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Association of aluminium tolerance with morphological traits in lucerne (*Medicago sativa* L.)

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The experiment was carried out at the Lithuanian Institute of Agriculture under greenhouse conditions in spring–summer 2008. The experimental material consisted of 37 accessions of *M. sativa* subspecies differing by aluminium tolerance, and three Lithuanian cultivars ('Augūnė II', 'Birutė' and 'Žydrūnė'). A statistically significant variation among the accessions was apparent for all the nine morphological traits studied ($P < 0.05$). These traits were plant rankness, amount of leaves, height, stem density, stem thickness, stem position, leaflet size, chlorophyll concentration, seed yield. The number of accessions that statistically significantly exceeded all Lithuanian cultivars varied from 1 to 20 out of 37 accessions, depending on trait. Accession 'PI 573140' was superior by the values of seven traits from those nine studied. Accession 'PI 419506' exceeded Lithuanian cultivars by values of five traits, and six accessions ('PI 573135', 'PI 502485', 'PI 499547', 'PI 310338', 'PI 452444' and 'PI 478550') were superior by values of four traits.

The paired regression analysis revealed that tolerance to Al in lucerne accessions was not statistically significantly negatively related with morphological traits. Al tolerance positively significantly ($P < 0.05$) correlated with height and seed yield, $r = 0.353$ and $r = 0.339$, respectively. Some positive tendency was observed in the case of rankness ($r = 0.207$), stem thickness ($r = 0.211$) and stem position ($r = 0.307$). A negative tendency was observed for the amount of leaves ($r = –0.239$).

Key words: lucerne, aluminium tolerance, morphological traits

INTRODUCTION

Lucerne (*Medicago sativa* L.) is one of the fodder plants widely grown worldwide. However, expanding of area under lucerne in some regions is limited by soil acidity and toxic aluminium (Al). Al toxicity, associated with acid soils, is particularly a growth-limiting factor due to restricted rooting depth and branching in plants, which in turn decrease drought tolerance and the use of subsoil nutrients. Liming increases the top-soil pH reaction, but the sub-soil reaction is changed insignificantly [1]. Lithuanian soils, especially in its western and eastern arable areas with a considerable share under grasslands earlier intensively limed, are becoming acidic and their pH gradually returns to the level before intensive liming [2].

Previous investigations of *Medicago* species showed its high sensitivity to Al [3, 4]. As whole populations or cultivars without individual selection were attempted to improve for Al tolerance, this approach has not been productive [5, 6]. However, the recent papers show the possibility of selecting and breeding populations with an increased tolerance to aluminium, methods of recurrent selection have been applied [7, 8]. Breeding for acid-tolerance and / or Al-tolerant cultivars appears to be the most economically viable solution to the acid soil problem, because genetic selection for acid soil tolerance offers an avenue for increasing plant production and reducing production costs [9].

Perspective breeding of Al-tolerant lucerne cultivars requires effective resistance donors [7, 10]. In some cases, resistance factors can be associated with poor agronomic traits. This is valid when resistance sources originate from a relatively small area with specific climate conditions or when resistance sources genetically differ from agronomically cultivated genotypes [6, 11–13]. Such possible relations suggest the necessity of evaluating the tolerance sources before using them for breeding. In this study, accessions of *M. sativa* subspecies differing by Al tolerance and intended for use in breeding were compared for a relation of Al tolerance with morphological traits.

MATERIALS AND METHODS

The experimental material consisted of 37 accessions of *M. sativa* subspecies and three Lithuanian cultivars – 'Augūnė II', 'Birutė' and 'Žydrūnė'. Seeds of accessions were obtained from the USDA-ARS Germplasm Resources Information Network (GRIN) centre. This institution provides information on plant germplasm resources, therefore, it was possible to select lucerne

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accessions with desirable traits. Accessions most tolerant to aluminium were selected. The tolerance indexes and the GRIN codes of the selected accessions are presented in Table 1. The tolerance indexes were obtained by comparing the reaction of accessions to acid soil containing Al with the reaction of the standard Al-tolerant check genotype GAAT [14]. The other selection criterion was the origination from temperate or harsh climate countries.

The accessions were seeded in April 2008 with scarified, *Rhizobium* not-inoculated 30 seeds separately in plastic pots filled with 10 l of a mix of soil and peat moss substrate pH 7.0 (1 : 1). Each accession was seeded into three pots and arranged together in a greenhouse. A greenhouse was isolated from insects-pollinators. Pests were controlled when appeared. Ventilation was adjusted to avoid air temperatures above 28 °C. Pots were watered to field moisture capacity on a daily basis. At the end of the 3rd week, seedlings were thinned; ten most vigorous seedlings in each pot were left for further evaluations. Plants were fertilized with a complex of nutrient elements. The mixture consisted of nitrogen, phosphorus, potassium, sulphur and magnesium (1.0 : 0.5 : 1.0 : 0.5 : 0.25). Fertilisation was done three times at the post-germination, mid-vegetative and early flower stages at a rate of 3 g per pot. Pollination was done by hand the during flowering period. Plants of each accession in pots were cross-pollinated between themselves, avoiding cross-pollination among accessions. The morphological characters such as rankness, amount of leaves, height, stem number, stem thickness, position of stems (prostrate – the lowest value or erect – the highest value) were evaluated at the early flower bud stage in scores 1–5, 1 being the lowest value. The length and width of the terminal leaflet were measured in mm [15, 16]. A SPAD-502 leaf chlorophyll (Chl) meter was used to obtain the Chl concentration of 20 upper well-developed leaves per accession; data are presented as values of this device [17]. Pods were harvested at ripe-seed pod stage, dried, hand-threshed and weighed in grams. Analysis of variance was conducted for evaluated traits. Data were analysed using STAT_ENG programme, author Dr. P. Tarakanovas. A correlation-regression analysis was used to evaluate the relationship among the tolerance to aluminium and morphological traits in lucerne.

RESULTS

Among the 37 accessions tested, *M. sativa* subsp *sativa* (28 accessions) prevailed, the other subspecies being *falcata* (4), *varia* (3), *caerulea* (1) and *tunetata* (1). A statistically significant variation among the accessions was apparent for all traits ($P < 0.05$) (Table 2). The number of accessions that statistically significantly exceeded all Lithuanian cultivars varied from 1 to 20 out of 37 accessions, depending on trait.

Accessions 'PI 502485', 'PI 310338', 'PI 573140', 'PI 419506', 'PI 478550', 'PI 467910' were characterized as forming more vegetative mass than the Lithuanian cultivars 'Augūnė II', 'Birutė' and 'Žydrūnė'. A higher amount of leaves was characteristic of accessions 'PI 561448', 'PI 573135' 'PI 502485', 'PI 467980', 'PI 499547', 'PI 573153', 'PI 573140'. Seventeen accessions out of 37 were taller than Lithuanian cultivars, but only accessions 'PI 467965' had a higher stem density. Six accessions – 'PI 310338', 'PI 573140', 'PI 452444', 'PI 419506', 'PI 478550', 'PI 449316' – had thicker stems and 20 accessions had more erect stems. A considerable number of accessions (18 out of 37) had bigger leaves, but only two ('PI 538985' and 'PI 573140') had a higher concentration of chlorophyll, and only two accessions ('PI 452463' and 'PI 452444') were characterized by a higher seed yield.

Table 1. **Subspecies of lucerne accessions and their Al tolerance index**

Accession No.	GRIN code	Subspecies of *Medicago sativa*	Al tolerance index	Accession No.	GRIN code	Subspecies of *Medicago sativa*	Al tolerance index
1	'PI 561439'	*sativa*	0.98*	21	'PI 310338'	*sativa*	1.12
2	'PI 561448'	*sativa*	0.99	22	'PI 452463'	*sativa*	1.13
3	'PI 214218'	*falcata*	0.99	23	'PI 573140'	*sativa*	1.14
4	'PI 538985'	*falcata*	1	24	'PI 440539'	*falcata*	1.14
5	'PI 502436'	*caerulea*	1	25	'PI 573153'	*sativa*	1.16
6	'PI 577460'	*sativa*	1	26	'PI 452444'	*sativa*	1.16
7	'PI 505865'	*sativa*	1.01	27	'PI 422567'	*varia*	1.16
8	'PI 573135'	*sativa*	1.02	28	'PI 420197'	*sativa*	1.19
9	'PI 577507'	*tunetata*	1.03	29	'PI 419506'	*sativa*	1.21
10	'PI 573173'	*varia*	1.04	30	'PI 478550'	*sativa*	1.21
11	'PI 577514'	*varia*	1.05	31	'PI 449316'	*sativa*	1.24
12	'PI 573166'	*sativa*	1.06	32	'PI 467922'	*sativa*	1.28
13	'PI 502485'	*sativa*	1.06	33	'PI 467910'	*sativa*	1.28
14	'PI 467980'	*falcata*	1.06	34	'PI 467901'	*sativa*	1.37
15	'PI 467965'	*sativa*	1.06	35	'PI 212104'	*sativa*	1.39
16	'PI 467888'	*sativa*	1.06	36	'PI 211609'	*sativa*	1.51
17	'PI 499547'	*sativa*	1.07	37	'PI 467916'	*sativa*	1.55
18	'PI 467899'	*sativa*	1.07	38	'Augūnė II'	*varia*	N**
19	'PI 505862'	*sativa*	1.07	39	'Žydrūnė'	*sativa*	N
20	'PI 467895'	*sativa*	1.11	40	'Birutė'	*sativa*	N

* Data received from GRIN centre; ** Not investigated

Table 2. **Mean values of the morphological characteristics of lucerne accessions**

Accession No.	Rankness, scores	Amount of leaves, scores	Height, scores	Stem density, scores	Stem thickness, scores	Position of stems, scores	Leaflet length × width, mm^2	Chlorph. conc., SPAD-502 values	Seed yield, g/pot
1	2.5	4.0	2.8	3.0	2.8	3.0	415*	45.0	1.2
2	2.5	5.0*	2.5	3.0	2.8	3.5*	348	51.2	4.0
3	3.0	4.0	1.8	4.0	3.0	2.0	436*	51.7	2.9
4	3.5	3.5	3.0	3.0	3.5	3.5*	459*	58.6*	4.1
5	4.0	4.0	2.5	3.5	4.0	2.5	306	48.9	0.4
6	4.0	4.0	3.0	3.5	3.0	2.5	318	47.4	3.0
7	3.0	3.5	3.5	3.0	3.0	3.0	415*	50.6	1.5
8	4.0	5.0*	4.0*	4.0	3.0	4.5*	425*	48.5	1.5
9	3.0	3.8	4.0*	4.5	3.5	3.8*	506*	46.3	1.9
10	4.0	4.0	4.5*	4.0	4.0	3.5*	567*	44.1	3.2
11	3.0	3.0	3.0	4.5	3.0	3.0	470*	48.3	0.6
12	2.8	3.0	2.8	4.0	3.5	2.0	449*	51.6	2.2
13	5.0*	4.5*	4.5*	4.0	4.0	3.8*	344	50.1	1.2
14	4.0	4.5*	3.8	4.0	3.5	3.5*	367	49.3	3.8
15	3.0	3.0	3.0	5.0*	3.0	2.8	340	46.9	0.6
16	3.5	4.3	3.0	4.5	3.0	3.3	375	46.9	2.0
17	4.0	4.5*	3.8*	3.5	4.0	3.5*	420*	46.6	2.5
18	4.0	3.3	3.5	4.0	3.5	3.3	519*	50.5	4.5
19	4.0	4.0	4.5*	3.5	3.5	4.3*	350	51.1	4.1
20	2.5	3.8	3.5	4.5	3.0	2.5	365	47.1	4.1
21	5.0*	3.3	5.0*	3.5	5.0*	4.5*	390	54.0	2.2
22	4.0	4.0	3.8*	4.0	3.5	3.0	329	49.8	5.5*
23	5.0*	5.0*	5.0*	4.0	4.5*	4.5*	493*	56.6*	4.4
24	2.0	3.0	2.0	3.0	2.0	2.0	362	49.3	1.1
25	3.5	4.5*	3.5	4.0	3.5	4.0*	513*	45.2	1.3
26	4.0	4.0	4.0*	4.0	4.5*	4.0*	416*	49.8	5.3*
27	1.5	3.0	1.5	4.0	2.5	1.5	416*	52.3	1.3
28	3.5	3.5	3.5	4.0	3.8	3.8*	404	52.3	3.4
29	4.5*	4.0	5.0*	3.5	4.5*	4.5*	408*	55.1	4.6
30	5.0*	4.0	5.0*	3.5	4.5*	5.0*	333	54.1	4.2
31	4.0	3.5	4.5*	3.5	4.5*	5.0*	379	54.0	3.5
32	3.5	3.5	4.0*	4.0	3.0	3.5*	380	51.1	1.8
33	4.5*	3.8	4.5*	4.5	3.3	3.5*	381	54.9	4.9
34	3.5	3.5	3.5	4.0	3.5	3.3	433*	49.3	3.3
35	3.5	3.0	3.5	3.5	3.3	3.3	398	44.3	4.2
36	4.0	3.5	4.5*	3.0	4	5.0*	372	53.0	3.3
37	4.0	4.0	4.0*	4.0	3.5	3.3	414*	45.0	4.0
38	3.5	3.5	3.5	4.0	3.5	3.0	367	52.8	4.0
39	3.5	3.5	3.0	4.0	3.3	3.0	389	50.8	3.3
40	4.0	4.0	3.0	4.0	3.3	3.0	380	51.7	3.8
Mean	3.6	3.8	3.6	3.8	3.5	3.4	404	50.2	3.0
LSD_{05}**	0.4	0.5	0.2	0.8	0.7	0.4	17.3	3.5	1.1

* Statistically significantly at P < 0.05 exceeded all Lithuanian cultivars. ** Least significant difference at P < 0.05.

Six accessions ('PI 502463', 'PI 577460', 'PI 467888', 'PI 467895', 'PI 440539' and 'PI 212104') did not exceed Lithuanian cultivars by the value of any trait. Accession 'PI 573140' was superior by the values of seven traits out of those nine studied. Accession 'PI 419506' exceeded Lithuanian cultivars by values of five traits, and six accessions ('PI 573135', 'PI 502485', 'PI 499547', 'PI 310338', 'PI 452444' and 'PI 478550') were superior by values of four traits. The rest genotypes exceeded Lithuanian cultivars by values of one, two or three traits.

Paired regression analysis revealed that the tolerance to Al of lucerne accessions was not statistically significantly negatively related (Table 3). Tolerance to Al positively significantly ($P < 0.05$) correlated with height and seed yield, r = 0.353*, $R^2 = 0.125$, Ft = 4.98, the equation of regression being $y = 1.057 + 2.253\ x$ and r = 0.339*, $R^2 = 0.115$, Ft = 4.54, the equation of regression being $y = -1.017 + 3.461\ x$. Some positive tendency was observed in the case of rankness (r = 0.207), stem thickness (r = 0.211) and stem position (r = 0.307). A negative tendency was observed in the case of the amount of leaves (r = –0.239).

Table 3. **Paired regression between tolerance index to aluminium (*x*) and other traits (*y*) of lucerne accessions**

Trait	Correlation coefficient	Fisher's test	Determination coefficient	Equation of a regression *y (x) = a + bx*
Rankness	0.207	1.59	0.043	2.246 + 1.218 x
Amount of leaves	–0.239	2.13	0.057	4.936 – 0.973 x
Height	0.353	4.98*	0.125	1.057 + 2.253 x
Stem density	–0.007	0.00	0.000	3.841 – 0.027 x
Stem thickness	0.211	1.63	0.044	2.417 + 0.967 x
Stem position	0.307	3.65	0.095	1.292 + 1.899 x
Leaflet length × width	–0.073	0.18	0.005	441.0 – 31.07 x
Chlorophyll conc.	0.0050	0.28	0.002	48.60 + 1.256 x
Seed yield	0.339	4.54*	0.115	–1.017 + 3.461 x

* $P < 0.05$.

DISCUSSION

The test accessions in many cases were equal to Lithuanian cultivars or superior by the study traits. All accessions exceeding the Lithuanian cultivars by the values of four and more traits were subspecies of *M. sativa* subsp. *sativa*. This relation considerably facilitates the breeding of new cultivars because populations obtained using for crosses *M. sativa* subsp. *sativa* are more stable and require a shorter period for cultivar development [18].

One of the most significant and difficult to achieve traits of lucerne cultivars is seed yield. High seed yielding is not important directly for many grassland keepers. However, the ability of cultivars to give a high and stable seed yield facilitates its spread and effective distribution to farmers due to a competitive selling price [19].

However, only two accessions were characterized by a higher seed yield. Results obtained under greenhouse conditions were similar to the previous results of screening under field conditions when only a few cultivars overyielded the elite Lithuanian cultivars by seed [20]. On the other hand, 18 accessions were statistically significantly similar to Lithuanian cultivars by seed yield, showing that the use of these germplasms for breeding possibly will not decrease the seed yielding ability of the newly developed derivatives.

Lithuanian cultivars stood out by chlorophyll concentration as only two accessions had the higher trait values; however, 19 accessions were characterized as similar by this trait. The leaflet size of Lithuanian cultivars was smaller than in half of accessions. Such relation can be explained by a compensatory mechanism [21] which is confirmed by the herbage yield of these cultivars [20], which was higher than in other cultivars.

Al tolerance of accessions did not negatively influence the development of vegetative parts of plants. Rankness, amount of leaves, stem density of the majority of accessions were similar to Lithuanian cultivars. The accessions tested are relatively old cultivars or landraces. However, recent investigations have revealed that the progress in herbage yield improvement during the past century was relatively low; however, resistance to biotic and abiotic factors was improved [22, 23].

Paired regression analysis showed that Al tolerance was not significantly negatively related with investigated morphological traits. Some negative tendencies were observed in the case of leaf amount. A direct physiological relation can hardly be explained. A possible negative influence was induced by other reasons. Also, positive correlations of Al tolerance with height ($r = 0.353$*) and seed yield ($r = 0.339$*) were possibly due to soil acidification around the roots of lucerne accessions, which later starts to depress the development of plants [24–26]. Such relations deserve attention as an adapted investigation technique could help select lucerne cultivars possessing some Al tolerance without scrupulous selection for Al tolerance under laboratory or greenhouse conditions [7, 8]. Moreover, intensive soil acidification around lucerne roots shows complications in a long-term cultivation of lucerne grasslands without cultivars possessing some Al tolerance.

Al tolerance was not negatively related with morphological traits; therefore, there are no obstacles for Al tolerance breeding, using these germplasms as regards the traits studied.

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LIUCERNOS (*MEDICAGO SATIVA* L.) ATSPARUMO ALIUMINIUI RYŠYS SU MORFOLOGINIAIS POŽYMIAIS

Santrauka

Bandymas vykdytas Lietuvos žemdirbystės instituto šiltnamyje 2008 m. pavasarį–vasarą. Tirta *M. sativa* porūšių 37 pavyzdžiai, kurie skyrėsi pagal toleranciją aliuminiui, ir trys lietuviškos veislės – 'Augūnė II', 'Birutė' ir 'Žydrūnė'. Pagal 9 morfologinius požymius – augalų vešlumą, lapuotumą, aukštį, stiebų kiekį, stiebų storį, lapų dydį, stiebų padėtį, chlorofilo koncentraciją, sėklų derlių – tarp pavyzdžių nustatyti statistiškai patikimi skirtumai (P < 0,05). Pavyzdžių skaičius, kuris statistiškai viršijo visas lietuviškas veisles, priklausomai nuo tirto požymio svyravo nuo 1 iki 20. 'PI 573140' pavyzdys pasižymėjo pagal 7 požymius, 'PI 419506' viršijo lietuviškas veisles pagal 5, o 'PI 573135', 'PI 502485', 'PI 499547', 'PI 310338', 'PI 452444' ir 'PI 478550' pavyzdžiai buvo išskirtiniai pagal 4 požymius.

Naudojant porinę regresinę analizę nustatyta, kad liucernos pavyzdžių Al tolerancija nebuvo statistiškai patikimai susijusi su tirtais morfologiniais požymiais. Al tolerancija patikimai teigiamai (P < 0,05) koreliavo su augalų aukščiu ir sėklų derliumi – atitinkamai r = 0,353* ir r = 0,339*. Teigiama tendencija nustatyta tarp Al tolerancijos ir vešlumo (r = 0,207), stiebų storio (r = 0,211) bei stiebų padėties (r = 0,307), neigiama – tarp Al tolerancijos ir lapuotumo (r = –0,239).

Raktažodžiai: liucerna, tolerancija aliuminiui, morfologiniai požymiai