THE RESPONSE OF SOME ROMANIAN ALFALFA GENOTYPES TO SOIL ACIDITY

Elena Petcu, Maria Schitea, Domnica Badea*

ABSTRACT

Soil acidity is a major limiting factor in alfalfa production. The effect of soil acidity on Romanian alfalfa genotypes in early stage of vegetation was investigated. The experiment was conducted under laboratory conditions with alfalfa plants grown in plastic boxes containing acid soil (pH = 4.4). Additionally, the same alfalfa genotypes were grown in plastic boxes containing neutral soil. Acid soil tolerance of each genotype was estimated by recording the root length, chlorophyll content and peroxidase activity under unlimed soil and comparing the data with the ones recorded in plants grown on normal soil.

A significant decrease of root length and chlorophyll content was registered in alfalfa plants grown on acid soil as well as on increase of peroxidase activity.

Results indicate genetic variability exists among Romanian alfalfa cultivars for soil acidity tolerance, which can be useful for alfalfa breeding program to develop alfalfa germplasm tolerant to this stress.

The increase of peroxidase activity in studied genotypes under soil acidity conditions might indicate a possible detoxification system in the leaves.

Keywords: alfalfa, resistance, screening, soil acidity

INTRODUCTION

Among the alfalfa growers's problems, acid soils are widespread and most limiting of all. This is because acid soils are found on every continent of the world and alfalfa is very sensitive to these conditions (Wambeke, 1976).

In Romania, the acid soils (pH < 6) occupy 1,627,000 ha (Răuță et al., 1985, quoted by Bunta, 1997).

It is known that the presence of aluminum ions becomes problematic in regions where the soil pH is acidic, as under low-pH conditions the metal is solubilized as free Al^{3+} ions (Blancaflor et al., 1998). Other limitative factors for wheat and alfalfa grown on these soils are increase of H^{+} and Mn^{+2} ions, defficiency in mobil phosphorus and molibden in soil (Devine et al., 1990). The result of aluminum toxicity is the inhibition of root elongation, which prevents crops form developing viable root system, making them more susceptible to drought conditions and decreasing nodulation in alfalfa.

A tolerant to soil acidity alfalfa germplasm (GA-AT) was developed (Bouton si Radcliffe, 1989), which showed enhanced topgrowth and nodulation over the check. However, the biomass yield loss in acid compared with limed soil in field plot demonstrated that higher levels of tolerance will be necesary to achieve economic succes. Therefore, identifying new sources of Al toxicity tolerance in alfalfa and undestanding the mechanism of resistance in order to establish adequate breeding strategy for aluminum tolerance, are main objectives.

NARDI Fundulea has for several years a plant breeding program whose objective is to develop alfalfa germplasms tolerant to the acid, Al-toxic soils.

The objective of this study was to establish a working protocol and to screen Romanian alfalfa collection for acid soil stress and/or Al tolerance response.

MATERIALS AND METHODS

The study was conducted under vegetation house and laboratory conditions with alfalfa plants grown in neutral (cambic chernozem, pH = 6.8) and acid soil (pH = 4.4). Two rows and 15 to 20 seeds were sown in each row/cup and covered with 60 g of sand. The cups were watered by weight to 70% field capacity with distilled water and rewatered to that level every 3 to 4 days and kept under laboratory conditions. One week after emergence, seedling were randomly thinned to 10 per row and than plants were grown in the vegetation house. Plants were harvested 35 days after sowing, roots were rinsed with stream flow water tap, and length of the main root (from the base of the stem to root tip), chlorophyll content (with 502 SPAD Minolta device) and peroxidase activity were measured. The relative root length (RRL) (the root length in acid soil/ the root length in neutral soil)*100 was scored.

RESULTS AND DISCUSSION

The analysis of variance regarding the effect of soil acidity on root lenght of alfalfa showed a very significant influence of the

* National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călăraşi County, Romania.
E-mail: petcue@ricic.ro

treatment, genotype and their interaction, but the variance of treatments was higher than the variance due to genotypes (Table 1).

Table 1. Analysis of variance for root length

Source of variance	DF	Sum of squares	Mean square	F value
Treatment (soil)	1	11516.54	11516.54	436.8433***
Error A	7	184.5417	26.3631	
Genotype	11	25470.69	2315.517	59.0906***
Interaction	11	3161.27	287.3882	7.3340***
Error B	154	6034.625	39.1859	

This suggested that acid soil had a strong inhibitory effect on studied Romanian alfalfa genotypes. Cultivars Selena, Granat, Adin, and F 1408 had relatively large reduction of root lenght (over 50 %) as compared with cultivars Cosmina, Sigma, Magnat, and Super (Table 2).

Table 2. Response of 12 alfalfa genotypes to soil acidity

Genotype	Experimental variants	Root length (mm)	Relative root length (%)
Satelit	Control	62.75	
	Treatment	52.75^{00}	84.06
Super	Control	78	
	Treatment	68^{0}	87.18
Selena	Control	66	
	Treatment	*36.67^{000}*	*55.56*
Sigma	Control	75.2	
	Treatment	65.2^{**}	86.70
Granat	Control	64	
	Treatment	41.5^{000}	64.84
Magnat	Control	75.67	
	Treatment	*65.67^{**}*	86.78
Adin	Control	60	
	Treatment	*34.33^{000}*	*57.22*
Cosmina	Control	87.1	
	Treatment	77.1***	88.52
F 1007	Control	84	
	Treatment	74**	88.10
F 1111	Control	60.11	
	Treatment	50.11^{00}	83.36
F 1408	Control	63	
	Treatment	*32.9^{000}*	*52.22*
F 1412	Control	72.1	
	Treatment	62.1**	86.13
Average	Control	72.1	
	Treatment	62.1	

*,**,*** positive significantly for P<0.5, 0.1 and 0.01
^{0},^{00},^{000} negative significantly for P<0.5, 0.1 and 0.01

There is a genetic variability for tolerance to soil acidity in alfalfa Romanian collection. So, the distribution of freqvency for 48 alfalfa genotypes showed that for 17% of them soil acidity decreased the root growth from 1.9% to 11.85% but for most of them this reduction was over 41.7% (Table 3).

Table. 3. The distribution of frequency for soil acidity tolerance in Romanian alfalfa collection

Class	Center of class	Frequency Absolute	Frequency Relative
1.9-11.85	6.87	8	0.17
11.86-21.81	16.83	15	0.31
21.82-31.76	26.78	4	0.08
31.77-41.72	36.74	7	0.15
41.73-51.68	46.70	9	0.19
51.69-61.64	56.60	5	0.10

Beside the negative effects on root growth, chlorophyll content was also negatively influenced by the soil acidity.

The analysis of variance regarding the effect of soil acidity on chlorophyll content showed a very significant influence of the treatment, genotype and their interaction (Table 4).

Table 4. Analysis of variance for chlorophyll content

Source of variance	DF	Sum of squares	Mean square	F value
Treatment (soil)	1	1515.3410	1515.3140	1963.67***
Error A	3	2.3151	0.7717	
Genotype	11	654.6404	59.5128	27.20***
Interaction	11	141.8932	12.8994	5.8970***
Error B	66	144.3724	2.1875	

Under neutral soil conditions the chlorophyll values ranged from 35 to 40 SPAD units (for cultivars Selena and F 1007) while under acid soil the range was from 23 SPAD units (Selena) to 34 SPAD units (for F 1007) (Figure 1).

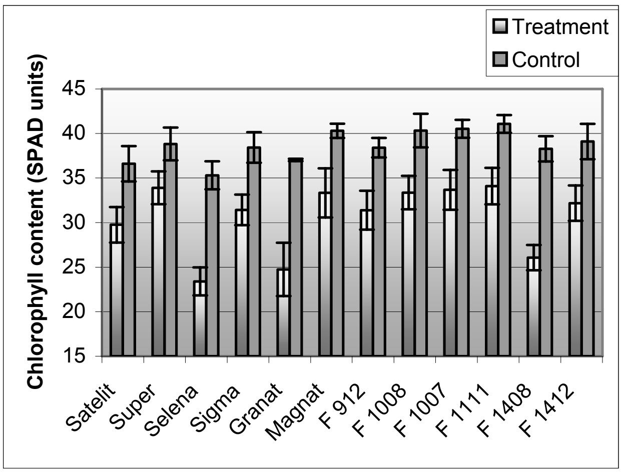

Figure 1. The effect of soil acidity on chlorophyll content of alfalfa genotypes

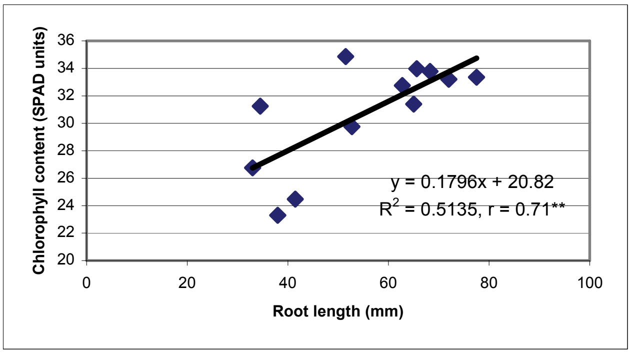

Figure 2. The relationship between root length and chlorophyll content of several alfalfa genotypes under soil acidity conditions

It was obvious that the genotypes for which soil acidity reduced more the root length had the lower chlorophyll content. There was a significant positive correlation between root length and chlorophyll content of alfalfa grown on acid soil (Figure 2), suggesting that those traits could be used in selecting soil acidity tolerant cultivars. A significant increase of peroxidase activity was registered in alfalfa plants under acid soil (Table 5).

Table 5. Analysis of peroxidase activity (µM guiacol/min/mg protein/ g d.m.)

Genotype	Control	Treatment
Satelit	6.43	13.50
Super	6.59	12.80
Selena	6.14	17.50
Sigma	6.93	9.50
Granat	5.23	12.10
Magnat	7.10	14.20
Adin	6.90	18.25
Cosmina	7.35	13.65

The increase of peroxidase activity in studied genotypes under soil acidity conditions might be indicate a possible detoxification system in the leaves.

CONCLUSIONS

The soil acidity inhibits the growth of roots, which caused obvious modification of photosynthesis (decrease of chlorophyll content) and intensification of enzymatic activity (peroxidase).

Results indicate a genetic variability of Romanian alfalfa germplasm for soil acidity tolerance, useful for alfalfa breeding program to develop alfalfa germplasm tolerant to this stress.

The protocol was simple and able to separates alfalfa genotypes for their relative ability to growth under soil acidity conditions.

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