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POSSIBILITIES FOR IMPROVING THE QUALITY OF ALFALFA SEED BY APPLYING TEMPERATURE TREATMENTS BEFORE SOWING

MOGUĆNOSTI POBOLJŠANJA KVALITETA SEMENA LUCERKE PRIMENOM TEMPERATURNIH TRETMANA PRED SETVU

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ABSTRACT

In addition to normal seeds, hard seeds of alfalfa have often been found after harvest. Such seeds prevent the penetration of water and gases into the seed interior, causing the reduction in germination. They do not tend to germinate even under ideal laboratory or field conditions, or they exhibit late germination. Consequently, they are of no relevance to planting crops. A decrease in the amount of hard seeds and an increase in germination can be achieved by scarification of acids, physical damage to the seed coat, hot water, cooling, etc. Temperature treatment scarification prior to sowing is a simple and inexpensive solution, safe for humans and the environment. The tests were carried out on three alfalfa cultivars: 'Medijana', 'Banat' and 'Zaječarska 83'. Their seeds were exposed to temperatures of 70 ^oC (for 10, 30, 60 and 90 minutes), 80 ^oC (for 10, 30, 60 and 90 minutes) and 90 ^oC (for 10, 30, 60 and 90 minutes). After the treatment under laboratory conditions, the germination rate and the share of hard/dormant seeds were investigated. The results obtained indicate that the increase in germination can be significant ($p \geq 0.05$) provided temperature seed treatments are applied. Furthermore, the optimal temperature treatment was found to be essentially dependent on the specific alfalfa cultivar.

***Key words**: germination, hard seed, cultivar, alfalfa, temperature treatment.*

REZIME

U semenu lucerke se, osim normalnog semena, javlja i seme sa tvrdom semenjačom koja onemogućava prodiranje vode i gasova u unutrašnjost i tako sprečava klijanje. Zbog toga, tvrda semena ne klijaju, iako su u idealnim laboratorijskim uslovima ili u polju. Dešava se i da klijaju naknadno, kada su klijanci iz normalnih semena razvijeni, ali kao takvi nemaju značaja za zasnivanje useva. Smanjenje broja tvrdih semena i povećanje klijavosti može se postići skarifikacijom semena kiselinama, fizičkim oštećenjem semenjače, toplom vodom, hlađenjem i dr. Skarifikacija primenom temperaturnih tretmana pred setvu je jednostavna, jeftina i bezbedna za čoveka i okolinu. Ispitivanja su sprovedena na tri sorte lucerke: Medijana, Banat, Zaječarska 83. Seme sve tri sorte je izlagano 10, 30, 60 i 90 minuta na temperaturama od 70, 80 i 90^{o}C. Nakon tretmana u laboratorijskim uslovima ispitivana je klijavost i tvrda-dormantna semena. Dobijeni rezultati ukazuju da se temperaturnim tretmanima semena može značajno ($p \geq 0.05$) uticati na povećanje klijavosti. Utvrđena je značajna interakcija sorta x temperaturni tretman, što ukazuje da je optimalni temperaturni tretman bio različit za različite sorte.

***Ključne reči**: klijavost, tvrdo seme, sorta, lucerka, temperaturni tretmani.*

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the most important fodder plant in Serbia. The great significance of alfalfa is reflected in the production of high-quality fodder (*Knežević, 2013*). Alfalfa seed is also required in domestic and foreign markets (*Terzić, 2010; Terzić et al., 2016*). In addition, alfalfa is a significant crop due to its strong nitrogen fixation and the ability to provide itself with the necessary nitrogen, thus reducing the cost of fertilizers and improving soil for the next crop. A total of 200,000 hectares is devoted to alfalfa in Serbia. The average length of crop stand is 4-5 years. This means that every year about 40 to 50 thousand hectares are devoted to alfalfa. The average sowing rate is about 20 kg/ha^{-1}, thus the seed requirements for alfalfa producers in Serbia approximate to 900 tons. On balance, Serbia is mainly an exporter of alfalfa seed, and a temporary importer (in extremely unfavorable years). Notwithstanding the varying market and climatic conditions in Serbia, the production of alfalfa seed is economically beneficial. This is confirmed by the economic indicators obtained in the period 2010-2014, according to which the production of alfalfa seed is characterized by relatively low investments per unit area (499.8 ha ha-1) (*Pajić and Marković, 2016)*.

Numerous studies have found that perennial forage legumes are characterized by the presence of hard-dormant seeds (*Zimmermann et al., 1998; Velijević et al., 2017*), which is associated with the reduction in germination. The seed coat of hard seeds contains more polyphenols, tannins, and other matters, thus possessing a different anatomical and morphological composition compared to normal seed which readily germinates (*Galussi et al., 2015*). According to *Karagić et al., (2011*) the seed germination in Serbia depends on the prevailing conditions during the year (ranging from 82 % to 91 %). The share of hard seed was from 3 % to 9 %. After a 5-year experiment conducted in Canada, *Fairey and Lefkovitch (1991*) reported that the presence of hard seeds in alfalfa ranged from 21 % to 36 %. According to *Čupić et al., (2005*), hard seed was present in alfalfa from 16 % ('Osječka 90') to 33 % ('Vuka') under the existing conditions in Croatia. According to

Zimmermann et al., (1998), under the existing conditions in Argentina, the presence of hard seed in alfalfa was 50 %. When planting alfalfa, farmers want to achieve a desired number of plants with as little seed as possible, which requires high germination. Germination and dormancy are complex seed processes attributable to a large numbers of genes and plant hormones (*Bewley, 1997*). Moreover, seed germination and dormancy are influenced by agroecological conditions, especially during seed maturation (*Fairey and Lefkovitch, 1991*). Owing to the impermeable seed coat, hard seeds exhibit late germination, and thus have no effect on the crop establishment (*Bass, 1988*).

Alfalfa hard seeds are impermeable to water and gases, and do not germinate even under idel laboratory and field conditions. In order to facilitate the reception of water and gases of the legume seed, it is possible to use acids, mechanical treatments, temperature treatments, laser and other methods (*Velijević et al., 2018, Đokić et al., 2017, Kimura and Islam, 2012*). Temperature treatments are easy to operate, economical, safe for workers, environmentally safe, and therefore acceptable. The purpose of this investigation was to apply temperature treatments to the seed of three alfalfa cultivars in a two-year experiment, with an emphasis on the optimal methods of reducing hard seeds and increasing seed germination.

MATERIAL AND METHOD

Alfalfa seed of three cultivars ('Banat', 'Medijana' and 'Zaječarska-83') was harvested in early August during a two-year investigation (2016 and 2017). The period of seed maturing and ripening in 2017 was characterized by high temperatures and low precipitation. The precipitation recorded was above average and with temperatures below the multi-year average in 2016 (weather conditions are not shown). Laboratory tastings was performed at the Seed Control Laboratory of the Institute for Plant Protection and the Environment, Belgrade. The seed samples were subsequently dried to a moisture content of less than 12 %. In both years, at the time of autumn seeding (September), the sample seed was exposed to three temperatures (T = 70 ^{o}C, 80 ^{o}C, and 90 ^{o}C) at exposure times of 10, 30, 60, and 90 minutes. The drying experiment was carried out in a laboratory "Binder" dryer. Furthermore, according to the standard procedure, the seed germination was examined using the filter paper with four replicates (*ISTA 2016*). The tetrazolium test was used to separate hard from dead seeds (*ISTA 2008*). The results were analyzed using the analysis of variance (ANOVA, F test). The Tukey's multiple range test was applied to establish the difference between the treatments applied. The relationship between the traits analyzed was established by the Pearson's correlation test. The germination data and dormancy percentages were computed using the arcsine transformation (√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

The seeds were exposed to temperature treatments to influence the permeability to water and gases, affect the reduction in hard seeds, and increase the germination. Using the F test, significant effects (P ≤ 0.001 to P ≤ 0.05) of the year, temperature treatments and cultivars were found on the seed germination and the presence of hard-dormant seeds. The interaction between years and temperature treatments exhibited a non-significant effect on seed germination and dormancy (P > 0.05), whereas other interactions were of significant importance to seed germination and dormancy (Table 1).

Table 1. Result of the analysis of variance (ANOVA, F test) on the effect of years, temperature treatments and cultivars on the seed germination and the presence of hard seeds

Source	df	Germinated seed (%)	Hard seed (%)
Years (A)	1	*	**
Temperature treatments (B)	12	***	***
Cultivar (C)	2	*	*
Interactions			
AxB	12	ns	ns
AxC	2	**	**
BxC	24	*	*
AxBxC	24	*	*

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.*

During the first year of the experiment (2016) involving the 'Banat' variety, the optimal temperature treatment for increasing the seed germination was 80 ^{o}C for 60 minutes (an increase of 16 %). Furthermore, an increase in germination of 16 % was achieved in the 'Medijana' variety, but after the temperature treatment of 80 °C for 90 minutes and 90 °C for 60 minutes (Table 2). The 'ZA-83' germination increased by 12 %, using treatments of 80 °C for 60 minutes and 90 °C for 30 minutes. Temperature treatments of 90 °C for prolonged periods (90 and 60 minutes) had the strongest impact on the reduction in hard seeds (2 % and 5 % for different cultivars). However, these temperatures did not achieve maximum germination. Compared to the first year of the experiment (with lower temperatures and higher precipitation), more hard seed (4 %) were recorded during the second year of the experiment. In this year, a significant interaction between the cultivars and the temperature treatments was established (Table 1 and Table 3). It was found that the amount of hard seeds is associated with the influence of higher temperatures in the seeding period. This is consistent with the results of *Fairey and Lefkovitch (1991*). The cultivar impact on the hard seed share during the first year varied as follows: CV = 32.7 % for the treatment with T90 ^{o}C lasting 60 minutes, CV = 28.3 % for the treatment with T90 ^{o}C lasting 90 minutes, and CV = 24.1 % for the treatment T90^{o}C lasting 30 minutes. In the second year, the influence of the alfalfa cultivars examined on the hard seed variability was significantly higher (CV = 100 % for the treatment with T = 90^{o}C lasting 90 minutes, CV = 50.0 % for the treatment T = 90^{o}C lasting 60 minutes, and CV = 34.6 % for the treatment with T = 80^{o}C lasting 90 minutes) (Table 2 and Table 3). Tukey's test, p≤ 0.01, ± standard error of mean, was applied to assess the significance by column a.b. (small letters) and row A.B. (capital letters) for influence of cultivar In the first year of the experiment, the highest variability of seed germination depending on the cultivar was determined for the treatment with T70 ^{o}C lasting 10 minutes (CV = 4.17 %) and the lowest for the treatment with T80^{o}C lasting 60 minutes (CV = 1.08 %).

In the second year, the germination variability ranged from CV = 5.03 % (T90 °C lasting 30 minutes) to CV = 0.00 % (T80 °C lasting 30minutes). In previous studies, different temperatures (below 40 °C to over 100 °C) and exposure times (one minute to 21h) were used in different studies on alfalfa seed cultivars. For example, heat treatments between 40 and 50 °C had little impact on the reduction in hard seed (*Rutar et al., 2001*). High temperatures (about 80 °C) can be effective in reducing hard-dormant alfalfa seed in relation to lower temperatures. However, negative effects were observed when the temperature was 80 °C or 90 °C, as well as with longer exposure times (*Rincker, 1954; Rutar et al., 2001*). The negative influence of high temperatures (90°C or 80°C) and longer exposure times is reflected in the increase in dead seeds (*Rincker, 1954, Rutar et al., 2001*). High seed damage (dead seeds) was detected during seed exposure to 104 °C for 1h and longer (*Rincker, 1954*). It was possible to increase the germination of red clover seed (by 16 %) by exposing the seed to an optimum temperature treatment of 80 °C for 30 minutes. The germination of six investigated red clover cultivars was increased by 14 %, 12 %, and 11 % using the temperature treatment of 80 °C and exposure times of 10, 60 and 90 minutes (*Velijević et al., 2018*).

Table 2. Influence of the air drying temperature on the quality of alfalfa seeds two months after harvest (2016)

Temperature (T, °C)	Germinated seeds (G, %)			Hard seeds (H, %)			$\bar{X}$ %		CV %	
	Banat	Medijana	ZA 83	Banat	Medijana	ZA 83	G	H	G	H
Control	$78^{\pm1.05cB}$	$79^{\pm1.10eB}$	$81^{\pm1.11cA}$	$15^{\pm1.12aB}$	$17^{\pm0.90aA}$	$13^{\pm1.12aC}$	79	15	1.93	9.18
T. 70°C 10'	$81^{\pm1.02bcB}$	$81^{\pm1.12deB}$	$87^{\pm0.95a\text{-}cA}$	$10^{\pm1.12bA}$	$9^{\pm1.12bA}$	$10^{\pm1.10abA}$	83	10	4.17	5.97
T. 70°C 30'	$84^{\pm1.09a\text{-}cB}$	$83^{\pm1.01c\text{-}eB}$	$88^{\pm1.10ab\ A}$	$8^{\pm1.11b\text{-}dA}$	$8^{\pm1.10bcA}$	$9^{\pm1.09bcA}$	85	8	3.11	6.93
T. 70°C 60'	$88^{\pm0.95abA}$	$86^{\pm0.98b\text{-}dA}$	$87^{\pm1.05a\text{-}cA}$	$7^{\pm1.00b\text{-}dB}$	$7^{\pm1.05bcB}$	$9^{\pm0.95bcA}$	87	8	1.15	15.1
T. 70°C 90'	$87^{\pm1.12abA}$	$89^{\pm1.03a\text{-}cA}$	$86^{\pm1.12bcA}$	$7^{\pm0.90b\text{-}dA}$	$8^{\pm1.07bcA}$	$7^{\pm1.12b\text{-}dA}$	87	7	1.75	7.87
$\bar{X}$	84	84	86	8	8	9	-	-	-	-
T. 80°C 10'	$88^{\pm1.05abB}$	$87^{\pm1.08b\text{-}dB}$	$90^{\pm1.04abA}$	$9^{\pm1.04bcA}$	$9^{\pm1.09bcA}$	$8^{\pm1.10b\text{-}dA}$	88	9	1.73	6.66
T. 80°C 30'	$92^{\pm1.08aA}$	$90^{\pm1.04abB}$	$92^{\pm1.10abA}$	$7^{\pm1.06b\text{-}dA}$	$8^{\pm1.07bcA}$	$7^{\pm1.11b\text{-}dA}$	92	7	1.26	7.33
T. 80°C 60'	$94^{\pm0.95aA}$	$92^{\pm1.09abB}$	$93^{\pm1.03aAB}$	$6^{\pm1.05c\text{-}eAB}$	$7^{\pm1.10bcA}$	$5^{\pm1.05b\text{-}fB}$	93	6	1.08	16.7
T. 80°C 90'	$92^{\pm1.11aB}$	$95^{\pm1.12aA}$	$90^{\pm1.00abB}$	$5^{\pm0.95d\text{-}fA}$	$5^{\pm1.12bcA}$	$3^{\pm0.98efB}$	90	4	2.73	26.7
$\bar{X}$	92	91	91	7	7	6	-	-	-	-
T. 90°C 10'	$92^{\pm1.12aA}$	$90^{\pm1.08abB}$	$92^{\pm1.12abA}$	$7^{\pm1.07b\text{-}dA}$	$8^{\pm1.05bcA}$	$7^{\pm1.12b\text{-}dA}$	91	7	1.26	7.87
T. 90°C 30'	$91^{\pm1.05aB}$	$94^{\pm1.10aA}$	$93^{\pm1.11aAB}$	$5^{\pm1.12d\text{-}fB}$	$8^{\pm0.90bcA}$	$6^{\pm1.12c\text{-}eAB}$	93	6	1.65	24.1
T. 90°C 60'	$89^{\pm1.08aC}$	$95^{\pm1.12aA}$	$92^{\pm1.12abB}$	$3^{\pm1.08e\text{-}fB}$	$6^{\pm1.12bcA}$	$5^{\pm1.10d\text{-}fAB}$	92	5	3.23	32.7
T. 90°C 90'	$88^{\pm1.11abB}$	$90^{\pm0.95abA}$	$88^{\pm0.98abB}$	$2^{\pm1.12fB}$	$3^{\pm1.12cA}$	$2^{\pm1.12fB}$	88	2	1.30	28.3
$\bar{X}$	90	92	91	4	6	5	-	-	-	-
$\bar{X}$ total	88	88	89	7	8	7	-	-	-	-
CV % g.	5.29	5.83	3.88	46.7	40.4	42.1	-	-	-	-

Tukey's test, p≤ 0.01, ± standard error of mean, was applied to assess the significance by column a.b. (small letters) and row A.B. (capital letters) for influence of cultivar

Table 3. Influence of the air drying temperature on the quality of alfalfa seed two months after harvest (2017)

Temperature (T, °C)	Germinated seeds (G, %)			Hard seeds (H, %)			$\bar{X}$ %		CV %	
	Banat	Medijana	ZA 83	Banat	Medijana	ZA 83	G	H	G	H
Control	$76^{\pm1.09bB}$	$78^{\pm1.22bB}$	$81^{\pm0.91bA}$	$20^{\pm1.01aA}$	$21^{\pm0.95aA}$	$17^{\pm1.127aB}$	78	19	3.21	10.8
T. 70°C 10'	$83^{\pm1.05abB}$	$85^{\pm0.89abA}$	$82^{\pm0.83bB}$	$9^{\pm1.02bB}$	$13^{\pm1.23bA}$	$15^{\pm1.14aA}$	83	12	1.83	24.8
T. 70°C 30'	$85^{\pm1.09abB}$	$88^{\pm1.02abA}$	$83^{\pm1.09bC}$	$8^{\pm1.21bA}$	$6^{\pm1.13cB}$	$8^{\pm1.10bA}$	85	7	2.95	15.7
T. 70°C 60'	$89^{\pm0.88abA}$	$89^{\pm0.93aA}$	$85^{\pm1.11abB}$	$6^{\pm1.21bB}$	$7^{\pm1.22cAB}$	$8^{\pm0.85bA}$	88	7	2.63	14.3
T. 70°C 90'	$90^{\pm1.11aB}$	$93^{\pm1.11aA}$	$88^{\pm1.09abC}$	$5^{\pm0.89bB}$	$6^{\pm1.11cAB}$	$7^{\pm1.02bA}$	90	6	2.79	16.7
$\bar{X}$	87	89	85	7	8	10	-	-	-	-
T. 80°C 10'	$86^{\pm1.51abB}$	$88^{\pm1.14abA}$	$88^{\pm1.24abA}$	$8^{\pm1.09bB}$	$9^{\pm1.14cA}$	$6^{\pm1.14bC}$	87	8	1.32	19.9
T. 80°C 30'	$89^{\pm0.78abA}$	$89^{\pm0.99aA}$	$89^{\pm1.13abA}$	$7^{\pm1.21bA}$	$8^{\pm1.21cA}$	$5^{\pm1.115bcB}$	89	7	0.00	22.9
T. 80°C 60'	$93^{\pm0.66aB}$	$95^{\pm1.21aA}$	$91^{\pm0.83aB}$	$4^{\pm1.09cAB}$	$6^{\pm1.14cA}$	$3^{\pm1.11cB}$	93	4	2.15	35.3
T. 80°C 90'	$95^{\pm1.22aA}$	$93^{\pm1.10aB}$	$93^{\pm1.09aB}$	$4^{\pm0.77cA}$	$4^{\pm1.15dA}$	$2^{\pm0.99cB}$	94	3	1.23	34.4
$\bar{X}$	91	91	90	6	7	4	-	-	-	-
T. 90°C 10'	$93^{\pm0.72aAB}$	$94^{\pm1.13aA}$	$92^{\pm0.56aB}$	$7^{\pm1.12bA}$	$6^{\pm1.21cAB}$	$5^{\pm1.03bcB}$	93	6	1.08	16.7
T. 90°C 30'	$85^{\pm1.12abC}$	$94^{\pm0.99aA}$	$90^{\pm1.23aB}$	$6^{\pm1.02dA}$	$5^{\pm0.96cA}$	$3^{\pm1.14cAB}$	90	5	5.03	32.7
T. 90°C 60'	$83^{\pm1.21abC}$	$92^{\pm1.29aA}$	$89^{\pm1.31abB}$	$2^{\pm1.11cB}$	$3^{\pm1.03dA}$	$1^{\pm1.14cC}$	88	2	2.21	50.0
T. 90°C 90'	$81^{\pm1.16bB}$	$83^{\pm0.79abB}$	$86^{\pm0.99abA}$	$1^{\pm1.02cB}$	$2^{\pm1.10dA}$	$0^{\pm1.15cC}$	83	1	3.02	100
$\bar{X}$	86	91	89	4	4	2	-	-	-	-
$\bar{X}$ total	87	89	88	6	6	5	-	-	-	-
CV % g.	5.51	4.89	3.99	63.5	61.4	77.5	-	-	-	-

Tukey's test, p≤ 0.01, ± standard error of mean, was applied to assess the significance by column, a.b. (small letters) and row A.B. (capital letters) for influence of cultivar

CONCLUSION

Considering that seed germination is of essential importance to the production of alfalfa seed, the optimal methods of reducing hard seed and increasing seed germination should be developed. Temperature treatments exerted a significant effect on the alfalfa seed germination and hard seed number ($P \leq 0.001$). Moreover, the interaction between alfalfa cultivars and treatment temperatures also had a considerable impact on these traits ($P \leq 0.01$). The interaction between planting years and treatment temperatures exhibited the lowest impact on the alfalfa germination and hard seed number (statistically non-significant). In both years, an increase in germination was obtained using the highest temperatures (80 and 90 ^oC). The lowest hard seed number was obtained with temperatures of 80 and 90 ^oC and seed exposure times of 30, 60 and 90 minutes. The use of the longest seed exposure time of 90 minutes and the highest treatment temperature of 90 ^oC resulted in the largest decrease in hard seed number, as well as in seed germination decrease in both years. In all the alfalfa cultivars examined, the best germination and lowest hard seed share was achieved using treatments temperatures of 80 and 90 ^oC in combination with exposure times of 30 and 60 minutes.

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