Agronomy Research **8** (Special Issue III), 663–672, 2010

Resistance of European lucerne accessions to aluminium

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Abstract. Aluminium toxicity is one of those factors limiting lucerne production on acid soils. Efficient method for selection of Al resistant plants should accelerate breeding of new cultivars. Reaction to Al of the 25 European lucerne cultivars was evaluated using Petri dish with filter paper moistened with $AlCl_3$ concentrations 0, 2, 4, 8, 16 mM. The tested lucerne cultivars did not differ considerably by resistance to Al in regard to the origin. It was clear that aluminium inhibited lucerne seed germination, seedling root and hypocotyl elongation depending on cultivar resistance. Germination rates at $AlCl_3$ concentrations 0, 2, 4 were similar for most cultivars, whereas $AlCl_3$ concentrations 8 and 16 mM highly inhibited germination of susceptible cultivars. Germination test was suitable for elimination of the most susceptible accessions. The seedlings hypocotyl elongation reaction to different $AlCl_3$ concentrations characterized cultivars better than root elongation rates. This method was suitable for selection of the most resistant accessions as only cultivars Magda, Vertus, Luna, Marova out of the 25 ones formed hypocotyls at $AlCl_3$ concentrations 16 mM.

Key words: *Medicago*, aluminium, resistance

INTRODUCTION

Aluminium (Al) toxicity is one of the main factors limiting crop production on strongly acidic soils. Toxic forms of Al are solubilized into the soil solution, inhibiting root growth and function, at soil pH values at or below 5. It has been estimated that over 50% of the world's potentially arable lands are acid; therefore Al negative impact is very important worldwide (Bot et al., 2000). The most of widely cultivated plant species are susceptible to Al (Wheeler et al., 1992). The valuable contribution of lucerne as a fodder plant is limited by its lack of tolerance to soil acidification and in turn to Al (Bouton, 1996). Liming is efficient only for plow layers improvement, but it does not affect sub-soil layers. Lucerne is characterized as plant possessing very deep roots, which allows efficient use of water and mineral elements from deeper soil layers. These traits raise lucerne over other legume grasses but only in neutral or alkaline soil. Lucerne breeding for higher soil acidity and Al tolerance is done for several decades (Devine et al., 1976; Kam-Glass et al., 1993; Chandran et al., 2008b). Results of previous researches were not optimistic (Devine et al., 1976; Bouton, 1996). The main problem was due to subjection of more tolerant populations for improvement not considering low number of tolerant plans. Individual plants were selected infrequently during initial growth stages therefore the number of later selected plants was too low to compound efficient populations (Zhang et al., 2007; Pan et al., 2008). Widely grown lucerne (*Medicago sativa*) is tetraploid plant and this genetic peculiarity impedes research of tolerance inheritance as well as transfer of tolerance from diploid *Medicago* species to cultivated tetraploid lucerne (Sledge et al., 2002: Narasimhamoorthy et al., 2007b; Chandran et al., 2008a). The genetics and physiology of lucerne tolerance to Al as well as tolerance screening techniques were comprehensively studied during the last decade (Kochian et al., 2005; Narasimhamoorthy et al., 2007a; Barone et al., 2008; Pan et al., 2008). Comparison of screening techniques revealed medium to high correlations of tolerance factors among them. It was determined that hydroponics, filter paper in dishes, natural soils and chemically modified soils, root staining methods (Dall'Agnol et al., 2000; Narasimhamoorthy et al., 2007a; Zhang et al., 2007; Pan et al., 2008) successfully and efficiently serve for search of tolerant populations as well as for development of more tolerant populations (Dall'Agnol et al., 2000; Charman et al., 2008; Scott et al., 2008). However, the main constrain for efficient development of populations possessing desirable tolerance level is deficiency of agronomically advanced populations with high tolerance level (Bouton, 1996; Sledge et al., 2002; 2005). Efficient selection level can be achieved only when several, if not tens of thousands (depending on initial tolerance level of population), plants are screened. Also the necessity to develop up to ten generations that should produce several thousands of seeds for subsequent screenings (Dall'Agnol et al., 2000; Scott et al., 2008) makes breeding process long lasting and expensive. This is sound reason for so slow development of Al tolerant lucerne populations. The necessity to develop new cultivars with other traits like resistance to diseases, stand stability, yielding capacity and feed quality also slows down development process. Information about Al tolerance of European lucerne cultivars is not sufficient for efficient selection of the most Al tolerant varieties for tolerance breeding. Screening of European lucerne should reveal possibilities of the use of this cultivars group for development of lucerne with improved Al tolerance.

The objective of this study was to screen lucerne accessions of European origin for Al tolerance and to identify Al tolerant accessions that could potentially be used for Al tolerant lucerne breeding.

MATERIALS AND METHODS

An experiment was carried out at the Institute of Agriculture during the 2009. The material (Table 1-3) subjected to Al resistance tests included lucerne accessions of distinct European origin. These countries were: Belarus (BY), Belgium (BE), Czech Republic (CZ), Estonia (EE), Denmark (DK), France (FR), Italy (IT), Lithuania (LT), the Netherlands (NL), Poland (PL), Slovakia (SK), Sweden (SE), Ukraine (UA).

The screening procedure was derived from Pan et al. (2008) with some modifications. Well-developed lucerne seeds of similar size were scarified and surface sterilized in solution of 10% sodium hypochlorite (NaClO) for 30 min and rinsed 3 times in distilled water. The seeds were sown in 90-mm sterile plastic Petri dishes containing two pieces of sterilized filter paper and 7 ml of sterilized 50 mM $CaCl_2$ (pH 4.5) with five levels of $AlCl_3$ (0, 2, 4, 8, 16 mM). Thirty seeds were placed on the filter paper, with three replicate dishes per treatment. The experiment was repeated twice. Petri dishes were incubated at 25°C in the dark. After four days, the photoperiod was adjusted to 12h/12h (day/night) at 25°C and 20°C, respectively. After three days, germinated seeds were counted, root and hypocotyls length of seedlings were measured. The percent of germination was counted as ration of germinated seeds to not-germinated ones. Relative germination rate (RGR), relative roots length (RRL), relative hypocotyls length (RHL) was calculated according to the following formula:

The value of relative trait at certain Al concentration = value of certain trait at certain concentration/value of certain trait at zero concentration x 100%.

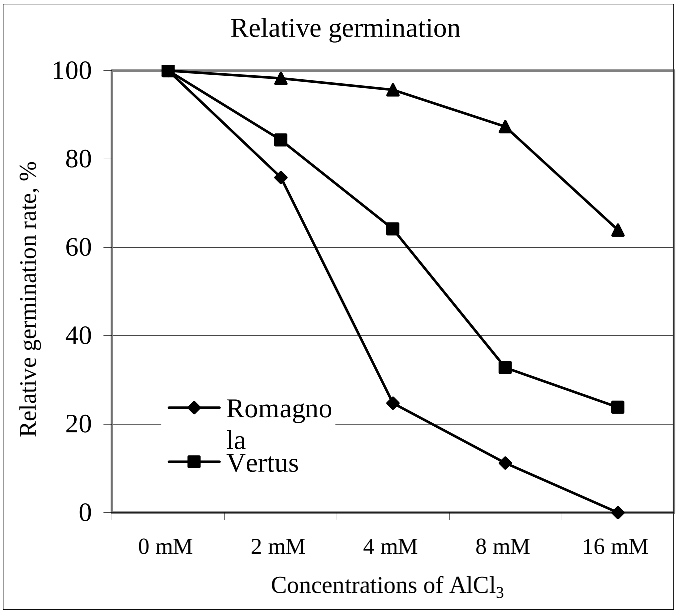
Duncan Multiple Range Test calculations were done at $P < 0.01$ using ANOVA from package SELEKCIJA (Tarakanovas & Raudonius, 2003).

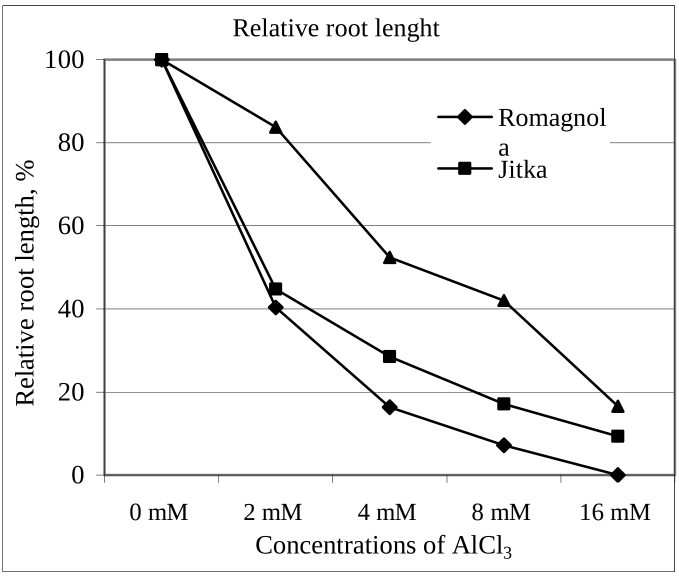
RESULTS AND DISCUSSION

Three cultivars the most divergent by relative germination, root length and hypocotyl length were selected to demonstrate differences of mentioned traits (Fig. 1). It was clear that aluminium inhibited lucerne seed germination, seedling root and hypocotyl elongation depended on cultivar resistance. Those effects became more evident with increased aluminium concentrations. These results are consistent with literature reports (Zhang et al., 2007; Pan et al., 2008).

At the lowest aluminum concentration, relative germination rate was not considerably different, although the most resistant cultivar Magda showed better germination in several percentages than the rest two. Generally, the cultivars in Fig. 1 were different for traits investigated as they showed different values of relative numbers mentioned above.

The cultivar Magda showed the best results for relative germination and relative hypocotyl length, whereas cultivar Romagnola possessed the lowest relative germination and relative root length. The seedling hypocotyl length was the most inhibited as the most susceptible cultivar Radius did not form hypocotyl at $AlCl_3$ concentration 8 mM, more resistant cultivar Janu did not form hypocotyl at 16 mM $AlCl_3$ and the most resistant cultivar Magda had only 19% of hypocotyl length at 16 mM $AlCl_3$. Aluminium also highly inhibited relative root length, with similar tendencies like relative hypocotyl length, however all the cultivars formed roots at 8 mM $AlCl_3$ and only the most susceptible did not form roots at $AlCl_3$ concentration 16 mM.


Relative germination
100
80
60
40
20
0
Relative germination rate, %
Romagno
la
Vertus
0 mM
2 mM
4 mM
8 mM
16 mM
Concentrations of AlCl3


Relative root lenght
100
80
60
40
20
0
Relative root length, %
Romagnol
a
Jitka
0 mM
2 mM
4 mM
8 mM
16 mM
Concentrations of AlCl3

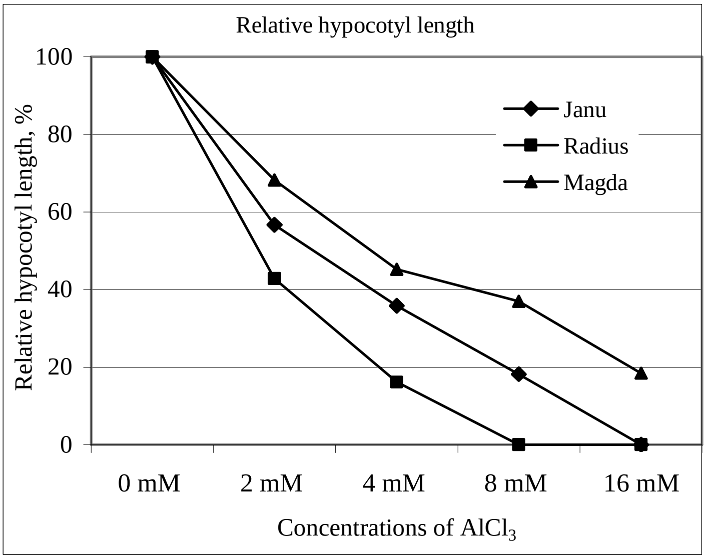


Figure 1. Aluminium inhibition of relative germination rate, root and hypocotyl length in different lucerne cultivars. All cultivars differed significantly (P < 0.01) from each other at all traits and concentrations.

The germination rates of 25 cultivars are presented in Table 1. The tested lucerne cultivars did not differ considerably by resistance to Al in regard to the origin. The lucerne cultivars showed relatively low germination rate as it ranged from 32.7 to 83.3% at $AlCl_3$ concentration 0 mM.

The resistant cultivars like Maria Odesskaya, Marova, Magda, Jarka, Luna, Viktoria, Janu, and Jitka showed similar germination rate at $AlCl_3$ concentrations 0, 2, 4 mM. The rest less resistant cultivars showed variations for reaction to $AlCl_3$ concentrations. Germination of some less resistant cultivars Mazhotnes, Niva, Vela decreased equally at increasing $AlCl_3$ concentrations, whereas germination of the cultivar Romagnola was low at $AlCl_3$ concentrations higher than 2 mM. The cultivars by Al resistance were the most different at $AlCl_3$ concentration 16 mM. The most resistant three cultivars Maria Odesskaya, Marova, Magda (12%) germinated 41.7, 36.7, 32.7%, respectively, whereas 28% of cultivars did not germinate at all.

Also germination rate was considerably different at $AlCl_3$ concentration 8 mM, but not low enough to detect the most resistant cultivars. Some cultivars (Maria Odesskaya, Viktoria, Antanė, Radius, Žydrūnė) showed higher germination rate at $AlCl_3$ concentration 2 mM than at 0 mM. Also some cultivars (Janu, Jitka, Lucia, Polder, Bella, Bobrava, Zuzana) germinated better at 4 mM than at 2 mM.

An average root length of the tested cultivars decreased from 21.2 mm at $AlCl_3$ concentration 0 mM to 9.6, 5.1, 3.4 and 2.0 mm at $AlCl_3$ concentrations 2, 4, 8, 16 mM, respectively (Table 2). Negative Al effect for root elongation was more considerable than for germination rates. The root elongation decline was similar for resistant and susceptible cultivars at $AlCl_3$ concentrations 0, 2, 4 mM. However, the difference was considerable at $AlCl_3$ concentration 8 mM.

The most resistant cultivars (28% of cultivars) had root length 4.2 mm whereas the most susceptible cultivars (28% of cultivars) had root length 3.1 mm. Some the most resistant cultivars (Marova, Lucia, Jarka, Viktoria, Magda) had similar root length at $AlCl_3$ concentrations of 8 and 16 mM. The most susceptible cultivars did not show such relation.

Table 1. The lucerne cultivars seeds germination rate at $AlCl_3$ concentrations 0, 2, 4, 8, 16 mM.

Cultivar	Country of origin	Concentrations of $AlCl_3$, mM				
		0	2	4	8	16
		Germination rate, %				
Maria Odesskaya	UA*	73.9 ab**	79.4 a	70.6 b	50.6 b	41.7 a
Marova	CZ	49.4 ef	48.3 de	45.6 ghi	36.7 efg	36.7 b
Magda	CZ	51.1 e	49.1 de	48.9 g	44.7 cd	32.7 c
Jarka	CZ	48.9 efg	46.7 def	44.8 ghi	41.3 bc	26.7 d
Luna	BE	65.6 c	56.7 cd	56.7 ef	45.0 c	26.1 de
Viktoria	CZ	45.6 g	50.7 cd	40 hij	35.0 fg	25 de
Janu	NL	76.7 a	68.9 bc	81.1 a	36.7 efg	23.9 def
Jitka	CZ	62.2 cde	41.1 ef	57.8 e	32.8 g	17.8 fg
Lucia	SK	32.7 ij	17.2 kl	17.8 m	11.7 lm	17.8 fgh
Vertus	SE	74.4 ab	62.8 bcd	47.8 gh	24.4 hi	17.8 fg
Antanė	LT	67.8 bc	80.0 a	66.0 c	57.7 a	14.4 ghi
Polder	FR	61.1 cde	42.2 ef	48.9 fg	37.8 ef	14.2 ghi
Bella	NL	51.1 e	30.6 hi	39.4 ij	27.8 gh	12.2 hi
Resis	DK	47.2 fg	40.6 f	41.7 hij	16.7 ijk	10.6 hij
Mazhotnes	BY	57.2 de	48.9 de	42.8 ghi	21.7 i	7.8 jk
Niva	CZ	41.7 h	28.9 ij	28.3 klm	14.4 jk	7.8 jk
Radius	PL	64.4 cd	72.2 b	41.1 hij	17.8 ij	5.6 kl
Vela	DK	83.3 a	68.7 bc	42.0 hi	22.2 i	3.9 lm
Žydrūnė	LT	65.6 c	67.8 bc	65.6 cd	42.2 de	0.0
Creno	DK	65.6 cd	48.9 de	38.3 ijk	27.8 gh	0.0
Bobrava	CZ	38.9 hi	30.6 hi	45 ghi	16.1 ijk	0.0
Zuzana	CZ	36.1 hi	24.4 ijk	29.4 kl	12.8 kl	0.0
Luzelle	FR	45.6 g	33.9 h	22 lm	14.4 jkl	0.0
Romagnola	IT	69.4 abc	52.6 cd	17.2 m	7.8 mn	0.0
Juurlu	EE	47.8 fg	33.3 h	15.0 n	6.7 mn	0.0
Average		56.9	48.9	43.3	28.4	13.7

*See Material and Methods, **Means followed by the same letters do not differ according to Duncan's Multiple Range Test at probability $P < 0.01$

The hypocotyl elongation inhibition by Al was similar to root elongation inhibition (Table 3). An average hypocotyl length of the tested cultivars decreased from 17.5 mm at $AlCl_3$ concentration 0 mM to 10.8, 7.0, and 3.6 and 0.3 mm at $AlCl_3$ concentrations 2, 4, 8, 16 mM, respectively. All cultivars suffered considerable hypocotyl length decrease at $AlCl_3$ concentrations 2 mM. Some resistant cultivars like Magda, Jarka, Bella, Lucia (mean decrease 0.78 mm) showed similar hypocotyl growth at $AlCl_3$ concentrations 2 and 4 mM, whereas susceptible cultivars like Romagnola, Radius, Antanė, and Vela showed higher decrease (mean decrease 7.48 mm) of hypocotyl growth. Only 4 cultivars Magda, Vertus, Luna, and Marova formed hypocotyls at $AlCl_3$ concentrations 16 mM. This shows that European lucerne cultivars possess low resistance to Al as well as this trend was shown in many of researches (Wheeler et al., 1992; Bouton, 1996; Sledge et al., 2002, 2005).

Table 2. The lucerne cultivars seedlings root length at $AlCl_3$ concentrations 0, 2, 4, 8, 16 mM.

Cultivar	Country of origin	Concentrations of $AlCl_3$, mM				
		0	2	4	8	16
		Root length, mm				
Marova	CZ*	18.1 fgh**	10.7 efg	5.8 bc	3.7 c	4.2 a
Lucia	SK	16.8 hi	6.3 ij	4.0 gh	3.5 def	4.2 a
Jarka	CZ	17.7 gh	11.1 de	5.2 de	4.3 bc	4.0 ab
Viktoria	CZ	17.3 h	9.0 g	5.4 cd	3.6 d	4.0 ab
Magda	CZ	19.6 ef	7.2 hij	5.1 def	3.6 d	3.1 b
Maria Odesskaya	UA	35.0 a	11.4 d	5.2 cd	4.4 b	3.0 b
Niva	CZ	17.5 h	7.7 hij	5.5 c	2.3 gh	3.0 bc
Bella	NL	18.3 fg	6.8 hij	5.1 def	4.1 bc	2.9 bc
Mazhotnes	BY	19.3 ef	8.4 ghi	5.0 def	3.3 efg	2.9 cd
Vertus	SE	22.5 de	8.3 hi	3.5 hi	3.0 fg	2.8 cde
Janu	NL	25.3 bc	8.6 gh	5.6 c	2.8 fgh	2.7 cde
Luna	BE	23.6 bcd	8.3 ghi	5.3 cd	3.3 ef	2.5 de
Antanė	LT	17.9 gh	16.4 a	7.6 a	6.1 a	2.4 def
Resis	DK	16.3 ij	6.3 ij	5.0 def	3.6 de	2.2 fg
Jitka	CZ	22.6 de	10.1 fg	6.4 b	3.9 c	2.1 fg
Polder	FR	16.6 hij	10.0 fg	4.8 ef	1.6 i	2.1 fgh
Vela	DK	34.1 ab	14.1 b	5.4 cd	4.5 b	1.6 ghi
Radius	PL	23.1 cd	5.7 jk	2.5 ij	2.9 fgh	1.1 hi
Žydrūnė	LT	18.9 fg	15.8 ab	7.7 a	5.8 ab	0.0
Luzelle	FR	18.4 fg	7.4 hij	4.5 efg	3.6 de	0.0
Zuzana	CZ	15.6 jk	8.6 gh	4.9 ef	3.2 efg	0.0
Creno	DK	22.9 cde	13.3 c	6.1 bc	2.6 fgh	0.0
Juurlu	EE	21.8 def	10.2 efg	3.2 hij	2.5 gh	0.0
Bobrava	CZ	23.2 cd	8.5 gh	4.4 fgh	2.2 ghi	0.0
Romagnola	IT	26.7 b	10.8 ef	4.4 fgh	1.9 hi	0.0
Average		21.2	9.6	5.1	3.4	2.0

*See Material and Methods, ** Means followed by the same letters do not differ according to Duncan's Multiple Range Test at probability $P < 0.01$

Generally, cultivars showed more or less similar Al resistance reaction to all 3 investigated traits. However, no cultivar was at the same position across all 3 traits. It seems that germination test using higher Al concentrations could be applied for rejection of the most Al susceptible accessions of lucerne as 7 cultivars out of the 25 ones did not germinated at $AlCl_3$ concentration 16 mM (Table 1). It was noted that germination rate of some cultivars was higher at $AlCl_3$ concentration 2 mM than at 0 mM as well as higher at 4 mM than at 2 mM. The same trends were found in some researches investigating the similar problems (Sledge et al., 2005; Pan et al., 2008;

Scott et al., 2008). However, explanation of such trend was not found. It can be presumed that low aluminium concentrations could stimulate germination of seeds of some cultivars.

Table 3. The lucerne cultivars seedlings hypocotyls length at $AlCl_3$ concentrations 0, 2, 4, 8, 16 mM.

Cultivar	Country of origin	Concentrations of $AlCl_3$, mM 0	2	4	8	16
		Germination rate, %				
Magda	CZ*	13.7 hi**	6.6 ij	6.2 ef	5.1 bcd	2.5 a
Vertus	SE	20.7 d	13.3 cd	6.2 ef	4.3 efg	2.3 ab
Luna	BE	21.3 bcd	11.0 efg	7.4 bcd	4.3 ef	1.8 b
Marova	CZ	10.1 klm	9.2 ghi	5.9 fg	3.2 h	0.8 c
Jarka	CZ	11.2 kl	9.4 fgh	7.0 cd	6.0 a	0.0
Viktoria	CZ	11.7 jkl	8.8 hi	6.5 def	5.4 b	0.0
Bella	NL	19.2 ef	7.5 ijk	7.6 bcd	5.3 b	0.0
Maria Odesskaya	UA	24.3 ab	16.3 b	9.4 a	5.2 bc	0.0
Jitka	CZ	23.2 b	13.0 cd	8.6 b	5.2 bc	0.0
Lucia	SK	14.7 ghi	6.2 ijk	6.6 def	5.0 bcd	0.0
Polder	FR	13.2 hi	9.9 fgh	8.0 bc	4.9 bcd	0.0
Luzelle	FR	13 ijk	9.1 ghi	6.7 de	4.7 d	0.0
Niva	CZ	18.3 fg	10.1 fg	8.2 bc	4.5 de	0.0
Creno	DK	15.8 gh	11.8 ef	7.9 bc	4.3 def	0.0
Mazhotnes	BY	19.9 e	10.8 fg	7.2 cd	4.3 ef	0.0
Žydrūnė	LT	21.2 bcd	16.1 b	8.7 b	4.1 fg	0.0
Bobrava	CZ	19.3 ef	9.3 gh	6.9 de	4.1 fg	0.0
Janu	NL	21.5 bc	12.1 de	7.7 bcd	3.9 g	0.0
Juurlu	EE	12.5 ijk	8.4 hij	4.7 hi	3.2 hi	0.0
Zuzana	CZ	12.5 jkl	8.3 hij	7.7 bcd	2.9 hij	0.0
Vela	DK	25.2 a	18.8 a	5.8 fgh	0.7 ij	0.0
Antanė	LT	13.2 hij	13.5 c	9.0 a	0.0	0.0
Radius	PL	21.5 bcd	9.2 gh	5.6 fgh	0.0	0.0
Resis	DK	18.6 fg	9.1 ghi	6.8 de	0.0	0.0
Romagnola	IT	21.6 bc	12.2 d	3.4 i	0.0	0.0
Average		17.50	10.8	7.0	3.6	0.3

*See Material and Methods, **Means followed by the same letters do not differ according to Duncan's Multiple Range Test at probability $P < 0.01$

The cultivars root elongation reaction to $AlCl_3$ concentrations was the same, as the same number of cultivars did not form roots? The trend of hypocotyls elongation to $AlCl_3$ concentration represented the highest inhibition level as only 4 cultivars out of the 25 formed hypocotyls at $AlCl_3$ concentration 16 mM. Considering all three tests, seed germination at high Al concentrations could be applied for rejection of the most susceptible accessions whereas hypocotyls length at high Al concentrations could be used for selection of the most resistant accessions.

The method showed an efficient possibility to select resistant to Al seedlings using simple materials and less work inputs as compared with other methods described in the literature (Devine et al., 1976; Sledge et al., 2005; Šlepetys et al., 2007; Barone et al., 2008). Pan et al., (2008) proposed a similar idea. Possibly, only hydroponic screening could be comparable by efficiency but only in the case of further growing of selected seedlings (Narasimhamoorthy et al., 2007a; Scott et al., 2008). The rest methods, especially based on soil techniques, are hardly suitable for screening of thousands of seeds per accession in temperate climate. However, these methods could be successfully applied for the final screening steps when several populations have been selected after multiple cycles of recurrent selection. The application of efficient selection of resistant seedlings is highly desirable as resistant accessions are infrequent (Scott et al, 2008). Development of a lucerne breeding population considering desirable agronomical traits requires at least several hundreds of plants. The relatively low number of plants resistant to biotic constrains makes breeders use several thousands of plants in order to compose the initial breeding populations. Selected Al resistant seedlings, which are grown in greenhouse, can be tested for disease resistance. This layout of screenings allows for selecting plants resistant to a couple of constrains. 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 resistance factors and receiving their seeds under the field conditions of temperate climate can take up to five years, even when screenings are done in the nurseries with artificial conditions.

CONCLUSIONS

The lucerne cultivars tested differed by Al resistance in germination, root length and hypocotyl length tests. However, the most cultivars showed rather similar resistance reaction to all three used tests. Germination test was suitable to eliminate the most susceptible accessions, whereas hypocotyl length at $AlCl_3$ concentration 16 mM was sufficient for selection of the most resistant accessions. Cultivars Magda, Vertus, Luna, Marova were the most resistant among the 25 cultivars by hypocotyl length test at $AlCl_3$ concentration 16 mM.

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