LEGUME-RHIZOBIAL SYMBIOSIS OF THE PANNONIAN CLOVER VARIETY ANIK USING COMPLEX MICROELEMENTS AND GROWTH REGULATORS

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Abstract

The article presents the scientific results of the influence of complex microelement fertilizers and growth regulators on the formation of the parameters of the symbiotic activity of agrocenoses and the productivity of the Pannonian clover variety Anik. During exogenous seed treatment with micronutrient fertilizers and bacterial preparations, the number and weight of active clover nodules of the first year of use in relation to control increased by 28.8-154.9 million units/ha and 77.2-240.8 kg/ha, respectively. With foliar application, the most effective method is double follicular treatment of Pannonian clover plants in the regrowth and budding phase with Megamix-Nitrogen complex fertilizer. The highest indicators of the symbiotic apparatus of the Pannonian clover crops were formed using tank mixtures of the Corsair herbicide with the drug Albit and Siliplant; according to the experimental variants, they amounted to 93-101 million units/ha of active nodules with a 50% reduction in the dose of the herbicides Corsair and Agritox.

Key words: nitrogen fixation, Pannonian clover, biological products, microelements.

INTRODUCTION

In solving the problem of increasing the production of feed and increasing their energy saturation, a significant role is assigned to perennial herbs. Therefore, it is relevant to organize adaptive fodder production through the introduction of new species that have ecological plasticity, longevity, high forage qualities, are distinguished by stable seed production, high winter resistance, heat and drought tolerance, and increase soil fertility. (Watson, 2012; Komainda et al., 2019).

In the Middle Volga region (Russia), a promising forage crop is the Pannonian clover (*Trifolium pannonicum* Jacq.), characterized by high ecological plasticity and adaptability, productive longevity of 10-12 years, drought resistance, winter hardiness, and resistance to diseases and pests, has stable seed production, increases soil fertility, and is valuable as a predecessor and a honey plant (Kshnikatkina et al., 2018).

An important element of modern agricultural technologies is growth regulators, complex micronutrient fertilizers and bacterial preparations that provide plants with deficient micronutrients and help to increase their resistance to environmental stress factors and pathogens (Watson, 2012; Ruiz-Navarro, 2019).

Of scientific and practical interest is the use of complex liquid mineral fertilizers with a rich composition of micro and macro elements and bacterial preparations to increase the productivity of the Pannonian clover variety Anik, which determined the relevance of the research (Mikula et al., 2020).

MATERIALS AND METHODS

The purpose of the research was to study the treatment of seeds with bacterial preparations and microelement fertilizers on the production process of the Pannonian clover. Experimental studies were conducted in 2014-2016. On the experimental field of Penza State Agrarian University (Penza, Russia).

The soil of the experimental plot is leached chernozem, heavy loam. The humus content in the arable layer is 6.5%, pH (KCl) - 5.2, the degree of saturation with bases - 80.8-82.3%, the availability of mobile forms of molybdenum, boron, manganese, copper, zinc and cobalt is low. Meteorological conditions during the years of research: 2014 - State Customs Committee - 0.59 units; 2015 - State Customs Committee - 0.5 units; 2016 - State

Customs Committee - 1.21 units. The object of research is the Pannonian clover (*Trifolium pannonicum* Jacq) variety Anik.

The area of the accounting plot is 10 m^2 . Agrotehnika is generally accepted in this region. Inoculation of seeds with bacterial preparations was performed on the day of sowing. Application rates recommended by manufacturers.

RESULTS AND DISCUSSIONS

Seed treatment before sowing by growth regulators has a multifunctional effect, since the seeds at the time of germination have high plasticity and are susceptible to changes in environmental conditions.

Our experiments have found that when treating clover seeds with the studied drugs, field germination according to the test variants increased on average by 0.6-13.9% over three years. The highest germination rate of 80.2% was observed during seed treatment with the Gumariz biopreparation together with the MegaMix-Semen microelement fertilizer, the excess in relation to the control was 13.9%, the plants were preserved for harvesting - 13.5%. The greatest winter-hardiness was noted during the processing of seeds Megamix-Seeds and Gumarizom. So, in the first year of use, the winter hardiness of clover plants was 94.6-99.8% according to the experimental variants (control 89.4%). After wintering, the largest number of clover plants (238.2 pcs/m^2) remained in the Gumariz + Megamiks-Semen variant, the excess in relation to control was 85.9 pcs / m^2 (10.4%).

When treating seeds with the agrochemicals Albite, Megamix-Seeds, Humate K/Na separately and together with Humaris, the Pannonian clover plants formed a more powerful root system, the mass of dry roots according to the experimental variants was 1.26-1.98 t/ha, which is 0.39-1.11 t/ha exceeds the control option.

The results of the analysis of the linear growth of the clover showed that in the 1st year of life, taller plants were formed in the variant with seed treatment with the Megamix-Semen preparation together with Gumariz. On average, over three years, the height of plants at the end of the growing season was 32.8 cm, in control - 10.2 cm. A similar trend continued in subsequent years.

The analysis of the formation of the symbiotic activity of the agrocenosis of the Pannonian clover 1st year of use showed that the largest number of active nodules and their mass formed in the budding phase during seed inoculation with the Gumariz biological product together with the Megamix-Semen microelement fertilizer. On average, over two years, the total number of nodules during the budding phase of the agrocenosis of the Pannonian clover 1st year amounted to 248.6 million units/ha with a mass of 992.6 kg/ha. active nodules - 219.3 million units/ha with a mass of 876.9 kg/ha, which exceeds the control variant by 3.9 and 2.87 times, with inoculation with rhizotorfin - by 1.8 and 1.3 times respecttively. A similar regularity in the formation of the parameters of symbiotic activity was also observed in the agrocenoses of the Pannonian clover 2nd year of use (Table 1).

It was found that bacterial preparations and microelement fertilizers have a significant effect on the formation of the leaf surface. The most intensive growth of the leaf surface is noted during the budding phase when treating clover seeds with Gumariz jointly with Megamix-Seeds. The area of leaves in the first year of use during the budding phase was 51.5 thousand m²/ha, which exceeds the control indicators by 24.1 thousand m²/ha (87.9%), with inoculation of seeds with risotorfin - by 14.7 thousand m²/ha (71.5%).

The highest values of photosynthetic potential (PP) and net productivity of photosynthesis (NPP) were noted when treating clover seeds with the micronutrient fertilizer Megamix-Seeds together with Gumariz PP - 2.14 million m^2d/ha , NPP - 4.83 g/m², in the risorotin + Megamix version Seeds - 2.02 million m^2dn/ha and 4.69 g/m² a day, when treated with Megamix-Seeds - 42.8 million m^2dn/ha , AF - 1.85 million m^2d/ha , NPP - 3.95 g/m² a day, in the control indicators the FP - 1.02 m^2d/ha , NPP - 1.92 g/m² a day. Moreover, in relation to the indicators of the 1st year of use, the parameters of photosynthetic activity of the 2nd year of use increased by 14.9-15.7.

Optimization of production conditions positively influenced the accumulation of clover dry matter (Table 2).

Option	1 st year (2015-2016)		2 nd year (2016)		
	number of tubers, the mass of number of tubers, th		the mass of tubers		
	(mln. pcs/ha)	tubers (kg/ha)	(mln. pcs/ha)	(kg/ha)	
No processing (control)	53.7	328.4	67.2	410.2	
Risotorfin + Albit	183.5	657.4	229.5	821.7	
Rizotorfin + Megamix-Seeds	208.6	823.5	260.7	1029.4	
Risotorfin + Humate K/Na	185.7	685.6	232.5	857.1	
Gumariz + Albit	201.5	863.5	252.4	1079.4	
Gumariz + Megamix-Seeds	219.3	876.9	264.5	1096.1	
Gumariz + Gumat K/Na	204.3	802.6	255.4	1003.2	

Table 1. The number and weight of active nodules of agrocenoses the Pannonian clover in the budding phase

Option	The collection of	f green mass, t/ha	Seed productivity, kg/ha		
	1 st year	2 nd year	1 st year	2 nd year	
No processing (control)	21.3	23.2	359.2	431.2	
Risotorfin + Albit	37.2	40.2	685.2	822.3	
Rizotorfin + Megamix-Seeds	40.6	44.3	703.8	896.2	
Risotorfin + Humate K/Na	36.6	39.1	678.9	814.7	
Gumariz + Albit	42.1	45.9	720.8	865.3	
Gumariz + Megamix-Seeds	47.5	51.8	776.4	931.7	
Gumariz + Gumat K/Na	40.8	44.5	714.7	857.6	
HCP ₀₅	2015 - 3.4; 2016 - 3.1	2016 - 2.4	2015 - 25.8; 2016 - 22.6	2016 - 3.34	

Table 2. The productivity of the Pannonian clover, 2015-2016

On average, over two years, the yield of green mass of the Pannonian clover 1st year of use, according to the experimental options, was 28.7-47.5 t/ha, dry weight - 7.18-11.87 t/ha. The greatest productivity of clover was obtained during the processing of Megamix-Seeds seeds together with Gumariz: green mass - 47.5 t/ha; dry matter - 11.87 t/ha, feed units - 8.54 t/ha, digestible protein - 1.32 t/ha, exchange energy - 95.48 GJ/ha (Table 2).

In the complex treatment of seeds with the Gumariz bio-preparation. together with microelement fertilizers, the most favorable conditions were formed for the formation of structural elements and seed productivity of the Pannonian clover. Thus, the number of generative shoots varied from 4.14 to 4.68 million units/ha, seeds in the head ranged from 41 to 46 pcs., The number of seeds per plant from 170 to 215 pcs., The productivity of an individual plant ranged from 0.67 to 0.90 g, weight of 1000 seeds - from 3.94 to 4.18 g. The average yield of clover seeds of the Pannonian clover 1st year of use for two years amounted to 528.3-776.4 kg/ha in the experimental variants, and in the control - 359.2 kg/ha. The most productive were clover agrocenoses during

complex seed treatment per year of sowing with the Gumariz biological product together with micronutrient fertilizers, the seed yield was 714.7-776.4 kg/ha, which is 2.0-2.2 times higher than the control. The maximum seed yield of 776.4 kg/ha was obtained in the variant Gumariz + Megamix-Seeds. In the second year of use, the seed productivity of clover increased and amounted to 634.3–931.7 kg/ha in the experimental variants. The highest yield of clover seeds was obtained on the Gumariz + Megamiks Seeds variant - 931.7 kg/ha, in the control - 431.2 kg/ha.

The use of micronutrient fertilizers for foliar top dressing contributed to an increase in the parameters of symbiotic activity, the value of which depended on the type of preparations and the timing of their use. It has been established that the most effective method is double treatment of clover plants in the growth and budding phase with the complex fertilizer Megamix-Nitrogen. So, the total number of tubers was 378-395 million units/ha, weight -1485-1625 kg/ha. The number of active tubers in the budding phase - 319-348 million units/ha, weight - 1238-1355 kg/ha, in the control variant - 108-130 million units/ha and 361-426 kg/ha, respectively (Table 3).

The best indicators of the productivity of the photo-synthetic apparatus were formed during the double treatment of vegetating plants of clover of the Pannonian clover microelement fertilizers in the phase of regrowth and budding. When foliar application with Azosol 36 Extra, the area of the assimilating surface of agrocenoses of the Pannonian clover 4-9th year of use was 70.8-77.8 thousand m²/ha, photosynthetic potential - 2.09-5.15 mln m²d/ha the net productivity of photo-synthesis is 4.36-5.57 g/m² a day. When used for feeding Megamix-

Profi PP - 2.34-2.95 mln m²dn/ha, NPP - 4.42-5.63 g/m² a day and the preparation Humate K/Na - PP - 2.20-2.90 mln m²d/ha, NPP - 4.15-5.36 g/m² a day. The crops of the Pannonian clover worked most productively during leaf dressing with Megamix-Nitrogen micronutrient fertilizer, the leaf area was 72.6-79.5 thousand m²/ha, and the NPP was 2.36-3.06 million m²/day, NPP - 4.47-5.68 g/m² a day.

The optimization of the mineral nutrition of the Pannonian clover plants by foliar feeding with complex microelement fertilizers positively influenced the formation of productivity (Table 4).

	Factor B –	Year of use						
Factor A - the drug	processing phase	4	5	6	7	8	9	
Without treatment (k)	•	<u>130</u> 426	$\frac{108}{418}$	$\frac{119}{410}$	<u>122</u> 401	<u>115</u> 389	<u>112</u> 361	
Azosol 36 Extra	regrowth	<u>231</u> 969	<u>209</u> 951	<u>220</u> 932	<u>223</u> 913	$\frac{216}{885}$	<u>213</u> 651	
Azosol 36 Extra	budding	<u>241</u> 978	<u>219</u> 959	<u>230</u> 940	<u>233</u> 921	<u>226</u> 893	<u>223</u> 657	
Azosol 36 Extra	regrowth + budding	<u>257</u> 1003	<u>235</u> 984	<u>246</u> 965	<u>249</u> 946	<u>242</u> 917	<u>239</u> 577	
Megamix-Profi	regrowth	<u>270</u> 1162	<u>248</u> 1140	<u>259</u> 1118	<u>262</u> 1095	<u>255</u> 1062	<u>252</u> 782	
Megamix-Profi	budding	<u>284</u> 1173	<u>262</u> 1150	<u>273</u> 1128	<u>276</u> 1105	<u>269</u> 1071	<u>265</u> 788	
Megamix-Profi	regrowth + budding	<u>300</u> 1204	<u>268</u> 1181	<u>279</u> 1158	<u>282</u> 1135	<u>275</u> 1100	<u>271</u> 815	
Megamix - Nitrogen	regrowth	<u>320</u> 1308	<u>282</u> 1283	<u>299</u> 1258	<u>302</u> 1232	<u>295</u> 1195	<u>291</u> 1098	
Megamix - Nitrogen	budding	<u>329</u> 1319	<u>291</u> 1294	<u>308</u> 1269	<u>311</u> 1243	<u>304</u> 1205	<u>300</u> 887	
Megamix - Nitrogen	regrowth + budding	<u>348</u> 1355	<u>340</u> 1329	<u>337</u> 1303	<u>330</u> 1277	<u>323</u> 1238	<u>319</u> 1286	
Humate K/Na	regrowth	$\frac{211}{882}$	<u>173/</u> 865	<u>190</u> 848	<u>188</u> 831	$\frac{186}{805}$	<u>182</u> 592	
Humate K/Na	budding	<u>232</u> 888	<u>194</u> 871	<u>211</u> 854	<u>209</u> 837	<u>207</u> 811	<u>203</u> 594	
Humate K/Na	regrowth + budding	<u>243</u> 1000	<u>205</u> 981	<u>222</u> 962	<u>220</u> 943	<u>218</u> 914	<u>214</u> 619	

Table 3. The number and weight of active tubers of agrocenosis of the Pannonian clover

*Note: the numerator is the number of tubers (million units/ha) the denominator is the mass of nodules (kg/ha)

Factor A - the drug	Factor B - processing phase	Year of use					
Tactor A - the drug		4	5	6	7	8	9
With	out treatment (k)	459.3	436.3	413.4	381.2	321.5	252.6
Azosol 36 Extra	regrowth	575.5	495.6	517.9	477.8	402.8	316.5
Azosol 36 Extra	budding	627.9	596.8	565.1	521.2	439.5	345.3
Azosol 36 Extra	regrowth + budding	791.6	752.9	712.4	657.0	554.1	435.4
Megamix-Profi	regrowth	523.7	497.5	471.2	434.7	366.7	290.0
Megamix-Profi	budding	565.2	536.9	508.7	469.1	395.6	310.9
Megamix-Profi	regrowth + budding	684.6	650.4	616.1	568.2	479.2	376.5
Megamix - Nitrogen	regrowth	542.6	515.6	488.3	450.4	379.8	299.4
Megamix - Nitrogen	budding	579.2	550.3	521.4	480.7	405.4	318.6
Megamix - Nitrogen	regrowth + budding	746.5	709.2	671.8	603.9	522.6	410.6
Humate K/Na	regrowth	520.8	494.8	468.7	432.3	364.6	86.4
Humate K/Na	budding	548.9	521.5	494.2	455.6	384.2	301.9
Humate K/Na	regrowth + budding	609.0	578.6	548.1	505.6	426.3	335.1
HCP05 Factor A		35.4	32.3	30.4	28.1	30.9	21.4
Factor B		43.2	45.4	38.6	37.1	36.4	32.8
Factor AB		68.3	71.2	67.9	70.4	66.3	68.2

Table 4. Productivity of the Pannonian clover seeds by years of use

The most productive agrocenoses of the Pannonian clover 4-9th year of use were when feeding with Megamix-Nitrogen complex fertilizer.

When feeding clover grass stands twice during the growing and budding phase, the yield of dry matter was 10.68 t/ha, feed units - 7.45 t/ha, digestible protein - 1.21 t/ha, exchange energy - 92.7 GJ. In relation to the control variant, the collection of dry matter increased by 3.27 t/ha (44.1%).

The yield of clover seeds of the Pannonian clover 4-9th year of use with foliar fertilizing with complex fertilizers according to the experimental variants was 286.4-791.6 kg/ha (control - 252.6-459.3 kg/ha).

The highest seed yield was obtained by double treatment of the grass stand with clover with Azosol 36 Extra in the regrowth and budding phase - 435.4-791.6 kg/ha, which exceeds the control variant by 182.8-332.3 kg/ha

A similar pattern is observed during the feeding of vegetation of the Pannonian clover microelement fertilizers Megamix-Profi, Megamix-Nitrogen and Humate K/Na.

The yield of the Pannonian clover seeds when fertilizing with Megamix-Nitrogen microelement fertilizer was approximately on par with that of Azosol 36 Extra (316.5-791.6 kg/ha) and varied from 299.4 to 746.5 kg/ha. The highest indices of the crop structure elements were formed during the double feeding of the seed grass-standing the Pannonian clover microelement fertilizer Megamix-Nitrogen: the number of generative shoots was 7.13 million units/ha, the number of seeds in the head was 55 pcs. 399 plants per plant, plant productivity 1.84 g, 1000 seeds weight 4.61 g, control - 5.68 million units/ha, 38 units, 216 units, 0.8 g and 3.78 g,

relatively. Almost equivalent indicators of the elements of the crop structure were formed when the fertilizer of the Pannonian clover 4-9th year of using Megamix-Nitrogen was double-fed.

The greatest economic effect was obtained by treating the seeds of clover of the Pannonian clover bacterial preparation Gumaris with Megamix-Seeds, while cultivating the seeds, the profitability was 185.0%, and for fodder purposes - 147.9%.

CONCLUSIONS

Thus, the use of microelement fertilizers and bacterial preparations for exogenous seed treatment significantly increased the indicators of symbiotic nitrogen fixation, photosynthetic activity and productivity of clover of the Pannonian clover variety - Anik.

REFERENCES

- Komainda, M., Küchenmeister, K., Küchenmeister, F., Breitsameter, L., Wrage-Mönnig, N., Kayser, M., & Isselstein, J. (2019). Forage legumes for future dry climates: Lower relative biomass losses of minor forage legumes compared to trifolium repens under conditions of periodic drought stress. *Journal of Agronomy and Crop Science*, 205(5), 460-469, doi:10.1111/jac.12337
- Kshnikatkina, A.N., Alenin, P.G., Galiullin, A.A., Kshnikatkin, S.A. (2018). Biological nitrogen fixation of Pannonian clover (Trifolium Pannonicum Jacq.) In the Middle Volga. *Bulletin of the Samara Scientific Center of the Russian Academy of Sciences*, 20(5-2), 226-233.
- Mikula, K., Izydorczyk, G., Skrzypczak, D., Mironiuk, M., Moustakas, K., Witek-Krowiak, A., Chojnacka, K. (2020). Controlled release micronutrient fertilizers for precision agriculture – A review. *Science of the*

Total Environment, 712, doi:10.1016/ j.scitotenv.2019.136365

- Pandey, A., Li, F., Askegaard, M., Olesen, J. E. (2017). Biological nitrogen fixation in three long-term organic and conventional arable crop rotation experiments in denmark. *European Journal of* Agronomy, 90, 87-95. doi:10.1016/j.eja.2017.07.009
- Ruiz-Navarro, A., Fernández, V., Abadía, J., Abadía, A., Querejeta, J. I., Albaladejo, J., & Barberá, G. G. (2019). Foliar fertilization of two dominant species in a semiarid ecosystem improves their ecophysiological status and the use efficiency of a water pulse. *Environmental and Experimental Botany*, 167 doi:10.1016/j.envexpbot.2019.103854
- Watson, C.A., Öborn, I., Edwards, A. C., Dahlin, A. S., Eriksson, J., Lindström, B. E. M., Walker, R. L. (2012). Using soil and plant properties and farm management practices to improve the micronutrient composition of food and feed. *Journal of Geochemical Exploration*, 121, 15-24, doi:10.1016/j.gexplo.2012.06.015