Romanian Biotechnological Letters *Vol.17, No.2, 2012*

ORIGINAL PAPER

Genotype, seed age and pH impacts on germination of alfalfa

Received for publication, April 27, 2011
Accepted, April 20, 2012

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Abstract

The aim of this investigation was to estimate the effects of pH levels of germination media (5, 6, 7 and 8) and seed age (9 and 1 years) on germination and early seedling growth in five Serbian alfalfa cultivars (K-22, K-23, NS Banat, NS Mediana and ZA-83). The experiment was conducted in the laboratory conditions of the Institute for Animal Husbandry in Belgrade in October 2010. Cultivars and seed age had significant effect ($p \leq 0.01$) on the germination energy (GE), germination (G), percentage of dead or infected seeds (DIS), percentage of hard seed (HS), normal (NS) and abnormal seedlings (AS), root length (RL), shoot length (ShL), seedling length (SeL), dry weight (DW) and seedling vigor index (SVI). The pH levels of germination media had significant effect ($p \leq 0.01$) on the GE, NS, AS, RL, ShL, SeL, fresh weight of seedling (FW), DW and SVI. Also pH had significant effect ($p \leq 0.05$) on the G and DIS. Results indicate genetic variability exists among Serbian alfalfa cultivars for pH tolerance, which can be useful for alfalfa breeding program to develop alfalfa germplasm tolerant to this stress.

Keywords: alfalfa, cultivars, germination, early seedling growth, pH

Introduction

Alfalfa is the most important perennial forage crops species in Serbia. Alfalfa is important for livestock production as components of pastures and in pure stands. This crop is grown on over 200 000 ha, which is about 4% of total agricultural area in Serbia [1]. Soil acidity is a major limiting factor in alfalfa production. In Serbia over 50% of agricultural soils are of acid reaction, of which about 30% is the strongly acidic, what is a limitation factor for successful growing of alfalfa on pseudogley with an acid reaction (pH 4.79) [2]. Alfalfa is mainly grown in low land regions on soils of neutral to slight acid reaction in Serbia [3]. The pH of the surrounding media is one of the environmental factors that can severely limited legume production [4]. The pH affects the growth and development of legumes, independent of other environmental factors [5, 6]. For the most crops other than alfalfa, the most appropriate nutritional conditions are from pH 6 to pH 6.5 [7]. Optimal pH for alfalfa has not been identified and extends from 6.5 to 7 [8], and from 6.5 to 7.5 [9] or 5.5 to 8.0 [10]. The most common problems in growing alfalfa on acid soils are aluminum toxicity [11],

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manganese toxicity, legume nodulation failures, increase in plant disease, molybdenum deficiency [12], phosphorus deficiency [13] and calcium deficiency [14]. Significant correlations between field and laboratory measurements of germination were demonstrated by many authors [15, 16, 17, 18]. The standard germination is usually higher than field emergence, especially when seed is sown in low-pH soil [19]. Seed germination and seedling growth of alfalfa genotypes is primarily influenced by water solution pH values [17, 18]. The pH of germination media significantly affected red clover germination [4]. The higher germination of red clover obtained at pH 4 and pH 5 than at pH 6 and pH 7 [20]. The effect of seed age on seed germination of alfalfa has been studied by many researchers [21, 22]. According to their results, seed germination decreased with seed aging. The germination of alfalfa was 35% in the 42-years old seeds while 96% in the control seeds (non-aged) [23]. Alfalfa seed may retain the ability of germination years 28-30, but with reduced of germination to 15-20% [24].

The aim of this investigation was to estimate the effects of pH levels of germination media (5, 6, 7 and 8) and seed age (9 and 1 years) on germination and early seedling growth in five Serbian alfalfa genotypes (K-22, K-23, NS Banat, NS Mediana and ZA-83).

Materials and Methods

The experiment was conducted in the laboratory conditions of the Institute for Animal Husbandry in Belgrade (Serbia) in October 2010. Seeds of five alfalfa genotypes, K-22, K-23, NS Banat, NS Mediana and ZA-83, produced in 2001 (seeds aged 108 months) and 2009 (seeds aged 12 months) were used as material. The seeds were stored in paper bags in laboratory room. Before germination, seeds were sterilized in 1% sodium hypochlorite solution during 5 min and washed three times by sterilized distilled water. After sterilization, seeds were mechanically scarified by rubbing them gently with fine quartz sand in a ceramic mortar.

Germination tests were carried out in sterile plastic germination boxes with lids (15 x 21 x 4 cm) on filter paper soaked with 10 ml of water media with various pH (5, 6, 7 and 8), using four replicates of 100 seeds. Distilled water has a pH of 7. Water pH was adjusted by addition of 0.1 M HCl to desired pH [4], and by addition of 0.1 M NaOH to desired pH [25]. Plastic germination boxes were arranged in a Randomized Complete Block Design (RCBD) in incubators set at 20 ± 1°C, in darkness.

Germination energy (GE) and germination (G) were tested according to the ISTA rules [26]. Seeds were considered fully germinated when root length was reached 2 mm [27]. Besides of normal seedlings (NS), abnormal seedlings (AS), the percentage of dead or infected seeds (DIS) and hard seeds (HS) were estimated. The primary root length (RL), shoot length (ShL), seedling length (SeL) and fresh weight of seedling (FW) were also measured after the final count for normal seedlings. Normal seedlings were dried in a hot air of oven at 80°C for 24 hours [28] and mean dry weight of seedling (DW) was recorded. GE was evaluated after 4 days. G, DIS, HS, NS, AS, RL, ShL, SeL, FW, DW and SVI were evaluated after 10 days. NS, AS, RL, ShL, SeL, FW and DW were determined on 25 randomly selected normal seedlings in each treatment and replication. Vigor index (SVI) was calculated as per equation by [29]:

Vigor Index = (Root length + Shoot length) x Germination percentage.

A three-way factorial ANOVA was used to test the impacts of genotype, seed aged, and pH levels, and the interaction between them. Statistical tests were performed using the Statistical Package for Social Sciences (SPSS) (version 5.0). Test of difference significance

between treatments were estimated by LSD. The significance level was set at $P \leq 0.05$ and $P \leq 0.01$ and means were compared using Duncan's multiple range test at $P \leq 0.05$ level.

Results and Discussion

Results of ANOVA indicated that the genotype affected the all studied traits highly significant (Table 1). The seed age affected the traits GE, G, DIS, HS, NS, AS, RL, ShL, SeL, DW and SVI highly significant, and trait FW non significant. The different pH values of germination media affected the traits GE, NS, AS, RL, ShL, SeL, FW, DW and SVI highly significant, traits DIS and G significant, and trait HS non significant. The interaction genotype × seed age affected the all studied traits highly significant. The interaction genotype x pH affected the traits RL, ShL, SeL, FW, DW and SVI highly significant, and traits GS, G, DIS, HS, NS and AS non significant. The interaction seed age x pH affected the traits AS and RL highly significant, and other studied traits non significant. The interaction genotype x seed age x pH affected the traits GE, G, DIS, HS, NS, RL, ShL, SeL and DW highly significant, trait FW significant, and traits AS and SVI non significant.

Genotype NS Mediana, in average for seed age and pH, had higher GE (68.8%) than genotypes NS Banat (65.4%), ZA-83 (60.8%), K-23 (60.3%) and K-22 (59.2%). The GE of alfalfa conditioned primarily genetic structure of varieties [17, 18, 30]. Alfalfa seeds produced in 2001 had significantly lower GE (51.5%) compared with the seeds produced in 2009 (74.3%). Many researchers reported that the older seed of legume lost more intensive GE than the younger one. The 33 months old seeds of field pea had significantly lower GE than 21 months old seeds [25]. Field pea seed after 21 months of storage had lower GE compared with the GE of seed after 9 months of storage [31]. Results showed that the pH levels of germination media from 6 to 8 significantly increased GE in comparison with pH 5. Minimal GE (58.9%) was at pH 5. However, some research showed that the pH has no effect on GE [18].

Genotypes NS Banat and ZA-83, in average for seed age and pH, had higher G (91.8% and 91.5%) than genotypes NS Mediana (87.7%), K-23 (83.7%) and K-22 (76.8%). Alfalfa seeds produced in 2009 had significantly higher G (92.6%) compared with the seeds produced in 2001 (80.0%). The aging in the same seeds decreased both germination capacity and seedling growth [21]. The long-term (42 years) storage alfalfa seed significantly reduced the G compared to non-aged seeds [23]. The G of the old *Trifolium repens* and *Trifolium pratense* seeds (40 years) were 32% and 17%, while freshly harvested seeds showed 99 and 96%, respectively [22]. Contrary results showed that the total percent of germinated seeds of alfalfa did not correlate with age of seeds but with genotype and storage conditions [32]. The G of alfalfa seed was remained unchanged during the period of 5 years under normal storage conditions [33]. The highest G was observed in mediums that were set at pH 7 (87.0%), with significantly lower G (85.3%) at pH 5. The highest G of alfalfa seed was at pH 4 [17]. The pH of germination media significantly affected red clover germination [4].

Table 1. Genotype, seed age and pH effects on alfalfa properties [(1)]

Factor	Properties: germination energy (GE), germination (G), dead or infected seeds (DIS), hard seed (HS), normal (NS) and abnormal seedlings (AS), root length (RL), shoot length (ShL), seedling length (SeL), fresh weight (FW) and dry weight (DW) of seedling, seedling vigor index (SVI)											
	Percentage						Length (cm)			Weight (mg)		Index
	Seed				Seedlings		Root	Shoot	Seedlings			
	GE	G	DIS	HS	NS	AS	RL	ShL	SeL	FW	DW	SVI
	Genotype effects: G1=K-22, G2=K-23, G3=NS Banat, G4=NS Mediana, G5=ZA-83											
G1	59.2^{c}	76.8^{d}	22.9^{a}	0.25^{b}	61.7^{d}	15.4^{b}	2.76^{c}	5.44^{b}	8.21^{c}	21.4^{a}	1.59^{b}	639^{d}
G2	60.3^{c}	83.7^{c}	16.1^{b}	0.16^{b}	65.2^{c}	18.5^{a}	2.85^{c}	5.79^{a}	8.64^{b}	22.7^{a}	1.63^{b}	729^{c}
G3	65.4^{b}	91.8^{a}	8.0^{d}	0.28^{b}	76.0^{a}	15.8^{b}	3.20^{a}	5.88^{a}	9.08^{a}	18.1^{b}	1.62^{b}	834^{a}
G4	68.8^{a}	87.7^{b}	12.3^{c}	0.12^{b}	74.0^{b}	13.7^{b}	3.19^{a}	5.97^{a}	9.16^{a}	21.2^{a}	1.76^{a}	805^{b}
G5	60.8^{c}	91.5^{a}	7.7^{d}	0.84^{a}	77.3^{a}	14.2^{b}	3.04^{b}	5.57^{b}	8.61^{b}	22.8^{a}	1.70^{a}	793^{b}
F test	**	**	**	**	**	**	**	**	**	**	**	**
	Seed age (SA: year of production) effects: 2009=12 months age, 2001=108 months age											
2009	74.3^{a}	92.6^{a}	6.8^{b}	0.51^{a}	81.3^{a}	11.5^{b}	3.14^{a}	5.92^{a}	9.06^{a}	21.3^{a}	1.60^{b}	841^{a}
2001	51.5^{b}	80.0^{b}	20.0^{a}	0.15^{b}	60.4^{b}	19.6^{a}	2.88^{b}	5.54^{b}	8.42^{b}	21.1^{a}	1.72^{a}	679^{b}
F test	**	**	**	**	**	**	**	**	**	ns	**	**
	pH of germination media effects: pH 5, pH 6, pH 7, pH 8											
pH 5	58.9^{b}	85.3^{b}	14.4^{a}	0.32^{a}	65.4^{c}	19.9^{a}	3.03^{b}	5.50^{b}	8.53^{c}	17.8^{d}	1.58^{b}	733^{b}
pH 6	62.8^{a}	86.4^{ab}	13.3^{ab}	0.38^{a}	70.6^{b}	15.9^{b}	3.17^{a}	5.73^{a}	8.90^{a}	20.7^{c}	1.69^{a}	773^{a}
pH 7	64.6^{a}	87.0^{a}	12.6^{b}	0.40^{a}	74.0^{a}	13.0^{c}	2.97^{bc}	5.84^{a}	8.80^{ab}	22.0^{b}	1.67^{a}	775^{a}
pH 8	65.4^{a}	86.5^{ab}	13.2^{ab}	0.22^{a}	73.2^{a}	13.3^{c}	2.86^{c}	5.86^{a}	8.72^{b}	24.4^{a}	1.70^{a}	759^{a}
F test	**	*	*	ns	**	**	**	**	**	**	**	**
	Interactions (F test): A=genotype, B=seed age, C=pH											
AB	**	**	**	*	**	**	**	**	**	*	**	**
AC	ns	ns	ns	ns	ns	ns	**	**	**	**	**	**
BC	ns	ns	ns	ns	ns	**	**	ns	ns	ns	ns	ns
ABC	**	**	**	**	**	ns	**	**	**	*	**	ns

[(1)] Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level ($p \leq 0.05$); **- significant at 1% level of probability, *- significant at 5% level of probability and ns - not significant

Genotype, seed age and pH have significantly affected on DIS. Genotypes NS Banat and ZA-83, in average for seed age and pH, had lower DIS than genotypes NS Mediana, K-23 and K-22. Seeds produced in 2009 had lower DIS (6.8%) than seeds produced in 2001 (20.0%). In average for genotypes and seed age, minimal DIS was recorded at pH 7 (13.2%).

The soil and climate characteristics as well as genetic factors contribute to HS formation in legumes [34, 35]. The alfalfa is a crop characterized by presence of hard seed that are viable but do not germinate in seed quality testing [36]. The genotype and seed age had significant effect on HS. ZA-83 had significantly higher HS (0.84%) than K-22 (0.25%), K-23 (0.16), NS Banat (0.28%) and NS Mediana (0.12%). Alfalfa seeds produced in 2009 had significantly higher HS (0.51%) compared with the seeds produced in 2001 (0.15%). The HS content usually decreases somewhat over time in storage [37]. This change is affected by the original environment, storage conditions, and genotype. The pH did not affect the HS. Results indicate that the percent of hard seed is under genetic control.

Genotypes NS Banat and ZA-83, in average for seed age and pH, had significantly higher NS (76% and 77.3%) than genotypes NS Mediana (74.0%), K-23 (65.2%) and K-22 (61.7%). Alfalfa seeds produced in 2009 had significantly higher NS (81.3%) compared with the seeds produced in 2001 (60.4%). The highest NS was observed in mediums that were set at pH 7 (74.0%) and pH 8 (73.2%), with significantly lower NS (65.4%) at pH 5.

The AS was significantly lower from K-23 (18.5%) compared with the others genotypes. The higher AS was obtained from seeds produced in 2001 (19.6%) than seeds produced in 2009 (11.5%). AS was decreased by increasing the pH levels of germination media. The lowest AS was at pH 7 (13.0%) and pH 8 (13.3%).

The genotype, seed age and pH had significant ($P<0.01$) effect on RL, ShL and SeL. Genotypes NS Banat and NS Mediana had higher RL (3.20 cm and 3.19 cm, respectively) and SeL (9.08 cm and 9.16 cm, respectively) than K-22 (2.76 and 8.21 cm), K-23 (2.85 cm and 8.64 cm) and ZA-83 (3.04 cm and 8.61 cm). The RL and ShL of alfalfa seedling was significantly influenced by genotype and pH [17,18], and significant decrease of root length in alfalfa plants under acid soil (neutral pH=6.8, and acid pH=4.4) [38]. Genotypes K-23, NS Banat and NS Mediana had higher ShL (5.79 cm, 5.88 cm and 5.97 cm, respectively) than K-22 (5.44 cm) and ZA-83 (5.57 cm). At average of genotype and pH of germination media, seeds produced in 2009 had a significantly longer RL, ShL and SeL (3.14 cm, 5.92 cm and 9.06 cm, respectively), than seeds produced in 2001 (2.88 cm, 5.54 cm and 8.42 cm, respectively). Germination media of pH 6 significantly increased RL (3.17 cm) and SeL (8.90 cm) compared with the germination media of pH 5 (3.03 cm and 8.53 cm, respectively). The height of seedlings derived from the stored seeds was significantly low, compared to that of controls [21]. Results showed that the pH levels of germination media of 6, 7 and 8, significantly increased ShL than pH levels of germination media of 5.

The genotype and pH had significant effect ($P<0.01$) on FW. NS Banat, in average for seed age and pH, had lower FW (18.1 mg) than genotype K-22 (21.4 mg), K-23 (22.7 mg), NS Mediana (21.2 mg) and ZA-83 (22.8 mg). The seed age did not affect the FW. Maximal FW (24.4 mg), in average for genotypes and seed age, was at pH 8, and minimal at pH 5 (17.8 mg).

The genotype, seed age and pH had significant effect ($P<0.01$) on DW. NS Mediana and ZA-83, in average for seed age and pH, had higher DW (1.76 mg and 1.70 mg, respectively) than genotype K-22 (1.59 mg), K-23 (1.63 mg) and NS Banat (1.62 mg). Seeds produced in 2009 had significantly longer DW (1.60 mg), than seeds produced in 2001 (1.72 mg). The DW was higher at pH 6 (1.69 mg), pH 7 (1.67 mg) and pH 8 (1.70 mg) than at pH 5 (1.58 mg).

Vigor comprises a set of characteristics that determine Vigor comprises a set of characteristics that determine seed vigor and is influenced by environmental conditions and handling during the stages of pre-harvest and post-harvest [39]. In addition to the above, determines the longevity of seed vigor, without adverse consequences [40]. The genotype, seed age and pH had significant ($P<0.01$) effect on SVI. NS Banat had higher SVI (834) than K-22 (639), K-23 (729), NS Mediana (805) and ZA-83 (793). Seeds produced in 2009 had significantly higher SVI (841) than seeds produced in 2001 (679). Some research suggests that aged seeds show decreased vigor and produce weak alfalfa seedlings [21]. However, old alfalfa seeds have high ability to maintain viability and vigor [41]. The SVI was higher at pH 6 (773), pH 7 (775) and pH 8 (759) than at pH 5 (733).

Conclusion

The traits GE, G, DIS, HS, NS, AS, RL, ShL, SeL, DW and SVI of alfalfa were significantly affected by genotype and seed age. The results showed that alfalfa old seed retain germination of 80% or at the level of commercial use. An increase in pH level from 6 to 8 had positive effects on GE, G, NS, ShL, SeL, FW, DW and SVI. Also, an increase in pH level has decreased the DIS, AS and RL. Results indicate genetic variability existing among Serbian alfalfa cultivars for pH tolerance. The testing of alfalfa genotypes at different pH levels of germination media in the earliest phases of growth is important for identification and selection of genotypes for particular soil.

Acknowledgements

The research was supported by the Ministry of Education and Science of Republic of Serbia, project TR 31053.

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