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Winterhardiness as the key factor for selecting accessions of *Medicago sativa* L. high-yielding germplasm

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The ultimate purpose of developing new varieties of *Medicago* spp. is selection of the initial genetic material characteristics responsible for the stabile yield of dry matter. The greatest shortcomings of conventional methods used to determine herbage yield are labour-consuming cuts and the processing of harvested green matter. The main task of the present studies was evaluation of the genetic material of *Medicago sativa* L. developed using germplasms received from different regions with an impact on winter hardiness and relations with this trait to other properties of accessions. The experiment was conducted at the Lithuanian Institute of Agriculture (LIA) during 2004–2006. Low winterhardiness highly negatively ($r = -0.85^*$, significant at $P = 0.05$) correlated with spring re-growth and negatively affected ($r = -0.65^*$) the dry matter yield of the first cut. Effective spring re-growth positively ($r = 0.41^*$) influenced plant height before the 1st cut and medium positively ($r = 0.66^*$) the yield of the 1st cut as well as total yield ($r = 0.62$). Analysis showed that the influence of winterhardiness on the yield of lines with different resistance was considerable. The least damaged lines yielded on average 8.67 t ha^{-1}, whereas most severely damaged lines yielded 5.43 t ha^{-1} or 37.4% less. Plant height before the 1st cut showed a weaker effect than winterhardiness and spring re-growth. Lines with highest differences in height produced a herbage yield whose differences varied within 25% for the 1st cut and 17% for total yield. Plant height sequentially decreased at every later regrowth and cutting time. Plants of populations with highest winterhardiness were on average slightly lower than those with poor winterhardiness. The highest plants (97 cm) at the 1st cut were found in populations LIA2104, LIA9//10, LIA2358, LIA 2311 with a medium winterhardiness level. The percentage of dry matter in herbage yield decreased with every cut. The highest decrease of dry matter content (25.4% to 20.3%) was observed in populations with a high winterhardiness. Winterhardiness and spring re-growth were the most effective indirect criteria for selecting high-yielding lines among the initial breeding material of *Medicago* spp.

Key words: *Medicago sativa***,** dry matter yield, spring re-growth, plant height

INTRODUCTION

Lucerne (*Medicago sativa* subsp. *sativa*) is one of the most important forage crops in the world. Lucerne has not only a high nutritional quality as animal feed, but also a potential to be used in soil bioremediation, as biofuel and for the production of industrial enzymes. The biological N_2 fixation of lucerne is the highest among cultivated leguminous plants and ensures production of cheap forages [1]. This trait is very significant in developing sustainable agriculture. It is comparatively expensive to establish stands again. Long-term persistence and sustained biomass yields are important to producers because they allow the costs of seeding to be amortized over a longer period. Persistence is a complex trait affected by a large number of factors, including growing technologies, biotic and abiotic stresses [2]. Desirable agronomic field traits include rapid re-growth, erectness, height, winterhardiness, early spring recovery [3]. Winterhardiness exerts a high impact on the later development and yield of lucerne crop stands in northern climates. Investigation of landraces and cultivars in countries highly differing by climatic conditions such as Italia, Mongolia, USA and Canada showed that winter mortality highly negatively correlated with dry matter yield [4–6]. A recent investigation of Estonian accessions with good winterhardiness in Lithuania revealed that only a few populations were superior by dry matter or seed yield over Lithuanian cultivars. These genotypes, adapted to a colder climate, were inferior in respect of the yield formation potential, but suitable for breeding [7]. Many of highly advanced lucerne genotypes are developed in a warmer climate than Lithuanian. This is problematic because newly developed populations in the breeding process are too susceptible to winterkill. Evaluation of the initial breeding material is often complicated due to the need to screen a plenty of populations in several years. Winterhardiness is one of essential traits in all overwintering plants. This trait is even more important for perennial plants such as alfalfa.

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MATERIALS AND METHODS

The experiment was carried out at the Lithuanian Institute of Agriculture (LIA) during 2004–2006 in the fields of a six-course rotation in forage grasses. The accessions tested were locally developed from the geographically broad material. Countries of the initial material origin were the USA (northern states), Canada, Russia, Estonia, Western European countries. In total, 160 populations were sown. Every breeding population was sown in two 5-meter long rows. The soil of the experimental site is *Endocalcari-Endohypogleyic Cambisol* CMg-n-w-can (pH 7.3, P_2O_5 200–270 mg kg^{-1} and K_2O 100–175 mg kg^{-1}, humus 2.46%). The lucerne nursery was sown after black fallow without a cover crop in July 2004. The sowing rate was 0.2 g (about 100 viable seeds) per 1 m. The distance between rows in a line was 0.5 m; the distance between the lines was 1.0 m. The initial number of plants was high (50–100 units per a 1-m row), therefore, every population was evaluated for all traits as one unit. Breeding nurseries of alfalfa were used for two years. In the first year, the crop stand was assessed for seed yield. In the second year, the breeding lines were evaluated for herbage yield. The nursery was evaluated for winterhardiness, spring re-growth, and plant height before seed and grass harvesting. In the second year, herbage yield was taken three times. Dry matter yield was evaluated as completely dry. Winterhardiness was evaluated after resumption of vegetation in scores 1–5, where 1 is the least damage by winterkill. Spring re-growth was evaluated two weeks after resumption of vegetation by scores 1–3, 1 being the lowest value. Plant height was measured in cm, before harvesting and at after two weeks of regrowth after cuts. Dry matter yield was measured in t ha^{-1}. The nursery of the second year was selected for analysis as more environmentally affected. The sowing year was favourable for establishment of plants. The first winter did not cause winterkill damage. The spring and summer of 2005 were favourable for the later establishment of crop stand. The dry and long autumn affected the autumnal re-growth of plants. The winter of 2006 was severe, and the winterhardiness of the genotypes screened was different. The spring and summer periods were dryer than usual and negatively affected plant growth. The nursery of the 2nd year was selected as the test populations were exposed to a broader range of environmental conditions. Correlation analysis was used to evaluate relationships among the traits. The obtained correlation coefficients were compared for significance level at P = 0.05.

RESULTS AND DISCUSSION

One of the problematic tasks in the sustainable production and long-term persistence of lucerne in Lithuania is maintenance of crop-stand with a uniform and high density of plants. Low winterhardiness highly negatively (r = –0.85*, significant at P = 0.05) correlated with spring re-growth (Table 1). The damage of plants negatively affected (r = –0.65*) the dry matter yield of the first cut. Later, the plants partially recovered as their winterkill damage did not correlate with the dry matter yield of the second and third cuts. However, the total dry matter yield was medium negatively (r = –0.58*) influenced by a lower winterhardiness. The effective spring re-growth weakly positively (r = 0.41*) influenced plant height before the 1st cut and medium positively (r = 0.66*) the yield of the 1st cut as well as the total yield (r = 0.62). Plant height before the 1st cut medium correlated (r = 0.51* – 0.62*) with the study traits, except for the yield of the 3rd cut (r = 0.41). Plant height before the other cuts correlated with the other traits less. Dry matter yield of the 1st cut medium correlated (r = 0.58*) with the yield of the 2nd cut. This trait very strongly (r = 0.94*) correlated with the total yield. This relation means that the yield of the 1st cut accounted for the largest share in the total yield.

The data shown in Table 2 reveal the effect of the screened traits on dry matter yield of all cuts. Analysis of the influence of winterhardiness on the yield of lines with different resistance showed a considerable effect. The differences were most significant among the yields of lines at the 1st cut. Lines with the lowest damage yielded on average 8.67 t ha^{-1}, whereas the most severely damaged lines yielded on average 5.43 t ha^{-1} or by 37.4% less. Some differences were noted among the lines when comparing them by the yield of the 2nd cut. In percentage, the lines with best and worst overwintering differed 24%. The yield of the 3rd cut lines was similar. Lines most different by overwintering differed by the total yield 1.34 times. Spring re-growth rate clearly characterized the lines by their yielding capacity, too. The highest differences (47%) among the lines were detected for the yield of the 1st cut. The differences for the total yield were less (27%). The yields of the 2nd and 3rd cuts weakly discriminated the lines.

Table 1. **Correlations of traits of lucerne accessions**

Trait	2	3	4	5	6	7	8	9
Winterhardiness	–0.85*	–0.33*	–0.65*	0.00	–0.29	–0.08	–0.07	–0.58*
Spring regrowth	-----	0.41*	0.66*	0.12	0.37*	0.14	0.17	0.63*
Plant height	-----	-----	0.51*	0.52*	0.59*	0.53*	0.41	0.62
Dry matter yield, 1st cut	-----	-----	-----	0.25	0.58*	0.20	0.19	0.94*
Plant height	-----	-----	-----	-----	0.57*	0.33*	0.30	0.39
Dry matter yield, 2nd cut	-----	-----	-----	-----	-----	0.29	0.34	0.76*
Plant height	-----	-----	-----	-----	-----	-----	0.48*	0.32
Dry matter yield, 3rd cut	-----	-----	-----	-----	-----	-----	-----	0.45*
Dry matter yield, total	-----	-----	-----	-----	-----	-----	-----	-----

* Significant at the probability level P = 0.05.

Table 2. **Detailed analysis of traits affecting yield of dry matter of lucerne germplasm**

Trait	Evaluation scale	Number of lines	Dry matter yield, t ha^{-1}			
			1st cut	2nd cut	3rd cut	Total
Winter-hardiness, scores	1	43	8.67	3.04	2.96	14.66
	2	23	8.14	3.01	2.98	14.13
	3	50	7.55	3.09	2.82	13.46
	4	31	6.27	2.82	2.94	12.03
	5	12	5.43	2.46	3.07	10.96
Spring re-growth, scores	1	35	5.86	2.70	2.99	11.55
	2	80	7.65	3.05	2.86	13.56
	3	44	8.62	3.03	2.98	14.63
Plant height before the 1st cut, cm	≤85	28	6.81	2.71	2.91	12.43
	86–90	36	7.33	2.87	2.87	13.07
	91–95	62	7.44	3.07	2.92	13.43
	≥96	33	8.52	3.09	2.98	14.59
Plant height before the 2nd cut, cm	≤60	56	7.47	2.76	2.97	13.21
	61–65	37	7.55	3.01	2.78	13.35
	66–70	36	7.58	3.04	2.96	13.57
	≥71	30	7.54	3.20	2.95	13.69
Plant height before the 3rd cut, cm	≤40	24	7.47	2.89	2.65	13.00
	41–45	63	7.65	3.02	2.89	13.55
	46–50	51	7.44	2.96	3.03	13.44
	≥51	21	7.43	2.92	3.08	13.42

Plant height before the 1st cut showed a weaker effect than winterhardiness and spring re-growth. Lines with greatest differences in height, according to the yield of the 1st cut differed by 25% and the total yield by only 17%. Plant height before the 2nd and 3rd cuts poorly characterized the lines' yielding capacity.

The populations presented in Table 3 were selected for analysis in every winterhardiness group by total dry matter yield ranging from the highest to the lowest. Plant height sequentially decreased at every later regrowth and cutting time. The population LIA9//10 with medium winterhardiness was highest at 2 weeks after spring regrowth (43 cm) and the population LIA2318 with poor winterhardiness was the lowest (23 cm). Populations with the highest winterhardiness were on average slightly lower than those with poor winterhardiness. The highest plants (97 cm) at the 1st cut were found in populations LIA2104, LIA9//10, LIA2358, LIA 2311 with a medium winterhardiness level. The later regrowth and cuts showed a similar tendency.

Data presented in Table 4 show that the percentage of dry matter in herbage yield decreased with every cut. The average highest decrease of dry matter content was observed for populations with a high winterhardiness (25.4% to 20.3%). Such tendency may depend on the decreasing reserves in roots because the share of the first cut in the total yield was larger than the sum yield of the rest cuts. The average decrease was lowest in populations with a poor winterhardiness (24.3% to 21.7%). The highest content of dry matter at the 1st cut was shown by population LIA2182 and LIA2348 (33.6% and 27.9%, respectively).

Table 3. **Winterhardiness, spring regrowth, plant height at different regrowth cuts of lucerne populations**

Accessions	Winter-hardiness, scores	Spring re-growth scores	Plant height, cm					
			14 d after re-growth	1st cut	14 d after 1st cut	2nd cut	14 d after 2nd cut	3rd cut
LIA278	1	3	31	74	25	54	15	39
LIA488	1	3	36	82	26	52	19	45
LIA219	1	3	35	96	35	71	16	40
LIA2321	1	3	39	94	30	60	19	50
LIA1180	1	3	40	93	39	61	25	58
LIA2182	1	3	38	96	32	64	16	50
LIA1919	2	3	32	91	30	61	10	44
LIA2104	2	2	36	97	39	54	19	49
LIA2306	2	3	38	93	25	54	16	56
LIA9/97	2	2	38	98	33	62	12	40
LIA2078	2	2	39	94	33	60	13	42
LIA1176	2	3	42	85	35	70	18	45
LIA62/18	3	2	35	93	31	62	17	50
LIA2200	3	2	33	91	28	56	17	53
LIA97/10	3	3	35	90	34	68	20	39
LIA2125	3	2	38	93	31	66	14	50
LIA2348	3	1	40	94	36	72	13	41
LIA9//10	3	2	43	97	32	64	10	42
LIA2058	4	1	32	96	25	51	12	47
LIA1//03	4	2	36	86	33	68	15	35
LIA2306	4	1	37	89	32	64	19	53
LIA2358	4	2	37	97	39	78	17	46
LIA2095	4	1	31	92	36	72	20	43
LIA2311	4	1	29	97	34	70	12	46
LIA11/87	5	1	26	81	31	64	10	41
LIA2318	5	1	23	82	24	50	10	42
LIA533	5	1	30	89	27	55	15	45
LIA1888	5	1	35	87	28	56	19	39
LIA2309	5	1	29	77	27	56	22	49
LIA2104	5	1	38	85	33	66	17	49

The ultimate purpose of developing a line is a high and stabile yield of dry matter. The greatest shortcomings of conventional methods used to determine herbage yield are labour-consuming cuts and the processing of harvested green matter. The determination of traits which allow breeders to select effectively under local conditions for high and stabile yield is highly desirable. Winterhardiness and spring re-growth were the best characterizing traits among the screened lines for dry matter yield at the 1st cut and for the total yield. Winterhardiness is the key trait for lucerne. The newly developed breeding numbers contain a plenty of

Table 4. **Herbage yield at different cuts of lucerne breeding populations**

Accessions	Yield of grass at 1st cut			Yield of grass at 2nd cut			Yield of grass at 3rd cut		
	Green, t ha^{-1}	Dry, t ha^{-1}	% of dry	Green, t ha^{-1}	Dry, t ha^{-1}	% of dry	Green, t ha^{-1}	Dry, t ha^{-1}	% of dry
LIA278	30.8	7.3	23.7	8.6	1.8	20.9	10.6	1.9	17.9
LIA488	32.2	7.2	22.4	13.2	2.8	21.2	14.2	3.0	21.1
LIA219	32.4	7.9	24.4	16.4	3.8	23.2	13.2	2.6	19.7
LIA2321	31.2	7.6	24.4	14.6	3.2	21.9	17.6	3.9	22.2
LIA1180	40.6	9.7	23.9	13.8	3.0	21.7	14.8	3.0	20.3
LIA2182	37.2	12.5	33.6	14.8	3.4	23.0	14.0	2.9	20.7
LIA1919	28.2	6.5	23.0	12.8	2.8	21.9	10.8	2.3	21.3
LIA2104	27.8	6.6	23.7	13.2	3.0	22.7	14.6	3.1	21.2
LIA2306	32.2	7.6	23.6	11.6	2.6	22.4	17.4	3.5	20.1
LIA9/97	37.4	8.5	22.7	14.6	3.3	22.6	12.0	2.4	20.0
LIA2078	35.0	8.7	24.9	15.0	2.8	18.7	15.2	3.3	21.7
LIA1176	37.6	10.1	26.9	16.0	3.6	22.5	19.2	4.2	21.9
LIA62/18	26.8	5.9	22.0	10.4	2.0	19.2	14.2	2.8	19.7
LIA2200	26.4	5.8	22.0	12.6	3.0	23.8	16.2	3.2	19.8
LIA97/10	29.4	7.2	24.5	11.8	2.7	22.9	12.4	2.8	22.6
LIA2125	32.8	7.5	22.9	16.6	3.6	21.7	13.8	2.5	18.1
LIA2348	29.4	8.2	27.9	14.6	3.6	24.7	12.2	2.8	23.0
LIA9//10	40.2	10.8	26.9	13.6	3.3	24.3	16.0	3.0	18.8
LIA2058	22.2	5.2	23.4	10.6	2.2	20.8	12.2	2.9	23.8
LIA1//03	24.4	5.9	24.2	10.2	2.5	24.5	13.8	2.8	20.3
LIA2306	26.2	6.4	24.4	11.2	2.5	22.3	14.8	2.8	18.9
LIA2358	25.0	6.1	24.4	12.4	3.2	25.8	12.6	2.9	23.0
LIA2095	28.0	6.6	23.6	13.6	2.9	21.3	14.6	3.3	22.6
LIA2311	30.6	7.8	25.5	14.8	3.4	23.0	14.4	3.1	21.5
LIA11/87	17.2	4.2	24.4	9.6	2.2	22.9	13.4	2.9	21.6
LIA2318	31.0	6.7	21.6	7.6	1.6	21.1	9.6	1.8	18.8
LIA533	21.8	5.3	24.3	11.4	2.5	21.9	13.0	2.7	20.8
LIA1888	27.2	6.3	23.2	11.0	2.4	21.8	15.2	2.7	17.8
LIA2309	25.4	6.5	25.6	12.4	2.7	21.8	14.4	3.2	22.2
LIA2104	25.4	6.2	24.4	11.8	2.5	21.2	18.0	3.9	21.7

different genotypes. Lucerne populations could be effectively selected for winterhardiness, if severe winter conditions occurred a few years in succession. During the test period, one of two winters was satisfactory for the characterization of lines. Grouping of lines by resistance scores and calculation of the average yield per group showed a considerable effect. However, climate warming results in a high unpredictability of weather conditions. The last decade has been characterized by mild winters which are a disadvantage for developing new cultivars with a high winterhardiness. In the absence of winterhardiness data, alfalfa breeders can predict the potential of genotypes by the related traits [8, 9]. The spring re-growth showed a good correlation with the dry matter yield of the 1st cut and the total dry matter yield. This trait is available every year, with minor fluctuations. Evaluation of this trait is very rapid and effective for selecting the highest-yielding lines when they are grouped by re-growth scores. Plant height showed a medium correlation with the majority of traits. However, this trait characterized lines by yielding capacity much less than did winterhardiness and spring re-growth. Considering our findings, we can suggest evaluation of winterhardiness and spring re-growth as the least labour-consuming criteria for selecting high-yielding lines among the initial breeding material of lucerne.

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ŽIEMKENTIŠKUMAS KAIP PAGRINDINIS VEIKSNYS *MEDICAGO SATIVA* L. GENETINĖS MEDŽIAGOS ATRANKAI

Santrauka

Kuriant naujas liucernų veisles svarbu atrinkti genetinę medžiagą, pasižyminčią dideliu ir stabiliu sausosios medžiagos derliumi. Didžiausias tradicinių metodų, nustatančių žolės derlingumą, trūkumas yra poreikis pjauti žolę kelis kartus per sezoną bei vėlesnis žalios masės apdorojimas. Lietuvos žemdirbystės institute 2004–2006 m. buvo tiriama liucernų genetinė medžiaga, sukurta naudojant genotipus, kurie gauti iš įvairių regionų akcentuojant žiemkentiškumą ir jo ryšius su kitais veiksniais. Prastas žiemkentiškumas neigiamai veikė (r = –0,85*, patikimas esant P = 0,05) pavasarinį ataugimą bei (r = –0,65*) pirmos pjūties sausosios medžiagos derlių. Efektyvus pavasarinis ataugimas teigiamai koreliavo (r = 0,41*) su augalų aukščiu prieš pirmą pjūtį, pirmos pjūties sausųjų medžiagų derliumi (r = 0,66*) bei bendru sausųjų medžiagų derliumi (r = 0,62*). Žiemkentiškumo analizė rodo, kad labiausiai pagal šį požymį besiskiriančios linijos taip pat stipriai skyrėsi ir pagal derlingumą. Atspariausių linijų grupės pirmos pjūties derlius buvo 8,67 t ha^{-1}, o labiausiai pažeistos linijos vidutiniškai užaugino tik 5,43 t ha^{-1}, arba 37,4% mažiau. Augalų aukštis prieš 1-ą pjūtį buvo silpniau susijęs su derlingumu nei žiemkentiškumas ir pavasarinis ataugimas. Augalų aukštis nuolat mažėjo kiekvieno ataugimo ir pjūties metu. Geriausiai peržiemojusios populiacijos vidutiniškai buvo žemesnės nei prasčiau peržiemojusios. Vidutiniškai peržiemojusių LŽI2104, LŽI9//10, LŽI2358, LŽI2311 populiacijų augalai buvo aukščiausi (97 cm) pirmos pjūties metu. Sausosios medžiagos kiekis mažėjo per kiekvieną vėlesnę pjūtį. Geriausiai peržiemojusių populiacijų sausosios medžiagos kiekis sumažėjo labiausiai: nuo 25,4 iki 20,3%. Žiemkentiškumo ir pavasarinio ataugimo vertinimas pareikalavo mažiausiai darbo sąnaudų atrenkant derlingas liucernų populiacijas.

Raktažodžiai: *Medicago sativa,* sausųjų medžiagų derlius, pavasarinis ataugimas, augalų aukštis