Variety and microbial nitrogen-fixing preparations in the yield formation of variegated alfalfa

E P Ivanova¹ and O M Skalozub²

¹FSBSI “Sakhalin Research Institute of Agriculture”, 22, Gorky Alley, Yuzhno-Sakhalinsk, 693022, Russia
²FSBSI "FSC of Agricultural Biotechnology of the Far East named after A. K. Chaika", 30 Volozhenin Street, Timiryazevsky stl., Ussuriysk, 692539, Russia

E-mail: kirena2010@yandexl.ru

Abstract. As a result of studies of two variegated alfalfa fodder varieties selected by the FWRC FPA (Previously All-Russian Williams Fodder Research Institute) and the local population variegated alfalfa variety, it was found that Vega 87 formed a lower yield of green mass in the first year of growth. The yield of the Pastbischnaya 88 variety of the local population was 16% higher; the superiority of both varieties over the variety Vega 87 was by 50 and 60% in the second year, and 13-15% in the third year of growth. Variegated alfalfa provided the following green mass harvest over the three years: Vega 87 - 31.55 t/ha (the lowest harvest), Pastbischnaya 88 - 39.55 t/ha, local population variegated alfalfa - 40.8 t/ha. The positive effect of inoculation with virulent active strains of the nodule bacteria on the total (per five cuttings) harvest of green mass was expressed in its increase in the experimental variants by 5.04-10.17 t/ha (6.8-13.7%) compared to the control variant. With the aim of adaptive intensification of fodder production, the creation of stable agrophytocenoses with variegated alfalfa with inoculation of seeds with virulent active strains of nodule bacteria is shown (the main production strain 425a showed best results).

1. Introduction

Alfalfa is one of the most important, most versatile crops in the world due to its superior feed quality. It is used as a fodder crop for various types of feed, biofuel, food and pharmacological purposes, etc. A third of the honey obtained in the USA is provided by alfalfa crops. The plant is characterized by high yields in a wide range of environments and high adaptability to various climatic conditions. The record harvest of alfalfa in the United States was 22 t/ha without irrigation and 54 t/ha of dry matter with irrigation [1-2].

Variegated alfalfa (Medicago sativa L. nothosubsp.varia (Martyn) Arcang) is a high-yielding crop resistant to unfavorable cultivation conditions, which is important for risky farming zones in Russia, as well as for the restoration of degraded soils (which is of high importance in the Far East).

To harmonize human relations with nature, ecology, and economics, it is necessary that there is a variety at the head of the biologization of agriculture, which is now the factor without which it is impossible to implement scientific and technological progress in agriculture and achieve effective development of production [3]. Variety is the most important factor in the formation of adaptive forage agroecosystems; it predetermines the sustainable productivity of phytocenoses, provides resource and energy efficiency, environmentally friendly production of feed while maintaining optimal environmental parameters [4]. Developing new varieties to improve natural hayfields and pastures is...
one of the main tasks of alfalfa breeding. Currently, a series of high-yielding varieties of alfalfa (Medicago sativa L.) and variegated alfalfa (M. varia Mart) of various types of use in different climatic conditions has been created [5-6]. As numerous studies have confirmed, there are significant differences in yield and quality between different varieties of alfalfa. This is the evidence that the genetic variability in varieties can be a good indicator for selecting the best varieties of alfalfa for future breeding programs to increase the yield and quality [7].

According to academician P.L. Goncharov, it is possible to increase the yield by 25% at the expense of variety, and because of the technology based on adapted varieties and high-quality seeds of local production - by another 45% (that is, yield and gross crop production can be doubled). Since the entire agricultural territory of the Far East is in extreme soil and climatic conditions with a shortage of heat and a short growing season, only varieties that combine high potential productivity with resistance to these stresses are able to ensure an increase in the size and quality of the crop, and reduce the cost of irreplaceable resources. Choosing the right varieties for the monsoon climate is paramount.

The yield of alfalfa largely depends on the success of the formation of a plant-microbial symbiotic system with nodule bacteria (rhizobia), which is capable of fixing atmospheric nitrogen [8]. The creation of microbiological technologies for ecologically acceptable agriculture through the development of microbial preparations that expand the adaptive potential of plants by optimizing the microbiome is topical [9]. Scientists and practitioners of the modern agro-industrial complex show great interest in the latest developments in agricultural technologies - biological products and growth stimulants.

Most plant species, especially legumes, entering into symbiosis with nodule bacteria, acquire new properties: fixing air nitrogen, increasing the absorbency of the root system, synthesizing biologically active substances and antibiotics; as a result, plants grow on low-fertile soil, resist drought and excessive moisture, and increase their resistance to diseases and pests [10]. Biological nitrogen fixation in plants is an essential mechanism for sustainable agricultural production and healthy ecosystem functioning. It plays an important role in reducing the use of synthetic nitrogen fertilizers in agriculture, increasing the nutrient content of plants, plant health (activating plant immunity and reducing problems caused by abiotic stress) and soils [11-12].

It is necessary to inoculate seeds before sowing to increase the fixation of atmospheric nitrogen [13]. In the forest-steppe of the Central Black Earth Region of Russia, inoculation of Voronezhskaya 6 variety with Pseudomonas sp., and inoculation of the other two varieties with Sinorhizobium meliloti strain 404b increased seed collection by 21-38% (over three years of use on average) and decreased the prevalence of plants affected mycoplasmosis up to 5-8%. With a moisture deficit during the growing season, pre-sowing inoculation of the alfalfa variety Voronezhskaya 6 with nodule bacteria increased seed yield by 7-12% [14].

The purpose of the study is to establish the significance of variety and inoculation of seeds with virulent active strains of rhizobia on the yield of the green mass of variegated alfalfa.

2. Materials and Methods
In the growing seasons of 2018-2020, in the fields of the selection crop rotation of the Fodder Production Department of the FSBSI "FSC of Agricultural Biotechnology of the Far East named after AK Chaika" and in the collection nursery of the Primorskaya State Academy of Agriculture, field experiments to assess the role of the variety (set up on June 18, 2018) and seed inoculation with virulent active strains of rhizobia (set up on July 4, 2018) on the productivity of variegated alfalfa according to the approved methods took place. Experimental design 1: 1. Pastbishchnaya 88 (FWRC FPA); Vega 87 (FWRC FPA); unnamed (local population). Experimental design 2: 1. No inoculation– control; 2 Strain 425а; 3. Strain 415; 4. Strain А-1; 5. Strain А-9.

3. Results
Meteorological conditions of the growing seasons in 2018-2020 were characterized by significant differences in the distribution of precipitation and temperature conditions. According to the
agrometeorological station "Timiryazevsky", the growing season of 2018 began on April 24, and in 2019 and 2020 – on April 16. The temperature regime was favorable for the growth and development of alfalfa. The sum of positive temperatures above 10 °C for the growing season in 2018 was 2625 °C, 2746 °C in 2019, and 2684 °C in 2020. The amount of precipitation for the studied period in 2018 was 714.1 mm, in 2019 - 472 and 2020 - 590.5 mm. Hydrothermal coefficient of the growing seasons in 2018-2020 equal 2.72; 1.72 and 2.2, respectively. The air temperature from April to September during the years of research was higher (by 0.3-2.7 °C), or at the same level with the average long-term values. The distribution of precipitation was uneven. The largest amount of precipitation occurred in August 2018 and 2019 (2.9 and 1.9 times more) and in June of 2020 (2.3 times more than the long-term average values).

Plant height is one of the important indicators of green mass yield (table 1).

| Hybrid                     | Pre-cutting plant height, cm |              |              |              | Green mass yield, t/ha |              |              | total |
|----------------------------|------------------------------|--------------|--------------|--------------|------------------------|--------------|--------------|-------|
|                            | Cut I | Cut II | Cut III | Cut I | Cut II | Cut III | total |
| Pastbischnaya 88 (FWRC FPA) | 55.2  | -      | -      | 5.1   | -      | -      | 5.1   |
| Vega 87 (FWRC FPA) (control)| 53.8  | -      | -      | 4.4   | -      | -      | 4.4   |
| Unnamed (local population)  | 61.6  | -      | -      | 5.1   | -      | -      | 5.1   |
| LSD05                      | -     | -      | -      | 0.6   | -      | -      | 0.6   |

Experiments carried out in the fields of selection crop rotation of the Fodder Production Department of the FSBSI "FSC of Agricultural Biotechnology of the Far East named after AK Chaika" showed that in the first year of alfalfa life, the height of plants before harvesting for green mass, depending on the variety, ranged from 53.8 to 61.6 cm. As a result of studies with two varieties selected by the FWRC FPA and the local population of alfalfa, it was found that the lower yield of green mass in the first year of growth was formed by the Vega 87 variety - 4.4 t/ha, 16% higher than the yield of Pastbischnaya 88 and the local population variety.

In the second and third year of growth, Vega 87 plants grew slower than the plants of Pastbischnaya 88 and unnamed varieties (local population). By the time of the first cutting in 2019, the Vega 87 plant height was 5.7 cm lower than the Pastbischnaya 88, by 6 cm at the second cutting and by 3.4 cm at the third cutting. The height of alfalfa plants in 2019 in the studied varieties varied depending on the cutting: before the I cutting - from 36.2 to 52.5 cm; before the II cutting - from 43.3 to 49.3 cm; before the III cutting - from 46.3 to 51 cm. On average, over three cuts, the height of the Vega 87 plants was 5 cm lower than of the Pastbischnaya 88 plants. The average height of alfalfa plants on the local population over three cuts was 50.6 cm and exceeded the height of the varieties Vega 87 and Pastbischnaya 88. From the sowing of the second year, the highest green mass yield of alfalfa for three cuttings in total was obtained from alfalfa of the local population - 15.9 t/ha, the variety Pastbischnaya 88 was slightly inferior (by 0.85 t/ha). The lowest yield of alfalfa in total of the three cuts was obtained from the crops of the Vega 87 variety and was 9.95 t/ha. Thus, the total yield
of Vega 87 green mass for three cuttings was 9.95 t/ha, which is 1.5 and 1.6 times higher than Pastbischnaya 88 and alfalfa of the local population.

The plant height in the third year of growth (vegetation period of 2020) of the studied varieties varied depending on the cutting: height before the first cutting - 60.1-77.6 cm; before the II cutting – 46.6-60.8 cm; before the III cutting - 38.7- 50.4 cm. On average, the plant height over three cuttings was the smallest in the Vega 87 variety and corresponded to 48.5 cm, in the Pastbischnaya 88 variety - 56.3 cm; the highest was in the local population alfalfa - 62.9 cm. Sowings of alfalfa in the third year of life formed the highest yield of green mass in three years - 17.2-19.8 t/ha. The maximum yield for three cuttings was obtained from the local population alfalfa.

Thus, alfalfa provided the following collection of green mass over three years in the conditions of Primorsky Krai: variety Vega 87 - 31.55 t/ha (the smallest), variety Pastbischnaya 88 - 39.55 t/ha, and alfalfa of the local population - 40.8 t/ha. The largest amount of plant mass in 2018-2020 was formed in the third year (by varieties from 48.5 to 54.5% of the total harvest for three years). It is advisable to use alfalfa of the local population to develop new varieties of alfalfa adapted for the monsoon climate of the Far Eastern zone.

The research carried out in the conditions of the collection nursery of the Primorskaya State Academy of Agriculture in the 2018-2020 showed that inoculation of alfalfa seeds of the Nakhodka variety with virulent active strains of Synorhizobium meliloty increases the green mass yield, as well as the height of alfalfa plants in the first and third years of growth (table 2).

| Variants | Cuttings | 2018 | 2019 | 2020 |
|----------|----------|------|------|------|
|          |          | h, cm | GM, t/ha | h, cm | GM, t/ha | h, cm | GM, t/ha |
| 1. No inoculation | 1 | 51.8 | 4.81 | 91.2 | 23.20 | 96.4 | 17.33 |
| Per 2 cuttings | 2 | - | - | 101.2 | 19.17 | 68.4 | 9.64 |
| 2. 425a | 1 | 58.1 | 5.75 | 96.7 | 28.03 | 100.6 | 18.63 |
| Per 2 cuttings | 2 | - | - | 110.8 | 21.83 | 72.5 | 10.08 |
| 3. 415 | 1 | 57.0 | 5.34 | 95.5 | 27.70 | 99.6 | 18.20 |
| Per 2 cuttings | 2 | - | - | 105.0 | 20.80 | 68.5 | 10.00 |
| 4. A-1 | 1 | 56.0 | 4.96 | 94.7 | 26.75 | 101.8 | 18.43 |
| Per 2 cuttings | 2 | - | - | 104.8 | 20.33 | 70.5 | 9.83 |
| 5. A-9 | 1 | 53.8 | 5.45 | 92.9 | 25.64 | 101.1 | 18.10 |
| Per 2 cuttings | 2 | - | - | 104.5 | 19.97 | 69.8 | 10.03 |

Note: h – plant height, cm; GM – green mass, t/ha.

According to the table 2 data, the increase in the green mass yield in 2018 amounted to 0.15-0.94 t/ha or 3.1-19.5% compared to the control (without inoculation) with the highest value in the variant with inoculation with strain 425a.

The increase in the green mass yield in the second year (2019) in the first cutting in the experimental variants varied from 2.44 to 4.83 t/ha (10.5-20.8%). The smallest increase was in the variant with inoculation with the virulent active strain of rhizobia A9, and the largest increase in the variant with inoculation with the 425 strain. The second cutting yield slightly decreased and varied
from 19.17 t/ha in the control to 19.97-21.83 t/ha in the experimental variants. The increase of the second cutting green mass was 0.8-2.66 t/ha (4.2-13.9%) compared to the control. As well as the first cutting, the maximum increase was in the variant inoculated with the 425 strain. The variant inoculated with the virulent active strain of rhizobia A9 showed the smallest increase (statistically insignificant). The total increase in the green mass yield in the experimental variants for two cuttings was 3.24-7.49 t/ha or 7.6-17.7% compared with to control [15]. The greatest increase was obtained in the variant inoculated with the main industrial strain 425a; the increments for other variants were lower, but exceeded the control.

The green mass yield increases in the first cutting of the third year (in 2020) in the experimental variants amounted to 0.77-1.30 t/ha or to 4.4-7.5%. The least increase was in the variant inoculated with the virulent active strain A9, and the largest was in the variant inoculated with 425. The amount of alfalfa plant mass formed in the second cutting was 1.8-1.9 times lower than the first cutting. Compared to the control, the green mass increase in the second cutting was 0.19-0.44 t/ha, or 2.0-4.6%. The maximum increase, as in the first cut, was in the variant inoculated with 425a, which was 4.6%. The smallest increase was in the variant inoculated with a virulent active strain of rhizobia A1. The total increase in the green mass yield in the experimental variants for two cuttings was 1.16-1.74 t/ha or 4.6-6.5% compared to the control. The largest increase was obtained in the variant inoculated with the main industrial strain 425a (6.5%).

An increase in plant height and green mass yield of alfalfa was established, which is variable when seeds are inoculated with virulent active rhizobia strains. In total, the yield for five cuttings over three years of growth in the control variant was 74.15 t/ha, in the experimental variants it varied from 79.19 to 84.32 t/ha or 6.8-13.7. The largest increase in the green mass of alfalfa from the first to the third years of growth was obtained in the variant inoculated with the main production strain 425a (13.7%), and the smallest increase was in the variant inoculated with the virulent active strain of rhizobia A9.

4. Conclusion

As a result of studies of two varieties of the FWRC FPA and the local population of alfalfa, it was found that the alfalfa variety Vega 8 formed a lower green mass yield in the first year, the yield of the variety Pastbischnaya 88 and the local population was 16% higher. The superiority of these varieties over Vega 87 was by 50 and 60% in the second year, and 13-15% in the third year of growth. Alfalfa provided the following collection of green mass over the three years: Vega 87 - 31.55 t/ha (the lowest harvest), Pastbischnaya 88 - 39.55 t/ha, and the local population variety - 40.8 t/ha. The positive effect of inoculation with virulent active strains of rhizobia on the total harvesting of green mass (for five cuttings) was expressed in its increase in experimental variants by 5.04-10.17 t/ha (6.8-13.7%) compared to the control variant. For the purpose of adaptive intensification of feed production, it is shown to create stable agrophytocenoses with variegated alfalfa and to inoculate the seeds with virulent active rhizobia strains (preferably 425a).

References

[1] Importance of alfalfa. USDA National Agricultural Statistics Service Retrieved from: https://www.nass.usda.gov/
[2] Moreira A and Fageria N K 2010 Liming influence on soil chemical properties, nutritional status and yield of alfalfa grown in acid soil R. Bas. Cl. Solo 34 1231-1239
[3] Amelin A V, Chekalin E I and Zaikin V V 2020 Problems of modern agricultural production and ways to solve them with the help of selection. Materials of the V All-Russian (National) Scientific Conference “The agrarian science in sustainable development of rural areas” Novosibirsk 3-6
[4] Juchenko A A 1990 Adaptive Plant production (Kishinev: Shtiintsa Publ) 432
[5] Kosolapov V M and eth. 2015 The basic species and varieties of fodder crops: Results of scientific activity of the Central breeding Center (FSBSI All-Russian Williams Fodder Research Institute. RAS. Moscow: Science Publ) 164
[6] Stepanova G V and Zolotarev V N 2015 The biotechnology of conjugated breeding of alfalfa for increased adaptive capacity *Adaptive Fodder Production* no 1 28-39

[7] Mussie S A 2020 The effect of cultivar difference in the yield and quality of alfalfa. *Materials of the All-Russian Scientific Conference “Plant production and meadow growing”* 111-115

[8] Rumyanceva M L, Vladimirova M E, Muntyan V S, Stepanova G V and eth. 2019 Highly effective root nodule inoculants of alfalfa (*Medicago varia* L.): molecular-genetic analysis and practical usage in cultivar selection. *Agricultural Biology* 54(6)1306-1323

[9] Shitikova A V 2020 Prospects for the development of organic production of crop production. *Materials of the All-Russian Scientific Conference “Plant production and meadow growing”* 804-808

[10] Kosolapov V M and Stepanova G V 2018 Renewable energy sources of plant-microbial interactions. *Problems of intensification of animal husbandry taking into account environmental protection and production of alternative energy sources, including biogas. Warsaw* 100-102

[11] Mahmud K, Missaou A, Makaju S and Ibrahim R 2020 Current progress in nitrogen fixing plants and microbiome research. *Plants* 9(1) 97

[12] Velasco-Jimenez A, Castellanos-Hernández O, Acevedo-Hernández G, Aarland R C and Rodríguez-Sahagún A 2020 Rhizospheric bacteria with potential benefits in agriculture. *Terra Latinoamericana* 38(2) 343-355

[13] Lazarev N N and Starodubceva A M 2018 Effect of inoculation on the productivity of various varieties of alfalfa and meadow clover. *Forage production* 1 25-28

[14] Stepanova G V 2015 Influence of pre-sowing seed inoculation with bacterial preparations on the adaptive capacity of alfalfa. *Education, science, and production* 3(12) 87-91

[15] Ivanova E P and Kurbonov F Sh 2020 Influence of bacterial preparations on the yield of variegated alfalfa in the conditions of the collection site of the Primorskaya Academy of Agriculture. *Materials of the Regional Scientific and Practical Conference “Actual problems of agricultural science and ways to solve them at the present stage”* 27-31