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Resistance of lucerne (*Medicago* spp.) germinating seeds to oxalic acid

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Resistance to oxalic acid (OA) of 37 lucerne and 3 red clover accessions was evaluated. The seeds of test entries were germinated in Petri dishes on filter paper moistened with 7 OA water solution concentrations: 0, 5, 7.5, 10, 15, 20, 30 mM. The test lucerne accessions did not differ by resistance to OA in regard to the origin of accessions or their development status. Accessions considerably differed by resistance at a concentration of 10 mM of OA solution. More than a third (42%) of lucerne accessions were totally susceptible to OA at conc. 30 mM. However, 0.0–23.8% of resistant seedlings of these accessions were found at OA conc. 20 mM. Lucerne accessions showing resistance (0.7–3.8%) at OA conc. 30 mM expressed a similar resistance level (3.1–26.7%) to OA conc. 20 mM as well as accessions totally susceptible to OA conc. 30 mM. The OA conc. 20 mM was adequate for selection of resistant seedlings in breeding material with a lower resistance, and conc. 30 mM was suitable for screening more resistant accessions. This method showed an efficient possibility to select seedlings resistant to OA and in turn to *Sclerotinia* spp. pathogens using simple materials and less work inputs as compared with methods described in the literature.

Key words: lucerne, resistance, oxalic acid, *Sclerotinia*

INTRODUCTION

Sclerotinia trifoliorum Eriks. and *S. sclerotiorum* (Lib.) de Bary are both important pathogens of lucerne and other forage legumes worldwide in countries with temperate climate. These pathogens cause Sclerotinia crown and stem rot (SCSR) and subsequent thin out of crop [1]. Lucerne as a perennial grass should maintain a stable plant density for at least several years. Stable crop density for a decade is a highly desirable trait of this plant. Cultivars of lucerne considerably differ by resistance to SCSR as well as plants consisting cultivars [1–4]. Lucerne plants of all ages can be damaged by this disease, but the damage is most severe at the seedling stage in cool and moist conditions [5]. Conventional resistance breeding based on field screenings is efficient when screenings are done under conditions of artificial infection in disease-favourable years. This technique is long-lasting and additionally highly depends on the year and environment interactions. Moreover, resistance breeding is efficient only when based on the recurrent selection strategy which in turn requires even more resources [6]. There are several methods developed which enable to screen breeding material in laboratory and greenhouse conditions [7, 8]. However, they are more suitable for the search of resistant material for creating new cross combinations. The later development of highly resistant cultivars possessing desirable agronomic traits can be done only when thousands of plants are tested during cycles of recurrent selection because of the tetraploidy and cross-pollination of lucerne [5, 9, 10]. Most desirable is selection of resistant plants just from their seed germination. Such selection can be done using inoculation of germinated seeds by *S. trifoliorum* mycelium [11, 12]. However, this technique has some limitations. One of them is variability of aggressiveness and pathogenicity of *S. trifoliorum* isolates [13]. It makes breeders to do additional screenings of local *S. trifoliorum* populations for selecting the most adequate isolates. Another limitation is that plants selected by resistance to *S. trifoliorum* will not be definitely resistant at the same level to other species of *Sclerotinia* [13–15]. However, all *Sclerotinia* species produce one general toxin – oxalic acid (OA) [12, 16, 17]. This allows plant breeders to select resistant plants using OA. Some researches concerning this technique were done with lucerne and clover [11, 12, 18]. Also, oxalic acid can be efficiently used for selection of cultivars and individual plants resistant to *Sclerotinia* spp. in many legumes [19–21] and other dicotyledonous species [22, 23].

This investigation was aimed to determine the reaction of germinating seeds of lucerne cultivars and populations to OA.

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MATERIALS AND METHODS

In 2008, the experiment were carried out at the Lithuanian Institute of Agriculture (LIA). The material subjected to OA resistance tests included mainly Lithuanian cultivars and breeding populations, Estonian wild populations and several accessions of lucerne from other countries characterized by temperate climate. Lithuanian red clover cultivars 'Arimaičiai', 'Sadūnai', 'Liepsna' with the known resistance to *S. trifoliorum* and oxalic acid [2, 18] were used to compare resistance level to OA in lucerne and red clover.

Resistance to OA in lucerne and red clover accessions was evaluated as a percentage of growing seedlings using the adapted method and materials used in the studies of Rowe [12] and Jančys and Vyšniauskienė [18]. Seeds were scarified and their surface was sterilized in a solution of 5% sodium hypochlorite for 10 min and rinsed 3 times in distilled water; 50 sterilized seeds were placed in one Petri dish on filter paper moistened with the equal amounts of water solution of OA. Three replications were used for each lucerne and red clover entry per one OA concentration. Seven concentrations of OA were used: 0, 5, 10, 7.5, 15, 20, 30 mM. Petri dishes with seeds were kept in the dark at 20 °C for 5 days. Germinated seedlings with active growing roots were considered as resistant.

The relative percentage of resistant seedlings was calculated for a detailed analysis of the reaction of lucerne and red clover accessions to OA. The percentage of germinated seeds in 0 solution of OA was equated to 100%. The relative percentage of resistant seedlings in other concentrations was calculated by dividing the percentage of resistant seedlings in each concentration by the percentage of resistant seedlings in 0 concentration of OA solution and multiplying by 100%. Statistical calculations were done using ANOVA from SELEKCIJA package [24].

RESULTS

The reaction of six lucerne accessions with the highest contrast of OA resistance is presented in Fig. 1. The selected accessions considerably differed by resistance at conc of 10 mM oxalic acid in a solution. The most resistant accession 'LIA2062' had 89.7% of relative germination, whereas the most susceptible breeding population 'LIA2097' had only 17.2% of relative germination, i. e. 5.2 times less. The resistance of these accessions at OA conc. 15 mM differed only slightly (16.5–28.9%), except 'LIA2097' (1.8%). The higher concentration (20 mM) of OA was highly toxic for susceptible entries as only 1.3% of seedlings of susceptible entries were relatively resistant. The relative percentage of resistant seedlings of the most resistant entries decreased about 2.2 times, whereas the negative effect was 10.2 times higher for susceptible entries. OA conc 30 mM was mortal for most of susceptible entries, whereas the most resistant entries 'Ventus', 'LIA1973' and '2062' had 4.6, 4.3 and 3.9 relative percentage of resistant seedlings.

The resistant red clover cultivars 'Arimaičiai' and 'Sadūnai' were a little more susceptible to OA than resistant lucerne accessions at OA concentrations 5 and 7.5 mM (Fig. 2). However, the susceptible cv. 'Liepsna' was much more susceptible

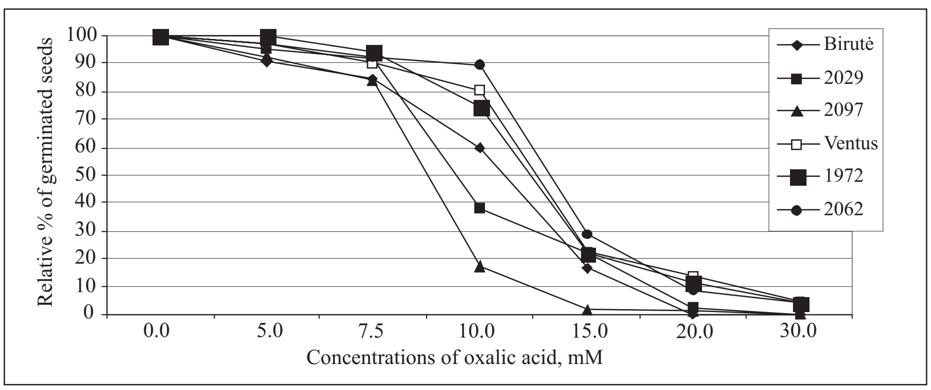

Fig. 1. Resistance of lucerne entries to different solutions of oxalic acid

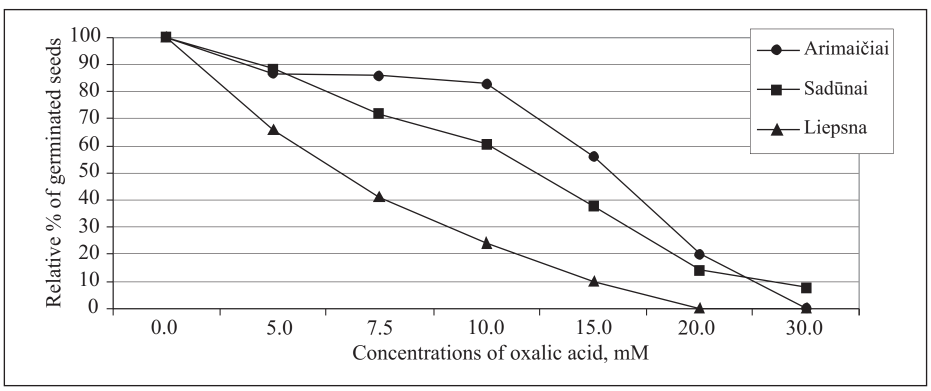

Fig. 2. Resistance of red clover entries to different solutions of oxalic acid

Table. **Effect of oxalic acid on seed germination in lucerne and red clover accessions**

Cultivar / breeding population	Status of accession	Origin country	Concentrations of oxalic acid, mM			
			0.0	15	20	30
			Resistant seedlings, %			
2062	B. p.***	LT**	97.2 i-m*	28.2 e-l	8.1 c-j	3.8 f-h
1972	B. p.	LT	86.7 c-i	19.4 b-j	10.0 d-l	3.7 e-h
'Ventus'	Cv.	SW	72.2 ab	15.6 a-h	9.8 d-k	3.3 d-h
'Derby'	Cv.	FR	84.3 c-g	13.3 a-f	11.2 f-l	3.0 d-f
2251	B. p.	LT	96.7 h-m	29.2 f-m	9.2 c-k	2.7 c-f
2456	W. p.	ES	94.3 g-m	17.0 b-i	7.9 b-j	2.4 b-f
'PGR12991'	W. p.	AF	100.0 m	19.4 b-j	5.2 a-g	2.3 a-f
'Polder'	Cv.	FR	90.9 f-m	16.7 b-i	7.6 b-j	2.1 a-f
'Augūnė II'	Cv.	LT	94.5 g-m	10.6 a-c	10.0 d-l	2.0 a-f
1825	B. p.	LT	82.5 c-f	34.4 i-o	15.3 j-n	2.0 a-f
2446	W. p.	ES	98.2 k-m	14.3 a-h	3.8 a-g	1.9 a-f
2427	B. p.	LT	85.5 c-h	44.8 l-o	19.1 l-p	1.8 a-f
2061	B. p.	LT	90.2 e-m	17.5 b-i	10.3 d-l	1.3 a-f
2060	B. p.	LT	89.7 d-m	27.8 d-l	7.8 b-j	1.2 a-f
2419	B. p.	LT	89.5 c-m	29.5 f-m	11.0 e-l	1.0 a-e
2125	B. p.	LT	91.3 f-m	40.4 k-o	14.1 h-m	1.0 a-e
'Tin Jin'	Cv.	CH	98.1 j-m	11.4 a-d	8.7 c-j	0.8 a-c
2182	B. p.	LT	79.4 b-d	47.5 m-o	16.3 j-n	0.8 a-d
'PGR8701'	W. p.	DK	89.4 c-m	12.0 a-e	3.1 a-g	0.7 ab
2447	W. p.	ES	92.7 f-m	23.8 b-k	6.0 a-h	0.7 ab
2125	B. p.	LT	87.3 c-k	31.1 g-m	26.7 p	0.7 ab
'Birutė'	Cv.	LT	84.8 c-g	14.3 a-g	0.0 a	0.0 a
2097	B. p.	LT	92.8 f-m	1.7 a	1.3 a-b	0.0 a
2029	B. p.	LT	88.2 c-k	18.5 b-j	2.1 a-c	0.0 a
2035	B. p.	LT	90.8 f-m	24.3 c-k	2.1 a-c	0.0 a
2249	B. p.	LT	91.3 f-m	15.4 a-h	2.7 a-e	0.0 a
'Orca'	Cv.	FR	88.9 c-m	19.2 b-j	2.7 a-f	0.0 a
2448	W. p.	ES	94.0 g-m	14.7 a-h	4.7 a-g	0.0 a
'Belfeuil'	Cv.	FR	91.5 f-m	28.9 f-l	6.3 a-i	0.0 a
2419	B. p.	LT	88.6 c-m	20.4 b-j	7.6 b-j	0.0 a
'Mireille'	Cv.	FR	69.4 a	10.3 a-c	8.7 c-j	0.0 a
1971	B. p.	LT	80.0 b-e	18.0 b-i	9.3 c-k	0.0 a
1970	B. p.	LT	89.9 e-m	24.3 c-k	9.5 d-k	0.0 a
2450	W. p.	ES	96.7 i-m	31.5 h-m	15.0 i-n	0.0 a
'PGR12425'	Cv.	USA	97.1 i-m	27.5 d-l	15.9 j-n	0.0 a
'Žydrūnė'	Cv.	LT	86.9 c-j	36.3 j-o	22.0 m-p	0.0 a
2454	W. p.	ES	96.0 h-m	33.2 i-o	23.8 n-p	0.0 a
Average			89.7	22.8	9.6	1.1
'Arimaičiai'	Cv.	LT	90.0 e-m	50.0 o	18.0 k-p	0.0 a
'Sadūnai'	Cv.	LT	79.3 bc	30.0 f-m	11.3 g-l	6.0 h
'Liepsna'	Cv.	LT	84.7 c-g	8.3 ab	0.0 a	0.0 a
Mean			84.7	23.7	9.6	0.8

* Means followed by the same letters do not differ according to Duncan's Multiple Range Test at 1% of significance.

** LT – Lithuania, ES – Estonia, SW- Sweden, FR – France, AF – Afghanistan, CH – Chine, DK – Denmark, USA – United States of America.

*** B. p. – breeding population, Cv. – cultivar, W. p. – wild population.

at these OA concentrations than the most susceptible lucerne accessions. Resistant cultivars 'Arimaičiai' and 'Sadūnai' at OA conc. 10 mM had 3 times more resistant seedlings than the susceptible cv. 'Liepsna'. The higher concentrations of OA were highly negative for cv. 'Liepsna': at 15 mM the relative germination percentage was 9.8%, and the other two OA concentrations were mortal. Cultivar 'Sadūnai' was slightly susceptible to OA at lower concentrations than cv. 'Arimaičiai'. Cultivars 'Sadūnai' showed a higher resistance (7.6%) at OA conc. 30 mM, whereas cv. 'Arimaičiai' was totally susceptible to OA at this concentration.

Considering the reaction of lucerne accessions to OA concentrations (Fig. 1), four OA concentrations (0, 15, 20, 30 mM) were selected as the best to demonstrate differences in accessions' resistance. Resistance of lucerne accessions to four OA concentrations (0, 15, 20, 30 mM) is presented in Table. The test lucerne accessions did not differ by resistance to OA in regard to the origin of accessions or their development status. More than a third (42%) of lucerne accessions were totally susceptible to OA at conc. 30 mM. However, 0.0–23.8% of resistant seedlings of these accessions were found at OA conc. 20 mM. Lucerne accessions showing resistance (0.7–3.8%) at OA conc. 30 mM expressed a similar resistance (3.1–26.7%) level to OA conc. 20 mM as well as accessions totally susceptible to OA conc. 30 mM. OA conc. 20mM was adequate for selecting resistant seedlings in breeding material with a lower resistance, and conc. 30 mM was suitable for the screening of more resistant accessions.

DISCUSSION

Comparison of lucerne and red clover accessions shows that lucerne accessions were more resistant to low OA concentrations (5 and 7.5 mM). These results correspond to data reported by Kanbe et al. [5] and Rowe [12] who found lucerne to be more resistant than red clover to *Sclerotinia* spp. and OA. However, at higher OA concentrations the resistance of less damaged lucerne accessions was similar to that of resistant red clover cultivars.

The method showed an efficient possibility to select seedlings resistant to OA, using simple materials and less work inputs as compared with methods described in the literature [11, 12, 18]. Such a situation is highly desirable as among 37 accessions tested by us, those most resistant had only 3.0–3.8% of resistant seedlings. Development of a lucerne breeding population with desirable agronomical traits requires at least several hundreds of plants. The relatively low number of plants resistant to at least one disease makes breeders use several thousands of plants in order to compose the initial breeding populations. Development of a population using such a number of plants selected for resistance allows testing young plants for resistance to other diseases under greenhouse conditions, for example, Fusarium crown rot [25, 26]. This layout of screenings allows for selecting plants resistant to a couple of diseases. Also, seeds of these plants are received in the same year if the plants are further kept in greenhouse conditions. Otherwise, selecting plants resistant to a couple of diseases and receiving their seeds in field conditions of temperate climate can take up to five years, even when screenings are done in nurseries with artificial infection [27, 28].

Considering the number of resistant plants among our accessions, such a population could be developed with screening only several hundred grams of seeds. This amount of lucerne seed is available only in advanced breeding populations in years favourable for seed setting. Therefore, such screening is more acceptable in case of selecting resistant plants from cultivars as parental breeding material for subsequent breeding cycles and development of the new populations. The lucerne populations with a lower seed quantity available could be tested for tentative determination of resistance to OA. If seeds are highly deficient or only several plants are available, other methods for OA application are available. Excised leaf tissues [29] and stem-tip methods [21, 30] could be used for testing a limited number of plants.

Considering the short duration of the experiment and seeds number tested in very limited space, the method should be successfully adapted for the development of lucerne resistant to OA and in turn to *Sclerotinia* spp.

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References

1. Pratt RG, Rowe DE. Plant Dis 1995; 79: 474–7.
2. Svirskis A. Yield increase of herbage legume species by plant breeding. Habil. D. thesis. Dotnuva, 1995. 156 p.
3. Saharan GS, Mehta N. Sclerotinia Disease of Crop Plants: Biology, Ecology and Disease Management. Dordrecht, The Netherlands, 2008: 485.
4. Khan UQ. Asian J Plant Sci 2002; 5: 596–8.
5. Kanbe M, Fujimoto F, Mizukami Y et al. Breed Sci 1997; 47: 347–51.
6. Halimi ES, Rowe DE, Aung M. Divergent selection in alfalfa for resistance to Sclerotinia crown and stem rot. Crop Sci 1994; 34: 1440–2.
7. Pratt RG, Rowe DE. Plant Dis 1998; 82: 1161–4.
8. Halimi ES, Rowe DE, Pratt RG. Crop Sci 1998; 38: 1179–82.
9. Halimi ES, Rowe DE, Aung M. Crop Sci 1994; 34: 1440–2.
10. Pierson PE, Martin SKSt, Rhodes LH. Crop Sci 1997; 37: 1071–8.
11. Callahan FE, Rowe DE. Phytopathology 1991; 81: 1546–50.
12. Rowe DE. Crop Sci 1993; 33: 1146–9.
13. Pratt RG, Rowe DE. Plant Disease 1995; 79: 474–7.
14. Pratt RG, Rowe DE. Phytopathology 2002; 92: 204–9.
15. Gilbert RG. Plant Dis 1987; 71:739–42.
16. Singh UP, Sarma BK, Singh DP et al. Can J Microbiol 2002; 48: 443–8.
17. Walz A, Zingen-Sell I, Theisen S et al. Eur J Plant Pathol 2008; 120: 317–30.
18. Jančys Z, Vyšniauskienė R. Biologija 2002; 3: 50–3.
19. Kolkman JM, Kelly JD. Crop Sci 2000; 40; 281–5.
20. Livingstone DM, Hampton JL, Phipps PM et al. Plant Physiol 2005; 137: 1354–62.
21. Chunk YS, Sass ME, Nienhuis J. Crop Sci 2008; 48: 2265–73.
22. Hu X, Bidney DL, Yalpani N et al. Plant Physiol 2003; 133: 170–81.
23. Dong X, Ji R, Guo X et al. Planta 2008; 228: 331–40.
24. Tarakanovas P, Raudonius S. Agronominių tyrimų duomenų statistinė analizė taikant kompiuterines programas *Anova, Stat, Spilit-plot*, iš paketo *Selekcija*. Akademija, 2003: 27–9.

25. Rhodes LH. 37th North American Alfalfa Improvement Conference, Abstracts. Madison, WI, USA 2000: 22.
26. Salter R, Miller-Garvin JE, Viands DR. Crop Sci 1994; 34: 1213–7.
27. Miller-Garvin JE, Viands DR. Crop Sci 1994; 34: 1461–5.
28. Nagy B. Czech J Genet Plant Breed 2003; 39: 282–4.
29. Pratt RG. Phytopathology 1996; 86: 923–8.
30. Halimi ES, Rowe DE. Crop Sci 1998; 38: 20–2.

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LIUCERNOS (*MEDICAGO* SPP.) DYGSTANČIŲ SĖKLŲ ATSPARUMAS OKSALO RŪGŠČIAI

Santrauka

Nustatytas 37 liucernos ir 3 dobilų pavyzdžių atsparumas oksalo rūgščiai (OR). Tirtų pavyzdžių sėklos buvo daiginamos Petri lėkštelėse ant filtrinio popieriaus, sudrėkinto 0, 5, 7,5, 10, 15, 20, 30 mM OR koncentracijomis. Įvairios kilmės ir patobulinti liucernos pavyzdžiai nesiskyrė pagal atsparumą OR. Šis atsparumas pradėjo skirtis esant 10 mM OR koncentracijai. Daugiau nei trečdalis (42%) liucernos pavyzdžių buvo labai jautrūs 30 mM OR koncentracijai. Tačiau, tiriant šių pavyzdžių atsparumą 20 mM OR koncentracijai, buvo rasta 0,0–23,8% atsparių individų. Liucernos genotipai, kurie turėjo atsparių individų (0,7–3,8%) esant 30 mM OR koncentracijai, buvo panašaus atsparumo (3,1–26,7%) ir esant 20 mM OR koncentracijai, taip pat kaip liucernos genotipai buvo labai jautrūs 30 mM OR koncentracijai. 20 mM OR koncentracija buvo tinkama atrenkant atsparius individus iš selekcinės medžiagos, pasižyminčios mažesniu atsparumu, o 30 mM OR koncentracija buvo tinkama atrenkant individus iš atsparesnių pavyzdžių. Naudotas metodas padėjo efektyviai atrinkti individus, atsparius OR ir *Sclerotinia* spp. patogenams, naudojant paprasčiausias laboratorines medžiagas bei įdedant mažiau pastangų, nei aprašyta literatūroje.

Raktažodžiai: liucerna, atsparumas, oksalo rūgštis, *Sclerotinia*