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EFFECT OF ACID STRESS ON GERMINATION AND EARLY SEEDLING GROWTH OF RED CLOVER

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Abstract: The aim of this investigation was to estimate the effect of four pH levels of germination media (4, 5, 6 and 7) on seed 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), fresh weight (FW) and dry weight (DW) of seedling, and seedling vigor index (SVI) in two red clover genotypes (cv. K-17 and cv. Kolubara). The experiment was conducted in the laboratory conditions of the Institute for Animal Husbandry in Belgrade in January 2011.

The pH levels of germination media had significant effect on the NS ($P<0.01$), AS ($P<0.01$), RL ($P<0.05$), ShL ($P<0.01$), SeL ($P<0.01$), FW ($P<0.01$) and SVI ($P<0.05$). The lowest GE, G, NS, ShL, SeL, FW and SVI were found at pH 4. Also, the highest DIS, HS and AS were found at pH 4. The genotype had significant effect on HS ($P<0.01$), RL ($P<0.01$), ShL ($P<0.01$), SeL ($P<0.01$), FW ($P<0.01$) and SVI ($P<0.01$). Genotype and pH levels of germination media did not affect on GE, G, DIS and DW significantly.

Key words: genotype, germination, early seedling growth, pH, red clover

Introduction

Red clover is planted on area of about 120.000 ha in Republic of Serbia, thanks to its adaptation to more acid and shallower soils, especially in mountainous regions. *Tomić et al. (2007)* stated that alfalfa is mainly grown in low land regions on soils of neutral to slight acid reaction, while red clover can be grown in hilly-mountainous regions on soils of poorer quality and slightly higher acidity. The pH of the surrounding media is one of the environmental factors that can severely limited legume production *(Agić et al., 2009)*. Red clover tolerates acid soils (pH 5.2 – 6.0) better than alfalfa *(Katić et al, 2006)*. *Duke (1983)* reported that red clover is better adapted than alfalfa at lower pH levels of 5.6 - 6.5. The optimum

pH range for red clover is 6.6 - 7.6, but a range of 4.5 – 8.2 is tolerated *(Sattell et al., 1998)*. *Taylor and Smith (1995)* reported that the red clover grows well at a pH of 5.0 to 6.0 if all nutrient needs are satisfied, but a pH above 6.0 and adequate Ca are needed for maximum yields. It is sensitive to Mn toxicity, which is a concern when pH is below 5.7 in some soils. Many researchers, *Tanaka et al. (1984), Yokota and Ojima (1995), Bukvic et al. (2007a; 2007b)*, reported that the pH might affect legume growth and development independently of other environmental factors. Significant correlations between field and laboratory measurements of germination have been examined in several leguminous crop species: alfalfa *(Klos, 1999; Bukvic et al. 2007a)*, soybean *(Szyrmer and Szczepanska, 1981)*, common bean *(Dickson and Boettger, 1984; Saminy et al., 1987)*, and white clover *(Bukvic et al., 2007b; Grljušić et al., 2008)*.

The aim of this investigation was to estimate the effects of various pH levels (4, 5, 6 and 7) on germination and early seedling growth in two red clover genotypes (cv. K-17 and cv. Kolubara).

Materials and Methods

Effects of four pH levels of germination media (4, 5, 6 and 7) on germination and early seedling growth of red clover were tested in laboratory conditions of the Institute for Animal Husbandry in Belgrade, in January 2011. Seeds of two red clover genotypes, cv. K-17 and cv. Kolubara, were used as material. Thousand seeds weight were 1.78 g (cv. K-17) and 1.93 g (cv. Kolubara). The seeds were stored in paper bags in laboratory room. Seeds were sterilized in 1% sodium hypochlorite solution during 5 min and washed three times by sterilized distilled water. Seeds were mechanically scarified by rubbing them gently with fine quartz sand in a ceramic mortar.

Germination tests were carried out at 20 ± 1°C, in sterile plastic germination boxes with lids (15 x 21 x 4 cm) on filter paper soaked with 10 ml of water media various pH (4, 5, 6 and 7), 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 *(Agić, et al., 2009)*. Plastic germination boxes were arranged in a Randomized Complete Block Design (RCBD). Germination energy (GE) was evaluated after 4 days. 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), fresh weight (FW) and dry weight (DW) of seedling, and seedling vigor index (SVI) were evaluated after 10 days. Seeds were considers fully germinated when root length measured 2 mm *(ISTA, 1999)*. NS, AS, RL, ShL, SeL, FW and DW were determined on 25 randomly selected seedlings in each treatment and replication. Seedlings were dried in a hot air an oven at 80°C for 24 hours *(Perry, 1977)* and mean dry weight of seedling (DW)

was recorded. Vigor index was calculated as per equation by *Abdul-Baki and Anderson et al. (1973)*:

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

Data were processed by ANOVA. Statistical tests were performed using the Statistical Package for Social Sciences (SPSS) (version 5.0) and Costat. Test of difference significance between treatments were estimated by LSD.

Results and Discussion

Results of ANOVA indicated that genotype had significant effect on HS ($P<0.01$), RL ($P<0.01$), ShL ($P<0.01$), SeL ($P<0.01$), FW ($P<0.01$) and SVI ($P<0.01$) (Table 1). The pH of germination media significantly affected the NS ($P<0.01$), AS ($P<0.01$), RL ($P<0.05$), ShL ($P<0.01$), SeL ($P<0.01$), FW ($P<0.01$) and SVI ($P<0.05$). Genotype and pH did not affect the GE, G, DIS and DW significantly.

GE, in average for pH and genotypes, was 94.03%. Genotype Kolubara, in average for pH, had higher GE (94.56%) than genotype K-17 (93.50%). Seeds of red clover were not as sensitive to low pH. Maximal GE (95.62%), in average for genotypes, was at pH 6, and minimal GE (92.00%) was at pH 4. These values were not significant. Seed was able to germinate at all tested pH levels of media. The result shows large environmental adaptation ability of red clover, which is in agreement with research of *Quesenberry et al. (1991)*. *Bukvić et al. (2009)* obtained the lowest average germination energy of red clover at pH 6.

Average G for pH and genotypes, was 96.56%. Kolubara had higher G (97.25%) than K-17 (95.88%). In average for genotypes, minimal G was recorded at pH 4 (95.25%). Maximal G was recorded at pH 5 (97.25%). These differences were not statistically significant. *Agić et al. (2009)* reported that pH of germination media significantly affected red clover germination ($P<0.05$). Their results indicated that highest seed G was found at pH 5. *Bukvić et al. (2009)* obtained higher G of red clover at pH 4 and pH 5 than at pH 6 and pH 7.

Normal seedlings are those show the capacity for continued development into normal plant *(ISTA, 2009)*. NS, in average for pH and genotypes, was 89.28%. In average for pH, K-17 had higher NS (89.44%) than Kolubara (89.12%). The difference was not statistically significant. pH has a significant effect on NS. The means in the Table 1 shows that at pH 7 the NS is 93.38%, at pH 6 is 91.25%, at pH 5 is 90.50% and at pH 4, this amount decreases to 82.00%. These values differed statistically significant.

Table 1. Effect of different pH levels on 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), fresh weight (FW) and dry weight (DW) of seedling, and seedling vigor index (SVI)

Genotype (A)	pH (B)	Traits											
		GE (%)	G (%)	NS (%)	AS (%)	DIS (%)	HS (%)	RL (cm)	ShL (cm)	SeL (cm)	FW (mg)	DW (mg)	SVI
K-17	4	91.50	94.50	83.50	11.00	3.0	2.5	2.40	4.88	7.28	13.70	1.42	688
	5	92.00	96.00	90.00	6.00	3.0	1.0	2.70	5.18	7.88	19.27	1.42	756
	6	95.00	96.00	91.00	5.00	3.0	1.0	2.33	5.55	7.88	17.75	1.41	756
	7	95.50	97.00	93.25	3.75	3.0	0	2.28	5.65	7.93	18.88	1.53	769
	M	93.50	95.88	89.44	6.44	3.0	1.12	2.43	5.32	7.75	17.40	1.44	742
Kolubara	4	92.50	96.00	80.50	15.50	4.0	0	2.80	5.05	7.85	15.97	1.43	753
	5	94.00	98.50	91.00	7.50	1.5	0	2.95	5.45	8.40	22.22	1.57	828
	6	96.25	97.50	91.50	6.00	2.0	0	2.53	6.15	8.68	23.88	1.63	847
	7	95.50	97.00	93.50	3.50	3.5	0	2.50	6.10	8.60	20.14	1.69	834
	M	94.56	97.25	89.12	8.12	2.75	0	2.70	5.69	8.38	20.55	1.58	816
M	4	92.00	95.25	82.00	13.25	3.50	1.25	2.60	5.00	7.57	14.84	1.42	720
	5	93.00	97.25	90.50	6.75	2.25	0.50	2.82	5.32	8.14	20.74	1.50	792
	6	95.62	96.75	91.25	5.50	2.50	0.50	2.43	5.85	8.28	20.82	1.52	802
	7	95.50	97.00	93.38	3.62	3.25	0	2.39	5.88	8.27	19.51	1.61	802
	M	94.03	96.56	89.28	7.28	2.88	0.56	2.57	5.51	8.07	18.98	1.51	779

LSD	GE			G			NS			AS		
	A	B	A*B	A	B	A*B	A	B	A*B	A	B	A*B
F test	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	**	ns
5%	2.35	3.32	4.67	2.06	2.91	4.09	3.02	4.27	6.00	2.33	3.30	4.63
1%	3.20	4.53	6.33	2.80	3.96	5.54	4.11	5.82	8.13	3.18	4.49	6.28
LSD		DIS			HS			RL			ShL	
F test	ns	ns	ns	**	ns	ns	**	**	ns	**	*	ns
5%	1.75	2.47	3.47	0.80	1.13	1.58	0.19	0.27	0.38	0.21	0.30	0.42
1%	2.38	3.37	4.70	1.08	1.53	2.14	0.27	0.37	0.52	0.28	0.41	0.57
LSD		SeL			FW			DW			SVI	
F test	**	**	ns	**	**	ns	ns	ns	ns	**	*	ns
5%	0.32	0.45	0.62	1.68	2.37	3.33	0.16	0.22	0.31	37.12	52.50	73.69
1%	0.43	0.61	0.85	2.29	3.23	4.52	0.21	0.30	0.42	50.54	71.48	99.86

** - significant at 1% level of probability, * - significant at 5% level of probability and ns - not significant

Abnormal seedlings are those which do not show capacity for continued development into normal plants when grown in good quality soil, under favorable condition of heat, light ad water supply *(ISTA, 2009)*. Average AS for pH and genotypes was 7.28%. The higher AS was obtained from Kolubara (8.12%) than K-17 (6.44%). The genotype did not affect the AS significantly. AS was decreased by increasing the pH from 4 (13.25%) to 7 (3.62%). pH has a significant effect on AS.

DIS, in average for pH and genotypes, was 2.88%. Genotype and pH did not significantly affect on DIS. Kolubara had lower DIS for 0.25% than K-17 (3.00%). In average for genotypes, maximal DIS was recorded at pH 4 (3.50%), while minimal DIS was recorded at pH 5 (2.25%).

The number of HS, which not germinate within 10 days after placement on germinator, is normally 7 - 15 % in red clover *(Chmelař, 1947)*. *Ünal (2004)* and *Elçi (2005)* concluded that the environmental and genetic factors contribute to HS formation in *Fabaceae* family. HS, in average for pH and genotypes, was 0.56%. The genotype had significant effect on HS ($P<0.01$). Kolubara had lower HS by 1.12% than K-17 (1.12%). The pH did not affect the HS. The HS ranged from 0% (pH 7) to 1.25% (pH 4). Results indicate that the hard seed characteristic is under genetic control.

Average RL for pH and genotypes, was 2.57 cm. Kolubara had higher RL for 0.27 cm than K-17 (2.43 cm). The difference was not statistically significant. In average for pH, differences in RL among tested pH levels were significant RL. The lowest value was recorded at pH 7 (2.39 cm). The highest RL value was recorded at pH 5 (2.82 cm). This result is in agreement with research of *Bukvić et al. (2009)*. Their results indicated that maximum RL at pH 5 and minimum RL at pH 7. *Agić et al. (2009)* found that higher RL at pH 5 and pH 6 than at pH 4 and pH 7. Their results show that RL on 9th germination day varied from 9.1 mm at pH 7 to 11.2 mm at pH 5 in average.

Average ShL for pH and genotypes, was 5.51 cm. Kolubara had higher ShL for 0.37 cm than K-17 (5.32 cm). The difference was statistically significant. The highest ShL was obtained at pH 7 (5.88 cm) and pH 6 (5.85 cm). These values were significant differed from the ShL at pH 5 (5.32 cm) and pH 4 (5.00 cm). *Bukvić et al. (2009)* obtained highest ShL at pH 4, and lowest ShL at pH 7. *Bukvić et al. (2008)* reported that RL and ShL of alfalfa significantly depended ($P<0.01$) on pH of water solution. They found the highest average values for both traits at pH 4.00. Significant effect pH on RL and ShL has been examined in several leguminous crop species: white clover *(Bukvic et al., 2007b)*, soybean *(Grljušić et al., 2007)* and field pea *(Bukvic et al., 2007c)*.

SeL, in average for pH and genotypes, was 8.07 cm. Kolubara had higher SeL for 0.63 cm than K-17 (7.75 cm). The difference was statistically significant. Significant differences were found for values obtained at different pH values. The highest SeL was found at pH 7 (8.27 cm) and pH 6 (8.28 cm), and the lowest at pH 4 (7.57 cm). SeL differences, at pH levels 7, 6 and 5 (8.14 cm) were not significant. *Bukvić et al. (2009)* found that highest average SeL at pH 4, and lowest SeL at pH 7.

FW, in average for pH and genotypes, was 18.98 mg. The genotype had significant effect on FW. Kolubara, in average for pH, had higher FW (20.55 mg) than genotype K-17 (17.40 mg). The pH levels had significant effect on the FW.

Minimal FW (14.84 mg), in average for genotypes, was at pH 4 and maximal FW (20.82 mg) at pH 7.

Average DW for pH and genotypes, was 1.51 mg. Genotype and pH did not affect on DW significantly. Kolubara had higher DW (1.58 mg) than K-17 (1.44 mg). In average for genotypes, minimal DW was recorded at pH 4 (1.42 mg). Maximal DW was recorded at pH 7 (1.61 mg).

The vigor comprises a set of characteristics that determine seed vigor and is influenced by environmental conditions and handling during the stages of pre- and post-harvest *(Vieira and Carvalho, 1994)*. In addition to the above, determines the longevity of seed vigor, without adverse consequences *(ISTA, 2009)*. SVI, in average for pH and genotypes, was 779. The genotype had significant effect on SVI. Kolubara had higher SVI (816) than K-17 (742). In average for genotypes, pH had significantly different effects on the SVI. The minimum SVI was found at pH 4 (720). The SVI was higher at pH 5 (792), pH 6 (802) and pH 7 (802) than at pH 4.

The interactions between varieties and pH levels were not significant for all studied traits.

Conclusion

The RL, ShL, SeL, FW and SVI of red clover were significantly affected by genotype and pH. Also, the pH levels had significant effect on the NS and AS, and the genotype had significant effect on HS. Red clover seed it is not sensitive to pH stress. The results of this experiment have showed the importance of pH on early seedling growth. Genotype and pH did not affect on GE, G, DIS and DW significantly. The lowest GE, G, NS, ShL, SeL, FW and SVI were found at pH 4. The highest DIS, HS and AS were found at pH 4. Results indicate on ability growth of seedling of red clover of different pH levels, especially in acidic environment. Also, results indicate that testing of genotypes of red clover in the early seedling growth at different pH levels, would be helpful in the identification and selection of genotypes for particular location (soil).

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Uticaj stresa usled promene pH na klijanje i porast klijanaca crvene deteline

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Rezime

Cilj istraživanja bio je da se odredi uticaj različitih nivoa pH vrednosti (4, 5, 6 i 7) na energiju klijanja (EK), klijavost (K), neklijala i bolesna semena (NB), tvrda semena (TS), normalne klijance (NK), abnormalne klijance (AK), dužinu korena (DK), dužinu hipokotila (DH), ukupnu dužinu klijanca (UDK), svežu masu klijanaca (SvMK), suvu masu klijanaca (SuMK) i vigor indeks klijanaca (VIK) kod dva genotipa crvene deteline (cv. K-17 i cv. Kolubara). Ogledi su izvedeni u laboratorijskim uslovima u Institutu za stočarstvo u Beogradu, u januaru 2011. godine.

pH vrednost imala je značajan uticaj na NK ($P<0.01$), NB ($P<0.01$), DK ($P<0.05$), DH ($P<0.01$), UDK ($P<0.01$), SvMK ($P<0.01$) i VIK ($P<0.05$). Najmanje vrednosti za EK, K, NK, DH, UDK, SvMK i VIK zabeležene su na pH 4. Takođe, najveća učestalost NB, TS i AK registrovana je na pH 4. Genotipovi su se značajno razlikovali za sledeće osobine: TS ($P<0.01$), DK ($P<0.01$), DH ($P<0.01$), UDK ($P<0.01$), SvMK ($P<0.01$) i VIK ($P<0.01$). Utvrđeno je da genotipovi i pH nemaju značajan uticaj na EK, K, NB i SuMK.

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