Biblid: 1821-4487 (2014) 18; 5; p 229-231
UDK: 633.31

Original Scientific Paper
Originalni naučni rad

APPLICATION OF DIFFERENT POLYETHYLEN GLYCOLE CONCENTRATIONS AND EVALUATION OF DIFFERENT METHODS FOR GERMINATION OF ALFALFA

PRIMENA RAZLIČITIH KONCENTRACIJA *POLYETHYLENE GLYCOLA* I OCENA RAZLIČITIH METODA NA KLIJAVOST SEMENA LUCERKE

Ratibor ŠTRBANOVIĆ, Rade STANISAVLJEVIĆ*, Lana ĐUKANOVIĆ*, Dobrivoj POŠTIĆ*, Jordan MARKOVIĆ**, Dragoslav ĐOKIĆ**, Nenad DOLOVAC**
**Institute for Plant Protection and Environment, 11040 Belgrade, Teodora Drajzera 9, Serbia*
***Institute for Forage Crops, Kruševac, 37251 Globoder, Serbia*
e-mail: ratibor.strbanovic@yahoo.com

ABSTRACT

This paper presents the germination three cultivars of alfalfa using different concentrations of PEG-6000 (Polyethylene glycol) and control. The aim of this study was to determine the tolerance of alfalfa under drought stress effects and the choice of optimal testing methods. Seed germination and establish the presence of hard seed alfalfa was done by the standard method without pretreatment and with pretreatment. Readout germination of alfalfa seeds was done after ten days, according to the Rules for testing seed quality of agricultural products. The concentration of PEG of 0.4 MPa has proven to be optimal for assessing the seed germination of alfalfa. The alfalfa cultivars have behaved differently towards different concentrations on PEG, indicating the possibility of correct selection of alfalfa cultivars for autumn sowing period (September) after seed harvest in August.

***Key words**: alfalfa, germination, PEG 6000, seed.*

REZIME

Lucerka je najvažnija višegodišnja i višeotkosna krmna biljka, koja daje visoke prinose i odličan kvalitet krme u različitim ekološkim uslovima. Gajena u kombinovanoj proizvodnji (seme/krma) tokom višegodišnjeg korišćenja veoma je varijabilna prema visini prinosa semena (CV = 29,4% do 47,5%) dok je prema visini prinosa krme sa nižom varijabilnšću (CV=17,1% do 25,7%), i najmanjom varijabilnošću za kvalitet semena (klijavost CV=5,4% do 6,4%). U radu je prikazana klijavost tri sorte lucerke primenom različitih koncentracija PEG-6000 (Polyethylene glycol) i kontrole. Cilj ovog rada je utvrđivanje tolerantnosti lucerke prema efektu stresa na sušu i izbor najoptimalnije metode ispitivanja. Ispitivanje klijavosti i utvrđivanje prisustva tvrdih semena lucerke rađeno je standardnom metodom bez predtretmana i sa predtretmanom. Očitavanje klijavosti semena lucerke urađeno je nakon deset dana, prema pravilniku za ispitivanje kvaliteta semena poljoprivrednog bilja. Koncentracija PEG-a od 0,4 Mpa pokazala se optimalnom za ocenu klijavosti semena lucerke. Sorte lucerke su se različito ponašale prema različitim koncentracijama na PEG, što ukazuje na mogućnost pravilnog izbora sorti lucerke za jesenji setveni rok (septembar) nakon žetve semena u avgustu mesecu. Sorte lucerke tolerantnije na PEG u jesenjem setvenom roku, kada je i najčešće nedostatak vlage u zemljištu u našim klimatskim uslovima bile bi uspešnije za zasnivanje lucerišta. Ove sorte bi bile pogodne i za uključivanje u proces selekcije radi stvaranja sorti tolerantnijih prema stresu na sušu.

***Ključne reči:** PEG 6000, lucerka, seme, klijavost.*

INTRODUCTION

Alfalfa is a nutrient rich, high-yielding and multi-cutting forage crop, adapted to diverse environmental conditions (*Đukić and Erić, 1995*). Depending on the genotypes characterized by high variability in forage yield (CV=28.6 %) (*Štrbanović, 2010*). Grown in a combined production (seed / fodder) during the years of use is very variable according to the amount of seed yield (CV=29.4 % to 47.5 %), while according to the amount of forage yield with lower variability (CV=17.1 % to 25.7 %), and lowest variability for seed quality (germination CV=5.4 % to 6.4 %) (*Stanisavljević et al., 2012*). In Serbia, seed production is mainly from the combined production of which is the harvest of seeds from the second cut in August (*Terzić, 2011*). The resulting seeds can be used for establishing alfalfa crop in the autumn sowing period, which is one to two months after the harvest of alfalfa seed. It can also be used in the spring or autumn sowing period in the coming years.

In the period of autumn sowing of alfalfa in the west Balkan area, the lack of rainfall is frequent, which leads to the shortage of soil moisture. In such circumstances there is a possibility of drought mitigation planting alfalfa seed which would require less moisture in the soil to germinate, which for practical conditions gave a high contribution to establishing alfalfa crop. Also, tolerance genotypes to drought is a very important trait for breeding programs and the creation of tolerant varieties under drought stress conditions in Southeast Europe (*Carmen and Nedelea, 2012*).

On the other hand in the time period between one to two months after seed harvest (August and September) in alfalfa is already hard seed (*Fairey and Lefkovitch, 1991; Hall et al., 1998; Čupić et al., 2005; Kimura and Islam, 2012*) which is attributed to seedlings to not allow the penetration of water and gas. On establishing the alfalfa crop, hard seeds germinate later, and can not withstand the competition already developed seedlings and have a great contribution to establishing alfalfa crop (*Albrecht et al., 2009*).

MATERIAL AND METHOD

Harvesting seeds of different cultivars of alfalfa was conducted in August 2013th year. After harvest, seed tests were conducted in September of the same year, 45 days after harvest. These tests were conducted on seeds of three different cultivars of alfalfa: Kruševačka 28 (N 45º34'47.18" E 20º35'36.52"; 72 m asl.), Osiječka 66 (N 45º00'13.06" E 13º58'29.65"; 172 m asl.), NS Mediana (N 45º32'15.56" E 20º03'13.17"; 71 m nv.).

Seed quality of alfalfa was done with three different methods:

A0 - Seeds were placed in Petri dishes on filter paper moistened with distilled water until complete saturation and placed on pretreatment (+4 ºC temperature) for 5 days. Then the seeds were transferred in hothouse at a temperature of 20 ºC and determined the energy germination (after four days) and total germination (after ten days) of the Rules of the quality of seeds of agricultural plants "Official Gazette of SFRY ", no . 47/87 Republic of Serbia.

A1 - the seeds were placed in Petri dishes on the filter paper, but without the addition of distilled water and placed in a pretreatment (+4 ºC temperature) for 5 days. Then the seeds were transferred in hothouse at a temperature of 20 ºC and determined the energy germination (after four days) and total germination (after ten days) of the Rules of the quality of seeds of agricultural plants "Official Gazette of SFRY", no. 47/87 Republic of Serbia.

A2- seeds were placed in Petri dishes on filter paper moistened with distilled water until fully saturated and placed in hothouse at a temperature of 20 ºC and determined the germination (after four days) and total germination (after ten days) of the Rules of the quality of seeds of agricultural plants "Official Gazette of SFRY", no. 47/87 Republic of Serbia.

Then germination was studied all the three cultivars of alfalfa with varying concentrations of Polyethylene glycol 6000 (PEG): Concentration I - 0.4 MPa (B1), Concentration II - 0.7MPa (B2), Concentration III - 1.0 MPa (B3) and control without the use of PEG (B0). PEG was applied on the basis of recommendations (*Villela et al., 1991*).

The Petri dishes are directly charged PEG 10 ml and seeds were transferred to the hot bed temperature of 20 ºC and determined the energy germination (after four days) and total germination (after ten days).

The obtained experimental data were processed by a mathematical statistical procedure using the statistical package STATISTICA 8.0 for Windows. The differences between the treatments were determined by analysis of the variance (ANOVA).

RESULTS AND DISCUSSION

The methods used to investigate the germination of alfalfa given different results, indicating the coefficient of variation for germination (K-28 CV=6.256 %, OS-66 CV=18.739 %, CV=19.794 % of the NS-Mediana), the presence of hard seed (K-28 CV=52,715 %, OS-66 CV=19.868 %, CV=26.051 % of the NS-Mediana), and fresh seed (K-28 CV=69,289 %, OS-66 CV=78 976 %, NS-Mediana CV=37.796 %). Generally in all cultivars of alfalfa significantly higher ($p \leq 0.05$) germination was determined by the standard method table 1. This was expected, because the pretreatment Ph (precooling) effect on the termination of dormancy and decrease the percentage of hard seeds, seedlings in these conditions has become more permeable to water and gases, which led to such normal seeds germinate table 1. On the long side of A2 is determined using significantly more hard seeds in relation to the method A1, and A1 is a method found significantly more hard seeds of standard methods resulting in reduced germination. These results variation of hard seeds depending on variety and location of production in the period after harvest (K-28 4%, OS-66 20 %, NS-Mediana 25 %) are consistent with the results (*Hall et al., 1998; Kimura and Islam, 2012*). In practical agronomic practices presence hard seeds is largely determined by the management and use of seeds (*Fairey and Lefkovitch, 1991; Čupić et al., 2005*).

On the tested cultivars, the standard method has been established and the difference in fresh seeds (K-28 1 %, OS-66 1 %, NS-Mediana 5 %) table 1.

Table 1. Impact methods (A0, A1, A2) evaluation of seed germination of three cultivars of alfalfa

Cultivar	Method	Seed		
		Germination %	Hard seed %	Fresh seed %
K-28	A0	94 ± 0.651 A	4 ± 6.232 C	1± 0.344 B
	A1	90 ± 0.705 B	7 ± 0.121 B	1± 0.101 B
	A2	83 ± 0.516 C	12 ± 0.321 A	3 ± 0.621 A
	CV %	6.256	52.715	69.289
OS-66	A0	77 ± 0.405 A	20 ± 0.356 C	1± 0.432 C
	A1	60 ± 0.296 B	26 ± 0.601 B	12± 0.563 B
	A2	54 ± 0.712 C	30 ± 0.198 A	15± 0.258 A
	CV %	18.739	19.868	78.976
NS-Mediana	A0	69 ± 1.236 A	25 ± 0.987 C	5 ± 0.321 AB
	A1	52 ± 0.986 B	42 ± 1.109 B	6 ± 0.321 A
	A2	48 ± 1.065 C	40 ± 1.258 A	10 ± 0.321 B
	CV %	19.794	26.051	37.796

Dancanov test, A, B,…Different letters between treatment denote significant differences ($p \leq 0.05$), Values are mean ± standard error of the mean (SEM).

Polyethylene glycol simulates drought stress conditions and is acceptable for this purpose in alfalfa seed (*Carmen and Nedelea, 2012; Tilaki et al., 2009; Tiryaki et al., 2009*). However the optimal concentration of seed of agricultural plants are different (*Villela, 1991*).

Table 2. The effect of different concentrations of PEG on the germination of alfalfa cultivars

Cultivars	PEG	Seed %	
		Germination	Hard seed
K-28	B0	94 ± 0.563 A	4 ± 0.452 A
	B1	62 ± 0.456 B	5 ± 0.423 B
	B2	35 ± 0.369 C	3 ± 0.235 C
	B3	0 ± 0.537 D	0 ± 0.652 D
OS-66	B0	77 ± 0.495 A	20 ± 0.478 A
	B1	71 ± 0.631 B	17 ± 0.452 B
	B2	29 ± 0.563 C	7 ± 0.429 C
	B3	0 ± 0.000 D	0 ± 0.000 D
NS-Mediana	B0	69 ± 0.665 A	25 ± 0.258 A
	B1	61 ± 0.456 B	20 ± 0.456 B
	B2	31 ± 0.236 C	4 ± 0.253 C
	B3	0 ± 0.425 D	0 ± 0.000 D

Dancanov test, A, B,…Different letters between treatment denote significant differences ($p \leq 0.05$), Values are mean ± standard error of the mean (SEM).

In our studies table 2 it has been found that the use of PEG at various concentrations is influenced to a significant reduction in germination compared to control. This is consistent with the results of (*Wang et al., 2003; Wang et al., 2009*). Variant with the strongest concentration of PEG-B3 led to a completely disable the germination of alfalfa seed in all three cultivars, and accordingly influenced the lack of hard seeds. So any stronger concentration of PEG was statistically significant ($p \leq 0.05$) effect on reducing germinated and hard seeds of alfalfa table 2.

According to our research for the evaluation of alfalfa seed germination optimum concentration was 0.4 MPa (B1).

Table 3. The use of optimal concentrations of PEG on the germination of alfalfa cultivars

PEG	Cultivar	Germinaton %
B1	K-28	62 ± 0.456 B
	OS-66	71 ± 0.631 A
	NS-mediana	61 ± 0.456 B

Dancanov test, A, B,…Different letters between treatment denote significant differences ($p \leq 0.05$), Values are mean ± standard error of the mean (SEM).

The cultivars of alfalfa with an optimal concentration of PEG in the germination differed by 10 % table 3. Obtained results from laboratory have to be confirmed in field conditions and in a number of varieties and locations in which they are produced, and seed lots.

CONCLUSION

In the period of 45 days after harvest real indication of germination is possible to establish a standard method for testing seed quality. The effect of PEG on germination of alfalfa optimal concentration of PEG was 0.4 MPa, with which it is possible to detect differences between the varieties of alfalfa. Alfalfa cultivars tolerant to PEG in the autumn sowing period, when it was most often a lack of moisture in the soil in our climate would be more effective for establishing alfalfa crop. These cultivars would be suitable for inclusion in the selection process in order to develop cultivars tolerant to drought stress.

ACKNOWLEGEMENT: The work was realized within the projects TR 31057 and TR 31018, Ministry of Education, Science and Technological Development of Republic of Serbia.

REFERENCES

Albrecht, K., Undersander, D., Dagenhart, N., Moutray, J., and McCaslin, M. (2009). Contribution of alfalfa hard seed to stand and yield in the field. *Forage and Grazinglands* doi:10.1094(*https://www.agronomy.org/.../2009-0114-01-RS)*.

Carmen, D., & Nedelea, G. (2012). The effect of genotype and water stress on germination ability of seeds in some alfalfa varieties. *Journal of Horticulture, Forestry and Biotechnology*, 16 (1), 153-156.

Čupić, T., Popović, S., Grljušić, S., Tucak, M., Andrić, L., Šimic, B. (2005). Effect of storage time on alfalfa seed quality. *Journal of Central European Agriculture*, 6: 65-68.

Djukic, D., Eric, P. (1995). *Lucerka*. Monografija. Poljoprivredni fakultet Novi Sad. Fairey, D. T. and Lefkovitch, L. P. (1991). Hard-seed content of alfalfa grown in Canada. *Canadian Journal of Plant Science*, 7l: 437-444.

Hall, J. W., Stout, D. G. and Brooke, B. M. (1998). Alfalfa seed germination tests and stand establishment: The role of hard (water impermeable) seed**.** *Canadian Journal of Plant Science*, 78: 295–300.

Kimura, E. and Islam, M. A. (2012). Seed Scarification Methods and their Use in Forage Legumes. *Research Journal of Seed Science, 5: 38-50.*

Stanisavljević, R., Beković, D., Djukić, D., Stevović, V., Terzić, D., Milenković, J., & Djokić. (2012). Influence of Plant Density on Yield Components, Yield and Quality of Seed and Forage Yields of Alfalfa Varieties. *Romanian Agricultural Research*, 29, 245-254.

Štrbanović, R. (2010). Genetička varijabilnost agronomskih osobina različitih genotipova lucerke (*Medicago sativa* L.). Magistarska teza, Poljoprivredni fakultet Zemun, Univerzitet u Beogradu, 1-99.

Terzić, D. (2011). The effect of cutting schedule, fertilization with micronutrients and plant growth regulation on yield and quality of alfalfa seed (*Medicago sativa* L.). Ph.D. Thesis. University of Belgrade, Faculty of Agriculture Belgrade.

Tilaki, G. A. D., Behtari, B. (2009). Effect of salt and water stress on the germınatıon of alfalfa (Medıcago sativa L.) seed. *Povolzhsk iy Journ al of Ecology*, 2, 158-164.

Tiryaki, I., Kizilsimsek, M., & Kaplan, M. (2009). Rapid and enhanced germination at low temperature of alfalfa and white clover seeds following osmotic priming. *Tropical Grasslands*, *43* (3), 171-77.

Villela, F. A., Doni Filho, L., & Sequeira, E. L. (1991). Tabela de potencial osmótico em função da concentração de polietileno glicol 6.000 e da temperatura. *Pesquisa Agropecuária Brasileira*, *26*(14), 1957-1968.

Wang, W. B., Kim, Y. H., Lee, H. S., Kim, K. Y., Deng, X. P., & Kwak, S. S. (2009). Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. *Plant Physiology and Biochemistry*, *47* (7), 570-577.

Wang, Y., Zhang, J., Liu, H., & Hu, X. (2003). Physiological and ecological responses of alfalfa and milkvetch seed to PEG priming. *Acta Ecologica Sinica*, *24* (3), 402-408.

Received: 28.02.2014. Accepted: 30.09.2014.