

Study of the agrobiodiversity of the Lotus corniculatus L. grassland under mountain conditions after the application of organic fertilizers

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Abstract. In a research experiment, carried out in 2020-2023 in the Research Institute of Mountain Stockbreeding and Agriculture-Troyan, on the grassland of *Lotus corniculatus* L., variety "Targovishte 1", the influence of fertilization with the organic fertilizers Blago 5 in doses of 300 and 600 ml/da (varieties 2 and 3) and Fertileader Axis in doses of 500 and 1000 ml/da (varieties 4 and 5) was tested in comparison with an unfertilized control (variety 1). Biodiversity of the untreated crop and agrobiodiversity by botanical groups and weed species composition were studied. The ecological effect of organic fertilization and its impact on the floristic composition of the grassland were determined. The percentage and species contribution of the main botanical groups (grasses, legumes, motley grasses) in the *Lotus corniculatus* L. grassland changed positively under the influence of fertilization, and the group of motley grasses including plants from different botanical families. There was an increased presence of the shade grass species *Lotus corniculatus* L. and a reduced number and percentage of representatives of motley grasses in the formed above-ground mass after treatment with the two biofertilizers. Fertileader Axis biofertilizer at a dose of 1000 ml/da proved to be the most effective, stimulating the development of *Lotus corniculatus* L. in the stand by 82.6% and reducing the number of weedy species (13.6%). The resulting higher relative proportion of *Lotus corniculatus* L. suggests better quality indicators of grass biomass. The results obtained define the applied biofertilizers as an effective agrotechnical practice, leading to an increase in the presence of economically important grass species and a decrease in the proportion of low-productive variable grasses.

Key words: *Lotus corniculatus* L., biodiversity, fertilization, agrobiodiversity, botanical composition.

Introduction

Species diversity of grasslands influences their productivity (Schmid, 2002). There is controversy regarding the cultivation of self-seeded or mixed grasses, and the number of components in them (Sanderson et al., 2004). Species composition in grasslands is variable and is directly related to the habitat of each species (Naydenova & Mitev, 2017). Conversion of an area to cultivation is associated with the destruction of primary species, decline in biodiversity, introduction of new species as a monoculture. For this purpose, the improved agrotechnical measures are applied on these areas.

These are in line with the adopted Convention on Biological Diversity (Ministry of Environment and Water of Bulgaria, 2020) and the Paris Agreement on Climate Change (2015), which recognize the importance of the integrity of all ecosystems and the conservation of biodiversity. As a part of the European Green Pact, new biodiversity and forestry strategies for the period up to 2030 aim to restore Europe's biodiversity (European fund, Operational program Growth Regions, 2020). The Farm to Fork Strategy, a key element of the European Green Pact, aims to ensure a healthier and more sustainable food

system of the European Union. This includes reducing the use of risky pesticides in agriculture, reducing the use of fertilizers and encouraging the development of organic farming (Ministry of Environment and Water of Bulgaria, 2019).

Sustainable agriculture includes the concept of agroecology or the ecology of agricultural production, which is a multifaceted, balanced approach to agricultural production that combines agronomic, zootechnical, ecological, human and social scientific solutions and requirements (Kirilov, 2016). In this regard, the cultivation of perennial grass forage species appears as an appropriate ecological agrotechnical measure (Kertikov et al., 2016). Forage from seeded meadow grasslands with perennial and legume species is easily digestible, rich in vitamins and economically effective due to the low cost of the resulting forage production (Khakbazan et al., 2009). Such a suitable grass species is *Lotus corniculatus* L. (bird's-foot trefoil), which is a leguminous nitrogen-fixing crop that enriches the soil with nitrogen, improving its fertility. The forage obtained from it combines favourably high protein and low fiber content (Uzun et al., 2016), which determines its high nutritional value (Bozhanska, 2020a), in accordance with the production requirements in the European Union.

Grass cover uses up a significant portion of carbon dioxide, one of the gases responsible for the greenhouse effect. The establishment and use of seeded meadows and pastures is linked to the respect of a number of conditions and requirements that determine the development of grasslands and the resulting production, as well as the efficiency of the costs incurred (Berentsen et al., 2000). This requires the application of appropriate agronomic practices that meet European standards, which are the basis for establishing sustainable and profitable forage production (Ching, 2018).

An important technological study in the field of fodder production and pasture farming is fertilization (Churkova & Bozhanska, 2016) and its impact on feed yield and quality. An essential element of forage production technology is the use of fertilizers of organic origin to achieve environmentally friendly plant and animal production.

The influence of biofertilizers on the dynamics of dry matter accumulation, changes in the

botanical composition of the grassland and the associated agrobiodiversity of *Lotus corniculatus* L. (Bozhanska, 2020b; Marinova et al., 2019), the identification of varieties suitable for cultivation in specific soil and climatic conditions is important for obtaining high yields and quality of forage (Churkova, 2019).

Technological improvements in this direction have a proven ability to sustainably increase productivity, restore soil fertility, improve agrobiodiversity with economically important grass species, and maintain yields over time (Ching, 2018). Agroecological technologies also have a high degree of climate resilience, contributing to climate change mitigation (Kirechev, 2017). The implementation of appropriate legal agricultural practices complies with laws on the protection of the environment, agricultural land, agricultural property and biodiversity through compliance with the National Standards for Good Agricultural and Environmental Condition of Land.

The aim of the study was to investigate the agrobiodiversity of seeded grassland from *Lotus corniculatus* L. under mountainous conditions after the application of organic fertilizers to improve the species composition of the crop stands.

Materials and methods

The research experiment is carried out in the period 2020 - 2023 in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture-Troyan (Bulgaria) on light grey pseudopodzolic soil. It was set up using the block method in four replications with a harvest plot size of 5 m² with *Lotus corniculatus* L., variety "Targovishte 1", treated with the biological products Blago 5 at doses of 300 and 600 ml/da (variants 2 and 3) and Fertileader Axis at doses of 500 and 1000 ml/da (variants 4 and 5) compared to a non-treated control (var. 1). The doses are recommended by the manufacturer of organic fertilizers.

The main ingredient of the Blago 5 biofertilizer was a highly concentrated foliar fertilizer of lake sapropel. Fertileader Axis is a product with the following composition: nitrogen - 3%, phosphorus - 18%, zinc - 5.7%, manganese - 2.5%.

Fertilizer application was done twice, by foliar spray, before harvesting of the undergrowth each year. The working solution was applied with a backpack sprayer during the period of active vegetation of the *Lotus corniculatus* L.

Species diversity is represented by the botanical composition of the grassland as a percentage by analysis of fresh mass samples taken at each cutting from each variant. These were weighed in an air-dry state, and the percentage of sown grass (*Lotus corniculatus* L.) and the presence of other species were determined by weight. The influence of species and biofertilizer rates on agrobiodiversity after the applied agrotechnical intervention - fertilization was determined. Data on grass species composition are presented by variation of the mean values of the mowings. A comparative analysis of the species biodiversity in the non-fertilized grassland (var. 1) was made and the agrobiodiversity after the application of two types of organic fertilizers at two doses each (variants 2, 3, 4 and 5) was followed.

Results and Discussion

Figure 1 shows the biodiversity of species in the grassland of bird's-foot trefoil without ferti-

lization applied. It can be seen that the seeded grassland of *Lotus corniculatus* L. consisted of cultivated legume species *Lotus corniculatus* L. (45.8%) which dominated the grassland of *Trifolium repens* L. (2.3%). The perennial group is represented by the species *Dactylis glomerata* L. and *Festuca rubra* L., whose presence is 8.7 and 7.9%, respectively. The number of species present in the grassland in the non-fertilized treatments are more than the fertilized treatments. The weeds are represented by the following six species: *Polygonum convolvulus* L. - 4.8%; *Galium aparine* L. - 7.8%; *Polygonum aviculare* L. - 6.5%; *Cirsium arvense* L. - 9.2%; *Convolvulus arvensis* L. - 1.8% and *Cynodon dactylon* L. - 5.2%.

Favourable temperature and moisture conditions during the years of the experimental period were a major factor for the insemination of the species, which predetermined their self-seeding, which is in line with the evidence of Czortek et al. (2021).

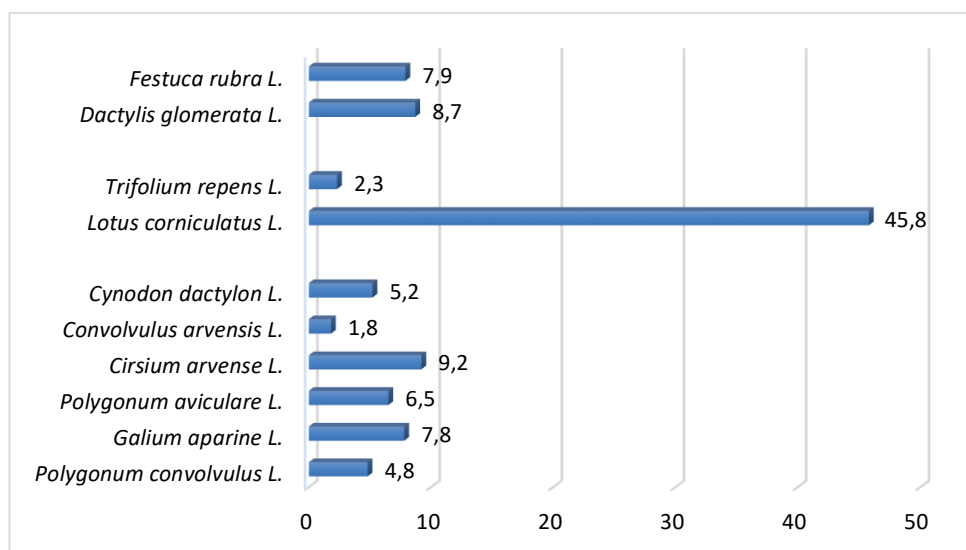


Fig. 1. Biodiversity of *Lotus corniculatus* L. grassland without fertilization, represented by grass species composition (%).

Figure 2 shows the variation of species under the influence of fertilization with the organic fertilizer Blago 5 at a rate of 300 ml/da (var. 2).

The benefits of fertilization and agrobiodiversity was demonstrated by the presence of the seeded grass species *Lotus corniculatus* L. in the grassland by 59.2%. *Trifolium repens* L. (2.9%) and *Trifolium pratense* L. (3.8%), which are probably self-seeded legumes, were observed. Of the perennial representatives, *Dactylis glomerata* L. (2.9%),

Festuca rubra L. (3.4%) and *Festuca arundinacea* Schreb. (4.8%) occur. The action of the biofertilizer at the indicated dose did not significantly affect the species composition of weeds. Their amount did not differ both in number and species compared to that of the unfertilized control. Fertilization with Blago 5 at a dose of 300 ml/da stimulates the seeded grass species *Lotus corniculatus* L., which determines the productivity and quality of forage.

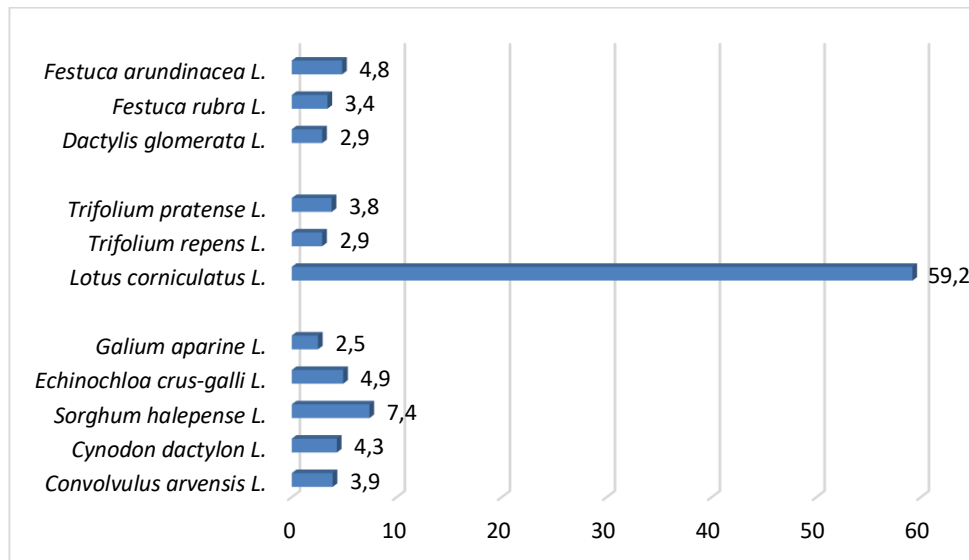


Fig. 2. Agrobiodiversity of seeded grassland of *Lotus corniculatus* L. treated with Blago 5 biofertilizer at 300 ml/da, represented by grass species composition (%).

The application of the organic fertilizer Blago 5 at double dose stimulated the development of seeded *Lotus corniculatus* L. up to 70.1% (Fig. 3) compared to 45.8% in the unfertilized control and 59.2% in the treatment with the same biofertilizer at 300 ml/da. *Cirsium arvense* L., which was present in variant 2, was absent from the weed com-

position. The relative proportion of weed species is represented as follows: *Convolvulus arvensis* L. - 3.9%, *Cynodon dactylon* L. - 3.0%, *Sorghum halepense* L. - 10.7%, *Echinochloa crus-galli* L. - 4.8% and *Galium aparine* L. - 4.6%. The presence of perennial grasses representatives (*Dactylis glomerata* L. - 1.2% and *Festuca rubra* L. - 1.6%) was insignificant.

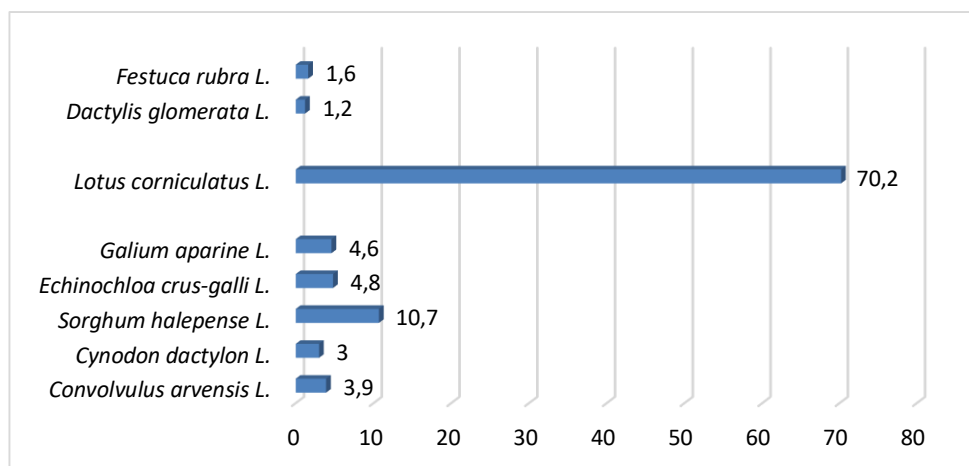


Fig. 3. Agrobiodiversity of seeded grassland of *Lotus corniculatus* L. treated with Blago 5 biofertilizer at 600 ml/da, represented by grass species composition (%).

When fertilized with Fertileader Axis at a rate of 500 ml/da (variant 4), an increase in the relative proportion of *Lotus corniculatus* L. in the grassland and the absence of co-occurring legume grasses was notable (Fig. 4). From the weedy species, *Cynodon dactylon* L. - 6.9%, *Echinochloa crus-galli* L. - 7.8% and *Cirsium arvense* L. - 3.7% dominated,

which were represented with negligible participation. The foliar-applied Fertileader Axis at a dose 500 ml/da had a significantly stronger positive effect on the relative proportion of seeded legumes compared to the biofertilizer Blago 5 applied at both rates. This is evident from both the reduced number of weed species and their overall

relative proportion, which was 18.4%. From the group of perennial grasses, only *Festuca rubra* L. is present, whose contribution is insignificant, respectively 2.7%. This action is probably the result

of the presence of nitrogen, phosphorus, zinc and manganese in the composition of the fertilizer, which affect the metabolic processes in plants and the movement of nutritional value.

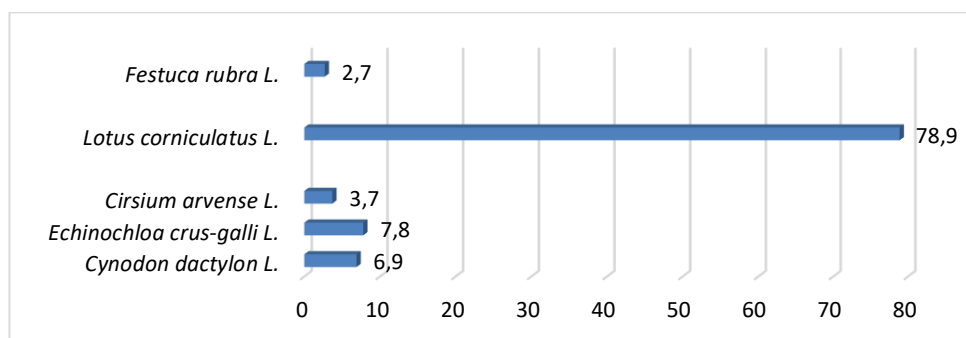


Fig. 4. Agrobiodiversity of seeded grassland of *Lotus corniculatus* L. treated with Fertileader Axis biofertilizer at 500 ml/da, represented by grass species composition (%).

The presence of different species in the grassland under the influence of fertilization with Fertileader Axis at a rate of 1000 ml/da is indicated in Fig. 5. From the data analysis, it can be seen that the predominant sown grass species *Lotus corniculatus* L. which was recorded at 82.6%. This fertilizer rate was found to be suitable for stimulating the development of the main crop in the stand, affecting its maximum presence and providing the lowest weed cover (13.6% overall) in the *Lotus corniculatus* L. crop. The number of weed species in the grassland was low (3 pieces) and their proportion insignificant, respectively

Cynodon dactylon L. - 7.5%, *Galium aparine* L. - 3.5% and *Cirsium arvense* L. - 2.6%. From the group of perennial grasses representatives, only *Festuca rubra* L. was recorded with participation of 3.8%. The results obtained are similar to those obtained by Vasileva (2015) on the positive effect of fertilization on the presence of legumes in the grassland. The dose of treatment with Fertileader Axis biofertilizer, did not affect the present participation of *Lotus corniculatus* L. (70.2% and 82.6%), which is confirmed by its relative share. All applications of both fertilizers had low relative weed contribution (18.4% to 13.6% total).

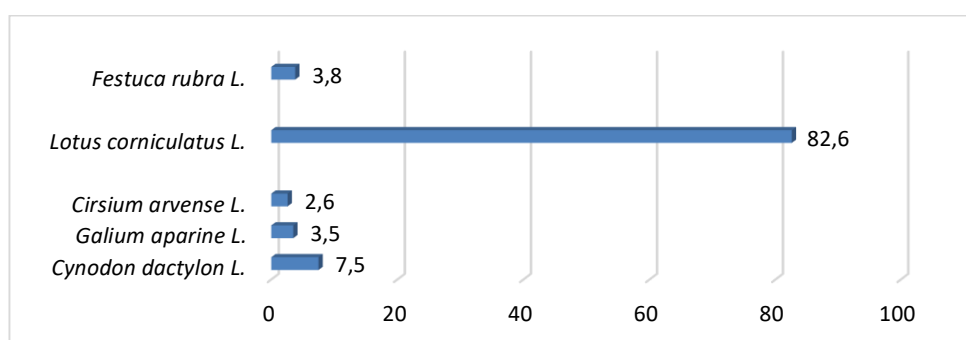


Fig. 5. Agrobiodiversity of seeded grassland of *Lotus corniculatus* L. treated with the Fertileader Axis biofertilizer at a dose of 1000 ml/da, represented by grass species composition (%).

The analysis of the results obtained from the botanical composition of the grassland confirms what Jankowska-Huflejt (2006) demonstrated that the application of fertilizers of organic origin to seeded grasslands determines the change of flo-

ristic composition in a desirable direction, and depending on the fertilizers used, the development of economically important grass species from the groups of perennial and legumes can be promoted, as well as the development of poorly

productive ones can be suppressed. The species diversity found in grasslands in the presence of a legume component determines their productivity and nitrogen-fixing capacity at the tuber formation level. This, in turn, affects the quality and especially the protein content and nutritive value of the forage (Iliev, 2018).

Incorporated organic fertilizers stimulate the growth of the plant root system, increase the utilization and uptake rate of hard-to-reach plant nutritional value (Datta et al., 2011). According to Iliev (2018), foliar organic fertilizers, actively improve and stimulate plant respiration and photosynthesis, as well as nitrogen and carbohydrate metabolism. As a result of the data obtained from the botanical analysis, we found different effects of the two biofertilizers included in the experiment on the species composition of grasses in the *Lotus corniculatus* L. crop depending on their doses. On average over the study period, the presence of seeded grass (*Lotus corniculatus* L.) in the grass association was affected to a higher positive extent by the biofertilizer Fertileader Axis applied at a dose of 1000 ml/da, which is explained by the higher fertilizer rate. This fertilizer and this dosage determined the high presence of *Lotus corniculatus* L. in the stand, improved the agrobiodiversity of the biocoenosis and reduced the competitive effect of weed species on the seeded grass. The agronomic measure applied proved to be suitable for improving the biodiversity of the seeded *Lotus corniculatus* L. grassland, which showed high yield and forage quality, as presented in another of our publications (Churkova & Churkova, 2024).

Conclusions

The applied fertilization with the biofertilizers Blago 5 and Fertileader Axis in the seeded grassland of *Lotus corniculatus* L. showed a high positive effect, which was manifested by a significant increase in the quantitative contribution of the seeded legume crop *Lotus corniculatus* L. and a reduced relative proportion of both the number and the species composition of weeds. This, in turn, makes it possible to increase the quality of forage, and determines the economic importance for forage production.

The composition of the applied organic fertilizer determines the ecological effect of fertilization. This improves soil composition by

increasing soil nutrients and increases the yield of forage grass, which in turn increases the economic effect of production. The action of the applied fertilizers is an expression of the reduction of the risk of loss of a variety of useful grass species, on the basis of a chosen appropriate agrotechnical practice, leading to a cost-effective production of forage, with reduced costs and a high environmental effect.

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