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BIOCHEMICAL CHANGES IN LUCERNE ATTACKED BY LONGHORN BEETLE AND ROOT ROT

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Abstract. Eight alfalfa genotypes were investigated at IASS "Obraztsov chiflik"–Rousse during the period 2006–2009. The objective of the study was investigation of biochemical changes in alfalfa roots, attacked by lucerne longhorn beetle (*Plagionotus floralis* Pall.) and root rot. Biochemical analysis of healthy roots, roots damaged by larvae of longhorn beetle, roots attacked by root rot and green mass were made. The healthy status of roots was evaluated according to the scale of Lyubenets and Shtukina (1968). A moderate negative correlation was determined between total phenols and sugars in plants damaged by longhorn beetle. The content of proteins, fiber, calcium, magnesium and total phenols in plants damaged by longhorn beetle and root rot increased compared to healthy ones and a decrease of sugars was observed. The highest number of healthy plants and high productivity were registered in Synthetic population (SP) N 7 and it could be appropriate in selection for the purposes of breeding.

***Keywords*:** alfalfa, *Plagionotus floralis*, root rot, biochemistry

Introduction

Penetration of pathogenic microorganisms and pests into plants–hosts was accompanied by affecting their biochemical processes which resulted in decreasing of the quantity and quality of forage and decreasing of the lucerne (*Plagionotus floralis* Pall.) persistence [ILIEVA and BLAZHEV, 1995].

Detection of valuable sources for breeding with expressed resistance to economically significant soil pests is of great importance in creation of new lucerne varieties [NIKOLOVA and KERTIKOVA, 2008].

The last years observations showed that main factor in Bulgaria causing thinning of stands were root rot [BLAZHEV, 1990; PETKOVA et al., 2004; ILIEVA and BLAZHEV, 1995] and lucerne longhorn beetle [PETKOLA et al., 2005; NIKOLOVA and KERTIKOVA, 2008; ZHEKOVA and PETKOVA, 2010].

The subject of investigation was following and detecting of variations and content in crude protein, fiber, calcium, phosphorus, magnesium, water soluble sugars, saponines and total phenols in alfalfa roots, attacked by longhorn beetle (*Plagionotus floralis* Pall.) and root rot.

Material and methods

The investigation was carried out in competitive variety experiment with 8 variants–7 new alfalfa germplasms and variety Prista 2–St.

The experiment was conducted in the experimental fields of IASS "Obraztsov chiflik"–Rousse during 2006–2009 in 4 replications, experimental plot of 10m^2. The experimental results for dry matter yield and seeds were officially interpreted and published [ZHEKOVA and PETKOVA, 2010].

At the end of the 4th year of alfalfa vegetation test plants from all the variants and replications were dug out from an area of 1m^2 and depth of soil 15–20 cm.

A biochemical analysis was made (content in percentage crude protein, fiber, calcium, phosphorus, magnesium, water soluble sugars, saponines and total phenols in relative units) in 4 variants of each investigated alfalfa genotypes: healthy roots, roots damaged by larvae of longhorn beetle, roots attacked by root rot and green matter.

The healthy status of roots was evaluated via cutting of root at 3 cm below alfalfa root neck, according to the scale of Lyubenets and Shtukina (1968) [LYUBENETS and

SHTUKINA, 1968], developed on the grounds of variations occurring in colour of vascular system of roots.

According to the authors the digital expression is presented via scale starting with 0 (healthy root) up to 5 (almost dead plants). One fungal (*Fusarium oxysporum*) and one bacterial (*Pseudomonas fluorescens*) agent were identified.

At the same root parts, presence or absence of moves of larva of Lucerne longhorn beetle were visually detected.

Contents of crude protein (CP) after Kjeldahl, fiber (F) via Veende analysis, soluble sugars after Ermakov [ERMAKOV, 1987], saponines after Jurzysta [JURZYSTA, 1979], Ca–complexometric, P–by hydroquinone, Mg after Sandev [SANDEV, 1979], phenols–as relative units after Swain and Hillis [SWAIN and HILLIS, 1959] were determined. Statistical processing of data was made by Excel/Windows 2000.

Results and Discussion

Content of crude protein in healthy roots varied from 9.93% to 11.03%, in damaged by longhorn beetle it varied from 12.77% to 14.42 %, in germplasms MM and Syn_1–73, respectively.

That fact was explained by the weak plants and the lowest yield of dry matter yield in MM and Syn_1–72 [ZHEKOVA and PETKOVA, 2010].

The lowest content of crude protein was confirmed again in first germplasm (MM)–10.87% and in damaged by root rot plants the limits varied from 10.78% to 12.82% (*Table 1*). Similar trend was observed about the content of fiber: average content in healthy plants was 24.83%, in damaged by pest–26.40% and 27.09% in healthy ones (*Figure 1*). The content of Ca and total phenols also increased in that direction. Opposite was the direction of the content of Mg and P (Figure 2).

Quantity of saponines showed a weak trend to decrease in diseased by root rot variants and more expressed in damaged by larvae of Lucerne longhorn beetle.

Comparing the results obtained for healthy roots to those, obtained from diseased by root rot and damaged by larvae of longhorn beetle, it was obvious through the average value that metabolic response of plants in both stress factors was similar.

The content of proteins (especially in damaged by longhorn beetle), fiber, Ca, Mg and total phenols increased, and the content of sugars decreased.

The differences between the effect of the disease and damages caused by the pest were that root rot causes a decrease in the content of crude proteins and saponines in roots and an increase of the mentioned substances in plants damaged by larvae.

The results showed significant non-specificity in responses of plants damaged by root rot and longhorn beetle.

The size and the character of the responses depended mainly on the degree of damage, caused by stress factors and the degree of resistance of plants [EDREVA, 1989].

Increase of Ca probably was connected with depolarizing of membranes under the influence of damages and the increase of secretion of peroxidase–an enzyme oxidizing phenols to quinones and suberin with protective function [GASPAR, 1985].

The plants of the SP №7 were of interest for the breeding. It showed the highest productivity; exceeding the standard variety Prista 2 concerning content of crude protein in dry matter by 0.83% average for 3 years (*Table 2*).

The difference of 0.99% was not statistically significant. Approximately the same was the excess of that trait in green matter in reporting of the healthy status at the end of the 4th year.

During the same year–2009, the content of crude protein in dry matter of SP № 7 was 1, 56% higher than the standard Prista 2–18.69%. The number of healthy plants of the same variant–324 was in support of the results about its productivity.

For the correlation analyses of the biochemical traits a moderate degree of negative relation was determined (r=–0.50) between total phenols in damaged by longhorn beetle plants and sugars in the same plants, which probably was due to the assimilated by larvae sugar substance and the defensive reaction of the plant, expressed in excretion of more phenols (*Table 3*).

The relation between sugars in diseased and damaged plants was positive (r=+0.50) in moderate, as their absolute values were lower than those in healthy plants (*Table 1*).

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Conclusions

The content of proteins, fiber, calcium, magnesium and total phenols in damaged by longhorn beetle and root rot plants increased compared to the healthy ones and the content of sugars decreased.

Moderate negative correlation was determined between total phenols and sugars in damaged by longhorn beetle plants.

The greatest number of healthy plants and the highest productivity were registered in SP №7 and it is appropriate in selection for the objectives of breeding.

Table 1.

Biochemical analysis alfalfa plants

№	Crude protein %DM	Fiber %DM	Soluble sugars %	Saponines %	Ca %DM	P %DM	Mg %DM	Total phenols r.u.
Healthy roots								
1	10.43	24.11	14.00	3.62	0.60	0.25	0.11	0.22
2	10.99	25.33	13.60	3.60	0.64	0.27	0.12	0.26
3	11.03	23.07	15.00	3.50	0.54	0.27	0.11	0.23
4	9.93	25.14	10.50	3.90	0.58	0.27	0.11	0.23
5	10.88	25.19	9.80	3.90	0.73	0.23	0.11	0.22
6	10.55	23.91	11.50	3.90	0.61	0.27	0.12	0.21
7	10.22	26.37	10.80	3.60	0.65	0.25	0.10	0.21
8	10.27	25.50	12.70	3.90	0.78	0.27	0.11	0.30
Damaged roots by *Plagionotus floralis*								
9	13.08	27.59	7.50	2.40	0.97	0.27	0.12	0.51
10	13.61	26.15	8.60	2.80	1.12	0.28	0.13	0.48
11	14.42	25.41	8.40	2.60	0.99	0.27	0.17	0.52
12	12.77	26.23	10.00	3.45	0.91	0.25	0.13	0.49
13	13.79	26.73	6.00	2.90	0.98	0.26	0.12	0.50
14	13.11	26.20	7.20	2.90	1.15	0.29	0.15	0.48
15	13.45	25.60	6.00	2.50	0.98	0.27	0.14	0.61
16	13.11	27.27	7.00	2.90	1.05	0.26	0.13	0.59
Diseased by root rot								
17	11.56	25.99	15.00	3.20	0.73	0.28	0.14	0.30
18	12.34	24.75	11.20	2.92	0.63	0.33	0.14	0.40
19	10.88	27.89	12.80	3.90	0.63	0.29	0.16	0.28
20	10.87	26.14	13.10	4.20	0.82	0.32	0.17	0.30
21	12.46	27.60	10.40	3.72	0.95	0.31	0.19	0.36
22	12.82	27.70	11.20	3.80	0.71	0.30	0.16	0.31
23	12.33	27.01	10.50	3.40	0.88	0.32	0.16	0.30
24	10.78	29.63	8.50	3.40	0.89	0.27	0.19	0.35
Green matter								
25	22.32	16.69	2.50	2.06	1.66	0.39	0.45	0.33
26	22.59	16.16	1.50	1.80	1.95	0.41	0.44	0.34
27	22.20	16.22	1.50	1.80	1.47	0.37	0.46	0.29
28	22.97	15.57	1.50	2.00	2.11	0.44	0.46	0.28
29	22.87	16.60	1.50	1.60	1.28	0.41	0.40	0.29
30	22.19	15.99	1.50	1.24	1.95	0.39	0.43	0.29
31	23.05	17.13	1.80	1.60	1.69	0.42	0.44	0.27
32	23.27	14.91	1.80	1.60	1.45	0.41	0.40	0.36

Table 2.

Content of crude protein and healthy status of alfalfa genotypes

No.	Genotype	Crude protein in dry matter (%)		Number of plants m^{-2}. 2009	
		2009	Average	Healthy	Damaged and diseased
	Prista 2–St	18.69	19.67	222	231
	Syn_1–72	19.69	20.40	186	252
	Syn_1–73	20.25	20.54	225	261
	MM	19.25	20.44	195	279
	6R 88	19.75	20.31	168	246
	SP OcMM	18.00	19.83	180	195
	SP №7	20.25	20.50	324	267
	SP 05	19.75	20.46	204	186
	GD 5%		0.99		
	GD 1%		1.35		
	GD 0.1%		1.82		

Table 3.

Correlation coefficients between the investigated traits

No.	Characteristics	Damaged and diseased plants	Total phenols in		Sugars in	
			damage by larva	diseased by root rot	damaged by larva	diseased plants by root rot
	Damaged and diseased plants	1.00				
	Total phenols in damage by larva	–0.09	1.00			
	Total phenols in diseased by root rot	–0.15	–0.21	1.00		
	Sugars in damaged by larva	0.38	–0.50	–0.09	1.00	
	Sugars in diseased plants by root rot	0.42*	–0.45*	–0.49*	0.50*	1.00
						*P=5%

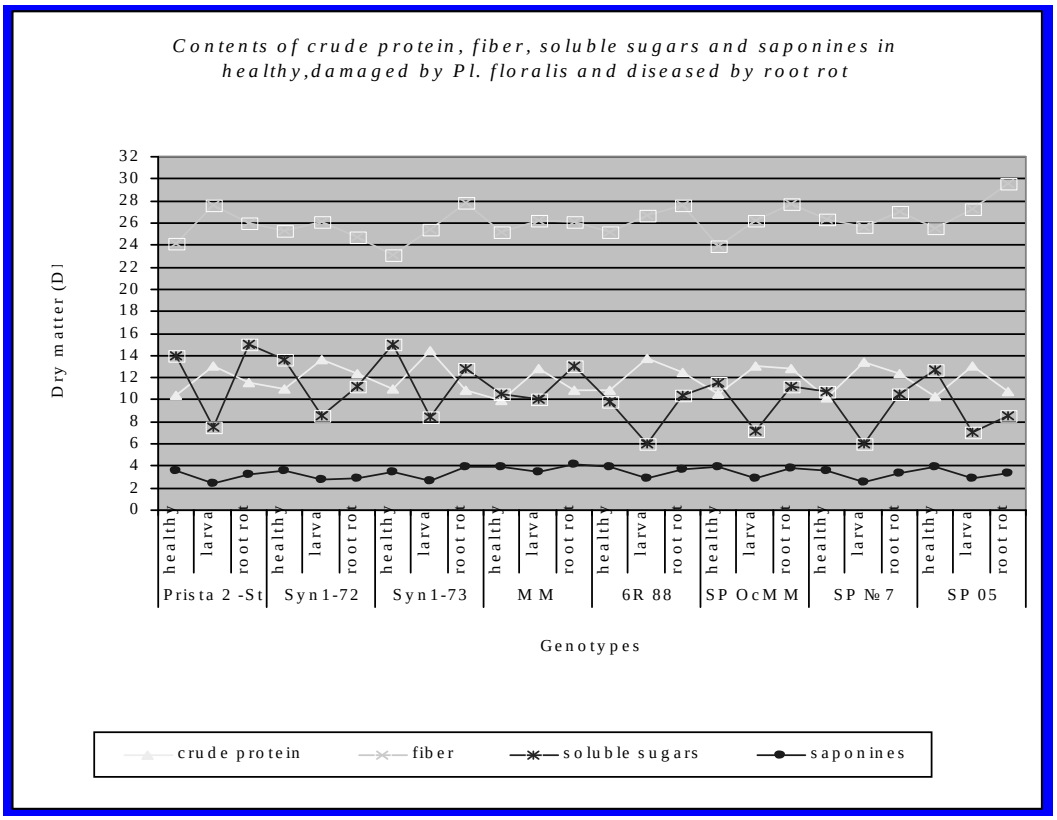

Figure 1.

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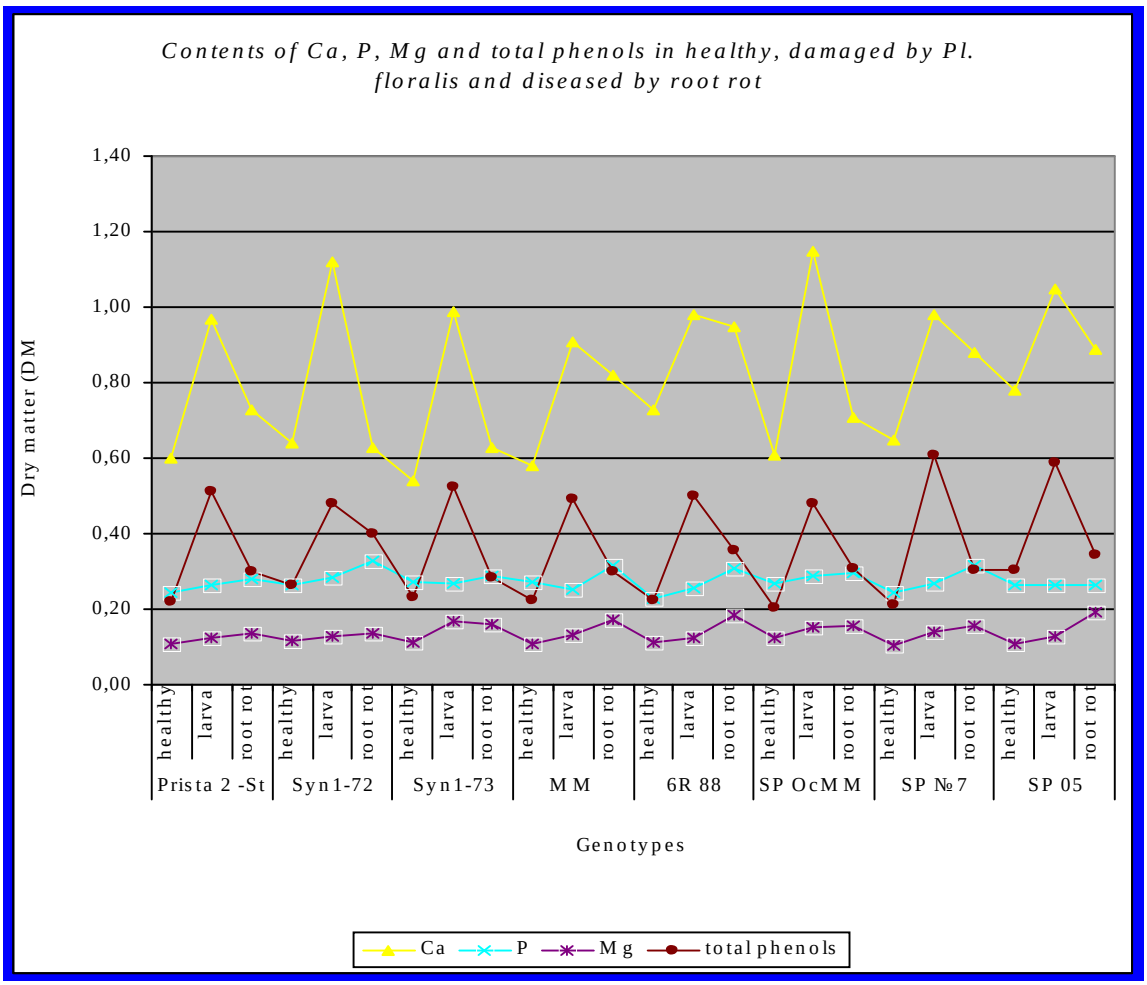

Figure 2.

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