

471



The amount of the amount of some tripeptide amino acid...
 and quantitative composition of free amino acid...
 in various leaves is by 3-4 orders lower than that...
 in various leaves and intensity of metabolic process...
 cell (Table 2). Variation by nodules bacteria of the...
 essentially changes quantitative relationship of free amino acid...
 20% increase by 10% strain and by 5% and 43% strain...
 10% increase by 10% strain and 4 times correspondingly in...
 maximum content. Maximum content of free amino acid...
 was found in the leaves of leaves of leaves (see Table 2)...
 was found when inoculating with MS strain for...
 and Table 2 shows. A comparative analysis of data on...
 plants grown during the leafy period of vegetation period within...
 increase for the second year of vegetation period within...
 depending on a strain. It was established that in

Table 2. Free amino acids in the leaves of leaves...
 when inoculating by various R. meliloti strains (see...
 vegetation period - the stage of budburst of leaves)

Amino acids	control	435a	412b	MS	MS
Arg	2	5	5	12	8
Val	14	15	17	38	18
Ile	1	1	1	15	15
Leu	7	13	13	32	17
Pro	1	1	1	1	7
Met	1	1	1	1	1
Thr	12	18	13	17	17
Asp	1	1	1	1	1
Glu	1	1	1	1	1
Ala	1	1	1	1	1
Asn	1	1	1	1	1
Pro	1	1	1	1	1
Trp	1	1	1	1	1
His	1	1	1	1	1
Other	1	1	1	1	1
Total	91	123	73	153	100
Arginine	102	117	68	159	181
Asp	80	40	40	85	130
Glutamic	1	3	3	11	7
Glu	30	82	89	132	191
Ala	30	38	31	52	50
Asparagine	10	14	14	14	14
Pro	1	1	1	1	1
Other	1	1	1	1	1
Total	91	123	73	153	100

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IMPROVEMENT OF LUCERNE AMINO ACID COMPOSITION BY BIOLOGICAL NITROGEN FIXATION

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1 INTRODUCTION

Lucerne is one of the perennial legumes which is of a paramount importance for forage crop production.¹ The quality of lucerne forage crop depends on the content easy to assimilate protein and amino acids, particularly, irreplaceable ones, the composition of which, primarily is defined by a variety and plant growth phase.^{2,3} Unlike other plants amino acid composition of lucerne shoot mass essentially depends on the intensity of nitrogen fixing process in root nodules, that is connected with the efficiency of *Rhizobium* strains.⁴

The present paper seeks to study the influence of *Rhizobium meliloti* strains of different efficiency on the qualitative and quantitative composition of bound and free amino acids in lucerne leaves.

2 MATERIALS AND METHODS

The field small plot experiments with lucerne (cv's Zaikievich and Yaroslavna) were carried out on dark grey podzol soil (pH 5,3, humus content 2,36) on the background of $N_{30}P_{60}K_{60}$. The experiments were replicated 3 times, the area of recorded plot was 4 square meters. The applied agrotechnics was that agreed for uncovered lucerne crops. Before sowing the seeds were inoculated by eight new *Rhizobium meliloti* strains of various efficiency. Control variants were inoculated by local races of *Rhizobium meliloti*. To carry out the analyses the plants were selected by lucerne growth stages. Hay cutting was made at the stage of budding - the initiation of flowering. The quantity of total nitrogen of lucerne shoot mass was determined by Kjeldal's method.⁵ The bound amino acid content was detected in acidic hydrolyzates of dry leaves on amino acid Biotronik LC 6001 analyzer.⁶ The amount of free amino acids was defined in fixed by ethanol plant material on AAAT 339 analyzer.⁷

3 RESULTS

The clearest differences between the strains of *Rhizobium* in respect of their influence on the green mass crop and protein content have been

revealed within the plants of the first year. The greatest harvest of green mass of lucerne cv Zaikievich has been received with inoculation by strain M4 (reliable gain to control is 29 %). Using inoculation by M6 strain maximum protein yield (11,6 c/ha) has been obtained (Table 1).

Table 1. *The inoculation effect of Rhizobium meliloti on green mass crops, nitrogen and bound amino acids content in lucerne leaves.*

Strains	Green mass		Nitrogen in air dry mass, %	Bound amino The sum of irreplaceabl e	acids, mg/g Total content
	c/ha	% to control			
Control	231	100	2.46	95.60	228.37
425a	228	98.7	2.55	105.00	264.38
435a	267	115.5	2.61	98.67	243.78
404b	246	106.5	2.49	99.00	248.30
412b	258	111.5	3.02	117.56	296.43
M3	269	116.4	2.55	115.48	288.34
M4	299	129.4	2.59	117.08	294.95
M6	272	117.7	2.99	112.65	283.73
M7	243	105.2	2.54	112.30	282.38
LSD 05	30.2				

The plants inoculated by M4 and 412b strains considerably exceeded control in respect of plant protein yield. It has been shown that inoculation of lucerne seeds by 5 from 8 tested *Rhizobium meliloti* strains favours both the increase of yield and protein harvest by 10 to 30 %. Our investigations show that the improvement of qualitative composition of green mass occurs when inoculating lucerne by nodule bacteria. The greatest number of bound amino acids including irreplaceable are found in lucerne leaves (cv Zaikievich) when inoculating by M3, 412b and M4 strains. The analysis of amino acids within 2 years of plant vegetation shows that in lucerne leaves of both cultivars 18 bound amino acids are permanently detected. Among them amides of aspartic and glutamic acids, phenylalanine, alanine, arginine, leucine and lysine predominate in all variants of the experiments.

One should note that quantitative composition of bound amino acids somewhat varies depending on a strain, stage, plant growth and cultivar. So, when inoculating by M4, M6, 435a and 425a strains the amount of phenylalanine in lucerne (cv Zaikievich) is increased by 13 to 20 % in comparison with noninoculated control. Leucine content is increasing by 7 to 14 % when inoculating by M4 and 425a strains correspondingly. Within the process of plant vegetation the number of bound amino acids is increasing during the transition from the stage of stemming to budding. Its content in the plants of the second year of growing is 1,2 to 1,5 times higher. In this period the content of asparagine, glutamine and alanine is increasing by 40 to 50%. It causes

the increase of the amount of some irreplaceable amino acids (lysine, arginine, histidine).

Qualitative and quantitative composition of free amino acids, which content in lucerne leaves is by 3-4 orders lower, than that of bound, is an evidence for the direction and intensity of metabolic process in a plant cell (Table 2). Inoculation by nodule bacteria of tested strains essentially changes quantitative relationship of free amino acids in leaves.

So, inoculation by M6 strain and by M3 and 425a strains increases lysin content 6 and 4 times correspondingly in comparison with noninoculated control. Maximum content of free amino acids, including irreplaceable ones in the leaves of lucerne (cv Zaikievich and cv Yaroslavna), is revealed when inoculating by M6 strain for the first and second years of growing. A comparative analysis of data on the stages of plant growth shows that the total number of free amino acids is 2 to 10 increased by the second year of vegetation period within both cultivars depending on a strain. It has been established that in

Table 2. Free amino acids in the leaves of lucerne (cv Zaikievich) when inoculating by various *R. meliloti* strains (the second year of vegetation period - the stage of budding-initiation of flowering), mg/kg

Amino acids	Strains of nodule bacteria							
	control	425a	435a	412b	M6	M4	M3	M7
Arg	5	6	2	5	12	8	9	6
Val	14	31	15	17	33	18	24	21
I le	7	13	4	10	15	15	17	10
Leu	7	12	3	13	22	17	23	11
Lys	3	13	1	5	18	7	12	5
Met	6	21	7	5	11	6	12	15
Thr	12	18	8	12	27	17	19	22
Phe	7	9	5	5	15	12	16	14
The sum of irreplaceable	61	123	45	72	153	100	132	104
Asparagine	105	117	66	159	182	181	30	104
Asp	60	40	40	55	93	120	53	41
Glutamine	1	8	3	5	11	7	2	5
Glu	89	82	89	138	146	192	124	132
γ -aminobutyric	20	25	21	32	51	50	48	36
Tyr	10	13	10	14	16	15	24	22
Cys	20	50	30	20	30	60	50	50
Total content	502	784	442	631	939	932	691	672

the process of development within lucerne leaves of both cultivars 26 free amino acids are found. Some of them (histidine, tryptophane and γ -aminobutyric acid) are revealed not at all stages of growth.

It should be noted that at the stage of budding in the leaves of lucerne (cv Zaikevich and cv Yaroslavna) the content of glutamine is lower by 1 to 2 orders than that of asparagine. It is an evidence for the worsening of carbohydrate exchange in plant leaves.

Thus, when inoculating lucerne (cvs Zaikievich and Yaroslavna) by active strains of *Rhizobium* not only the yield of green mass and protein harvest is increased by 10 to 30 %, but the quality of forage protein is as well improved due to the increase of the content of some bound amino acids including irreplaceable ones. Maximum amino acid content in leaves of lucerne of both cultivars is found at the stage of budding of the second year of plant vegetation. That is why hay cutting is recommended to carry out exactly in this period.

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