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QUALITY OF ALFALFA SEEDS FROM DIFFERENT REGIONS OF SERBIA DURING STORAGE UP TO 42 MONTHS

KVALITET SEMENA LUCERKE IZ RAZLIČITIH REGIONA SRBIJE U PERIODU ČUVANJA DO 42 MESECA

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ABSTRACT

This study presents the findings from assessing the quality of alfalfa seeds collected from three Serbian regions: in each region, batches of seeds from six places were analyzed. The following criteria were investigated: the proportion of dormant seeds, the germinated seed content, the proportion of dead seeds, and the percentage of aberrant seedlings. Seed quality was assessed after six, eighteen, thirty, and forty-two months of storage. The highest seed quality was obtained after thirty and eighteen months of seed storage (91% and 89% of germination, respectively). During the 42-month storage period, the percentage of dead seeds (8%) and abnormal seedlings (7%) increased, while germination declined to 84%. In a storage period of six months, the participation of dormant seeds of 21% affected the germination rate, which was 77%, regardless of the low proportion of dead seeds (1%) and abnormal seedlings (2%).

***Keywords**: alfalfa, quality, seed, storage.*

REZIME

U ovom istraživanju su prikazani rezultati ispitivanja kvaliteta semena lucerke kolekcionisane iz tri regiona Srbije: u svakom regionu su ispitivane partije semena sa šest lokaliteta. Ispitivani su sledeći parametri: udeo semena; dormantnog, klijalih, mrtvog% i udeo nenormalnih klijanaca (%). Analiza kvaliteta semena je rađena nakon čuvanja šest, osamnaest, trideset, i četrdeset dva meseca. Najbolji kvalitet semena je ostvaren nakon trideset (klijavost 91%) i osamnaest meseci čuvanja semena (klijavost 89%). U periodu čuvanja od 42 meseca povećalo se učešće mrtvog semena (8%) i nenormalnih klijanaca (7%), a klijavost je opala na 84%. U perodu čuvanja od šest meseci učešće dormantnog semena od 21% je uticalo na klijavost koja je bila 77%, bez obzira na nizak udeo mrtvog semena (1%) i nenormalnih klijanaca (2%).

***Ključne reči**: čuvanje, kvalitet, lucerka, seme.*

INTRODUCTION

The most prevalent type of seed dormancy in nature is physiological seed dormancy which causes the inability of the embryo to elongate due to the thick and impenetrable endocarp that prevents elongation and/or hormonal imbalance *(Baskin and Baskin 2021; Galussi et al., 2016)*. In nature, this feature occurs in the seeds of plants from the *Fabaceae* family over a long period of time *(Jaganathan, and Berry, 2023)*. This characteristic was considerably decreased or eliminated during the breeding process, resulting in improved seed output. Also, it is positive that seed dormancy promotes germination when environmental conditions are favorable, ensuring the survival of many species in nature *(Cuena Lombraña, et al., 2024*).

In alfalfa seeds, physical dormancy is present and still expressed in different climatic zones *(Čupić et al., 2005; Zimmermann et al., 1998; Fairey and Lefkovitch 1991)*. According to *Harrison et al., (2021), Xie et al., (2023)*, alfalfa seeds are characterized by a strong seed coat that does not allow the absorption of water and gases.

In agricultural practice, the term "hard seed" is frequently used instead of "physical dormancy." These seeds grow only when dormancy is broken owing to external factors; however, these seedlings are insignificant for the establishment of alfalfa fields because they cannot compete with mature plants (Bass et al., 1988). A reduced share of dormant seeds and an increase in the percentage of germination can be achieved by scarification: less damage to the seed coat enables faster water absorption *(Stanisavljević et al., 2018)*. However, in the process of scarification, it can easily damage other parts of the seed, including the embryo, and reduce germination *(Bukvić et al., 2001, Kimura and Islam 2012)*. Seed damage can also allow pathogenic and saprophytic organisms to enter the seed and affect seed decay.

To establish an alfalfa field, it is necessary to have seeds with predictable, fast, and high germination. However, there are numerous factors that can negatively affect seed quality and high germination. If seeds with low germination are used, a higher sowing rate is required, which makes the establishment of the crop even more expensive. In agriculture, laboratory germination correlates strongly with field germination *(Frischie et al., 2020; Knežević et al., 2019)*.

In agronomic practice, the achievement of maximum seed germination is considered the most important factor, which is achieved by reducing seed dormancy, but without a significant increase in dead seeds and abnormal seedlings.

After reaching the maximum germination of alfalfa seeds, there follows a period of seed aging. The moment of the beginning of seed aging depends on the plant species, the structure and composition of the seed *(Rawlins et al., 2012)*, the material in which the seed is stored *(Tiwari et al 2022)*, and even the genus within the same species *(Tiwari and Das 2014)*. The most important thing is to obtain such a quality of alfalfa seeds that can enable the reduction of dormancy but at the same time not allow seed aging to occur, which affects the quality of the seedlings. In all aspects of seed storage, it should be taken into account that humidity and temperature in the storage space are very important factors for maintaining seed germination during the storage period *(Nagel and Börner 2010)*.

MATERIAL AND METHODS

The material for this research is seed lots obtained from seed production from different locations in three regions of Serbia: Southeast, Central Serbia and Banat. In each region, seed samples were taken from six localities. At each locality, 3 to 4 different alfalfa varieties and/or populations were represented (Tab. 1).

Table 1. Regions, localities and lots from which the seeds of the test varieties were collected

Region	Variety or population	Seed lot from
Eastern and Southern Serbia	a 1	Negotin
	a 2	Zaječar
	b 1	Boljevac
	c 1	Niš I
	a 2	Niš II
	b 2	Niš III
Central Serbia	c 1	Kragujevac
	a 2	Gornji Milanovac
	a 1	Batočina I
	b 1	Batočina II
	c 2	Rača I
	c 3	Rača II
Banat	a 1	Zrenjanin
	b 1	Srpska Crnja I
	b 2	Srpska Crnja II
	a 2	Srpska Crnja III
	b 3	Vršac
	c 1	Jaša Tomić

The seeds from these locations - batches were examined after different storage periods: 6 months, 18 months, 30 months, and 42 months. During this period, the seeds were stored in three-layer paper packaging in the ambient conditions of the seed storage typical for Serbia *(Stanisavljević et al., 2020)*. The following seed quality parameters were examined: dormant, germinated, dead and abnormal seedlings (%). The test was conducted in accordance with the rulebook for testing the quality of seeds of the Republic of Serbia (Official gazette, No. 47/87, 1987; and supplement to 34/2013).

Tukey's multiple range and coefficient of variability (CV %) were used to determine the effect of treatment. The germination data and dormancy percentages were computed using the arcsine transformation ($\sqrt{x}/100$) (Snedecor and Cochran 1980) prior to the variance analysis. The Minitab 16.1.0 software was used for data processing.

RESULTS AND DISCUSSION

In our trials after six months of seed storage, alfalfa seed dormancy ranged from 20.0 to 21.7% with a variability of CV% = 4.17, depending on the region. Depending on the variety and/or population, it ranged from 20 to 25%. A high percentage of dormant seeds affected the reduction of seed germination (average for the region 78.7 to 75.5%), regardless of the low percentage of participation of dead seeds (average for the region 0.7 to 0.8%) and abnormal seedlings (average for the region 1.5 to 2.2%); (Table 2). After eighteen months of alfalfa seed storage, depending on the region, the share of dormant seeds decreased from 8.0 to 7.2%, germinating seeds increased from 88.2 to 89.8%, dead seed content 1.2 to 1, 6%, and abnormal seedlings from 1.4 to 2.0%. In this period of testing by region, the following variability was determined: for dormant seeds, CV=5.26; germinating seed CV=0.934; for dead seeds CV=15.7%; for abnormal seedlings CV=21.7%. For all examined regions and for all traits, the influence of seed lot was statistically significant ($P \leq 0.05$); (Table 3).

Table 2. Quality of seed lots from three regions after six months of storage

Time of storage	Region	Seed lots from	Seeds %			Abnormal seedlings %
			Dormant	Germinated	Dead	
Six months	Southeastern Serbia	Negotin	25 a	73 c	1 ab	1 b
		Zaječar	22 b	75 b	2 a	1 b
		Boljevac	21 bc	76 ab	1 ab	3 ab
		Niš I	20 c	77 a	1 ab	2 b
		Niš II	22 b	76 ab	0 b	2 b
		Niš III	20 c	76 ab	0 b	4 a
		Average for the region	21.7	75.5	0.8	2.2
		Min	20	73	0	1
		Max	25	77	2	3
	Central Serba	Kragujevac	20 ab	78 ab	1 a	1 b
		Gornji Milanovac	23 a	76 b	1 a	3 a
		Batočina I	19 b	80 a	0 b	1 b
		Batočina II	22 a	77 b	0 b	1 b
		Rača I	18 b	80 a	1 a	1 b
		Rača II	18 b	81 a	1 a	0 b
		Average for the region	20.0	78.7	0.7	1.2
		Min	18	76	0	1
		Max	23	80	1	3
	Banat	Zrenjanin	22 ab	76 b	0 b	2 ab
		Srpska Crnja I	20 bc	75 b	2 a	3 a
		Srpska Crnja II	18 c	80 a	0 b	1 b
		Srpska Crnja III	23 a	74 b	1 ab	2 ab
		Vršac	21 b	79 a	0 b	0 b
		Jaša Tomić	23 a	75 b	1 ab	1 b
		Average for the region	21.2	76.5	0.7	1.5
		Min	18	74	0	0
		Max	23	80	2	3
CV % for the average of the region			4.17	2.13	7.87	31.4

Different small letters in the columns (a, b… x) have significant effect; $P \leq 0.05$; Tukey's Multiple Range test

In the period of seed storage after thirty months, seed germination of over 90% was achieved, regardless of the region of origin, batch, variety, or population (Table 4). This enabled a reduced share of dormant seed (1 to 4%, taking into account all regions and lots). In this period, the presence of dead seeds from 2 to 5% and abnormal seedlings from 1 to 4% was determined; also taking into account all regions and tested seed batches (Table 4). During a seed storage period of forty months, seed dormancy decreased (0 to 2%), for all regions and seed patties examined. In this period, a decrease in seed germination was recorded to 83.7 to 83.8% for the averages of the examined regions with very low variability (CV%=0, 207). The realized difference caused by the influence of parties within the region was from 82 to 86% for the region of central Serbia to 82 to 85% for the two other investigated regions.

Table 3. Quality of seed lots from three regions after eighteen months of storage

Time of storage	Region	Seed lots from	Seeds %			Abnormal seedlings %
			Dormant	Germinated	Dead	
Eighteen months	Southeastern Serbia	Negotin	8 ab	88 c	1 b	1 b
		Zaječar	5 c	90 b	3 a	2 ab
		Boljevac	9 a	87 c	1 b	2 ab
		Niš I	7 b	90 b	1 b	2 ab
		Niš II	9 a	86 d	2 ab	3 a
		Niš III	4 c	92 a	2 ab	2 ab
		Average for the region	7.6	88.2	1.60	2.00
		Min	5	86	1	1
		Max	9	90	3	3
	Central Serba	Kragujevac	6 bc	91 ab	2 ab	1 b
		Gornji Milanovac	9 a	89 bc	1 b	1 b
		Batočina I	7 b	88 c	3 a	2 a
		Batočina II	5 c	92 a	1 b	2 a
		Rača I	9 a	89 bc	1 b	1 b
		Rača II	8 ab	90 b	1 b	1 b
		Average for the region	7.20	89.8	1.60	1.40
		Min	5	88	1	1
		Max	9	92	3	2
	Banat	Zrenjanin	5 b	91 a	2 a	2 a
		Srpska Crnja I	9 a	88 b	1 b	2 a
		Srpska Crnja II	8 ab	90 ab	1 b	1 b
		Srpska Crnja III	9 a	89 ab	1 b	1 b
		Vršac	9 a	89 ab	1 b	1 b
		Jaša Tomić	6 b	92 a	1 b	1 b
		Average for the region	8.00	89.4	1.20	1.40
		Min	5	88	1	1
		Max	9	91	2	2
CV % for the average of the region			5.26	0.934	15.7	21.7

Different small letters in the columns (a, b… x) have significant effect; P ≤ 0.05; Tukey's Multiple Range test

Table 4. Quality of seed lots from three regions after thirty months of storage

Time of storage	Region	Seed lots from	Seeds %			Abnormal seedlings %
			Dormant	Germinated	Dead	
Thirty months	Southeastern Serbia	Negotin	3 ab	91 b	3 b	3 ab
		Zaječar	2 b	93 a	3 b	2 b
		Boljevac	4 a	91 b	3 b	2 b
		Niš I	2 b	91 b	5 a	2 b
		Niš II	2 b	91 b	4 ab	3 ab
		Niš III	1 b	93 a	3 b	4 a
		Average for the region	2.60	91.4	3.60	2.40
		Min	2	91	3	2
		Max	4	93	5	3
	Central Serba	Kragujevac	2 b	92 ab	3 ab	3 ab
		Gornji Milanovac	3 ab	90 b	4 a	3 ab
		Batočina I	2 b	93 a	2 b	3 ab
		Batočina II	3 ab	92 ab	3 ab	2 b
		Rača I	3 ab	90 b	3 ab	4 a
		Rača II	4 a	91 b	2 b	3 ab
		Average for the region	2.60	91.4	3.00	3.00
		Min	2	90	2	2
		Max	3	93	4	4
	Banat	Zrenjanin	2 b	90 bc	4 a	4 a
		Srpska Crnja I	3 ab	91 b	3 ab	3 ab
		Srpska Crnja II	4 a	92 ab	2 b	2 b
		Srpska Crnja III	4 a	89 c	4 a	3 ab
		Vršac	2 b	93 a	4 a	1 c
		Jaša Tomić	2 b	91 b	3 ab	4 a
		Average for the region	3.00	91.0	3.40	2.60
		Min	2	89	2	1
		Max	4	93	4	4
CV % for the average of the region			8.45	0.253	9.17	11.5

Different small letters in the columns (a, b… x) have significant effect; P ≤ 0.05; Tukey's Multiple Range test

Table 5. Quality of seed lots from three regions after forty-two months of storage

Time of storage	Region	Seed lots from	Seeds %			Abnormal seedlings %
			Dormant	Germinated	Dead	
Forty-two months	Southeastern Serbia	Negotin	1 a	85 a	8 b	8 a
		Zaječar	0 b	84 ab	10 a	6 b
		Boljevac	1 a	83 ab	9 ab	7 ab
		Niš I	0 b	85 a	10 a	5 b
		Niš II	0 b	83 ab	9 ab	8 a
		Niš III	1 a	82 b	10 a	7 ab
		Average for the region	0.500	83.7	9.33	6.83
		Min	0	82	8	5
		Max	1	85	10	8
	Central Serba	Kragujevac	1 a	82 c	9 a	8 a
		Gornji Milanovac	1 a	85 ab	9 a	5 b
		Batočina I	0 b	84 b	8 ab	8 a
		Batočina II	0 b	83 c	9 a	8 a
		Rača I	0 b	86 a	7 b	7 ab
		Rača II	1 a	84 b	8 ab	9 a
		Average for the region	0.500	83.8	8.33	7.50
		Min	0	82	7	5
		Max	1	86	9	9
	Banat	Zrenjanin	0 b	85 b	9 ab	6 bc
		Srpska Crnja I	1 ab	82 c	10 a	5 b
		Srpska Crnja II	2 a	84 bc	7 bc	7 c
		Srpska Crnja III	1 ab	83 bc	8 b	8 b
		Vršac	1 ab	87 a	5 c	9 ab
		Jaša Tomić	0 b	83 bc	7 bc	10 a
		Average for the region	0.833	83.7	7.67	7.50
		Min	0	82	5	5
		Max	2	87	10	10
CV % for the average of the region			31.5	0.207	9.90	5.32

Different small letters in the columns (a, b… x) have significant effect; $P \leq 0.05$; Tukey's Multiple Range test

After this period of seed storage, the content of dead seeds increased: 7.63% (average for the Banat region) and 9.33 (average for the lots of the Southeast Serbia region). An increase in abnormal seedlings was also determined: 6.83 (average of batches from the region of Southeast Serbia) and 7.50% (average for the regions of Banat and Central Serbia).

The maximum achieved germination after thirty-eight months of seed storage is at the level of the average of the examined seed germination for different regions and seed lots. After forty-two months of storage, germination decreases (Table 5).

Table 6. Changes in seed quality during the investigated storage period (average of regions and seed lots)

Months of storage	Seeds %			Abnormal seedlings %
	Dormant	Germinated	Dead	
Six	21	77	1	2
Eighteen	8	89	1	2
Thirty	3	91	3	3
Forty-two	1	84	8	7
CV %	112	7.35	105	70.5

This indicates that the aging process of the seed has begun. The reduction of seed vitality and quality during the seed storage period depends on three important factors: the moisture content of the seed in balance with the relative humidity of the atmosphere, the storage temperature, and the gaseous environment *(Corbineau 2024; Nadarajan et al., 2023; Acharya et al., 1999)*.

CONCLUSION

The highest usable value of alfalfa seeds is achieved after storage periods of 18 and 30 months. Longer storage results in an increase in the proportion of dead seeds and abnormal seedlings, which reduces the percentage of germinating seeds. In a storage period of less than 6 months, the proportion of dormant seeds greater than 20% significantly affects reduced germination.

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REFERENCES

Acharya S. N., Stout D. G., Brooke B. and Thompson D. (1999). Cultivar and storage effects on germination and hard seed content of alfalfa. Can. J. Plant Sci., 79: 201–208

and seed sprouting. Agronomy Monograph, 29: 961-983

Baskin J.M., Baskin, C.C. (2021). The great diversity in kinds of seed dormancy: A revision of the Nikolaeva–Baskin classification system for primary seed dormancy. Seed Sci. Res., 31: 249–277

Bass L.N., Gunn C.R., Hesterman O.B. and Roos E.E. (1988). Seed physiology, seedling performance and seed sprouting. Agronomy Monograph 29: 961-983

Bass, L.N., Gunn, C.R., Hesterman, O.B. and Roos, E.E. (1988). Seed physiology, seedling performance

Bukvic Z., Pacek D., Popovic S., Rutar R., Stjepanovic M. Effect of temperature on germination and hard alfalfa seed. In : Delgado I. (ed.), Lloveras J. (ed.). Quality in lucerne and medics for animal production . Zaragoza : CIHEAM, 2001. p. 137-139 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 45)

Corbineau, F. (2024). The Effects of Storage Conditions on Seed Deterioration and Ageing: How to Improve Seed Longevity. Seeds, 3: 56–75.

Cuena Lombraña A., Dessì L., Podda L., Fois M., Luna B., Porceddu M., Bacchetta, G. (2024). The Effect of Heat Shock on Seed Dormancy Release and Germination in Two Rare and Endangered *Astragalus* L. Species (Fabaceae). Plants, *13*, 484. https://doi.org/10.3390/plants13040484

Čupić T., Popović S., Grljušić S., Tucak M., Andrić L., Šimić B. (2005). Effect of storage time on alfalfa seed quality. Jour. of Cen. Europ. Agric., 6: 65- 68.

Fairey, D.F., Lefkovitch, L.P. (1991). Hard seed content of alfalfa grow in Canada. Can. Jour. of Plant Scii., 71: 437-444.

Frischie S, Miller A.L, Pedrini S, Kildisheva O.A (2020). Ensuring seed quality in ecological restoration: native seed cleaning and testing. Rest. Ecol., 28: 239-248

Galussi, A.A., Argüello, J.A., Cerana, M.M., Maximino, M., Moya, M.E. (2016). Anatomical and chemical characteristics of the seed coat of Medicago sativa L. (alfalfa) cv. Baralfa 85 seeds and their association with seed dormancy. Phyton, Inter. Jour. of Expe. Botany, 84(1): 163-175.

Harrison R.J, Howieson J.G, Yates R.J, Nutt B.J. (2021). Long-term storage of forage legumes greatly alters the hard seed breakdown pattern in situ. Grass Forage Sci., 76: 72–81.

Jaganathan, G.K., Berry K. (2023) Climate Change during Cretaceous/Paleogene as a Driving Force for the Evolutionary Radiation of Physical Dormancy in Fabaceae. Seeds, 2: 309-317.

Kimura, E., Islam A. M. (2012). Seed scarification methods and their use in forage legumes. Rese. Jour. of Seed Sci., 5: 38-50.

Knežević J., Tomić D., Jovanović D., Tmušić N., Štrbanović R., Poštić D., Stanisavljević R. (2019): Seed Quality of Oilseed Rape Varieties with Different Size and Colors After Three and Fifteen Months Storage. Tarım Bilimleri Dergisi-Jour. of Agric. Scie. 25: 449–458.

Nadarajan J., Walters C., Pritchard H.W., Ballesteros D., Colville L. (2023). Seed longevity—The evolution of knowledge and a conceptual framework. Plants, 12, 471.

Nagel M and A Börner (2010). The longevity of crop seeds stored under ambient conditions. Seed Scie. Res., 20(1): 1-12.

Official gazette SFRJ, No. 47/87. Rule on the quality of seeds of agricultural plants. 1987 and supplement to 34/2013)

Rawlins J.K., Roundy B.A., Davis S.M., Egget D. (2012). Predicting germination in semi-arid wildland seedbeds. I. Thermal germination Models. Environ. Exp. Bot., *76*: 60–67.

seed germination and viability of some medicinal plants. Afr. J. Agric. Res. 20

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

Stanisavljević R., Poštić D., Milenković J., Đokić D., Tabaković M., Jovanović S., Štrbanović R. (2018). Possibilities for improving the quality of alfalfa seed by applying temperature treatments before sowing. Journal on Proc. and Ene. in Agric., 22(2):76-9.

Stanisavljevic R., Poštić D., Štrbanović R., Tabaković M., Jovanović S., Milenković J., Đokić D., Terzić D. (2020): Effect of seed storage on seed germination and seedling quality of Festulolium in comparison with related forage grasses. *Trop. Grass.-Forrajes Tropicales* 8(2): 125-132.

Tiwari R.K.S. and Das K. (2014). Impact of differential storage conditions on seed germination and viability of some medicinal plants. African Jour. Agric. Res., 9 (20): 1578-1585.

Xie J., Li Y., Jiang G., Sun H., Liu X., Han L. (2023). Seed color represents salt resistance of alfalfa seeds (*Medicago sativa* L.): Based on the analysis of germination characteristics, seedling growth and seed traits. Front Plant Sci. 2023 doi: 10.3389/fpls.2023.1104948.

Zimmermann L.R., Galussi A.A., Martinelli A.H.M., Fernández A.P., García A.H., Pitter J.R., Zecchin A.R. and Dechanzi D.I. (1998). Hard seed viability in *Medicago sativa* L., *Lotus corniculatus* L., *Trifolium repens* L., *T. pratense* L. and *Melilotus alba Med*. Seed Sci. Technol., 26: 271-273.

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