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IMPROVING THE BIOLOGICAL VALUE OF (*VICIASATIVAL*.) BY TREATMENT WITH GROWTH REGULATORS

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Abstract.

A three-year field trial was carried out with two Bulgarian common vetch cultivars – Dobrudzha and Obrazets 666. The experiment was performed in the Training and Experimental Fields of the Agricultural University – Plovdiv on alluvial-meadow soil, in four replications, the size of the experimental plot being 10 m^2. The studied growth regulators RENI and RENI D, as well as the commercial products Bormax, Mn chelate and Molybdenite were applied once in the season, at the budding stage of vetch. The results obtained show that treatment with the growth regulators contributed to the increase of the starch content, the effect being more pronounced in Obrazets 666 – an increase of 3,2 to 3,5% compared to the untreated variant. What is more, the application of the products increased the protein content in both studied common vetch cultivars – Dobrudzha responded better to Bormax and RENI D, while Obrazets 666 responded to three growth regulators (Bormax, RENI and RENI D). A change in the elemental and amino acid composition of the harvested grain was also reported, which is essential for the production of feed of higher biological value for the farm animals. In Dobrudzha cultivar, a 12,0% increase of the lysine content in grain was found after treatment with RENI. Obrazets 666 responded with a sharp increase in the values of the sulfur-containing amino acid methionine in the grain protein in the variants treated with RENI D and Bormax – 47,2 and 35,2% on average.

Keywords: common vetch, growth regulators, biological value of feed, cereals and legumes, protein quality

INTRODUCTION

When choosing cereals and legumes to be grown in a certain region, the yield and the amount of crude protein per unit area are important, but they depend to some extent on the applied agricultural practices. Common vetch is the main grass forage crop in our country. In terms of crude protein content, vetch is not inferior to alfalfa and its grain ranks one of the first places among the legumes after soybeans and lupins. Vetch grain contains 30,64% of crude and 27,18% of pure protein, 0,77% of crude fat, 3,28% of crude fiber, 3,27% of ash, 0,18% of calcium, 0,46% of phosphorus. Green biomass, hay and straw are rich in calcium and grain is rich in phosphorus.

In recent years, there have been many research studies on the effect of various exogenous substances (growth regulators, foliar fertilizers, etc.) on the yield and quality of legumes. The application of such products is considered to have a positive effect on symbiotic nitrogen fixation, which also leads to an increase in grain yield and quality.

Molybdenum, which is the major component of RENI, is an element that builds the nitrogenase complex and has a direct effect on nitrogen fixation, affecting also the uptake of other nutrients – zinc, cobalt, copper, nickel (Atkins et al., 1984) and phosphorus (Huber et al., 1992). It is absorbed by plants as an anion (MoO_4^{2-}) and is involved in the active site of nitrogenase. In addition, it catalyzes the transamination reactions that produce new amino acids.

According to Reguera et al. (2010) boron is a particularly important element for nitrogen fixation and bean-rhizobial symbiosis. Boron deficiency reduces the stability of macromolecules in the nodules, which are responsible for the infection with nodule bacteria and for regulating the oxygen concentration. Boron can be attributed to the group of trace elements, the need for it being well established. Its action is closely related to the formation of plant tissues, with redox processes, with the metabolism of carbohydrates, proteins and nucleic acids, with the uptake of mineral elements by plants, etc. (Gorbanov et al., 2005).

Kolasinska and Grzelak (1993) announced that treatment of the pea seeds with Neoxin L or Polycrust resulted in stronger sprouts and fewer sprouts with deviations. The biostimulatorCherkaz (organosilicon product) led to an increase in grain yield by 9-19% (Kirsanova, 2001).

Saimbhi et al. (1975) reported an increase in the content of Ca and Mg in the fresh and dry biomass of peas after pre-sowing treatment of the seeds with 2-chloroethylphosphonic acid. In her studies, Wyzskowska (1999) pointed out that L-tryptophan, a precursor of auxins and indole-acetic acid, applied to soil and as a foliar spray, had a minimal effect on the chemical composition of the seeds, pods, stems and leaves of broad beans, increasing only the content of K in the pods and the content of N and Ca in the leaves.

An increase in the nitrogen, protein and amino acid contents in the pea grain was announced by El-Beheidi (1995) and Stakhova (2000) after foliar application of folic acid, increasing mainly the content of the amino acids glutamine, glycine and methionine.

Zhelyazkova and Pavlov (2003) found out that treatment of vetch, Obrazets 666 cv., with three different biostimulants(N-40, HP-55 andG-31) applied at different rates, increased the yield of grain and dry biomass, due to the higher values of the structural elements of the yield – number of pods and seeds per plant, weight of 1000 seeds.

MATERIAL AND METHODS

A field trial was carried out to establish the effect of RENI, applied separately and in a combination with boron, as well as the commercial products Bormax, Manganese Chelate and Molybdenite, on the quality characteristics of common vetch cultivars. The experiment was conducted in the period 2008-2010 by the split-plot design method in four replications, the size of the experimental plot being 10 m^2. Both cultivars were treated in the budding stage at the following concentrations: RENI – 0,5%; RENI D – 0,5%; Manganese chelate – 0,4%; Molybdenite – 0,2%; Bormax – 0,4%.

Plant Analysis

• Dry matter in vetch grain (%) – determined by drying in an oven at 105 ºC until constant weight of the sample.

• Crude protein (%) – determined by mineralization of the plant samples with concentrated sulphuric acid and perhydrol used as a catalyst, following the method of Kjeldahl, with subsequent distillation of the mineralized sample using Parnassus-Wagner apparatus and multiplying the obtained values of total nitrogen by a coefficient of 6,25.

• Essential amino acids (%) – determined by a Knauer type amino analyzer in the grain of vetch after hydrolysis of the plant material with 6n HCl.

• Starch (%) – determined by the polarimetric method. A sample of 2-5 g was treated with 50 ml of 1% HCl and 4 ml of hydrofluoric acid.

• Elemental composition – the content of Fe, Mn, Ca, Mg and Mo (mg/kg) in the grain was determined by dry combustion in the presence of HCl and reporting by AAS (BDS 11374-8 – 1986, Sofia).

Common Vetch Cultivars Used In the Study

Common vetch, cultivar Obrazets 666 was developed by Prof. M. Pehlivanov and tested by the State Varietal Testing system for biological and economic qualities in 1970-1973 and for distinctness, uniformity and stability – in 2001-2002. The cultivar has a rapid development rate, early flowering and it matures before the summer droughts. The absolute seed weight is 80-100 g and the hectolitre weight – 80-85 kg. The average yield is 2326 kg/ha.

Common vetch, cultivar Dobrudzha was established at Dobrudzha Agricultural Institute in General Toshevo and tested by the State Varietal Testing system for biological and economic qualities in 2001-2002 and for distinctness, uniformity and stability – in 2002-2003. The vegetation period is 81 days on average. The height of the first pod is 33 cm. The cultivar is susceptible to lodging, as is the standard cultivar. The weight of 1000 seeds is 62,99 g. The hectoliter weight varies from 76,9 to 85 kg. The protein content is 31,37% of the absolute dry matter. It is moderately resistant to ascochitosis and resistant to powdery mildew and rust.

Products Used

RENI products are combinations of molybdenum, manganese and magnesium ions in different concentrations and ratios, which are additionally and purposefully combined with agents with a biochemical and physiological action, such a strace elements, synthetic regulators of cytokinintype, basicmetabolites, etc.

RENI-D contains the main elements of RENI products with B (boron) added.

Manganese chelate is a foliar fertilizer for fertigation, hydroponics and foliar application in manganese deficiency. It is applied at a concentration of 0,2-0,4% solution. Its application can be combined with foliar nutrition with an aqueous solution of urea. In the present study Mn is in a chelated form (EDTA), which is easily absorbed by plants.

Molybdenite–afoliar fertilizer for fertigation, hydroponics and foliar application in crops with high molybdenum requirements: potatoes, cabbage,

broccoli, beans, peas, tomatoes. Leaf application is at a concentration of 0,1 – 0,2%.

Bormax– a foliar fertilizer for all the crops with high boron requirements – maize, beets, fruit, potatoes, legumes, vegetables and flowers. Foliar application is at a concentration 0,3-0,4% and the application rate is 1 l/ha.

Pearson correlation analysis has been used to calculate the relationships between the variables.

RESULTS AND DISCUSSION

Crude Protein Content

In Dobrudzha cultivar, the crude protein content in the studied variants varied from 27,06 g/100 g to 32,44 g/100 g of dry matter in the period 2008-1010. Obrazets 666 cultivar was characterized by a lower protein content in the grain – from 25,07 to 30,06 g/100 g dry matter.

In cultivar Dobrudzha, the analysis of the results by years shows the highest protein content in the last year of the experiment (2010), the values being from 30,44 g/100 g (Control) to 32,44 g/100 g (treatment with RENI D). The lowest protein content was reported in the first experimental year (2008) with values ranging from 27,06 g/100 g (treatment with Bormax) to 29,47 g/100 g (treatment with RENI D). The grain of Obrazets 666 cultivar had the highest values of that characteristic in 2009 – from 29,06 g/100 g to 30,63 g/100 g and the lowest in the first year, with an average content of 27,06 g/100 g to 29,47 g/100 g (Table 1).

Table 1. Crude protein content in the grain of common vetch cultivars Dobrudzha and Obrazets 666 by years, g/100 g dry matter

Variants	Crude Protein, Dobrudzha cultivar, g/100 gdry matter					
	2008	%	2009	%	2010	%
Control	28,27	100,0	29,94	100,0	30,44	100,0
RENI	27,60	97,6	29,06	97,1	30,69	100,8
RENID	29,47	104,2	30,63	102,3	32,44	106,6
Bormax	27,06	95,7	30,50	101,9	31,38	103,1
Mn chelate	27,56	97,5	29,50	98,5	31,75	104,3
Molybdenite	28,20	99,7	29,94	100,0	31,50	103,5
-	Crude Protein, Obrazets 666 cultivar, g/100 gdry matter					
Control	25,68	100,0	28,63	100,0	26,06	100,0
RENI	25,85	100,7	28,69	100,2	29,94	114,9
RENID	25,65	99,9	28,81	100,6	29,00	111,3
Bormax	25,60	99,7	30,06	105,0	26,75	102,6
Mn chelate	25,93	101,0	28,06	98,0	27,81	106,7
Molybdenite	25,07	97,6	29,69	103,7	29,44	113,0

In the first experimental year (2008), the lowest values of crude protein were recorded. In the other two years (2009 and 2010), characterized by a deficit of moisture during the critical stages of vetch development, a higher protein content in the grain of both studied cultivars was observed.

The data analysis shows that the application of the tested growth regulators had a positive effect on the protein content of both cultivars. That is a positive step towards increasing the nutritional value of vetch grain feed.

In Dobrudzha cultivar, the crude protein content increased more significantly (4,4%) only when treated with RENI D. In the variants with the application of the other growth regulators the differences to the control were insignificant (Fig. 1).

Obrazets 666 responded to the applied regulators with a more significant increase in crude protein content compared to Dobrudzha. In the former cultivar all the variants had higher values compared to the control. The protein content was the highest after treatment with RENI and Molybdenite with values of 28,16 g/100 g and 28,07 g/100 g, i.e. 5,1% and 4,8% higher than in the control variant. An increase was also observed after the application of RENI D and Bormax, the protein content being 27,82 g/100 g and 27,47 g/100 g, respectively, i.e. exceeding the control by 3,8 and 2,5%.

The analysis of the data obtained shows that Dobrudzha has a higher protein content in the grain compared to Obrazets 666. The effect of the application of the studied products in Dobrudzha cv.

was lower and only the boron-containing product RENI D had a positive effect on the crude protein content.

In Obrazets 666, which is characterized as a cultivar with a lower protein content, the foliar-applied growth regulators had a more pronounced effect. In that cultivar, the introduction of RENI, Molybdenite and RENI D can become an important part of the cultivation technology, aiming at an increase of the protein content in the grain.

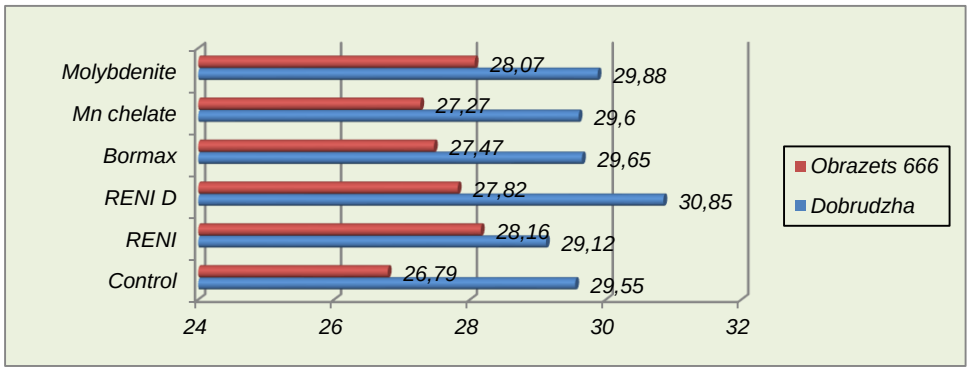

Fig.1. Crude protein content in the grain of common vetch, cultivars Dobrudzha and Obrazets 666, on average for the period 2008-2010, g/100 g dry matter

The analysis of the data obtained shows that Dobrudzha has a higher protein content in the grain compared to Obrazets 666. The effect of the application of the studied products in Dobrudzha cv. was lower and only the boron-containing product RENI D had a positive effect on the crude protein content.

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Chemical Composition of Grain

Table 2 presents the average data of the content of dry matter, P, K and starch (in percentages) in the grain of common vetch of the two studied cultivars for the period 2008-2010.

Data show that the foliar applied growth regulators did not have a significant effect on the dry matter accumulation in both cultivars.

The grain of Dobrudzha cultivar in all the variants of the experiment had a higher dry matter content in comparison with Obrazets 666. In both cultivars there was a slight increase in the dry matter content after application of Bormax. Changes in the content of P and K in the grain of both common vetch cultivars were controversial, and, no trend in the accumulation of the two elements could be determined.

The starch content in the grain of both cultivars ranged from 46,69 to 48,83%. It increased, although slightly (by 3,3%), only in the grain of Dobrudzha cv. after treatment with RENI D. In Obrazets 666 cv., it increased by 3,2% and 3,5% in the variants treated with RENI and RENI D, respectively. In the other variants the values were around and below those of the control. The results of correlation analysis showed no correlation between the content of K and three of the measured parameters (dry biomass, a protein and phosphorus) at R> 0.6 (Table 3). The strongest relationship is between the protein content and the dry mass of the seeds of the rests (R = 0.88). It is negative, because in well-developed grains the content of nitrogen-free substances increases at the expense of protein. However, according to the analysis, the starch content does not depend on the other four indicators. Potassium and phosphorus have a synergistic effect on the accumulation of dry matter, including that in seeds. The correlation between them is positive at R> 0.6.

Elemental Composition Of the Grain

The elemental composition of the grain gives an idea of its biological value as an ingredient in combined feed for farm animals. In the present study, the contents of Fe, Mn, Ca and Mg in the grain of both cultivars were determined.

The analysis of the results (Table 4) shows that the iron content in the grain of Dobrudzha cv. increased in all the variants of the experiment. The most significant increase was reported in the variants treated with Molybdenite and Mn chelate – 62,0 and 50,2%, respectively, compared to the control. A clear increase was also observed after treatment with RENI D, where iron was 32.2% more than in the untreated variant.

In Obrazets 666, an opposite tendency was observed – the iron content in the grain decreased

in all the studied variants. Iron is an extremely important trace element for the animal body, as it is part of hemoglobin and an oxygen carrier to the tissues. Feeds rich in iron, greatly improve the physiological status of the farm animals.

Table 2. Content of absolute dry matter, P, K and starch in the grain of common vetch, Dobrudzha and Obrazets 666 cultivars (on average for the period 2008-2010)

Composition Variant	Dry matter, %	%	P	%	K	%	Starch,%	%
Dobrudzha cultivar								
Control	88,2	100,0	0,46	100,0	0,42	100,0	46,69	100,0
RENI	88,4	100,2	0,28	60,6	0,38	92,0	46,69	100,0
RENID	88,5	100,3	0,24	52,9	0,37	90,0	48,24	**103,3**
Bormax	88,6	100,5	0,13	28,8	0,37	90,0	46,72	100,1
Mn chelate	88,5	100,3	0,21	46,2	0,42	100,0	45,92	98,4
Molybdenite	88,5	100,3	0,50	**109,6**	0,41	98,0	46,69	100,0
Obrazets 666 cultivar								
Control	89,7	100,0	0,41	100,0	0,42	100,0	47,19	100,0
RENI	89,5	99,8	0,43	104,3	0,45	**108,0**	48,72	**103,2**
RENID	89,8	100,1	0,39	95,7	0,45	**108,0**	48,83	**103,5**
Bormax	89,5	99,8	0,43	104,3	0,45	**108,0**	47,20	100,0
Mn chelate	89,6	99,9	0,49	**120,4**	0,43	104,0	46,69	98,9
Molybdenite	89,7	100,0	0,50	**121,5**	0,41	98,0	47,00	99,6

Table 3. Correlation analysis between the variables (r Pearson coefficient).

Variables	Protein	DM	P	K	Starch
Protein	**1**	**-0,880**	-0,527	**-0,677**	-0,161
DM	**-0,880**	**1**	0,487	**0,642**	0,460
P	-0,527	0,487	**1**	**0,623**	0,123
K	**-0,677**	**0,642**	**0,623**	**1**	0,310
Starch	-0,161	0,460	0,123	0,310	**1**
Values in bold are different from 0 with a significancelevelalpha=0,05					

Manganese content increased in the grain of both common vetch cultivars in all the variants of the study. The most significant increase was reported in the grain of Dobrudzha cultivar after the application of Mn chelate and RENI D and in Obrazets 666 cv. – in the variants treated with RENI D and Bormax.

The calcium and magnesium content was not significantly affected by the application of the tested products. Magnesium content increased only after treatment with Bormax and Mn chelate in Dobrudzha cv. and with RENI D and Bormax in Obrazets 666. It is known that highly productive adolescent animals have the greatest need for magnesium. Magnesium deficiency causes various diseases and that makes the feeds of higher magnesium content preferable.

Content of EssentialAminoAcids

The data presented in Table 5 show that the total concentration of essential amino acids in the protein ofDobrudzha cultivar was higher in all the variants of the experiment compared to the control.

In Dobrudzha cv., the greatest increase in the essential amino acid content was found in the variants treated with RENI and RENI D – 5,5 and 3,4% on average, compared to the control. The products Bormax, Mn chelate and Molybdenite slightly increased the content of the essential amino acids in the grain of the common vetch cultivar Dobrudzha (2,5 to 2,9%).

The lysine content increased most significantly (12,0%) after treatment with RENI. In the other variants, the lysine content also increased (6,9 to 8,6%). The application of RENI resulted in an increased content of phenylalanine by about

16%. That is especially valuable in terms of the biological value of the feed. Feed is usually poor in lysine content and that is one of the main problems from a nutritional point of view.

There was a tendency to an increase of the threonine content in all the variants of the study and the highest values were determined in the variants treated with Mn chelate, RENI and Molybdenite.

Table4. Elemental composition of grain, mg/kgof dry matter.

No.	Fe	%	Mn	%	Ca	%	Mg	%
Dobrudzha cultivar								
Control	129,0	100,0	8,4	100,0	592,1	100,0	673,9	100,0
RENI	142,1	110,2	9,0	107,1	526,3	88,9	679,4	100,8
RENID	170,6	132,2	10,0	119,1	592,1	100,0	686,7	101,9
Bormax	136,1	105,5	9,2	109,5	592,1	100,0	700,0	103,9
Mn chelate	193,8	150,2	10,8	128,6	592,1	100,0	706,1	104,8
Molybdenite	209,0	162,0	9,7	115,5	657,9	111,1	679,4	100,8
Obrazets 666								
Control	163,5	100,0	8,8	100,0	592,1	100,0	700,5	100,0
RENI	138,8	84,9	9,3	105,7	592,1	100,0	689,4	98,4
RENID	148,4	90,8	9,9	**112,5**	592,1	100,0	712,2	**101,7**
Bormax	131,5	80,4	9,5	**108,0**	592,1	100,0	711,1	**101,5**
Mn chelate	153,6	93,9	9,4	106,8	526,3	88,9	695,0	99,2
Molybdenite	116,7	71,4	8,9	101,1	493,4	83,3	707,2	101,0

Table 5. Content of essential amino acids in the grain of common vetch, Dobrudzha and Obrazets 666 cultivars (% to protein), on average for the period 2008-2009.

Amino acid	Variants					
	Control	RENI	RENI D	Bormax	Mn chelate	Molybdenite
	Dobrudzha					
Lysine	6,27	**7,02**	6,70	6,81	6,73	6,70
Phenylalanine	4,48	5,34	4,76	4,55	4,55	4,91
Leucine	7,23	7,29	7,69	7,51	7,55	7,43
Isoleucine	4,46	4,51	4,52	4,42	4,56	4,35
Methionine	0,473	0,393	0,181	0,247	0,368	0,288
Valine	5,16	5,20	5,33	5,27	5,28	5,21
Threonine	3,04	3,17	3,09	3,18	3,09	3,14
Total	31,21	32,92	32,27	31,99	32,13	32,03
	Obrazets 666					
Lysine	6,70	6,91	6,90	6,91	6,71	6,52
Phenylalanine	4,22	4,59	4,51	4,49	4,37	4,45
Leucine	7,50	7,58	7,53	7,64	7,55	7,43
Isoleucine	4,54	4,43	4,46	4,49	4,58	4,46
Methionine	0,199	**0,203**	**0,293**	**0,269**	0,165	0,155
Valine	5,26	5,38	5,35	5,39	5,26	5,38
Threonine	3,16	3,13	3,00	3,19	3,12	2,98
Total	31,58	32,22	32,04	32,38	31,76	31,38

As in Dobrudzha cv., the application of the growth regulators also changed the content of the essential amino acids in the grain protein in Obrazets 666 cv. in a positive direction, the best expression of the effect being observed after treatment with Bormax, RENI D and RENI.

It should be noted that the values of the sulfur-containing amino acid methionine in the grain protein in the variants treated with RENI D and Bormax increased sharply – 47,2 and 35,2% on average. Methionine is also one of the limiting amino acids and increasing its content in feed is particularly important for its nutritional value. An

increase in the content of phenylalanine and valine was also reported in all the variants (Table 5) compared to the control.

The tendency to an increase in the content of the limiting amino acid lysine, although to a lesser extent, was observed in Obrazets 666 cultivar. Lysine was on average 3,0% higher in the variants treated with RENI, RENI D and Bormax.

CONCLUSIONS

The foliar application of growth regulators led to an increase in the content of crude protein in common vetch grain in both studied cultivars. Dobrudzha cv. responded most strongly to Bormax and RENI D. In the other vetch cultivar (Obrazets 666) three growth regulators had a pronounced effect on the protein yield – Bormax, RENI and RENI D.

The studied products applied in the budding stage of vetch, increased the content of the elements important for the health status of the farm animals. The most significant increase of iron was reported after the application of Molybdenite and Mn chelate in Dobrudzha cultivar, the concentration increasing by 62,0 and 50,2% compared to the control. A significant increase was also observed after treatment with RENI D, the iron content increasing by 32.2%. There was also an increase of magnesium content in grain in both common vetch cultivars in all the variants of the experiment. The highest values were established in the grain of Dobrudzha cv. after treatment with Mn chelate and RENI D and in Obrazets 666 – after treatment with RENI D and Bormax.

In Dobrudzha cultivar, a 12,0% increase of the lysine content in grain was found after treatment with RENI. In the other variants, the lysine content also increased by 6,9 to 8,6%. That is especially valuable because lysine is usually a limiting amino acid in ruminant feed. The application of RENI also led to about a 16% increase of the phenylalanine content. There was a tendency to an increase of the threonine content in all the variants of the experiment, the greatest increase being observed in the variants treated with Mn chelate, RENI and Molybdenite. That also contributed to the improvement of the biological value of feed.

Obrazets 666 responded with a sharp increase in the values of the sulfur-containing amino acid methionine in the grain protein in the variants treated with RENI D and Bormax (47,2 and 35,2% on average). That is also quite significant from a nutritional point of view, improving the nutritional value of feed. An increase in phenylalanine and valine content was also reported in all the treated variants compared to the control.

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