

CORRELATION DEPENDENCES BETWEEN ESSENTIAL AMINO ACIDS IN ABOVE-GROUND BIOMASS IN ALFALFA (*Medicago sativa* L.)

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Abstract

The present study aims at evaluating the influence of Reni preparations content on essential amino acids in aboveground biomass from the alfalfa varieties *Multifolium 1* and *Legend* using correlation analysis. Three -year data from field experience conducted in the experimental field of the Agricultural University - Plovdiv in the period 2017-2019 were used.

As a result of the analysis, correlations were found between the studied indicators in both varieties For *Multifolium 1* the strongest positive correlation was reported between the amino acids valine and isoleucine ($r = 0.981$), threonine and phenylalanine ($r = 0.980$); and for *Legend* variety - between the indicators isoleucine and leucine ($r = 0.995$), lysine and threonine $r = 0.966$).

The correlations between the accumulation of methionine and other amino acids were mathematically unproven.

Keywords: alfalfa, amino acids, correlation

INTRODUCTION

Alfalfa green mass and hay are characterized with high protein content and essential amino acids, the main indicators that determine the quality of resulting biomass. A number of studies have shown that the application of various growth regulators has a positive effect on the quality of alfalfa feed (Buck et al., 1988; Wang et al., 2003 Radu et al., 2010). The protein-genic amino acids in the vegetative mass of alfalfa are related to the biological value and balance of proteins. Their amount in the individual protein fractions is under genetic control and difficult to change, but the ratio of protein fractions, and through them the quality of the protein can change under external influences.

The treatment with Reni preparations has a positive effect on the total content of protein-genic amino acids in *Multifolium 1* variety. The variety is a representative of alfalfa latest generations. It has been created by an author team from IZZ "Obraztsov Chiflik" - Ruse, VSI - Plovdiv and IGI - Kostinbrod. Over 50% of plant leaves have from 5 to 7 petals in a single petiole. This feature distinguishes it from all Bulgarian and foreign alfalfa varieties listed in the National List of the country. The rich leaf mass guarantees 1.5% higher crude protein content compared to standard varieties. The plants are strongly branched, dark green, with predominant

purple inflorescences.

In terms of yield of dry vegetative mass, the variety is as good as the best Bulgarian varieties. The plants are resistant to leaf diseases and root rot.

RENI preparations have been created at the Agricultural University - Plovdiv, as a means of regulating nitrogen metabolism. RENI preparations are combinations of the microelements molybdenum, manganese, magnesium in different concentrations and ratios.

RENI preparations are used for foliar treatment on alfalfa with a solution containing the components. Methods of the experiment conduction along with the studied indicators are available in our other works (Popov et al., 2007, Popov et al., 2010). The content of some nutrients in plants and the activities of the enzymes nitrogenase, nitrate reductase, glutamine synthetase and asparagine synthetase serve as a diagnostic sign for determining the exact concentration and ratio of the individual components in the various formulations.

RENI preparations increase the activity of the main enzyme systems related to the metabolism of nitrate ions. As a result, the yield is increased, the obtained production quality is improved, and their application is harmless to humans and animals. The treatment with RENI is performed once a year in the budding phase of each slope with a dose of

200 ml / da. The use of different Reni preparations on the variety increases the content of essential amino acids in only two of all options - Reni + Co by 14.52% and Reni by 4.8%, while Reni + B and ½ Reni + variant (½ Reni + B) it is lower.

The purpose of the present study is to establish the existence of a correlation between the studied qualitative indicators in order to make a more objective assessment; namely - to show the degree of influence of each indicator in the formation of yields of alfalfa (*Medicago sativa L.*).

MATERIALS AND METHODS

For the purpose of the study, there were used three-year data from a field experiment conducted on the experimental field of the Agricultural University - Plovdiv in the period 2017-2019. It was conducted in order to establish the influence of RENI preparation, applied alone, and in combination with added microelements, on the enzyme activity, the manifestation of multifoliate features, the productivity and the quality of multifoliate alfalfa varieties. The experiment was based on the method of fractional plots, in 4 repetitions with the size of the experimental plot - 10 m².

The evaluation of the tested options was performed by comparing the following indicators determining the quality of alfalfa: X₁ - lysine, X₂ - threonine, X₃ - valine, X₄ - methionine, X₅ - isoleucine, X₆ leucine, X₇-phenylalanine.

A correlation analysis was performed in order to establish the presence of statistically significant correlations between the studied indicators.

Based on the obtained results, the accuracy in assessing the influence of nitrogen metabolism regulators on the accumulation of some amino acids can be increased, which is a prerequisite for increasing the accuracy in making specific decisions in practice and the actual treatment of alfalfa.

The experimental data were processed by correlation analysis (Barov, 1982), with the help of which the relationship between the studied indicators was established and evaluated. It is expressed by the correlation coefficient *r*, determined by the statistical program SPSS. The same approach was used to establish the relationship between important agronomic indicators in wheat (Kuneva, 2015), rye (Kuneva, 2018) and soybean (Matev, 2014).

RESULTS AND DISCUSSION

The correlation coefficients expressing the relationship between the studied indicators are indicated in the correlation matrix (Table 1).

A strong positive correlation was found between the amino acids lysine and leucine, threonine and phenylalanine; valine and isoleucine, respectively, with correlation coefficients - *r* = 0.960; *r* = 0.980 and *r* = 0.981. The correlation between the indicators was less expressed between lysine and phenylalanine (*r* = 0.920). All these correlation coefficients were statistically proven at the level of significance.

Table 1. A correlation matrix for Multifolium 1 variety

X _i	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇
X ₁	1.00	0.828	0.633	0.565	0.726	0.960**	0.920*
X ₂		1.00	0.872	0.034	0.864	0.691	0.980**
X ₃			1.00	-0.246	0.981**	0.588	0.810
X ₄				1.00	-0.095	0.624	0.230
X ₅					1.00	0.716	0.833
X ₆						1.00	0.804
X ₇							1.00

High positive values were also reported for the Legend variety, where the interaction between lysine and threonine made the most serious impression (*r* = 0.966); isoleucine and leucine (*r* = 0.995); the correlation between the indicators was less expressed: valine and lysine, threonine with coefficients *r* = 0.898, respectively; *r* = 0.944; valine and isoleucine, leucine with *r* = 0.898, respectively; *r* = 0.910, phenylalanine and threonine, valine, methionine *r* = 0.931; 0.942; 0.896. This analysis can be used to predict the quality indicators of the samples and possibilities for their change, as well as the advantages of each of them.

Table 2. A correlation matrix for Legend variety

X _i	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇
X ₁	1.00	0.966**	0.898*	0.699	0.708	0.754	0.812
X ₂		1.00	0.944*	0.804	0.728	0.753	0.931
X ₃			1.00	0.875	0.898*	0.910*	0.942*
X ₄				1.00	0.867	0.837	0.896
X ₅					1.00	0.995**	0.764
X ₆						1.00	0.755
X ₇							1.00

The presence of a cross-correlation between valine and isoleucine, leucine, phenylalanine suggested the presence of a multicorrelation model, which can be used to explain the relationship between the individual amino acids in their accumulation. After the variation analysis of the biomass chemical composition and on the basis of the data for the minimum, maximum and average values, the error of the means, the standard deviation and the coefficient of variation of the studied quality indicators, the different variability of the studied indicators was taken into account.

From the data presented in Tables 3 and 4 it can be seen that the variability of the studied indicators was clearly expressed and was represented by the coefficient of variation (VC) as average.

Table 3. Variation analysis of amino acids of alfalfa in the variety Multifolium

	Mean	Min	Max	SE	SD	VC-%
lysine	1.70	1.56	1.92	0.06	0.15	9
threonin	1.16	1.12	1.25	0.02	0.05	4
valine	1.23	1.12	1.35	0.04	0.09	7
methionine	0.11	0.09	0.13	0.01	0.02	18
isoleucine	0.93	0.84	1.05	0.04	0.08	9
leucine	1.70	1.57	1.86	0.06	0.13	8
phenylalanine	1.26	1.19	1.45	0.05	0.11	9

It is noteworthy that in the narrowest range the amino acids threonine and methionine changed, and in the widest range - lysine for the variety Multifolium 1, while in the case of the Legend variety in the narrowest range is again methionine, and in the widest range – leucine.

Table 4. Variation analysis of amino acids of alfalfa in the Legend variety

	Mean	Min	Max	SE	SD	VC-%
lysine	1.66	1.52	1.86	0.06	0.12	7
threonin	1.09	0.97	1.23	0.04	0.09	8
valine	1.27	1.09	1.46	0.06	0.14	11
methionine	0.06	0.03	0.09	0.01	0.02	33
isoleucine	0.98	0.88	1.11	0.05	0.10	10
leucine	1.68	1.51	1.91	0.08	0.17	10
phenylalanine	1.22	1.11	1.31	0.04	0.08	7

The present study contributes to a better understanding of the relationships between individual biochemical parameters and the influence of these indicators on the accumulation of amino acids in the aboveground mass of alfalfa. On one hand, it explains the relationship between the accumulation of individual amino acids, and, on the other hand, it is an evidence how the synthesis of amino acids in alfalfa biomass can be regulated. Thus, we can model the quality of alfalfa and produce feed for different uses and to different groups of animals.

CONCLUSION

The proposed mathematical approach allows to increase the objectivity in the assessment of the complex influence of the factors related to the synthesis and accumulation of amino acids during the vegetation of alfalfa varieties. This analysis can be used to predict the quality indicators of the two most common Bulgarian alfalfa varieties and the possibilities for their change, as well as the advantages of each of them.

As a result of the conducted correlation analysis, correlations between the studied indicators were established. The strongest positive correlation was reported for the amino acids valine and isoleucine ($r = 0.981$), threonine and phenylalanine ($r = 0.980$) in the *Multifolium 1* variety, and in the *Legend* variety - between the indicators isoleucine and leucine ($r = 0.995$), lysine and threonine $r = 0.966$).

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