THE INFLUENCE OF FERTILIZATION ON THE QUALITY AND BIOLOGICAL GROWTH OF LOLIUM PERENNE

Cristian BOSTAN[1], Nicolae Marinel HORABLAGA[1,2], Luminita COJOCARIU[1,2], Ramona Loredana TOPORAN[2,*], Christianna ISTRATE-SCHILLER[1,2], Ionel SAMFIRA[1,2,*]

[1]Lovrin Agricultural Research and Development Station, Romania

[2]University of Life Sciences "King Mihai I" from Timisoara, Romania

[*]Corresponding authors: ramo27lore@yahoo.com; ionelsamfira@usvt.ro

Abstract. *Perennial ryegrass has been the subject of breeding activity focused on intensive breeding, especially in recent decades. Breeding has focused on population improvement and obtaining synthetic cultivars that show strong increases in biomass yield. This in the conditions where the production of biomass, respectively the green mass/forage is one of the most important productive features of forage plants. The ability to increase the yield of the whole plant through reproduction reaches an optimal index of leaf area quite quickly during the growth of the forage plant support. Varieties were created and selected with short growth, greater density, persistence and resistance to cutting. Although phenotypic differences between these functional groups are evident, a genome-wide analysis of the degree of differentiation and biological pathways, or genes under selection, has not been previously reported. Productive success has been achieved in recent decades as summer and autumn dry matter yield has increased between 0.3 % and 0.9% annually over the past 40–50 years, and spring dry matter yield has remained almost unchanged. Breeders have developed forage ryegrass crops with different maturity groups, from early to late season types, adapted to a long grazing season that are being used for permanent pastures, due to growth in both summer and autumn. The purpose of the paper is to highlight the influence of mineral fertilizers (nitrogen, phosphorus, potassium, sulfur) on the biological development, production and quality of the Lolium perenne species. Databases with specialized literature, published works and research results were used as materials and working methods. 324 papers that met the selected criteria were analyzed and processed.*

Keywords*: perennial ryegress, grasslands, productive capacity, plant quality, fertilization*

INTRODUCTION

It is well known that the genetic progress of perennial ryegrass in the last 60 years is due to fertilization, the improvement of technological practices, and the use of varieties with superior genetics. Meadows are considered to be one of the most diverse and species-rich ecosystems, however, the intensification of agriculture in recent decades has led to a decrease in area of (semi-) natural grasslands associated with a concomitant decrease in their biodiversity (Copăcean et al., 2019; Toporan et al., 2019, 2021; Samfira et al., 2023a, 2023b).

Modern agricultural systems have reached unprecedented production levels. Still, they are dependent on external inputs such as mineral fertilizers. The negative environmental impact of these agroecosystems has led to growing concern about their sustainability (Van Dobben et al., 2019). In this context, permanent meadows and fodder plants represent the backbone of sustainable agriculture, contributing significantly to the world economy.

Perennial ryegrass or *Lolium perenne* is a fodder species that ensures a production with superior quality and productivity, being adapted to the current climate of the cultivation areas in Europe. Current climate change is believed to improve forage production conditions in the future because the growing season will be longer (1-3 months), with milder and rainier autumns, and dominating winters (Nakamura et al., 2008; Blackmore et al., 2016). Grasslands dominated by this species represent a major livelihood resource for approximately one billion people, fulfilling a wide range of functions (Hopkins et al., 2014). It is recognized that areas in natural and semi-natural regimes actually support "life" and implicitly socio-economic development (Giraldo et al., 2018).

Lolium perenne (perennial ryegrass) stands out, as the most important forage species in European agriculture and the dominant species in meadows exploited by sheep and cattle grazing (Berthier et. al., 2009). Perennial ryegrass is thus used for sowing new meadows and pastures, lawns, and sports fields, and is also cultivated as green manure or for energy purposes. Even the seed production in this species is high which is most desirable for farmers who cultivate the plant specifically for seed production (Jakusek et. al., 2020).

Due to these considerations, it is considered an important forage species in Europe, New Zealand, some regions of Japan, Australia, South Africa, and South America. They are affixed in this way as objectives of scientific research and genetic improvement, for the improvement of the productivity and quality of production. To achieve these goals, and increase quality, additional knowledge is needed about the balanced use of mineral fertilizers.

MATHERIAL AND METHOD

Literature search.

The study of the specialized literature related to the biological development and the analysis of the interdependence between the physico-chemical development of the plant under the influence of the main macro and microelements in the soil was carried out by accessing electronic databases. These investigated electronic databases were mainly represented by Google scholar (2000–2023), Web of Science (2012–2017) and Scopus (2012-2024).

The search terms (title, abstract, keywords) used were: perennial ryegrass, grasslands, productive capacity, plant quality, fertilization.

Based on our previous experience in conducting bibliographic research some works could not be found using the search terms (Toporan et al., 2023). Pursuing the goal of maximizing the quantity and quality of relevant literature, we used in the search some terms and expressions associated with key words such as: decadal genetic progress, quality dynamics of perennial ryegrass, the response of perennial ryegrass to chemical fertilization applied to a pure crop or a plant association built by this species.

Inclusion and exclusion criteria

The inclusion criteria were: studies relating the biological development, production and quality of ryegrass with the application of chemical fertilization published in English and Romanian. The exclusion criteria were represented by results regarding the participation of perennial ryegrass in the construction of the plant communities that constitute the permanent meadows, studies on the behavior and response of the growth of the species to grazing with different species and animal loads per hectare (LSU), studies that define the biological response to perennial ryegrass in extreme living conditions both in terms of the climate and the soil.

Study selection

Some duplicates of research on perennial ryegrass were also eliminated, analyzing the evolution of the species over time, its botanical classification, etc. 324 published research papers from all searched databases that matched the desired criteria were initially processed. Eligibility and initial relevance were checked starting from the attractiveness of the title and abstract, the evaluation of the full text was carried out after this stage and the studies that did not meet the inclusion conditions set out previously were eliminated. Thus, in the end, a total of 45 papers were included in this review.

RESULT AND DISCUSSIONS

The current context of mineral nitrogen fertilization

The change in agricultural policies caused the intake of chemical fertilizers to be reduced. In the future, the change in fertilization practices will have clear influences on the composition and productivity of pastures (Mosquera-Losada et. al., 2004).

The dependence of green mass production on nitrogen fertilization is becoming a major sustainability issue in modern agriculture. The main reason is the dependence on fossil fuels and the associated economic and ecological costs, and the sustainable production of green mass will depend on the successful development of varieties that allow high productivity and quality in low-nitrogen soil environments.

Knowing the characteristics of the forage varieties of *Lolium perenne* is of great importance for growers, since the variety of forage plants must correspond to the soil and climate requirements for forage production in any climate, on all types of soil, irrigated or non-irrigated, which can be covered by zoning the forage plant species and within each species. Experiences with varieties or hybrids have a permanent character, because for most cultivated species, new varieties and hybrids appear every year, both nationally and internationally, and there is a need to test them before introducing them into the culture of certain areas (Corcheş et. al., 2009; Toporan et al., 2021).

The quality of forage species of *Lolium perenne* is dependent on factors such as the species and variety of plants, age, type of fertilizer applied, and type of soil (especially the concentration of mineral nutrients) (Bumane, 2010; Butnariu and Cauni, 2013).

Also, high digestibility and grazing tolerance are the main objectives of improving the species (Wilkins, 1991; Smit and Elgersma, 2004). In these conditions, the chemical composition of fodder plants is dependent on factors such as: species and variety, plant development phase, soil fertility (especially with nitrogen), fertilizers, weather conditions, harvesting time, and harvesting technology.

Results of fertilization studies

The application of organic fertilizers is a necessity of sustainable agriculture that can improve the physical, chemical, and microbiological aspects and soil properties (Balla et. al., 2017). Studies on the quality of forage species suggest that the development phase is the most important factor influencing protein content in DM, it also significantly influences the dose and time of application of nitrogen fertilizers. Nitrogen is a macronutrient found in a wide range of cellular compounds, including proteins, nucleic acids, amino acids, and lipids, and nitrogen levels regulate many aspects of plant metabolism, growth, and development (Foito et. al., 2013). Nitrogen (N) contributes to physiological functioning of the plant by stimulating the growth of plant shoots and roots and is essential for the consumption of carbohydrates in plants.

Nitrogen required for leaf tissue synthesis is provided by remobilization of nitrogen from remaining tissues until uptake of recovered nitrogen (Macduff et al., 2002). Heterotrophic tissues in the zone of meristematic cell division and the closed elongation zone of the immature laminae of the foraging species depend on the supply of new carbon fixed from mature autotrophic laminae to sustain growth. Chlorophyll and thylakoid proteins account for about 25% of the total N content in mature leaves, and after severe defoliation by herbivores or agricultural machinery, mineral N uptake declines very rapidly in perennial ryegrass. Frequent defoliation of ryegrass by livestock grazing or mowing leads to the removal of photosynthetic tissues that have the role of fixing CO_2 and deprives tissue growth of sugars. Depletion of sugars induces a shift from carbon storage to low molecular weight sugars to support rapid elongation of the immature leaf limb as well as to form new photosynthetic tissue essential for plant growth (Liu et al., 2015).

Frequent defoliation of *Lolium perenne* leads to acquired adaptations such as the accumulation of water-soluble carbohydrate reserves and less developed root systems than in less frequently defoliated plants, thus reducing survival beyond the summer months (Clayton et al., 2008). Root growth and lifespan is an important factor determining nutrient and carbon fluxes in terrestrial ecosystems. However, the effect of temperature on root longevity is not yet fully understood. Iron (Fe) is an essential nutrient for almost all organisms due to its central role in vital cellular reactions. The redox properties of iron confer an essential catalytic function for fundamental metabolic pathways in DNA synthesis, respiration and photosynthesis. Therefore, the control of iron homeostasis is an essential function, and organisms have developed complex strategies for iron uptake, utilization, transport, and storage (Jonhnson et al., 2013). In forage plants, such as *Lolium perenne L*., the ability to grow after cutting or grazing is important for persistence, and defoliation and grazing practices alter both the metabolism and the pattern of C allocation in these forage plants, producing a reduction of nutrient uptake and an increase in organic C exudation from roots.

For these reasons, fertilization programs are generally built around this element (Ianculov et al., 2005; Hakki Akdeniz and Hosaflioğlu, 2016; Nizam, 2009). Nitrogen recycling, especially through the remobilization of nitrogen from the organs of mature plants for the emission of new tissues or in the development of the leaf apparatus, is due to the migration of this element from the mature organs (especially leaves) of the plant.

Intraspecific and interspecific genetic variation in N remobilization is important in this process, offering the potential for genetic manipulation to improve N use efficiency. Free amino acids play an important role in nitrogen storage in *Lolium perenne* and soluble proteins are also true nitrogen reservoirs for leaf regeneration. Most of the remobilized N from mature leaves reside in chloroplasts (the photosynthetic enzyme Rubisco), (Khaembah et. al., 2013).

Phosphorus (P) deficiencies adversely affect plant growth because this element supports winter survival, cold resistance, and dry matter production. Potassium (K) plays a key role in root growth, disease resistance, and cold resistance (Ihtisham et al., 2018). Acidic soils comprise more than 50% of the world's potentially arable land, and low availability of phosphorus (P) is considered the main limiting factor in production. Phosphorus (P) also has a vital role in plant cellular processes (energy metabolism, signal transduction, photosynthesis) and is the main constituent of phospholipids, ATP, and nucleic acids, being an essential macronutrient that contributes to plant growth.

As a more environmentally friendly alternative to P fertilization, phospho-bacteria are proposed as bio-inoculants, and studies show that this inoculation process is beneficial for development, nutrition, and alleviation of oxidative stress. As a result, phosphorus fertilization in the *Lolium perenne* species provides higher biomass production, increased P content, and less cellular damage in inoculated plant tissues (Barra et. al., 2019).

Potassium (K) is a major nutrient required along with nitrogen (N) and phosphorus (P) for plant growth. Unlike N and P, K fertilizers are applied at a much lower rate and less than 50%, therefore, large agricultural areas of the world are deficient in K ratio (Li et. al., 2015).

The nutrient accumulation characteristics and mineral content of plants depend on several factors, such as crop and site characteristics, environmental conditions and soil nutrient levels. In other words, nutrient interactions are quite complex and can play an important role, and the phosphorus (P)-potassium (K) interaction is a part of the cation-anion balance in plants. Good P supply can have a beneficial influence on K uptake, and plant K concentration, and plant P and K accumulation is closely related to dry matter production potential (Sárdi et. al., 2012).

The influence of fertilization on quality in the species *Lolium perenne*

Also, determining characteristics in the expression of productive potential such as the longevity and production capacity of forage plants was studied in various experiences with long-term fertilization with doses of NPK between 0-400 kg ha^{-1}. In these conditions, the application of mineral fertilizers changed the productivity ratio of perennial ryegrass mixtures, but also the botanical composition of permanent meadows exploited by grazing or mowing. In many of these studies, the $N_{200}P_{100}K_{300}$ fertilization dose provided the highest dry matter yield 7.32 - 12.0 t ha^{-1} (Berzins et. al., 2011).

The study of the interaction of nutrients, nitrogen (N), phosphorus (P), potassium (K), and sulfur (S), demonstrated the increase in productive yield and crude protein content (CP) in direct correlation with the doses used. N application increases the concentration of non-protein nitrogen (NPN), P fertilization does not affect the NPN level, while K fertilization decreases the NPN content.

The content of non-structural carbohydrates (NSC) decreases with the application of N, but is not affected by the application of P, K and S. Also, the content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) is not affected by the type of fertilizer applied (Findlay, 2010). In an intensive culture system, perennial ryegrass is preferred due to its quick response to nitrogen (N) as well as high productive yield, fertilization with high doses of nitrogen as well and harvesting at a young stage of development increases the crude protein content (Tas, 2007).

The influence of fertilization on biological development in the species *Lolium perenne*

The application of nitrogen fertilizers at the optimal time during the growing season is a valuable tool for managing the reduction of nitrogen losses as well as for increasing the efficiency of nitrogen use in grasslands by synchronizing nitrogen supply with nitrogen demand (De Boer et. al., 2016).

Root system characteristics, such as density and rooting depth, play a critical role in nitrogen (N) interception and uptake because the three-scythe-per-year harvesting system implies an earlier first harvest in the spring. This three-scythe harvesting system without nitrogen fertilization has proven to be a suitable practice for managing botanically complex grasslands contributing to optimizing the productivity and sustainability of these ecosystems (Brumt et al., 2009; Toporan et al., 2021, 2022). From the experimental data, we can distinguish the idea that the season and the dose of nitrogen (N) fertilization in perennial ryegrass vary depending on the destination of the production. In fodder production, it is necessary to apply high doses from spring, and in the case of a crop intended for seed production, no positive effect of applications has been demonstrated (Gislum et. al., 2003; Kominko et. al., 2017).).

CONCLUSIONS

Among the fodder species, we can distinguish Lolium perenne, currently considered one of the important species, having a high productive yield associated with a very good quality.

Due to these considerations, in the last decades the obtaining of new perennial ryegrass cultivars that are compatible with modern agriculture has been linked to the increase in the importance of chemical fertilization along with the improvement of technological practices.

Many studies are published regarding fertilization practices with independence on some morpho-productive elements of the species such as variety, phenotypic development phase, soil fertility, climate conditions, season and harvesting technology. Many of the published studies show that the dose along with the time of application of nitrogen fertilization are very important elements and influence the protein content of the plant as well as DM production.

It is shown that nitrogen fertilization is also important in the conditions where the perennial ryegrass is a plant that is frequently grazed by animals or frequently mowed for fodder and the fertilization doses and practices are built around this chemical macroelement. Nitrogen thus intervening in the recovery of the active leaf part, storing in the root system some reserve substances that allow a rapid regeneration after grazing.

Along with nitrogen, the influence of phosphorus (P) is noticeable, especially in the survival rate over the cold season of perennial ryegrass plants. In most ryegrass cultivation areas, the natural lack of phosphorus in the soil is a limiting factor, ensuring a normal phosphorus content is beneficial to produce more biomass by stimulating nutrition processes or developing resistance to oxidative stress.

The response of perennial ryegrass to fertilization is different and has been studied in several long period fertilization experiments, using doses of NPK between 0-400 kg ha^{-1}. The best results obtained when fertilization doses of $N_{200}P_{100}K_{300}$ were used, which statistically ensured productions of 7.32 - 12.0 t ha^{-1}, are scientifically substantiated.

In conclusions, global population growth will increase the demand for agricultural and food production leading to an increasing dependence on fertilizer inputs, the world demand for NPK is estimated to increase by 1.4-2.6% annually, and P is known as a non-renewable resource which is exhausted in the next 50-130 years. In this context, it is necessary to reduce mineral fertilizer losses, a phenomenon supported especially by the increase in soil fertility.

ACKNOWLEDGMENTS

The authors thank the ARDS Lovrin and its partners for facilitating this study.

REFERENCES

1. Balla K. A., Kremper R., Kincses I., Orosz-Tóth M., Juhász E. - Changes of yield, nutrient contents of ryegrass (Lolium perenne) due to different organic fertilizers application. Annals of the University of Oradea, Fascicle: Environmental Protection, 2017, Vol. XXVIII, 9-16.
2. Barra P.J., Pontigo S., Delgado M., Parra–Almuna L., Duran P., Valentine A.J., Jorquera M.A., de la Luz Mora M. - Phosphobacteria inoculation enhances the benefit of P–fertilization on Lolium perenne in soils contrasting in P–availability, Soil Biology and Biochemistry, 2019, Vol. 136, Article number: 107516, https://doi.org/10.1016/j.soilbio.2019.06.012.
3. Berthier A., Desclos M., Amiard V., Morvan-Bertrand A., Demmig-Adams B., Adams W., Turgeon R., Prud'homme M.P., Noiraud-Romy N. - Activation of Sucrose Transport in Defoliated Lolium perenne L.: An Example of Apoplastic Phloem Loading Plasticity, Plant and Cell Physiology, 2009, Vol. 50, Issue 7, 1329–1344.
4. Berzins P., Rancane S., Svarta A. - The productive longevity of perennial grasses swardsdepending on the NPK fertilizer rates. Proceedings of the 8th International Scientific and Practical Conference, 2011, Vol. II, 244-251.
5. Blackmore T., Thorogood D., Skøt L., McMahon R., Powell W., Hegarty M, 2016 - Germplasm dynamics: the role of ecotypic diversity in shaping the patterns of genetic variation in Lolium perenne, Scientific Reports, 6, Article number: 22603.
6. Brum O.B., López S., García R., Andrés S., Calleja A. - Influence of harvest season, cutting frequency and nitrogen fertilization of mountain meadows on yield, floristic composition and protein content of herbage, Forage Crops, 2009, Zootec. 38 (4), https://doi.org/10.1590/S1516-35982009000400002.
7. Bumane S. - The influence of NPK fertilization on Lolium perenne L. forage Quality, Agronomy Research 8, 2010, (Special issue III), 531-536.
8. Butnariu M., Caunii A. - Design management of functional foods for quality of life improvement, Annals of agricultural and environmental medicine, 2013, Vol. 20, Issue: 4, 736–741.
9. Clayton S. J., Read D.B., Murray P. J., Gregory P.J. - Exudation of Alcohol and Aldehyde Sugars from Roots of Defoliated Lolium perenne L. Grown Under Sterile Conditions. Journal of Chemical Ecology, Vol. 34, 1411–1421.
10. Copacean Loredana, Zisu I., Cojocariu Luminita - Analysis of land use changes and their influence on soil features. case study: Secaș village, Timiș county (Romania), Present Environment and Sustainable Development, 2019, Vol.2., 157-166.
11. Corcheş M., Moisuc A. - Study of production characters of three foreign Lolium perenne varieties in the Timisoara's climatic conditions, Research Journal Of Agricultural Science, 2009, No. 41 (3), 26-31.
12. De Boer H.C., Deru J.G.C., Hoekstra N.J., van Eekeren N. - Strategic timing of nitrogen fertilization to increase root biomass and nitrogen-use efficiency of Lolium perenne, Plant and Soil, 2016, vol. 407, 81–90.
13. Dineen M., Delaby L., Gilliland T., McCarthy B. - Meta-analysis of the effect of white clover inclusion in perennial ryegrass swards on milk production. Journal of Dairy Science, 2018, Volume 101, Issue 2, 1804-1816.
14. Findlay N.J. - The effect of application of nitrogen, phosphorus, potassium and sulphur fertilisers to a perennial ryegrass sward on yield, quality and apparent intake by dairy cows. Master of Science (Agriculture), School of Conservation and Biological Sciences Faculty of Science and Agriculture University of KwaZulu-Natal Pietermaritzburg, 2010, http://hdl.handle.net/10413/10827.
15. Foito A., Byrne S.L., Hackett C.A., Hancock R.D., Stewart D., Barth S. - Short-term response in leaf metabolism of perennial ryegrass (Lolium perenne) to alterations in nitrogen supply. Metabolomics, 2013, vol. 9, 145–156.
16. Giraldo Paula Andrea, Elliott C., Badenhorst P., Kearney G., Spangenberg G.C., Noel O. I. Cogan, Smith K.F. - Evaluation of endophyte toxin production and its interaction with transgenic perennial raigras (Lolium perenne L.) with altered expression of fructosyltransferases. Springer Link, Transgenic Research, 2018, Vol. 27, 397–407.
17. Gislum R., Wollenweber B., Boelt B., Jensen E.S. - Uptake and Distribution of Nitrogen in Perennial Ryegrass: Effect of Additional Applications at Vegetative Growth. Journal of Plant Nutrition, 2003, Vol. 26, Issue 12, 2375-2389.
18. Hakkı Akdeniz H., Hosaflioğlu I. - Effects of Nitrogen Fertilization on Some Turfgrass Characteristics of Perennial Ryegrass (Lolium perenne L.). Journal of Agricultural Science and Technology B 6, 2016, pag. 226-237, doi: 10.17265/2161-6264/2016.04.002.
19. Hopkins A., Collins R.P., Fraser M.D., King V.R., Lloyd D.C., Moorby J.M., Robson P.R.H. - EGF at 50: The Future of European Grasslands. Proceedings of the 25th General Meeting of the European Grassland Federation Aberystwyth, Wales, Grassland Science in Europe, 2014, Vol. 19, 753-755.

20. Ianculov I., Palicica R., Butnariu Monica, Dumbrava D. - Gergen I., Achieving the crystalline state of chlorophyll of the Fir–tree (Abies alba) and the pine (Pinus sylvestris), Revista de chimie, 2005, 56(4), 441–443.
21. Ihtisham M., Fahad S., Luo T., Larkin R.M., Yin S., Chen L. - Optimization of Nitrogen, Phosphorus, and Potassium Fertilization Rates for Overseeded Perennial Ryegrass Turf on Dormant Bermudagrass in a Transitional Climate. Frontiers Plant Science, 2018, https://doi.org/10.3389/fpls.2018.00487.
22. Jakusek M., Brennensthul M., Markowska J., Wolski K., Sobol L. - Effect of a Micronutrient Fertilizer and Fungicide on the Germination of Perennial Ryegrass Seeds (Lolium perenne L.) in Field Conditions. Agronomy, 2020, 10(12), 1978; https://doi.org/10.3390/agronomy10121978.
23. Johnson L., Koulman A., Christensen M., 2013 - An Extracellular Siderophore Is Required to Maintain the Mutualistic Interaction of Epichloë festucae with Lolium perenne.10.1371/journal.ppat.1003332.
24. Khaembah E.N., Irving L.J., Thom E.R., Faville M.J., Easton H.S., Matthew C. - Leaf Rubisco turnover in a perennial ryegrass (Lolium perenne L.) mapping population: genetic variation, identification of associated QTL, and correlation with plant morphology and yield. Journal of Experimental Botany, 2013, Vol. 64, Issue 5, 1305–1316.
25. Kominko H., Gorazda K., Wzorek Z. - The Possibility of Organo-Mineral Fertilizer Production from Sewage Sludge. Waste and Biomass Valorization, 2017, volume 8, 1781–1791.
26. Li T., Wang H., Wang J., Zhou Z., Zhou J. - Exploring the potential of phyllosilicate minerals as potassium fertilizers using sodium tetraphenylboron and intensive cropping with perennial ryegrass. Scientific Reports, 2015, vol. 5, Article number: 9249, https://doi.org/10.1038/srep09249.
27. Liu Q., Jones C. S., Parsons A. J., Xue H., Rasmussen S. - Does gibberellin biosynthesis play a critical role in the growth of Lolium perenne? Evidence from a transcriptional analysis of gibberellin and carbohydrate metabolic genes after defoliation, Frontiers in Plant Science, 2015, https://doi.org/10.3389/fpls.2015.00944.
28. Macduff J.H., Humphreys M.O., Thomas H. - Effects of a Stay-green Mutation on Plant Nitrogen Relations in Lolium perenne During N Starvation and after Defoliation, Annals of Botany, 2002, Vol.89(1), 11–21.
29. Mosquera-Losada M.R., González-Rodríguez A., Rodríguez A.R. - Fertilization with nitrogen and potassium on pastures in temperate areas. Journal of Range Management, 2004, 57(3), 280-290.
30. Nakamura Ryoji, Naoki Kachi, Jun-Ichirou Suzuki - Root growth and plant biomass in Lolium perenne exploring a nutrient-rich patch in soil, Journal of Plant Research, 2008, Vol. 121, 547–557.
31. Nizam, I. - Effect of nitrogen fertilization on seed yield and some plant characteristics of perennial ryegrass (Lolium perenne L.). Journal of Tekirdag Agricultural Faculty, 2009, Vol.6 No.2 pp.111-120 ref.19.
32. Samfira I., Barliba C., Butnariu Monica - Management of the grasslands surfaces for a better farming and landscape protection. A descriptive case in Banat Plain Romania, Jurnal International Multidisciplinary Scientific GeoConference: SGEM, 2017, Vol. 17, 881-886.
33. Samfira I., Horablaga N.M., Barliba C., Istrate-Schiller Christiana, David G., (b) - Dynamics of grasslands soil productivity under the altitudinal influence. case study of the Sureanu Mountains area in Romania, International Multidisciplinary Scientific GeoConference: SGEM, 2023, Vol. 23(3.1), 201-208.
34. Samfira I., Horablaga N.M., David G., Barliba C., Agapie Alina Laura, (a) - Elements of correct fertilization of the meadows in the high plain area of Banat Romania, International Multidisciplinary Scientific GeoConference: SGEM, 2023, Vol. 23(3.1), 231-238.
35. Sárdi K., Balázsy Á., Salamon B. - Interrelations in Phosphorus and Potassium Accumulation Characteristics of Plants Grown in Different Soil Types. Communications in Soil Science and Plant Analysis,2012, Vol. 43, Issue 1-2, 324-333.
36. Smit H.J., Elgersma A. - Diurnal fluctuations in vertical distribution of chemical composition in a perennial ryegrass (Lolium perenne L.) sward during the season. Land Use Systems in Grassland Dominated Regions, 2004, 951-953.
37. Tas B. - Nitrogen utilization of perennial ryegrass in dairy cows. Fresh Herbage for Dairy Cattle: the Key to a Sustainable Food Chain, 2007, Volume 18, https://library.wur.nl/ojs/index.php/frontis/article/view/1249.
38. Toporan Ramona Loredana, Horablaga N.M., Samfira I. - Perennial Lolium species as a germoplasm resource and biodiversity, Research Journal of Agricultural Science, 2021, Vol.52 (4).
39. Toporan Ramona Loredana, Horablaga N.M., Samfira I. - Influence of intraspecific variation of perennial ryegrass on quality and level of production, Research Journal of Agricultural Science, 2022, Vol.54 (2); ISSN: 2668-926X 181.
40. Toporan Ramona Loredana, Horablaga N.M., Samfira I. - Analysis of the frequency and distribution of genetic variation in perennial ryegrass populations, Jurnal Research Journal of Agricultural Science, 2022, Vol. 54 (1); ISSN: 2668-926X.
41. Toporan Ramona Loredana, Samfira I. - The impact of perennial Lolium on the biodiversity and the structure of the grasslands, Jurnal Research Journal of Agricultural Science, 2021, Vol. 53(1).

42. Toporan R.L., Horablaga M., Samfira I. - Breeding review on the Trifolium repens, 2023, Research Journal of Agricultural Science 55 (2).
43. Van Dobben H.F., Quik C., Wieger Wamelink G.W., Lantinga E.A. - Vegetation composition of Lolium perenne-dominated grasslands under organic and conventional farming. Basic and Applied Ecology, 2019, Vol. 36, 45-53.
44. Wilkins P.W. - Breeding perennial ryegrass for agriculture. Euphytica, 1991, Vol. 52, 201–214.
45. Woodfield D.R., Caradus J.R. - Genetic Improvement in White Clover Representing Six Decades of Plant Breeding, Crop Science - Crop Breeding, Genetics & Cytology, 1994, https://doi.org/10.2135/cropsci1994.0011183X003400050011x, https://acsess.onlinelibrary.wiley.com/doi/abs/10.2135/cropsci1994.0011183X003400050011x.