensibilityand cell size.The same authors have found significant positive correlation of seed mass with plant height and shoot weight. Results of Evans (1970) have also shown that smaller seeds give smaller root and shoot masses.

Total plant dry weight

Total plant dry weight varied from 1.34 to 8.59 g (Table 3). Crush (2009) has found total plant dry weight from 0.594 to 2.845 g for wild types and from 1.146 to 2.900 g for inbred lines of perennial ryegrass grown 110 days in a glasshouse.

When averaged over cultivars, the greatest total plant dry weight ($p<0.01$) was found in plants grown from the seeds stored at -20°C. The lowest total plant dry weight was found in seeds stored at 20°C. Cultivar ‘Calibra’ has shown greater total plant dry weight at storage at -80 than at 10°C.

When averaged over seed storage temperatures, total plant dry weight was greater in tetraploid ‘Calibra’ than in diploid ‘Bartwingo’which was in accordance with previous findings of Neuteboom et al. (1988).

CONCLUSIONS

The research of different storage temperatures during five-year seed storage has revealed significant effects ($p<0.01$) of storage temperature and cultivar on all investigated plantlet traits. The highest values for number of developed plants, number of leaves per plant, shoot and total dry weight were achieved upon storage at -20°C. Number of stems per plant didnot differ between -20°C and 10°C storage temperatures. Root dry weights didnot differ between -20°C and -80°C storage temperatures. The lowest values of all investigated traits were observed upon storage at 20°C. Diploid cultivar ‘Bartwingo’ had greater average values for number of stems and leaves per plant while tetraploid ‘Calibra’ had greater average number of developed plants, root, shoot and total plant dry weight.

Seed quality loss during the seed storage period is indivertible. In order to maintain the seed quality during longer storage periodsit is required to choose the most favorable conditions. These conditions will depend on the plant species, cultivar and ploidy level, environment temperature, and seed moisture and storage space. Results of this research have shown that the perennial ryegrass seeds have maintained their viability during long storage period of five years. Though, storage at lower temperatures has resulted in greater biomass production in both perennial ryegrass cultivars.

BIOMASA KULTIVARA ENGLESKOG LJULJA ZASIJANIH NAKON 5 GODIŠNJEG SKLADIŠTENJA SJEMENA NA RAZLIČITIM TEMPERATURAMA

SAŽETAK

Sjeme dva kultivara (diploidni 'Bartwingo' i tetraploidni 'Calibra') engleskog ljulja (*Lolium perenne* L.) je bilo uskladišteno u hermetički zatvorenim staklenkama na četiri različite temperature (-80, -20, 10 i 20°C) tijekom pet godina. Nakon razdoblja skladištenja sjeme je zasijano u kontejnere s komercijalnim supstratom. Početni rast i razvoj odvijali su se pod prirodnim svjetlom i uz ručno zalijevanje radi održavanja optimalne vlažnosti supstrata. Nakon 60 dana vegetacije razvijene biljke su uzete iz supstrata, prebrojene su, korijenje im je oprano i potom su im izmjerene suhe mase izdanka, korijena i cijelih biljčica. Izbrojan je i broj stabljika i listova po biljci. Istraživanje je pokazalo značajne učinke ($p<0.01$) temperature skladištenja, kultivara i njihovih interakcija na sva ispitivana svojstva. U prosjeku po kultivarima, najveće vrijednosti svojstava ustanovljene su nakon skladištenja na -20°C, osim za svojstvo suhe mase korijena koja se nije razlikovala između -20 i -80°C. Najniže vrijednosti svih ispitivanih svojstava ustanovljene su nakon skladištenja na 20°C. S obzirom na temperature skladištenja diploidni kultivar je u prosjeku imao veći broj stabljika i listova po biljci, a tetraploidni je imao veću suhu masu korijena, suhu masu izdanka i ukupnu biomasu.

Ključne riječi: engleski ljulj, starost sjemena, temperatura, suha masa biomase

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