The effectiveness of the use of *Sinorhizobium meliloti* in the cultivation of variable alfalfa (*Medicago varia* Mart.)

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Abstract. As a result of three-year studies, we have established a growth in the yield and feed advantages of variable alfalfa in the variants with inoculation of seeds with virulent active strains of rhizobia. The total yield augmentation of green mass in the experimental variants over the three years of alfalfa life were 6.8–13.7 % compared to the control ones. The positive effect of inoculation with virulent active rhizobia strains on the total collection of dry matter was expressed in its increase in experimental conditions by 12.6–21.7 %. The highest yield of green mass, as well as dry matter was obtained in the variant with the inoculation of alfalfa seeds with the main production strain 425. The researched factor has a positive effect on the collection of feed units, digestible protein and feed protein units from 1 ha. The collection of feed units per hectare in the experimental versions increases by 1.1–1.3 times, the collection of digestible protein – by 1.2–1.4 times. The maximum substance of feed units and digestible protein per hectare was observed in the version with seed inoculation with strain 425a. The provision of a feed unit with digestible protein increases by 10.44–18.18 g or by 6.1–10.6 %.

1. Introduction

Successful management and biologization of feed production requires extensive development of grass sowing. According to statistics, the largest areas in the world in the crops of fodder legumes for mowing use is occupied by alfalfa. Alfalfa, usually known as the “Queen of Forages”, is the main source of vegetable protein to meat and milk production systems worldwide. This legume is extremely rich in proteins due to its highly efficient symbiotic association with nitrogen-fixing strains [1, 2]. According to the FAO, the contribution of biological nitrogen fixation to agricultural industry exceeds nitrogen fertilizers by 2 times nitrogen fertilizers. Biological nitrogen is a factor in the formation of soil fertility and environmental protection, the only environmentally friendly way to supply plants with bound nitrogen [3]. The presence of a specific virulent active strain of rhizobia is significant in plant productivity and soil fertility, plant protection from stress, increasing plant resistance to adverse conditions and to diseases and pests. The maximum effect from the use of microbiological preparations can be obtained by careful selecting them for specific soil and climatic conditions.
The yield of alfalfa largely depends on the success of the formation of a plant-microbial symbiotic system with nodule bacteria (rhizobia) capable of fixing atmospheric nitrogen. According to modern research on symbiogenetics, the effectiveness of symbiotic systems is determined by the complementarity of the genomes interaction of a plant and its microsymbiont [4].

Many Russian and foreign scientists are engaged in studying plant microbial interactions of nodule bacteria and legumes. Studies conducted in different regions of the Russian Federation have noted a positive effect of pre-sowing inoculation of seeds with active strains of nodule bacteria [5]. The pre-sowing inoculation of seeds with complementary strains of nodule bacteria increased the yield of alfalfa green mass in the Northwestern region by 30–177 %, in the Central region by 45–102 % [6], in the conditions of the Lower Volga region – by 18 % [7], in the conditions of the Leningrad region allows plants to accumulate 18–20% more dry matter, on alfalfa 1–3 years of life – by 1.1-2.6 times [8], in the conditions of the foothill zone of the Russian Federation the inoculation increases the collection of dry matter by 18-20 % [9]. In the forest-steppe of the Central black soil region of the Russian Federation, it was shown that the inoculation of alfalfa with the 404b strain Sinorhizobium meliloti increased seed collection by 21–38 %, and also reduced the infestation of plants with mycoplasmosis by 5–8 % [10]. N N Lazarev, A M Starodubtseva found that the inoculation with preparations of nodule bacteria contributed to an increase in the yield of legume-cereal grass mixtures by 20 % [11]. In 2009–2012, in the conditions of the Primorsky Territory, we conducted studies and obtained positive results on the effect of defecate and inoculation of seeds with virulent active rhizobia strains on the yield of alfalfa of the Vega 87 variety [12].

Foreign researchers also note the positive role of seed inoculation by strains of nodule bacteria Sinorhizobium meliloti [13–18]. It is necessary to find out what species of S. meliloti bacteria are the best for the specific alfalfa cultivar [19]. As noted by Serbian researchers, the use of rhizobial inoculants with highly effective strains is needed in alfalfa production. They showed that shoot dry weight increased by 42-77 % in inoculated alfalfa, while the amount of fixed nitrogen increased by 35 %. Alfalfa inoculation with effective strains is an alternative approach to improving the long-term productivity of alfalfa [20]. In Saudi Arabia the field experiment showed that the inoculation of alfalfa with effective strains of alfalfa rhizobia resulted in increases of 3.6–12.1 % in the dry matter production and 6.8-27.6% of crude protein [21]. Using Sinorhizobium meliloti-based liquid inoculants for alfalfa and the application of the pre-inoculation technique can increase the quality of alfalfa crops and reduce cultivation cost [22].

The purpose of this work is to study the effect of seed inoculation with virulent active rhizobia strains on the yield and quality of alfalfa of the Nakhodka variety.

2. Materials and Methods
In 2018–2020, in the conditions of the south of Primorsky Territory, on the territory of the collection site of the FSBEI Primorskaya State Agricultural Academy (experiment scheme: 1. Without inoculation – control; 2. Strain 425a; 3. Strain 415; 4. Strain A-1; 5. Strain A-9), we laid small-scale field experiments to study the effect of various strains of nodule bacteria Sinorhizobium meliloti on the growth, development and productivity of alfalfa changeable. The strains of nodule bacteria Sinorhizobium meliloti were provided by the Ecology Laboratory of symbiotic and associative rhizobacteria of the All-Russian Research Institute of Agricultural Microbiology (St. Petersburg). The soil is meadow-brown bleached. The experimental studies, records and observations were carried out according to the Methodological Guidelines for conducting field experiments with forage crops [23]. The experimental data were processed by the method of analysis of variance [24].

3. Results and Discussion
Weather conditions during the years of research were generally favorable for the growth and development of alfalfa. The sum of the active temperatures of the growing seasons was: in 2018 – 2625°C, in 2019 – 2746°C, in 2020 – 2684°C with an average annual indicator of 2533°C. The
hydrothermal coefficient (GTC) in 2018 was 2.72 (excessively wet), in 2019 – 1.72 (wet) and in 2020 – 2.20 (excessively wet).

The studies conducted in the conditions of the collection site of the Primorskaya State Agricultural Academy have shown that the inoculation of alfalfa seeds of the Nakhodka variety with Synorhizobium meliloti strains increases the yield of green and dry alfalfa mass of the 1st-3rd years of life (Table 1).

**Table 1.** The effect of bacterial preparations on the yield of green and dry mass of alfalfa variable (2018-2020).

| Variants | Cuttings | 2018 | 2019 | 2020 |
|-----------|----------|------|------|------|
|           |          | GM\(^a\) | DM\(^b\) | GM | DM | GM | DM |
| 1. No inoculation | 1 | 4.81 | 1.19 | 23.20 | 5.42 | 17.33 | 4.55 |
| | 2 | - | - | 19.17 | 4.51 | 9.64 | 2.64 |
| | Per 2 cuttings | - | - | 42.37 | 9.93 | 26.97 | 7.19 |
| 2. 425a | 1 | 5.75 | 1.52 | 28.03 | 6.86 | 18.63 | 5.25 |
| | 2 | - | - | 21.83 | 5.74 | 10.08 | 2.92 |
| | Per 2 cuttings | - | - | 49.86 | 12.60 | 28.71 | 8.17 |
| 4. 415 | 1 | 5.34 | 1.40 | 27.70 | 6.58 | 18.20 | 5.00 |
| | 2 | - | - | 20.80 | 4.93 | 10.00 | 2.77 |
| | Per 2 cuttings | - | - | 48.50 | 11.51 | 28.20 | 7.77 |
| 4. A-1 | 1 | 4.96 | 1.30 | 26.75 | 6.74 | 18.43 | 5.12 |
| | 2 | - | - | 20.33 | 5.22 | 9.83 | 2.79 |
| | Per 2 cuttings | - | - | 47.08 | 11.96 | 28.26 | 7.91 |
| 5. A-9 | 1 | 5.45 | 1.42 | 25.64 | 6.27 | 18.10 | 5.01 |
| | 2 | - | - | 19.97 | 5.09 | 10.03 | 2.82 |
| | Per 2 cuttings | - | - | 45.61 | 11.36 | 28.13 | 7.83 |

LSD\(_{0.05}\) 0.38 1.50 0.35

\(^a\) green mass, t / ha.
\(^b\) dry mass, t / ha.
\(^c\) no data available.

We have established an increase in the yield of the green mass of alfalfa variable when inoculating seeds with virulent active strains of rhizobia. In total, over the three years of alfalfa life, the yield of green mass in the control variant was 74.15 t / ha, in the experimental variants it varied from 79.19 to 84.32 t / ha or by 6.8–13.7 %. The growth in the green mass of alfalfa in the first-third years of life was obtained in the variant with inoculation with the main production strain 425a (13.7 %), and the smallest – in the variant with inoculation with the virulent active strain of rhizobia A9 [25].

A similar pattern was established for the output of dry matter. The total yield of dry matter for the three years of alfalfa life in the experimental variants varied from 18.31 t/ha in the control variant to 20.61–22.29 t/ha in the experimental variants, i.e. it increased by 12.6–21.7%. The maximum yield of dry matter was also noted in the variant with inoculation with the main production strain 425a.

The research results indicate a rise in the quality and nutritional value of alfalfa plant mass when using biological preparations (Table 2).
Analyzing the data in table 2, we found a sufficiently high content of feed units in 1 kg of dry matter of the plant mass of alfalfa of the Nakhodka variety with the highest value in the variant with inoculation with the main production strain 425a. The explored factor has a positive effect on the collecting of feed units, digestible protein and feed protein units from 1 ha. The collection of feed units per hectare in the experimental versions increases by 1.1–1.3 times, the collection of digestible protein – by 1.2–1.4 times. The maximum quantity of feed units and digestible protein per hectare was obtained in the variant with seed inoculation with strain 425a.

The availability of a feed unit with digestible protein is high for all variants and varies from 172.09 in the control to 182.53–190.27 g, i.e. it raises by 10.44–18.18 g or by 6.1–10.6 %.

Thus, the results of the three-year studies have shown that the inoculation of seeds with virulent active strains of rhizobia enhances the yield and quality of the plant mass of alfalfa of the Nakhodka variety. The total yield of alfalfa green mass for three years of life in the control variant was 74.15 t / ha, and in the experimental variants it was boosted to 79.19–84.32 t / ha or by 6.8–13.7 %. The largest raise in the green mass of alfalfa of the 1st-3rd years of life was received in the variant with inoculation with the main production strain 425a (13.7%), and the smallest-in the variant with inoculation with the virulent active strain of rhizobia A9. The total yield of dry matter for three years of life of alfalfa in the experimental variants differed from 18.31/t/ha in the control variant to 20.61–22.29 t / ha in the experimental conditions, i.e. increased by 12.6–21.7 %. The maximum yield of dry matter, as well as green mass, was noted in the case with the inoculation with the main production strain 425a. The inoculation of alfalfa seeds *Syrnorhizobium melloti* elevated the feed value of alfalfa plant mass, increasing the content of feed units in 1 kg of dry matter of plant mass, the collection of feed units and digestible protein from 1 hectare, as well as the provision of a feed unit with digestible protein.

### 4. Conclusion

As a result of the three-year studies, we have established a growth in the yield and feed advantages of alfalfa variable in the variants with the inoculation of seeds with virulent active strains of rhizobia. The total yield augmentations of green mass in the experimental variants over the three years of alfalfa life were 6.8–13.7 % compared to the control ones. The positive effect of the inoculation with virulent active rhizobia strains on the total collection of dry matter was expressed in its increase in experimental conditions by 12.6–21.7 %. The highest yield of green mass, as well as dry matter was obtained in the variant with the inoculation of alfalfa seeds with the main production strain 425. The researched factor has a positive effect on the collection of feed units, digestible protein and feed protein units from 1 ha. The collection of feed units per hectare in the experimental versions increases by 1.1–1.3 times, the collection of digestible protein – by 1.2–1.4 times. The maximum substance of feed units and digestible protein per hectare was observed in the version with seed inoculation with strain 425a. The provision of a feed unit with digestible protein increases by 10.44–18.18 g or by 6.1–10.6 %. The use of biological preparations for the pre-sowing
treatment of alfalfa seeds is agronomically and economically justified, it increases plant productivity, the ecological resistance of plants to stressful conditions and, in general, the ecological stability of the agricultural landscape.

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