Biblid: 1821-4487 (2019) 23; 1; p 10-13
UDK: 581.48

Original Scientific Paper
Originalni naučni rad

EFFECT OF SEED AGING ON THE SEED QUALITY AND SEEDLING GROWTH OF TIMOTHY GRASS (Phleum pratense L.)

UTICAJ STAROSTI SEMENA NA KLIJAVOST I PORAST KLIJANACA MAČJEG REPA (*Phleum pratense* L.)

Rade STANISAVLJEVIĆ[*], Dobrivoj POŠTIĆ[*], Jasmina MILENKOVIĆ[**], Dragoslav ĐOKIĆ[**], Dragoljub BEKOVIĆ[***], Ratibor ŠTRBANOVIĆ[*], Snežana JOVANOVIĆ[****], Marijenka TABAKOVIĆ[****]

[*]Institute for plant protection and the environment, Teodora Drajzera 9, 11000 Belgrade, Serbia
[**]Institute for forage crops, Kruševac, Globoder 37251, Serbia
[***]University of Priština, Faculty of Agriculture, 38219 Lešak bb, Serbia
[****]Maize research institute Zemun Polje, Slobodana Bajića 1, 11000 Belgrade, Serbia
**stanisavljevicrade@gmail.com*

ABSTRAKT

Timothy grass is a fodder grass mostly grown as a pure stand or in mixed stands with fodder legumes at higher elevations. It is cultivated for its high yields and the supreme quality of roughage containing Timothy grass. There are different methods for preserving the seed quality of fodder grasses relative to the following parameters: species, variety, harvest methods, postharvest treatments, as well as the storage temperature and air humidity. Poor seed germination is often caused by the presence of dormant seeds which fail to germinate despite ideal conditions in the field or laboratory. After a certain storage period, dormant seeds start germinating, but prolonged storage periods result in seed aging which is associated with reductions in seed germination, seedling growth and the total mass of the root system. The seed quality features of four Timothy grass seed lots (the 'Foka' cultivar), at ages of 3, 15 and 27 months, were examined in this study according to the share of dormant, germinated and dead seeds, as well as abnormal seedlings. Upon assessing seed germination, the stem length (cm), radicle length (cm) and fresh weight (g) of seedlings were measured. Different seed lots were found to exert no effect on the seed quality parameters examined and the growth of seedlings. Conversely, the seed age had a significant impact ($P<0.001$ to $P<0.05$) on the seed quality parameters examined and the seedling growth.

Key words: *seed quality, Timothy grass, seedling growth*

REZIME

Mačji rep je krmna trava koja se uglavnom gaji u smeši sa krmnim leguminozama ili kao čist usev na većim nadmorskim visinama. Njegovim gajenjem se ostvaruju visoki prinosi i odličan kvalitet kabaste stočne hrane. Za razliku od većine krmnih trava, mačji rep nije sklon osipanju pa se ubiranje semena obavlja kada je seme sa nižim sadržajem vlage što je zbog značajno zbog dužeg održanja kvaliteta semena. Postoje razlike u očuvanju kvaliteta semena između krmnih trava. U značajnije faktore spadaju: vrsta, sorta, način ubiranja, posležetveni tretmani semena, kao i temperatura i vlažnost vazduha u skladištu. Smanjenu klijavost često uzrokuje prisustvo dormantnog semena koje ne klija iako postoje idealni uslovi u laboratoriji ili na polju. Nakon određenog perioda skladištenja dormantno seme postaje klijavo. Produženjem vremena skladištenja dolazi do starenja semena, što se ogleda u smanjenoj klijavosti a vrlo često i u smanjenom porastu klijanaca i ukupnoj masi korenovog sistema. Ova istraživanja su izvedena na četiri partije semena mačjeg repa sorte Foka. Analiziran je kvalitet semena (udeo dormantnog, klijavog, mrtvog semena i nenormalnih klijanaca) kod semena starog tri, petnaest i dvadeset sedam meseci. Nakon utvrđivanja klijavosti, na klijancima su izmereni stabaoce (cm), korenak (cm) i masa svežih klijanaca. Partija semena nije imala značajnog uticaja na ispitivane parametre kvaliteta semena, kao i ni na porast klijanaca. Sa druge strane, starost semena je imala značajan uticaj ($P<0.001$ do $P<0.05$) na ispitivane parametre kvaliteta semena.

Ključne reči: *kvalitet semena, mačji rep, porast klijanca*

INTRODUCTION

Timothy grass is a fodder grass mostly grown as a pure stand or in mixed stands with fodder legumes at higher elevations. It is widespread in cool regions of Europe, Asia, North Africa and North America. In Serbia, Timothy grass is widely cultivated in mountainous areas, favoring damp valleys and mountain meadows of the *Arrhenatheretea type.* On balance, its cultivation under appropriate climatic conditions results in high yields and high-quality fodder (Knezević and Stanisavljević, 2018). Unlike most fodder grasses, Timothy grass is not susceptible to seed shattering, so Timothy grass seeds can be harvested with low moisture contents, which is of paramount importance to the preservation of seed quality. The methods for preserving the seed quality of fodder grasses depend on the following parameters: species, variety, harvest methods, postharvest treatments, as well as the storage temperature and air humidity (*Stanisavljević et al., 2016, 2017*). Poor seed germination is often caused by the presence of dormant seeds which fail to germinate despite ideal conditions in the field or laboratory (*Adkins et al., 2002*). After a certain storage period, fodder and ornamental grass seeds undergo complex physiological and biochemical processes, during which dormant seeds start germinating, i.e. achieve the maximum germination and agricultural use-value.

In many intensively cultivated crops, dormant seeds have been minimized or virtually non-existent (Adkins et al., 2002). However, fodder grasses exhibit no significant difference in the share of dormant seeds between cultivated and wild populations within the same species (Stanisavljević et al., 2012). Under natural conditions, dormant seeds delay germination, awaiting favorable conditions for the survival of the seedlings (Bewley, 1997). However, prolonged storage periods result in seed aging which is associated with reductions in seed germination, seedling growth and the total mass of the root system. The purpose of this paper is to examine the seed quality features of four Timothy grass seed lots (the 'Foka' cultivar), at ages of 3, 15 and 27 months, according to the share of dormant, germinated and dead seeds, as well as abnormal seedlings. Upon assessing seed germination, the stem length (cm), radicle length (cm) and fresh weight (g) of Timothy grass seedlings were measured as well.

MATERIAL AND METHOD

The seed quality features of four Timothy grass seed lots (the 'Foka' cultivar), at ages of 3, 15 and 27 months, were examined in this study according to the share of dormant, germinated and dead seeds, as well as abnormal seedlings. After harvest, the seeds were dried to moisture contents of 10 to 12 %, and placed in two-layer paper bags. The seeds of the following ages were analyzed: three months (AGS1 – sown and harvested in the same year), fifteen months (AGS2 – sown and harvested with one year apart) and twenty seven months (AGS3 - sown and harvested with two years apart). The following parameters of germinated seeds were measured using the method described by Stanisavljević et al. (2011): stem length (cm), radicle length (cm), and fresh seedling weight (g). The seed quality assessment was performed in accordance with the official seed quality rules (Official Gazette of SFRJ, 47/87) and the ISTA Rules (ISTA 2002-2018). The analysis of variance (ANOVA, F test) was applied for examining the seed lot and age parameters. Treatment differences were determined using the Tukey's multiple range tests. The variability for each seed quality feature was expressed by the coefficient of variation (CV, %), whereas the dependence between the features examined was expressed by the simple correlation coefficient (r). The percentages of germination and dormancy obtained were arcsine transformed ($\sqrt{x}$ / 100) before subjected to the analysis of variance (Snedecor and Cochran, 1980). The Minitab 16.1.0 program was used for data processing.

RESULTS AND DICUSSION

On the basis of the analysis of variance (F test) performed, the SL seed lot and the SL x AGS interaction were found to exert no significant effects on the seed quality indicators (Tab.1).

Table 1. Analysis of variance for dormant seeds (DS), germinated seeds (GS), dead seeds (MS), abnormal seedlings (AS), stem length (S), radicle length (R) fresh seedling weight (FS). Sources of variation: seed age (AGS), seed lot (SL).

Source	DS	GS	MS	AS	R	S	FSM
AGS	***	***	***	***	**	**	*
SL	NS	NS	NS	NS	NS	NS	NS
Interaction AGSxSL	NS	NS	NS	NS	NS	NS	NS

*NS = Not Significant F tests at the P > 0.05 level of significance, *Significant F tests at the P < 0.05 level of significance, **Significant F tests at the P < 0.01 level of significance, ***Significant F tests at the P < 0.001 level of significance*

According to Baskin and Baskin (2004), the dormancy of seeds is classified as primary and secondary: the primary seed dormancy is inherent only in the harvested seed and develops during seeding, whereas the secondary seed dormancy is the consequence of the environmental influence. The authors describe the possibility of having adequate and secondary dormancy in the same seed. A number of studies on fodder grass have argued that the appearance of seed dormancy is pronounced (Adkins et al., 2002), as confirmed by Stanisavljević et al. (2016, 2017). According to Hill and Watkin (1975), a total of 30 to 40 % of dormant seeds were found in Timothy grass immediately after harvest. The same authors associate the presence of dormant seeds with seed moisture contents during harvest. However, Havstad and Aamlid, (2013) indicate that Timothy grass seeds can be dormant for several months, regardless of seed moisture contents during harvest. Upon comparing a number of fodder grasses, Stanisavljević et al. (2011) suggest that dormant seeds were present in Timothy grass to a lesser extent than in orchard grass and Italian rye-grass. The dormancy of seeds is undesirable in fodder and ornamental grasses because it impedes fast and uniform germination, especially if the grass is mixed with fodder legumes.

Table 2. Effect of the seed age on the share of dormant seeds (DS), germinated seeds (GS), dead seeds (MS) and abnormal seedlings (AS)

Seed age	Seed			
	DS %	GS %	MS %	AS %
AGS1	8 ± 0.356 a	90 ± 0.878 a	0 ± 0.000 c	2 ± 0.878 a
AGS2	1 ± 0.016 b	87 ± 0.542 b	8 ± 0.467 b	4 ± 0.878 b
AGS3	0 ± 0.000 b	79 ± 0.357 b	13 ± 0.249 a	8 ± 0.878 c
Mean	3.0	85.3	7.0	4.7
Coefficient of variation (CV= %)	145.4	6.664	93.68	65.47

a, b…x (different lower-case letters) significant effect (P < 0.05; Tukey's multiple range test) for the column values. Values are the mean ± the standard error of the mean.

Three months after harvest, a total of 8 % of dormant seeds were present in the AGS1 seed lot. By the next fall, the seeding percent of the AGS2 dormant seeds was 7 % lower than that recorded in the AGS1 seed lot. In the AGS1 seed lot, a germination rate of 90 % was recorded, which is 3 % higher than that recorded in the AGS2 seed lot. A statistically significant difference (P > 0.05) in germination rates was not found between AGS1 and AGS2. This suggests a high utilization value of Timothy grass at seed ages ranging from three to fifteen months.

A significant (P ≤ 0.05) decrease in germination (79%) was recorded in the AGS3 seed lot, as well as increases in dead seeds (13%) and abnormal seedlings (8%), compared to both AGS1 and AGS2. This indicates that the aging process of the seed was in progress, adversely affecting the seed use-value. According to the official seed quality rules in Serbia (Official Gazette SFRJ 47/87), seeds meet the criteria of germination even after 27 months of storage, which is the final acceptable age limit for germinating seeds.

Table 3. Seedling quality parameters: stem length (S), radicle length (R) and fresh seedling weight (FS) at different seed ages

Seed age	S (cm)	R (cm)	FS (g)
AGS1	3.14 ± 0.029 a	2.84 ± 0.125 a	0.0081 ± 0.009 a
AGS2	3.04 ± 0.105 a	2.74 ± 0.066 a	0.0078 ± 0.113 a
AGS3	2.76± 0.099 a	2.49 ± 0.178 b	0.0070 ± 0.069 b
Average	2.98	2.69	0.0077
Coefficient of variation (CV= %)	6.610	6.702	7.449

a, b, …x (different lower-case letters) significant effect (P < 0.05; Tukey's multiple range test) for the column values. Values are the mean ± the standard error of the mean.

Table 4. Simple correlation coefficients (r) between germination (G), dormant seeds (DS), germinated seeds (GS), dead seeds (MS), abnormal seedlings (AS) and seedling quality parameters (namely stem length (S), radicle length (R), and fresh seedling weight (FS)) after three, fifteen and twenty seven months of storage (AGS1, AGS2, AGS, respectively) (n=4)

Seed age		Seed quality parameters			Seedling quality parameters - vigor		
	G I	DS II	MS III	AS IV	S (cm) V	R (cm) VI	FS (g) VII
AGS1	I	-0.999 ***	-0.941 *	-0.665 ns	0.991 **	0.956 *	0.935 *
	II	-	0.456 ns	-0.235 ns	-0.875 ns	-0.568 ns	-0.498 ns
	III	-	-	-0.025 ns	-0.996 **	-0.999 ***	-0.996 **
	IV	-	-	-	-0.654 ns	-0.587 ns	-0.612 ns
	V	-	-	-	-	0.995**	0.930 *
	VI	-	-	-	-	-	0.933 *
AGS2	I	-0.993 *	-0.932 *	-0.565 ns	0.963 *	0.932 *	0.930 *
	II	-	0.287 ns	-0.136 ns	-0.643 ns	-0.342 ns	-0.498 ns
	III	-	-	-0.198 ns	-0.933 *	-0.935 *	-0.939 *
	IV	-	-	-	-0.359 ns	-0.456 ns	-0.369 ns
	V	-	-	-	-	0.934 *	0.933 *
	VI	-	-	-	-	-	0.941 *
AGS3	I	-0.941 *	-0.930 *	-0.565 ns	0.941 *	0.936 *	0.937 *
	II	-	0.258 ns	-0.199 ns	-0.542 ns	-0.198 ns	-0.268 ns
	III	-	-	-0.055 ns	-0.928 ns	-0.905 ns	-0.916 ns
	IV	-	-	-	-0.301 ns	-0.295 ns	-0.156 ns
	V	-	-	-	-	0.931 *	0.933 *
	VI	-	-	-	-	-	0.933 *

*ns Not significant F tests at the P > 0.05, *Significant F tests at the P < 0.05 level of significance, **Significant F tests at the P < 0.01 level of significance, ***Significant F tests at the P < 0.001 level of significance*

Timothy grass is often grown in mixed stands with fodder legumes, characterized by the complementary and rapid initial growth of Timothy grass and fodder legume seedlings which allows for a more successful competition with weeds (Vučković 2004). In the present study, the differences between the seedling quality parameters of AGS1 and AGS2 were found to be statistically non-significant. However, the differences between the seedling quality parameters of AGS1 and AGS2, on one hand, and the seedling quality parameters of AGS3, on the other, were found to be statistically significant (P <0.05) (Table 3).

The coefficients of correlation (r) obtained emphasize the dependence between the seed and seedling quality parameters at three seed ages and four seed lots under consideration (Table 4). A negative correlation was found between the germinated and dormant seeds tested (r = -999, P <0.001), as well as between the dead seed and the radicle length of seedlings (r = -999, P <0.001). Furthermore, a negative correlation was also found between the dead seed, the stem length and the fresh seedling weight (by r = -996, P <0.01) for seeds at three months of age (AGS1). Conversely, a positive correlation was established between the germination rate and the stem length (r = -991, P <0.01), as well as between the stem length and the radicle length (r = -995, P <0.01). A positive correlation was also found between the germination rate and the radicle length, as well as the germination rate and the fresh seedling weight (P <0.05) for seeds at all ages under consideration (AGS1, AGS2, AGS3; Table 4). A similar dependence between the seed quality parameters and the seedling growth of fodder grasses has been reported in the literature. In the instance of English ryegrass, the dependence between the germination rate and the stem length was r = 0.989, between the germination rate and the radicle length was r = 0.988, and between the stem length and the radicle length was r = 0.978 (Bukvić et al 2018). In the case of Italian ryegrass, the dependence between the germination rate and the stem length was r = 0.604 to r = 0.806, depending on the variety (Velijević et al., 2018). In the instance of tall fescue, the dependence between the germination rate and the stem length was r = 0.389, between the germination rate and the root length was r = 0.395, and the germination rate and the fresh seedling weight r = 0.305. In the case of red fescue, the dependence between the germination rate and the stem length was r = 0.245, between the germination rate and the radicle length was r = 0.235, between the germination rate and the fresh seedling weight was r = 0.288, between the germination rate and the stem length was r = 0.299, between the germination rate and the radicle length was r = 0.326, as well as between the germination rate and the fresh seedling weight was r = 0.313 (Stanisavljević et al. 2014).

CONCLUSION

The Timothy grass seeds examined at three and fifteen months of age exhibited the highest germination rates, i.e. 90 % and 87 % respectively. As a result of seed aging, the germination rate amounted to 79 % over the period of twenty-seven month, which is close to a minimum seed germination rate of 75 % according to the official seed quality policy in Serbia. The seeds at all three ages under consideration were found to exhibit high germination rates accompanied by strong seedlings, as indicated by the correlation coefficients obtained between the germination rates and the stem lengths.

ACKNOWLEDGEMENTS: This paper is a result of the projects TR.31057 and TR.31018, which were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

REFERENCES

Adkins, S.W., Bellairs, S.M., Loch, D.S. (2002). Seed dormancy mechanisms in warm season grass species, Euphytica, 126(1), 13-20.

Baskin, J.M., Baskin, C.C. (2004). A classification system for seed dormancy, Seed Science Research, 14: 1-16

Bewley, J.D. (1997). Seed germination and dormancy. The Plant Cell 9, 1055–1066,

Bukvić, G., Gantner, R., Agić, D., Popović, B., and Stanisavljević, A. (2018). Svojstva klijanaca i biljaka engleskog ljulja nakon 5 godina skladištenja sjemena na različitim temperaturama, Poljoprivreda, 24(1), 18-24

Havstad, T. L., and Aamlid, S., T. (2013). Influence of harvest time and storage location on the longevity of timothy *(Phleum pratense L.)* seed, Acta Agriculturae Scandinavica, Section B - Soil and Plant Science, 63:5, 453-459.

Hill, M.J., Watkin, B.R. (1975). Seed production studies on perennial ryegrass, timothy and prairie grass. 2. Changes in physiological components during seed development and method of harvesting for maximum seed yield, Journal of the British Grassland Society, 30:131-140.

Knežević, J., Stanisavljević R. (2013). Posebno ratarstvo, industrijsko i krmno bilje. Univerzitet u Prištini, Poljoprivredni fakultet, Kosovska Mitrovica – Lešak.

International Seed Testing Association. International Rules for Seed Testing, edition 2002-2018. ISTA Basserdorf, CH.

Pravilnikom o kvalitetu semena poljoprivrednog bilja (Sl. list SFRJ 47/87).

Sanisavljević, R., Đokić, D., Milenković, J., Terzić, D., Stevović, V,. Tomić, D., Dodig, D. (2014). Drying of forage grass seed harvested at different times and its utility value in sowing periods, Zemdirbyste-Agriculture, 101, 169-176.

Snedecor, G.W., Cochran, W.G. (1980). Statistical Methods, 7th edition. The Iowa State University Press, Ames, Iowa, USA

Stanisavljević, R., Milenković, J., Štrbanović, R., Poštić, D., Velijević, N., Jovanović, S., Tabaković, M. (2017). Variability italian ryegrass and perennial ryegrass seed quqlity produced in two different region, Journal on Processing and Energy in Agriculture, 21,(2), 124-126.

Stanisavljević, R., Vučković, S., Simić, A., Marković, J., Lakić, Ž., Terzić, D., Đokić, D. (2012). Acid and temperature treatments result inincreased germination of seeds of three fescue species, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 40, 220-226.

Stanisavljević, R., Milenković, J., Đokić, D., Terzić, D., Beković, D., Poštić, D., Štrbanović R. (2016). Influence of storing seeds on seed vigor and seedling meadow and tall fescue after five years, Journal on Processing and Energy in Agriculture, 20, 111-113

Stanisavljevic, R., Đjokic, D., Milenkovic, J., Đukanovic, L., Stevovic, V., Simic, A., Dodig, D. (2011). Seed germination and seedling vigour of Italian ryegrass, cocksfoot and timothy following harvest and storage. Ciencia e agrotecnologia, 35(6), 1141-1148.

Velijević, N., Simić, A., Vučković, S., Živanović, LJ., Poštić, D., Štrbanović, R., Stanisavljević, R. (2018). Influence of different pre-sowing treatments on seed dormancy breakdown, germination and vigour of red clover and italian ryegrass. International Journal of Agriculture and Biology, 20, 1548–1554.

Vučković (2004). Travnjaci, Univerzitet u Beogradu, Poljoprivredni fakultet Beograd

Received: 18. 02. 2019. Accepted: 15. 03. 2019.