Hop-like alfalfa as an object of introduction into culture

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Abstract. It was found that the most promising strains of nodule bacteria XM1 and 412b are the most promising for inoculation of the Mira variety, the former increased the collection of dry matter by 96%, the latter by 81%, seeds by 115 and 73%. Strain XM1 was isolated from the nodules of the same wild population of alfalfa in the hop-like Moscow region from which the variety Mira was formed by the method of chemical mutagenesis. Strains 412b and XM 1 can be recommended for pre-sowing inoculation of the Mira variety when cultivated for feed and seeds, and the XM6 strain - when cultivated for seeds. The latter increases the seed yield by 84%, and the green mass by only 31%. Strains XM2, XM5 and XM6 shift the metabolism of the alfalfa-rhizobial system towards an increase in the number of seeds. The proportion of seeds increases to 24-31% of the mass of the entire plant, in other symbiotic systems this figure is 19-22%. A promising breeding number of hop alfalfa VIC 26 has been created, which is 58% more productive than the Mira variety when grown in the traditional way and by 65% when inoculated with the A2 strain.

Keywords: alfalfa hop-like (black medic), strain, nodule bacteria, symbiosis efficiency, yield

1. Introduction

Hop-like alfalfa (Medicago lupulina L.) - the most widespread polymorphic species of the genus Medicago, belongs to the subgenus Lupularia Grossh., includes varieties: vulgaris Koch u Willdenowii Bönn., having a one-year life cycle, represented by monocarp plants, as well as a variety perennans Grossh., which includes biennial and perennial monocarpic and bicarpic forms of winter and intermediate type. Hop-like alfalfa is an obligate self-pollinator. In natural conditions, there are diploid and tetraploid forms (2n= 16, 32) [1; 2]. Wild hop alfalfa is widespread throughout Europe (except for the Arctic), Siberia, the Far East, Central and Asia Minor, and North Africa. Secondary distribution centers are located in North America and Australia [1; 3].

Hop-like alfalfa grows in various environmental conditions. It grows on slopes, embankments along railway tracks, highways and field roads, on pebbles, in river valleys, meadows and pastures. It is well eaten by all kinds of animals. It is characterized by good regrowth after mowing and grazing, resistance to trampling, long growing season, increases soil fertility, is an excellent ground cover plant. Hay yield can reach 4 t / ha and more, seeds 200-450 kg / ha. Hop-like alfalfa is characterized by cold resistance and frost resistance in spring and autumn [1; 4]. A small longevity in pastures is compensated by good self-seeding, which constantly renews the herbage [4; 5].

Hop-like alfalfa surpasses many leguminous grasses in terms of fodder merits. The nutrient content depends on the variety, age, growing conditions. In the research of the Federal Research Center "VIK
im. V.R. Williams "it was found that the yield of annual spring forms was 0.5-4.4 t / ha dry matter, 170-1100 kg / ha of seeds, protein content 19.7-20.1%. Yield of biennial specimens of the variety *perennans* reached 4.4-5.6 t / ha dry matter, 800-1800 kg / ha of seeds, protein content - 22.2-24.8% [6]. It should be noted that a valuable fodder property of hop alfalfa is the lower intensity of accumulation of fungal metabolites (mycotoxins) compared to other types of alfalfa and clovers [7].

Hop-like alfalfa is valuable when used as a green fertilizer. According to P.F. Medvedev and A.I. Smetannikova (1981), up to 7 t / ha of dry matter of aboveground and root biomass is formed per season, which contains 250 kg of nitrogen, including 70-75% symbiotic, 50 kg of phosphorus and 230 kg of potassium. This accumulation of nutrients is equivalent to the application of 740 kg / ha of ammonium nitrate, 130 kg of double superphosphate and 380 kg of potassium chloride. A significant amount of trace elements accumulates in the roots: 87-90 mg / kg manganese, 47-49 mg / kg zinc, 8-10 mg / kg copper, 5-7 mg / kg magnesium, 0.1-0.2 mg / kg iodine [1]. According to the Federal Research Center "VIK im. V.R. Williams", the use of hop-like alfalfa variety Mira as a green manure, when plowing the grass stand in the budding phase, provided the accumulation in the soil of 10.35 t / ha of dry matter, 373 kg / ha of nitrogen, 39 kg / ha of phosphorus, 275 kg / ha of potassium and 148 kg / ha calcium [8]. The yield of winter wheat variety Moskovskaya 39, sown in the traditional way, amounted to 3.98 t / ha of grain. When sowing according to the bed turnover of the hop variety Mira, the yield increased by 0.45-0.92 t / ha, and according to the turnover of the bed of alfalfa inoculated with the endomycorrhizal fungus preparation *Glomus intraradices*, –by 0.77-2.08 t / ha [9].

It has been established that the yield and environment-forming ability of hop alfalfa increases when seeds are inoculated with preparations of soil-fertilizing microorganisms. For example, pre-sowing inoculation with the preparation of nodule bacteria A1 increased the collection of dry matter of the hop-like alfalfa variety *perennans* on average for three years by 22-55%, seeds - by 10-50%, and varieties *vulgaris* – by 20-25 and 110-150%. The high responsiveness of the variety Mira to pre-sowing inoculation with the strain of rhizobia A1 was revealed. On average, over three years of research, the increase in the productivity of aboveground biomass reached 67%, and that of seeds - 50%, compared with the option without inoculation. The accumulation of total nitrogen in the aboveground biomass was 150–180 kg / ha, and the share of symbiotic nitrogen was 75–80% [10].

In conclusion, I consider it necessary to cite the result of the study by A. K. Simonsen and J.R. Stinchcombe (2014), who write: “The results of diallelic crosses showed that the heritability of the Hop trait (Host preference) in hop alfalfa (*Medicago lupulina*) is high enough for selection to enhance this trait in plant populations” [11].

Consequently, there is a preferable inoculation of plants with certain genotypes of rhizobia, manifested in mixed infection, that is, plants sort of reject rhizobia that do not fix atmospheric nitrogen. Therefore, it is quite possible to find the initial genotypes of hop alfalfa, on the basis of which it is possible to create varieties capable of providing high yields that are stable over the years due to effective symbiosis with complementary rhizobia genotypes with a high nitrogen-fixing ability, which alfalfa plants select from a mixture of active and inactive races (strains).

The purpose of our research is to evaluate the Mira variety and a new breeding material of hop alfalfa according to the symbiotic properties.

2. Methods of the research

As a macrosymbiont, we used a variety of alfalfa khmelevidny Mira, created at the V.N. V.R. Williams, as well as breeding numbers of hop alfalfa related to the variety *perennans*: VIC 8, VIC 9, VIC 40, VIC 51, VIC 61, created using mass positive selection from wild populations of various origins; VIC 26 was created using chemical mutagenesis; VIK95 - selection for resistance to trampling from samples VIC 8, VIK 9, varieties of the World; VIC 97 - selection from VIC 26 for increased winter hardiness; MYTH - selection against an infectious background of genotypes with increased resistance to root rot from the Mira variety. Microsymbionts: in the first experiment, we used strains of rhizobia from VNIIMS 415b, 412b, 408b, as well as strains XM1, XM2, XM5 and XM6, isolated at the Federal Research Center "VIK im. V.R. Williams "from wild-growing hop alfalfa nodules from different regions of the Russian
Federation. Strain XM1 was isolated from wild alfalfa nodules in the Moscow region. In the second experiment, the A2 strain created at the All-Russian Research Institute of Agriculture was used.

The assessment of the breeding material was carried out in the field on poorly cultivated soil. The humus content was 1.60-1.93%, pH 4.33-4.95, total nitrogen 0.131-0.142%, mobile phosphorus 14.8-18.78, potassium 8.4-10.17 mg / 100 g of soil. Sowing continuous, row, plot area 5m², repetition is 3 times. Control is a variant without inoculation. Sowing was carried out in May 2015 and 2018.

3. Results of the research

In a sowing experiment in 2015, we studied the responsiveness of the hop-like alfalfa variety Mira to inoculation with rhizobia strains of various origins. In the year of sowing, a total of 330 g / m² was obtained for two cuts dry matter of alfalfa in the control (without inoculation), and in variants with inoculation, the yield of symbiotic systems was 252-648 g / m² dry matter. Five of the seven strains significantly increased the collection of dry matter by 31-96%. The most promising strains for the inoculation of the variety Mira were strains XM1 and 412b, the former increased the collection of dry matter by 96%, the latter by 81%. Strain XM1 was isolated from the nodules of the same wild population of alfalfa in the hop-like Moscow region from which the variety Mira was formed by the method of chemical mutagenesis. Therefore, they are the most genetically complementary and provide the maximum effectiveness of symbiosis. In the second year of herbage life, the collection of seeds of the variety Mira in the control was 67 g / m², and inoculation with active strains of rhizobia increased the yield to 69-144 g / m². As before, the most complementary were strains XM 1 and 412b, inoculation with these strains increased the collection of seeds by 115 and 73%, bringing it to 144 and 116 g / m².

Inoculation with strains XM 2 and XM 6 increased the productivity of variety-rhizobial systems to 116 and 123 g / m², the efficiency of symbiosis reached 73 and 84%. Strains 412b and XM 1 can be recommended for pre-sowing inoculation of the Mira variety when cultivated for feed and seeds, and strain XM 6 - for cultivation for seeds. The efficiency of symbiosis with the latter strain when cultivated for feed was only 31%. In addition, it should be noted that strains XM2, XM5, and XM6 increase the adaptive ability of the alfalfa variety Mira, shifting the metabolism of the alfalfa-rhizobial system towards an increase in the number of seeds, which contribute to the expansion of the range of this population. The proportion of seeds in plants inoculated with the aforementioned strains increases to 24-31% of the total plant weight, while in other symbiotic systems this figure is 19-22%.

In the 2018 sowing experiment, new breeding samples of alfalfa of the hop perennans variety, created recently, were tested. The Mira variety was used as a standard for comparing the yield of a new breeding material. In the year of sowing, all new breeding material turned out to be significantly more productive than the standard variety of the World. In the variant without inoculation, the yield of the breeding material was 13-58% higher than the variety Mira. The collection of green mass was 2.80-3.93 kg / m², Mira varieties 2.48 kg / m², and in the variant with inoculation with active strain A2 - 3.25-4.89 kg / m², Mira varieties - only 2.96 kg / m², which is 10-65% higher compared to the Mira variety. The most promising for further use can be considered selection numbers VIK 26, VIK 40 and VIK 51, which are more productive than the Mira variety with the traditional cultivation method by 29-58% and by 31-65% when inoculated with the A2 strain.

In the second year of life of alfalfa herbage in the variant with pre-sowing inoculation, 15-78% of the original number of plants remained, in the variant without inoculation much less plants overwintered (from 0 to 35%). Winter hardiness of the Mira variety was 25% in the control variant and 70% in the experimental one. Without inoculation, the variety Nordol, numbers VIK 51 and VIK 26, significantly yielded to the standard in terms of winter hardiness. Inoculation with the A2 strain increased the winter hardiness of these breeding numbers to 55-65%.

In the second year of life, in the variant with inoculation in terms of the yield of the aboveground biomass in the phase of full seed ripening, only the number VIC 26 significantly surpassed the variety Mira by 29%. The same number turned out to be 31% higher than the standard in terms of seeds, it provided 103.7 g / m² seeds, variety Mira - only 79.3 g / m².
4. Conclusion

Inoculation of alfalfa of the hop-like variety Mira with strains of nodule bacteria 412b and XM1 increases the collection of dry matter in the year of sowing by 81 and 96%, seeds in the second year of life - by 73 and 115%. Inoculation with strains XM 2, XM 5 and XM 6 increases the proportion of seeds to 24-31% of the total plant weight.

A promising breeding number of hop alfalfa VIC 26 has been created, which is 58% more productive than the Mira variety when grown in the traditional way and by 65% when inoculated with the A2 strain.

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