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Full Length Research Paper

Relationships among alfalfa resistance to Sclerotinia crown and stem rot, *Sclerotinia trifoliorum* and oxalic acid

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Sclerotinia crown and stem rot (SCSR) of alfalfa caused by *Sclerotinia trifoliorum* is one of the main constraints for efficient alfalfa cultivation in temperate climate all over the world. The resistance of 200 alfalfa accessions to Sclerotinia crown and stem rot was evaluated during 2010 to 2011 in the field nursery established in 2009. The resistance of alfalfa accessions germinating seeds to mycelium of *Sclerotinia trifoliorum* and oxalic acid (OA) concentrations of 10, 20, 30 mM was screened under laboratory conditions. The statistically significant differences at *P<0.05* were determined among evaluated alfalfa accessions resistance to all screened factors. The reactions of alfalfa accessions to disease under field conditions showed that majority of the non-adapted accessions were heavily diseased, whereas the resistant accessions had only negligible disease severity. The germination of accessions seeds at oxalic acid concentration of 30 mM showed strong correlation (r = -0.817, *P<0.01*) with SCSR severity in 2011. Among OA concentrations, this one showed the highest correlation rate with SCSR as well as was the least time consuming method. This method of seeds germination on mycelium of *S. trifoliorum* was unfit due to its weak correlation with SCSR and higher inputs.**

Key words: Alfalfa, resistance screening, *Sclerotinia trifoliorum*, oxalic acid.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the world's leading legume forage grass. As perennial plant, it should maintain stable plant density for at least several years. Stable crop density for decade is a highly desirable traits of this plant. *Sclerotinia trifoliorum* Erikss. is an important pathogens of alfalfa and other forage legumes worldwide in countries with temperate climate. The pathogen causes Sclerotinia crown and stem rot (SCSR), resulting in thinning or destruction of stand and reduced yield as well as increased number of weeds (Elgin et al., 1988). Alfalfa plants of all ages can be damaged by this disease but the damage is most severe at the seedling stage under cool and moist conditions (Kanbe et al., 1997). The damage usually is evident in spring after resumption of vegetation. When cultivars are very susceptible, dead plants are evident just after resumption of vegetation. More resistant plants showed slower disease developing symptoms such as wilting plants or partially damaged crowns later in spring (Pratt and Rowe, 1995). Cultivars of lucerne considerably differ by resistance to SCSR as well as plants consisting cultivars (Pratt, 1996; Pratt and Rowe, 1998; Khan, 2002). Conventional resistance breeding based on field screenings is efficient when screenings are done under artificial infection pressure in favorable to disease years.

This technique is suitable for evaluation of cultivars resistance. However, development of resistant cultivars is long lasting and highly depends on year x environment interactions using this technique. Moreover, resistance breeding is considerably efficient only when based on recurrent selection strategy which in turn requires even

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more resources (Halimi et al., 1994; Kanbe et al., 1997).

There are several methods developed which enables the screening of breeding material under laboratory and greenhouse conditions in short period (Pratt and Rowe, 1998; Halimi et al., 1998). However, they are more suitable for searching resistant material for creating new cross combinations. 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 tetraploidy and cross-pollination of lucerne (Halimi et al., 1994; Kanbe et al., 1997; Pierson et al., 1997).

The most desirable is the selection of resistant plants just from their seed germination. Such selection can be done using exudates of mycelium of *S. trifoliorum* (Callahan and Rowe, 1991; Rowe, 1993). However, this technique has some limitations. One of them is variability of aggressiveness and pathogenicity of *S. trifoliorum* isolates (Pratt and Rowe, 1995). It makes breeders to do additional screenings of local populations of *S. trifoliorum* for selection of 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* (Gilbert, 1987; Pratt and Rowe, 2002).

However, all *Sclerotinia* species produce one general toxin; oxalic acid (OA) (Singh et al., 2002; Saharan and Mehta, 2008; Walz et al., 2008). This allows plant breeders to select resistant plants using OA. Some researches concerning this technique were done with lucerne and clover (Callahan and Rowe, 1991; Rowe, 1993; Jančys and Vyšniauskienė, 2002). Also, it was found that OA can be efficiently used for selection of resistance to *Sclerotinia* spp. cultivars and individuals plants in many legumes (Kolkman and Kelly, 2000; Livingstone et al., 2005; Chung et al., 2008) and other dicotyledonous species (Hu et al., 2003; Dong et al., 2008).

This investigation was aimed to determine the relationships between alfalfa accessions reactions to Sclerotinia crown and stem rot, *Sclerotinia trifoliorum* and oxalic acid.

MATERIALS AND METHODS

Experimental site and plant material

The experiments were carried out at the Institute of Agriculture, during the period of 2009 to 2011. The alfalfa material was subjected for investigation of resistance to *S. trifoliorum,* OA under laboratory and resistance to SCSR under field conditions in mainly Lithuanian, European and USA cultivars and populations. A total number of 200 accessions were investigated.

Evaluation of resistance to OA

Alfalfa resistance to OA was evaluated as percent of germinated seeds using adapted method and materials used in the researches of Rowe (1993) and Jančys and Vyšniauskienė (2002). Seeds were scarified and surface sterilized in solution of 5% sodium hypochlorite for 10 min and rinsed three times in distilled water. 50 sterilized seeds were placed in one plastic Petri plate (diameter 9 cm) on filter paper moistened with 5 ml of water solution of OA. The three replications were used for each alfalfa entry per one concentration of OA. The seven concentrations of OA used were: 0, 5, 10, 7.5, 15, 20, 30 mM. Petri plates with seeds were maintained in the dark at 20°C for five days. Germinated seeds with active growing roots were counted as resistant. Relative percent of germination was calculated for detailed analysis of alfalfa accessions reaction to OA. Percent of germinated seeds in 0 mM solution of OA was equated to 100%. Relative percent of germinated seeds in other concentrations was calculated as:

$$RG = (GS_{OAx}/GS_{OA0}) \times 100$$

Where, RG is the relative percent of germinated seeds; GS_{OAx} is the germinated seeds % in corresponding OA concentration; GS_{OA0} is the germinated seeds % in OA concentration 0 mM.

Evaluation of resistance to *Sclerotinia trifoliorum*

Germination of alfalfa seeds on mycelium of *S. trifoliorum* was done with local population of pathogen. Pathogen isolates were produced from sclerotia collected in alfalfa breeding nurseries of Institute of Agriculture in spring of 2009. Sclerotia were sterilized in solution of 10% sodium hypochlorite for 20 min and rinsed three times in distilled water. Sterilized sclerotia were plated on potato dextrose agar (4%) and grown at 20°C in continuous darkness until formation of mycelium. The further mycelium cultivations were done under the same conditions. The medium virulent isolate was selected as it was recommended in the study of Pratt and Rowe (1995). The growing mycelium was transplanted to fresh medium. Plates with formed sclerotia were selected and placed in a refrigerator at 2°C. The new mycelium was grown for resistance tests until it covered all surface of medium. The seeds preparation, germination and evaluation were the same as for testing of OA resistance.

Field experimental design

The study of alfalfa resistance to Sclerotinia crown and stem rot (SCSR) was done at the Institute of Agriculture situated in the center of Lithuania (55°23‘N, 23°51‘E). The alfalfa nursery for the field experiments was sown after black fallow without a cover crop in July of 2009. The soil of the experimental site was calcaric shallow gleyic Cambisol (Endocalcari-Epihypogleyic Cambisol, CMg-p-w-can) light loam. It contained 2.46% humus, available phosphorus (P_2O_5) ranging from 201 to 270, available potassium (K_2O) from 101 to 175 mg kg^{-1} and pH from 6.5 to 7.2. $P_{60}K_{90}$ was applied pre-sowing. Every alfalfa accession was sown in two 5 m long rows in three replications. The sowing rate was 50 scarified seeds per 1 m. The distance between the rows of accession was 0.5 m; the distance between accessions was 1.0 m.

The herbicide Basagran 480 and insecticide Karate Zeon 5 CS (active compound bentazon 480 g/l and lambda-cihalotrin 50 g/l, respectively) was applied at the rate of 2.0 and 0.15 l/ha when germinated alfalfa plants reached 5 cm height in 2009. The herbicide Fenix 600 SC (active compound aklonifen 600 g/l) was applied at the rate of 3.0 l/ha in spring when alfalfa plants reached 10 cm height in 2010 and 2011. Alfalfa accessions were grown under natural infection pressure and used as the seed crop.

Evaluation of resistance to SCSR

Resistance to SCSR was evaluated two weeks after vegetation

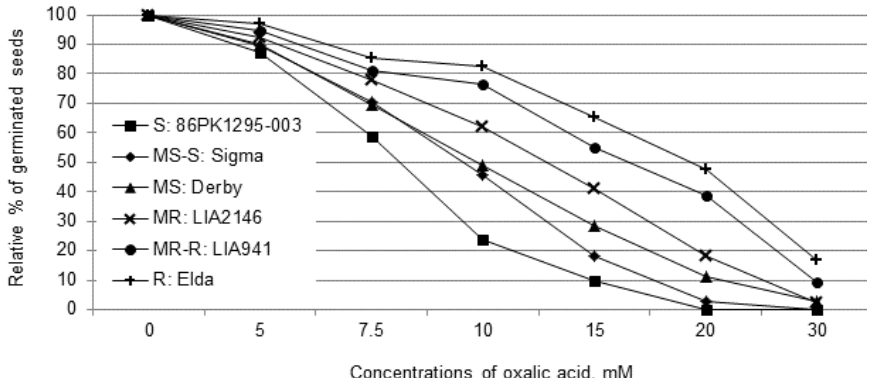

Figure 1. Resistance of alfalfa accessions to different concentrations of oxalic acid.

resumption of alfalfa. The disease severity was measured on a scale of 0-9 scores, where 1 is the lowest value. The following grouping by disease severity scores was used to estimate varietal resistance:

≤3.0 = resistant (R); >3.0 – 4.0 = medium resistant - resistant (MR-R); >4.0 – 5.0 = medium resistant (MR); >5.0 – 6.0 = medium susceptible (MS); >6.0 – 7.0 = medium susceptible - susceptible (MS-S); >7.0 = susceptible (S).

Meteorological conditions

The sowing year was favorable for establishment of alfalfa. The first experimental season was unfavorable for disease development due to long cold with scarce snow winter. The second experimental season was highly favorable for disease development due to warm autumn and long winter with heavy snow.

Statistical analyses

All experiments were performed in triplicate. Duncan's Multiple Range Test was used to evaluate the significant differences between alfalfa genotypes reactions to different treatments at the *P<0.05* probability level. Correlation-regression analysis were performed among treatments at a significance level of *P<0.05* (*) and *P<0.01* (**) to determine the most suitable method. Analyses were done with STATISTIKA 7 statistics software.

RESULTS

The reaction to OA of six alfalfa accessions which belong to different SCSR resistance groups is presented in Figure 1. The selected accessions started considerably and were clearly differentiated by resistance at OA concentration 7.5 mM. The RG of the most susceptible and the most resistant accessions differed 1.45 times. However, the highest differences were found at OA concentrations 10 and 15 mM. The most resistant accession Elda had 82.6% of RG, whereas the most susceptible accession 86PK1295-003 had only 23.8% of RG at OA concentrations 10 mM. The resistance of these accessions at OA concentration 15 mM differed by 65.1 to 9.8% of RG. The higher concentration (20 mM) of OA was very toxic for two most susceptible accessions 86PK1295-003 and Sigma (RG 0.0 and 2.9%, respectively). The two most resistant accessions Elda and LIA941 had rather high RG, 47.6 and 38.5%, respectively. OA concentration 30 mM was mortal for susceptible entries; whereas the most resistant accessions had 16.7 and 9.5% of RG.

The main evaluation criterion was alfalfa resistance to SCSR under field conditions. Eight of the least damaged accessions (4%) were selected per each resistance group (Table 1). The accessions were sorted in ascending order of SCSR severity. Only second growth season showed considerable difference of accessions resistance. Considering the reaction of alfalfa accessions to OA, three OA concentrations (10, 20, 30 mM) were selected as the best to demonstrate differences in accessions resistance. Significant (P<0.05) differences by resistance between selected 48 accessions were determined for all testing methods. Accessions by resistance were sorted to 6 groups from resistant (≤3.0 score) to susceptible (≥7.0 score). Disease severity ranged from 2.3 (resistant) to 8.0 scores (susceptible) in 2011. However, disease severity was much lower (from 1.0 to 2.7 scores) in 2010. It shows that alfalfa SCSR resistance testing under natural infection pressure is not reliable during short time period. *Sclerotinia trifoliorum* mycelium test showed rather low differentiation of accessions resistance. Accessions RG ranged from 12.5 to 85.2%, but RG in resistance groups differed in 1.64, 1.84, 3.54, 5.20, 2.38, 3.10 times for R, R-MR, MR, MS, MS-S, S groups, respectively. All the three OA concentrations considerably differentiated the tested accessions. Their reaction to OA concentrations 10, 20, 30 mM ranged from 12.5 to 85.2, 0.0 to 50.0, and 0.0 to 18.4%, respectively. However, low number of highly resistant plant and low selection intensity in many populations was reported by Kanbe et al. (2002) and Jančys and Vyšniauskienė (2002). Also, our study shows similar results in cases of OA concentration 30 mM as it eliminated all susceptible to SCSR accessions and RG in

Table 1. Resistance of selected alfalfa accessions to Sclerotinia crown and stem rot, oxalic acid and *Sclerotinia trifoliorum.*

Resistance	Accesion	Country of origin	Resistance to SCSR		Relative seed germination			
					Oxalic acid concentrations			Mycelium of *S. trifoliorum*
			2011	2010	10 mM	20 mM	30 mM	
			Scores		%			
R	Karlu	EE	2.3^{a}	1.0^{a}	81.3^{hi}	50.0^{hi}	17.5^{gh}	70.0^{fgh}
R	Kunsmme	EE	2.3^{a}	1.3^{ab}	74.3^{fg}	47.3^{ghi}	10.9^{ef}	72.1^{gh}
R	LIA2448	LT	2.3^{a}	1.0^{a}	69.4^{efg}	39.2^{gh}	14.9^{fg}	71.0^{fgh}
R	Romagnola	IT	2.7^{a}	1.3^{ab}	78.4^{ghi}	35.8^{fgh}	18.4^{gh}	65.2^{fg}
R	Eerik Saare	EE	2.7^{a}	1.3^{ab}	83.6^{hij}	48.2^{ghi}	18.2^{gh}	85.2^{ij}
R	Saartepola	EE	2.7^{a}	1.3^{ab}	82.8^{hij}	48.0^{ghi}	13.3^{ef}	58.2^{ef}
R	LIA2450	LT	2.7^{a}	1.0^{a}	75.4^{fgh}	28.0^{fg}	15.7^{fgh}	80.0^{hi}
R	LIA1147	LT	2.7^{a}	1.0^{a}	77.9^{gh}	26.0^{efg}	10.3^{ef}	52.1^{def}
R-MR	Augūnė	LT	3.3^{b}	1.0^{a}	65.4^{ef}	18.6^{def}	8.6^{def}	72.8^{gh}
R-MR	Nadežda II	RU	3.3^{b}	1.3^{ab}	73.2^{efg}	46.3^{ghi}	8.9^{def}	85.0^{ij}
R-MR	LIA2051	LT	3.3^{b}	1.0^{a}	79.5^{ghi}	58.5^{hij}	17.4^{gh}	82.1^{hi}
R-MR	LIA2145	LT	3.3^{b}	1.0^{a}	77.1^{gh}	23.8^{def}	8.0^{def}	65.4^{fg}
R-MR	LIA859	LT	3.3^{b}	1.0^{a}	75.2^{fg}	24.8^{ef}	7.2^{def}	46.2^{def}
R-MR	LIA971	LT	3.3^{b}	1.0^{a}	57.7^{de}	26.5^{efg}	8.6^{def}	70.0^{fgh}
R-MR	Verko	HU	3.7^{b}	1.0^{a}	75.6^{fgh}	29.5^{fg}	16.4^{fgh}	82.3^{hij}
R-MR	LIA1754	LT	3.7^{b}	1.0^{a}	92.7^{ij}	61.9^{ij}	6.2^{de}	65.8^{fg}
MR	Antanė	LT	4.3^{c}	1.0^{a}	82.0^{hij}	42.1^{gh}	5.8^{cde}	65.4^{fg}
MR	Radius	PL	4.3^{c}	1.7^{bc}	59.1^{def}	26.5^{efg}	6.7^{de}	56.2^{ef}
MR	Viktorija	CZ	4.0^{c}	1.3^{ab}	69.4^{efg}	36.2^{fgh}	7.9^{def}	65.0^{fg}
MR	Creno	DK	4.3^{c}	1.7^{bc}	79.8^{ghi}	44.4^{ghi}	5.9^{cde}	62.5^{efg}
MR	LIA1772	LT	4.0^{c}	1.0^{a}	89.9^{ij}	58.3^{hij}	5.7^{cde}	62.3^{efg}
MR	LIA2146	LT	4.0^{c}	1.0^{a}	62.2^{ef}	18.2^{def}	2.4^{bc}	19.8^{abc}
MR	LIA2182	LT	4.3^{c}	1.0^{a}	81.2^{ghi}	46.7^{ghi}	6.2^{de}	63.2^{efg}
MR	LIA1172	LT	4.3^{c}	1.0^{a}	76.9^{gh}	47.8^{ghi}	16.4^{fgh}	70.0^{fgh}
MS	Vertus	SE	5.3^{d}	1.7^{bc}	54.6^{cde}	9.8^{cd}	3.3^{bc}	43.6^{de}
MS	Janu	NL	5.3^{d}	2.0^{cd}	82.3^{hi}j	17.5^{de}	6.3^{de}	65.0^{fg}
MS	Derby	NL	5.3^{d}	1.3^{ab}	48.9^{cd}	11.2^{cde}	3.0^{bc}	54.2^{def}
MS	Europe	FR	5.3^{d}	1.3^{ab}	76.6^{fgh}	22.7^{def}	1.3^{b}	12.5^{a}
MS	Sitel	NL	5.3^{d}	1.3^{ab}	45.0^{cd}	18.2^{def}	4.7^{cde}	52.3^{def}
MS	LIA1775	LT	5.3^{d}	1.3^{ab}	81.9^{hi}	2.6^{bc}	0.1^{ab}	28.6^{cd}
MS	LIA1985	LT	5.3^{d}	1.3^{ab}	85.4^{hij}	32.6^{fgh}	0.7^{ab}	40de
MS	Belfenil	FR	5.7^{de}	1.3^{ab}	58.7^{de}	12.1^{cde}	0.2^{ab}	23.1^{bc}
MS-S	PI 214218	DK	6.3^{e}	1.7^{bc}	27.7^{abc}	9.7^{cd}	0.6^{ab}	42.8^{de}
MS-S	PGR 12489	USA	6.3^{e}	2.3^{de}	60.4^{def}	17.2^{de}	0.5^{ab}	75.0^{ghi}
MS-S	PGR 12404	USA	6.3^{e}	2.0^{cd}	32.2^{bc}	9.9^{cde}	1.4^{b}	65.0^{fg}
MS-S	Szarvasi	HU	6.3^{e}	2.3^{de}	44.9^{bcd}	10.3^{cde}	0.2^{ab}	42.3^{de}
MS-S	Vertibenda	PL	6.3^{e}	2.0^{cd}	59.5^{def}	1.7^{b}	0.0^{a}	32.9^{cde}
MS-S	PGR 12410	USA	6.3^{e}	2.0^{cd}	59.8^{def}	4.9^{c}	0.2^{ab}	51.0^{def}
MS-S	PI 212104	AF	6.3^{e}	2.3^{de}	38.9^{bc}	4.9^{c}	0.2^{ab}	31.5^{cd}
MS-S	LIA1986	LT	6.3^{e}	1.3^{ab}	40.6^{bcd}	5.9^{c}	0.0^{a}	40.0^{de}
S	LIA1769	LT	7.2^{f}	1.7^{bc}	23.8^{ab}	1.0^{b}	0.0^{a}	23.5^{bc}
S	PGR 12431	USA	7.3^{fgh}	2.3^{de}	23.8^{ab}	0.0^{a}	0.0^{a}	45.0^{def}
S	PI 211609	AF	7.3^{fgh}	2.3^{de}	44.6^{bcd}	2.3^{bc}	0.0^{a}	37.9^{de}
S	Katinka	RO	7.3^{fgh}	2.7^{ef}	44.6^{bcd}	1.3^{b}	0.0^{a}	31.0^{cd}
S	Mireille	RO	7.7^{gh}	1.7^{bc}	28.9^{abc}	0.0^{a}	0.0^{a}	14.5^{ab}
S	PGR 10268	USA	7.7^{gh}	2.7^{ef}	15.9^{a}	0.0^{a}	0.0^{a}	22.8^{bc}
S	86PK1295003	PK	8.0^{h}	2.0^{cd}	23.8^{ab}	0.0^{a}	0.0^{a}	21.9^{bc}

Table 1. Cont'd.

S	X 910041	CH	8.0^{h}	2.3^{de}	13.4^{a}	0.3^{ab}	0.0^{a}	29.7^{cd}

AF, Afganistan; EE, Estonia; CH, China; CZ, Czeck Republic; DK, Denmark; FR, France; HU, Hungary; IT, Italy; LT, Lithuania; NL, Netherlands; PK, Pakistan; PL, Poland; RO, Romania; RU, Russia; SE, Sweden; USA, United States of America. Means followed by the same lower case letter in the same column are not significantly different at the *P<0.05* probability level, based on Duncan's Multiple Range Test.

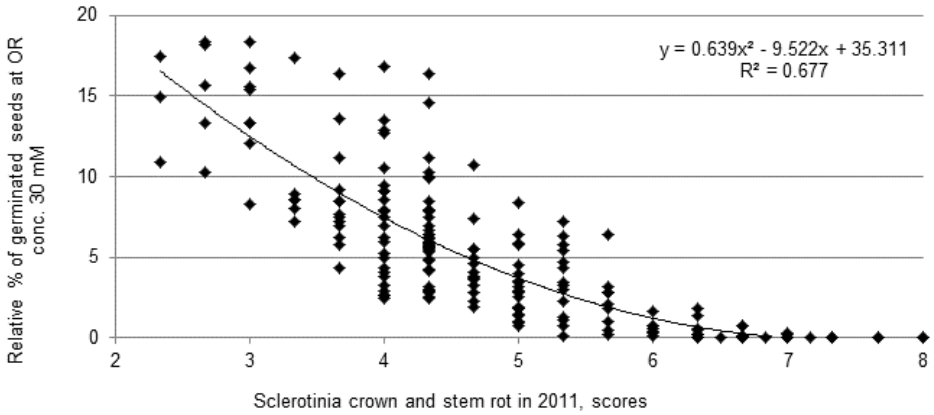

Figure 2. Relationship of alfalfa accessions resistance to Sclerotinia crown and stem rot and oxalic acid.

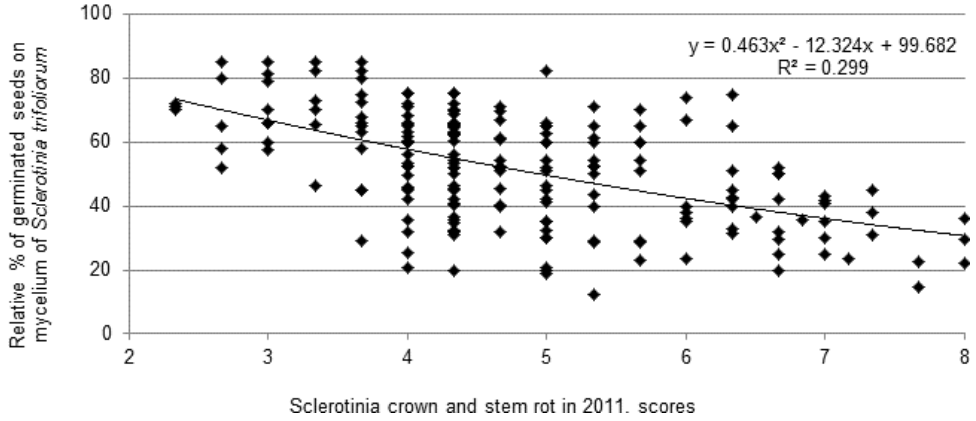

Figure 3. Relationship of alfalfa accessions resistance to Sclerotinia crown and stem rot and *Sclerotinia trifoliorum.*

groups of MS-S and MS was very low too. OA concentration 30 mM was suitable for the selection of individual seedling from more resistant accessions, whereas concentration 20 mM was favorable for screening of less resistant material.

The country of origin showed considerable impact on resistance to SCSR as well as to OA. Resistant and resistant-medium resistant accessions clearly dominated Lithuanian and Estonian accessions. Accessions with medium SCSR reaction belonged to different European countries. The most susceptible accessions originated from geographically distinct countries such as USA, Afghanistan and Romania.

The accessions showed highly varying percent of RG in OA concentration 30 mM at the same resistance to SCSR group (Figure 2). The accessions RG percent per resistance groups differed in 2.2, 6.9, 23.4, 7.2, 1.8 and 0.0 times with averages of 14.5, 7.9, 5.1, 2.5, 0.2 and 0.0% for R, R-MR, MR, MS, MS-S, S groups, respectively. However, correlation between resistance to SCSR and OA was strong (r = 0.817**, P<0.01).

The accessions showed considerably lower variation of RG percent on mycelium at the same resistant to SCSR group (Figure 3). Accessions RG differed by 1.6 to 5.9

Table 2. Coeficients of correlation among alfalfa resistance to Sclerotinia crown and stem rot, relative seed germination at oxalic acid concentrations and mycelium of *Sclerotinia trifoliorum.*

Trait	SCSR (2011)	SCSR (2010)	OR (10 mM)	OR (20 mM)	OR (30 mM)
SCSR, 2010	0.747**				
OR, 10 mM	-0.715**	-0.595*			
OR, 20 mM	-0.679**	-0.520*	0.631**		
OR, 30 mM	-0.817**	-0.481*	0.509**	0.656*	
S. trifoliorum mycelium	-0.545*	-0.291	0.359*	0.433*	0.641*

* and ** significant at *P<0.05* and *P<0.01*, respectively. SCSR, severity of Sclerotinia crown and stem rot; OR, oxalic acid.

times and the mean of RG per resistance group ranged from 29.1 to 69.9%. Correlation between resistance to SCSR and mycelium was medium (r = 0.547*, P<0.05).

The alfalfa reaction to SCSR in 2011 showed the highest rate of correlations with other traits (Table 2). The strong correlations were between this trait and disease severity in 2010, and RG at OA concentration 10 and 30 mM (r = 0.747**, -0.715**, -0.782**, respectively). Medium correlations were calculated for RG at OA concentration 20 mM and *S. trifoliorum*. The lowest correlation (weak to medium) rate with other traits showed mycelium of *S. trifoliorum*.

DISCUSSION

The goal of this research was to test whether laboratory resistance screening methods can be applied for rapid and reliable screening of alfalfa resistance to SCSR. Differentiation of tested accessions by SCSR resistance was very considerable and ranged from resistant to susceptible. It shows that crop of non-adapted cultivars of alfalfa under Lithuanian conditions can be destroyed in the second exploitation year only by one disease SCSR under natural disease pressure. Also, the other very harmful diseases such as downy mildew (*Peronospora trifoliorum* de Bary) and spring black stem and leaf spot (*Phoma medicaginis* var. *medicaginis* Malbr.) destroys alfalfa crop (Liatukienė et al., 2011). Therefore, SCSR resistant accessions should be selected due to further resistance selection stages as well as selections for other agronomical traits. Correlations of accessions reaction to SCSR in 2010 and 2011 with accessions RG at different OA concentrations and *S. trifoliorum* mycelium showed that alfalfa should be tested with OA concentration 30 mM. Lower concentrations (10 and 20 mM) showed very similar correlation level, but seedlings counting lasted longer due to higher RG. OA concentration 30 mM was highly favorable for fast screening and elimination of susceptible and medium susceptible accessions due to high mortality of seedling. This method showed efficient possibility to select resistant to OA seedlings using simple materials and less inputs compared with those described in literature methods (Callahan and Rowe, 1991; Rowe, 1993; Pratt and Rowe, 1998). *S. trifoliorum* mycelium test showed low advantages for rapid and reliable resistance testing. Possibly, lower correlations of accessions RG in this test with SCSR under field conditions was related to plant infection process differences in the laboratory and field. The primary infection source under field conditions is ascospores and not the mycelium, which is the secondary infection source (Dijkstra, 1964). However, the study of Pratt (1996) and Pratt and Rowe (1998) showed that *S. trifoliorum* mycelium was suitable for screening and selection of resistant alfalfa accession as well as single plants. These researches were done with excised leaves of different ages but germinating seeds were not involved. Possibly, methods of these authors could be used for complementation of further selection cycles with seedlings that survived OA concentration 30 mM. Such complementation could be favorable for selection of plants possessing very high complex resistance to *Sclerotinia* spp. The study of Callahan and Rowe (1991) showed complex effect of pathogen mycelium exudate on growth of seedlings. Among tested 200 accessions, only 16 accessions (8%) were resistant. These alfalfa accessions showed RG from 8.3 to 18.4% at OA concentration 30 mM with an average of RG 14.5%. Such RG level is favorable for further maintenance of survived seedlings as prolonged negative effect of OA is very likely (Saharan and Mehta, 2008).

Development of lucerne breeding population with desirable agronomical traits requires at least several hundreds of plants possesing desirable traits. The relative low number of plants resistant to at least one disease makes breeders to compose initial breeding populations using several thousands of plants. Development of population using such number of selected resistance plants allows testing young plants for resistance to other disease under greenhouse conditions, for example, Fusarium crown rot (Salter et al., 1994). This layout of screenings makes possibility for selecting plants resistant to couple of diseases. Also, seeds of these plants are received twice in the same year if plants are further maintained under greenhouse conditions. One seed yield per year could be received if plants are planted outside at appropriate time. Otherwise, selecting

plants resistant to couple of diseases and receiving their seeds under field conditions of temperate climate can take up to ten years even when screenings are done in nurseries with artificial infection (Kanbe et al., 2002; Nagy, 2003).

Considering number of resistant plants among our accessions, such population could be developed only when at least 100 g of seeds would be screened. Therefore, such screening is more acceptable in the case of the selection of resistant plants from cultivars as parental breeding material for subsequent breeding cycles and development of the new populations. Also, improvement of advanced populations are possible too. The lucerne populations with lower seed quantity available could be tested for tentative determination of resistance to OA.

CONCLUSION

These investigations showed that oxalic acid successfully can be applied for screening of alfalfa accessions resistance to Sclerotinia crown and stem rot. The method used should be successfully adapted for development of new alfalfa cultivars resistant to Sclerotinia crown and stem rot as laboratory experiments lasted just several days and hundreds of seeds per accession were tested in very limited space.

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