PERENNIAL LOLIUM SPECIES AS A GERMOPLASM RESOURCE AND BIODIVERSITY

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Abstract.*The purpose of this paper is to conduct a pathological study to identify and collect ecotypes of Lolium perenne as a function of germplasm in the process of improvement "in situ" and "ex situ" in the Danube Meadow, Protected Areas of Mehedinți County and the Western Plain of Timiș County. A study is performed on areas (altitudes, types of meadows) where the species Lolium perenne is present with accompanying vegetation and biogeomorphological characterization. The collection field for the study is made at the Lovrin Agricultural Research Station, where the biological development of the collected material is followed. The study of Lolium perenne species is performed by determining the ecological indices and biodiversity and performing the analysis of plant-soil quality of the soils in the collected area. Productive characteristics of Lolium perenne as a cultivated species and productive / breeding potential for use as feed, seed production, quality of green hay mass, net lactation energy and evolution of crude protein content. Seed yield is a measure of the total marketable seed recovered from the harvesting and processing of a seed crop. An economic level of seed yield is vital for the successful production and marketing of any forage plant. However, seed yield in perennial ryegrass, as in other fodder plants, has received less attention from plant breeders than feed production and quality, as these species are mainly used as fodder.Lolium perenne seed production is still low, uncertain and little is known about the production capacity of seeds of different varieties. The delimitation of the area with the help of satellite maps and the description of the area studied through the administrative and geographical description but also from the point of view of the meadows that make it up. Description of the plant associations from which the biological material of Lolium perenne was taken.*

Keywords*:perennial lolium, variety, grasslands, biodiversity,germolpasm.*

INTRODUCTION

Meadows cover 70% of the world's agricultural area and support crop and animal husbandry systems that contribute to the livelihoods of more than 800 million people worldwide (2013). In addition, they produce feed for many of the animals that graze in both developed and developing countries, provide crucial ecosystem services, including carbon sequestration, soil protection and enrichment, and biodiversity conservation (NORTON M. R. ET AL., 2016).

Lolium perenne is a predominant forage plant for temperate pastoral production globally because it grows rapidly (LEE J. M. ET ALL., 2010) providing dense assortments of highly nutritious and easily digestible fodder that can be transformed into healthy meat and animals for human consumption. However, their use is restricted because they lack persistence, especially in areas and marginal locations that are subject to summer and winter stress. Species with close relatives of the genus *Festuca* are much better adapted to abiotic stresses, but, on the other hand, do not compare well in providing animal feed with *Lolium* species, because they have poor stabilization and lower quality characteristics. *Lolium* and *Festuca* species hybridize naturally and have high frequencies of gene exchange in the hybrid state. Intergeneric hybrids (*Festulolium*) between *Lolium* and *Festuca* species are used to broaden the genetic basis and provide the plant breeder with options to combine high-quality traits with broad adaptations to a range of environmental constraints (YAMADA T. ET ALL., 2005).

In perennial forage species, the seeds were marketed in Europe before World War II, but these seeds were produced from strains with acceptable yield without regard to forage performance. Moreover, at that time, farmers used to sow ryegrass seeds harvested from local natural stems. However, early fodder breeding programs began in the United Kingdom as early as 1919 and the first variety was launched there in 1931. More important efforts to reproduce perennial fodder plants were initiated in the 1950s and 1960s by public institutes and breeding companies, mainly in Europe, North America, New Zealand and Australia. Since these first improved strains, recurrent selection programs have cyclically recombined elite genotypes and launched increasingly improved varieties to date (SAMPOUX J. P. ET ALL., 2011).

Meadows, pastures and hayfields are natural ecosystems and are dominant elements of the rural environment, having a great biological diversity compared to cultivated areas. In Romania, these natural ecosystems still have a large share, given that only in the mountainous area there are 3.2 million hectares of agricultural land, of which about 2.5 million hectares of permanent meadows. Nationally, there are over 4.8 million hectares of permanent meadows and they are an important source of fodder for the livestock sector in agriculture. In the Oltenia region, from the agricultural area of about 1.9 million hectares, about 465 thousand hectares presenting pages, having a large share in the hill and mountain regions (CIOBOATĂ M. N., 2010, http://cis01.ucv.ro/lucrari_dr/docs/79_rez-ro.pdf).

Meadow and depression meadows have grassy vegetation dominated by the following species: *Agrostis stolonifera* (field grass), *Alopecurus pratensis* (foxtail), *Poa pratensis* (thread), *Lolium perenne* (lawn grass, perennial ryegrass), *Arrhenatherum elatius* (oats) and *Festuca pratensis* (orchard fescue), as well as other very valuable forage species that have already been introduced into the culture. The pastoral value is good to very good, with productions of 7.5-15 t / ha MV, depending on the type and mode of maintenance ((https://lege5.ro/Gratuit/gu3dinrwgm/principalele-tipuri-de-pajisti-si-raspandirea-lor-hotarare-78-2015?dp=g42dqnbsgqzdi). On the higher hills there are meadows in the forest area made up of grasses: forest fescue (*Agrostis tenuis*), sheep fescue (*Festuca pseudovina*), English ryegrass (*Lolium perenne*), being very widespread. There are also grasses such as: orchard fescue (*Festuca pratensis*), twig (*Poa pratensis*), bramble (*Dactylis glomerata*) and others (http://www.madr.ro/attachments/article/223/ADER-1111-faza- 1.pdf).

Pastures based on *Lolium perenne* and *Trifolium repens* are the foundation of production and profit in pastoral sectors, and the improvement of these species offers direct opportunities to increase the performance of the sector, such as the forage value index (Barrett B. A. et al., 2014). The compatibility of these two forage plants is defined as the ability of the two species to grow together and produce large crops of forage plants, by optimizing the benefits of nitrogen fixation and superior feed quality (COLLINS R. P. ET AL., 2003).

Lolium perenne is not only a high yield and good quality fodder, but also a kind of lawn suitable for home lawns, parks, golf courses, but currently the global water supply is declining and soil salinity is growing, and the cultivation of perennial ryegrass with a higher salt tolerance would have important economic and social benefits (WU Y. ET ALL., 2005). Is an important source of nutrition for ruminants, but it is known that they do not provide enough nutrition to meet the genetic potential of production in animals. One of the reasons for this deficiency is the inaccessibility of feed fibers which are structural components of forage plants and are a source of energy for animal grazing, due to the cell wall content of polysaccharides (hemicellulose and cellulose), (FAVILLE M. J. ET AL., 2010).

Reproductive techniques are used to produce varieties with desired traits, such as high water-soluble carbohydrate content, crude protein content and digestibility. In addition to the yield of fodder and seeds, resistance to pests and diseases a special and relevant interest for this study, are sugar-rich varieties that have high levels of fructans.

Fructans are the main storage carbohydrates in perennial ryegrass and are made up of different grades and complexities of linear or branched fructose polymers, denoted by the degree of polymerization that directs the accumulation of fructan and therefore the total sugar content. Varieties with high sugar content are proposed to increase milk and meat production through increased use of protein by ruminants (SUBBARAJ A.K., ET AL., 2019).

Perennial ryegrass yield is a complex trait influenced by several interactional morphological features, which include leaf appearance interval (ALf), leaf elongation duration (LED), leaf elongation rate (LER), leaf blade length (LL) , rudder weight (TW), chain number (TN) and seat filling (Fs). All these characters are quantitative traits and are interdependent in their contribution to feed growth. Identification and selection of component traits has been proposed as a means to increase genetic gain for complex traits in plant reproduction (SARTIE A. M. ET ALL., 2011). It is essential that the yield of perennial ryegrass increases, requiring a doubling of the rate of increase in yield in order to support the world's population, which will be increased by 2050. *Lolium perenne* production was first tested in late 1970, initially obtaining only embryos and albino plants, until the first green regenerants were reported in 1984.

In the following decades, contemporary protocols for barley and wheat were adapted for the use of perennial ryegrass to optimize pre-culture temperature stress, a source of carbohydrates in vitro, by adding growth regulator and culture conditions such as light and temperature. Thus, the total number of regenerated plants increased, although the percentage of bees remained high, and genotypes capable of producing green plants by androgenesis were described as rare exceptions. For example, only 71 of 229 genotypes, derived from 15 varieties, produced regenerants and only one genotype produced plants (BEGHEYN R. ET ALL., 2016).

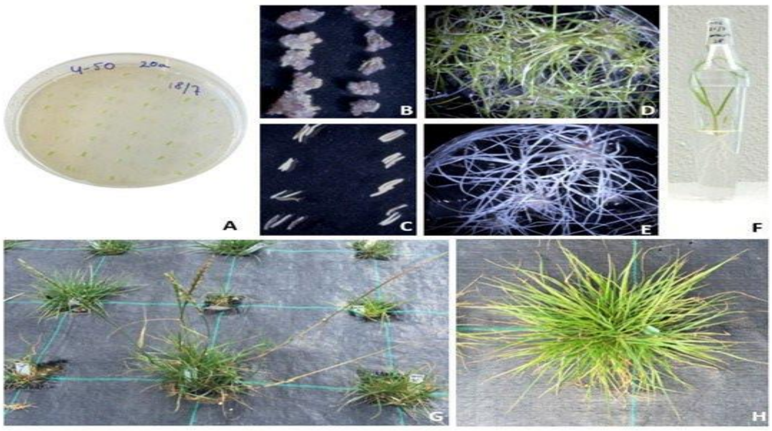

Figure 1. Different stages of perennial ryegrass *(Lolium perenne* L.) anther culture (AC) and doubled haploid (DH) production. (A) Fresh anthers on DH induction medium; (B) anthers of a responsive donor genotype producing many embryo-like structures (ELS) on DH induction medium, six weeks post AC; (C) anthers of an unresponsive donor genotype, six weeks post AC; (D) green and albino putative DH plantlets growing from ELS cultured on DH regeneration medium, four weeks post subculture; (E) albino plantlets growing from ELS, four weeks post subculture; (F) putative DH plantlet on regeneration medium; (G) flowering DH plant in the field; and (H) a vigorous vegetative DH plant in the field (photographs by BEGHEYN R. ET ALL., 2016).

Improving the tolerance to grazing and drought of temperate perennial ryegrass is also an important component of improvement programs, which are best based on an understanding of the physiology of forage growth and development. It was concluded that the density of cultivator and buds could be increased for new varieties because some existing varieties probably have too low a density of the sower (KEMP D. R., CULVENOR R. A., 1994). Over the last 40 years, major investments have been made in the fodder industry to produce new varieties and independent testing systems designed to identify and list the best performance. The yield of the dry matter and the density of the new varieties deposited from 1973 to 2013 and the digestibility of the forage plants from 1980 to 2013 were compared in the management of storage and simulated grazing. Dry matter yields showed a significant average annual increase (p <0.001) average of 0.52% in conservation conditions and 0.35% in the case of simulated grazing. These rates were not constant over time, and the periods without gain took place in different groups of varieties. Only at the beginning of the twentieth century began to appear grass varieties with good agronomic performance, the growth of perennial ryegrass being focused mainly on yield and persistence since the 1970s (MCDONAGH J. ET ALL. , 2016).

Of the fodder and with the production of biomass. However, the senescence of the leaves occurs naturally with the aging of the leaves and can be induced or accelerated by abiotic stress, which can negatively affect plant growth and productivity. Understanding the molecular mechanisms that control leaf senescence is of great importance for perennial forage plants harvested for green leaves with high nutritional values (XU B. ET ALL., 2019).

The genetic structure and morphological diversity of forage species may result from restricted gene flow. Direct methods of estimating gene flow are problematic and can provide estimates only for a relatively restricted area and over a short period of time. For the past 30 years, isozyme markers have been widely used in the study of genetic structure in plant populations, and forage species, which dominate many temperate agricultural and semi-natural areas, have been no exception. Most studies on the genetic variation in the population of forage species have analyzed those species that are important for agriculture that are widely sown as fodder, and perennial ryegrass is probably the most important species of forage grass and has experienced massive amounts of gene flow. by sowing selected varieties (WARREN J. M. ET ALL., 1998). Identifying and selecting relevant traits is a means of enhancing genetic gain in plant reproduction by selecting positively correlated components with sufficient heredity. As a molecular strategy for accelerating trait improvement in forage plants, genomic selection, supported by low-cost single nucleotide polymorphism labeling systems, will replace marker-assisted selection using quantitative trait loci-linked molecular markers (QTL) (SARTIE A. M. ET ALL ., 2018).

Seed yield is a measure of the total marketable seed recovered from the harvesting and processing of a seed crop. An economic level of seed yield is vital for the successful production and marketing of any forage plant. However, seed yield in perennial ryegrass, as in other fodder plants, has received less attention from plant breeders than feed production and quality, as these species are mainly used as fodder.

Seed storage leads to certain physiological and biochemical processes that result in aging and decreased seed germination and conservation depends on plant species and storage conditions (STANISAVLJEVIC R., ET AL., 2011) .

Production of Lolium perenne seeds is still low, uncertain and little is known about the production capacity of seeds of different varieties. Therefore, the genetic variation for seed yield was studied for three years in two locations, consisting of plots with nine diploid varieties, with late flowering, in four replications where significant genetic differences were detected for seed yield and seed weight, and the classification of varieties for seed yield was different from that for seed weight. Seed production levels and weight were affected by environmental factors such as year, soil type and year of crop production, but the interactions of these factors with varieties were generally not significant. The highest yielding variety was superior to a wide range of media, and the seed yield of the poorest variety was on average only 64% of that of the best variety (ELGERSMA A., 1990).

The Iron Gates Natural Park is a protected area established by Law no. 5/2000 on the approval of the National Territorial Plan - Section III - protected areas. It is an important area for geoconservation in Romania, as its geological heritage is among the richest in the Southern Carpathians. Crossed by the Danube, it has unique features from a paleontological, structural and morphological point of view (POPA M. E., 2003) with beautiful landscapes and biological diversity, where unaltered traditions predominate (BOENGIU V., 2012).

The Iron Gates Natural Park is located in southwestern Romania, located on the administrative territories of Caraş-Severin and Mehedinți counties. It covers an area of approximately 115,665.8 ha, most of which includes the geographical region known as the Danube Gorge or Danube Gorge. The main form of relief are the mountains, it includes the southern extensions of the Banat Mountains (Locvei Mountains and Almăjului Mountains), Mehedinți Mountains and part of the Mehedinți Plateau. It is one of the largest natural parks in Romania (2nd place), with 18 reservations (https://ro.wikipedia.org/wiki/Parcul_Natural_Por%C8%9Bile_de_Fier).

The primary vegetation of the meadows is very much modified by the anthropic interventions, and the meadows are quite limited in area, being interspersed between forests and agricultural lands. The group of xeromesophilic associations is located on slopes and on other morphological surfaces with varied exposure, at altitudes of over 300 m, on the place of cleared gorunetes and beeches. There is the typical xeromesophilic variant for the sunny and strongly inclined slopes and the xeromesophilic variant on the semi-sunny and low inclination slopes. The meadows of this genus are those with obsiga-*Brachyopodium pinnatum*, zâzanie-*Lolium perenne* and, especially, with sardine-*Chrysopogon gryllus* (www.mmediu.ro).

Plateau Mehedinţi Geopark is a protected natural area with a territory where the remarkable beauty of landscapes and biological diversity can be capitalized while preserving traditions, and improving the quality of life of communities to be the result of economic activities of inhabitants, carried out in harmony with nature. (http://www.geoparcmehedinti.ro/despre-geoparcul-platoul-mehedinti).

Figure 2. *Lolium perenne* vegetation in Eibenthal (original photo)

The flora of the national park consists of plant species (trees, shrubs and grasses) distributed in accordance with the geological structure, soil and climate characteristics, geomorphological structure or altitude (https://ro.wikipedia.org/wiki/Geoparcul_Platoul_Mehedin%C8%9Bi#Flor%C4%83)

Blahnița Plain is a subdivision of the Romanian Plain, located in the southwestern part of Romania, is part of the Oltenia Plain, has a general orientation NW-SE and includes eight terraces of the Danube that are covered with sand dunes (https: // ro.wikipedia.org/wiki/C%C3%A2mpia_Blahni%C8%9Bei) and includes the following localities: Vânători, Rogova, Vânjuleț, Pătulele, Livezile, Poroina Mare, Bălăcița, Vlădaia, Padina, Corlățel, Punghina, Oprișor. Mehedinți), Vînju Mare (city, Mehedinți county). In the southeast lies the lowest stage - that of the Oltenia Plain (Blahnita Plain, with altitudes between 200-400m), (https://www.ropedia.ro/judetul/Mehedinti). From the category of mesophilic intrasonal meadows reunited in this alliance, areas occupied by *Lolium perenne* with *Trifolium repens* have been identified, which are found in flat and slightly trampled places, on uneroded soils and with more pronounced humidity (RĂDUȚOIU D. ET ALL., 2010).

Timiş County is located in the west of the country, it is bordered on the north by Arad County, on the east by Hunedoara County, and on the south by Caraş-Severin County (file:///C:/Users/Ramona/Downloads/Monografie_Timis_2012.pdf).

The Western Plain occupies over 85% of the territory of Timiş County, with the subunits of Timiş Plain, Mureș Low Plain, Arancai Plain, Jimboliei Plain (low plains), Vingăi Plain, Lugoj Plain, Bârzaaltei Plain (https: ropedia.ro / county / Timis). In the low plains there are grassy plants (fescue and colilia), clumps of misty oak, fluffy and pedunculate oak, locally there are meadows that love moisture, salt or sand (MESAROȘ C., 2013).

MATERIALS AND METHODS

The study of plant associations is performed by delimiting the area with the help of satellite maps and collecting the biological material of *Lolium perenne*, but also the description of plant associations. The ecotypes come from two regions of Romania: Oltenia Region, southwestern area, Mehedinți County and Banat Region, Timiș County, located in the west.

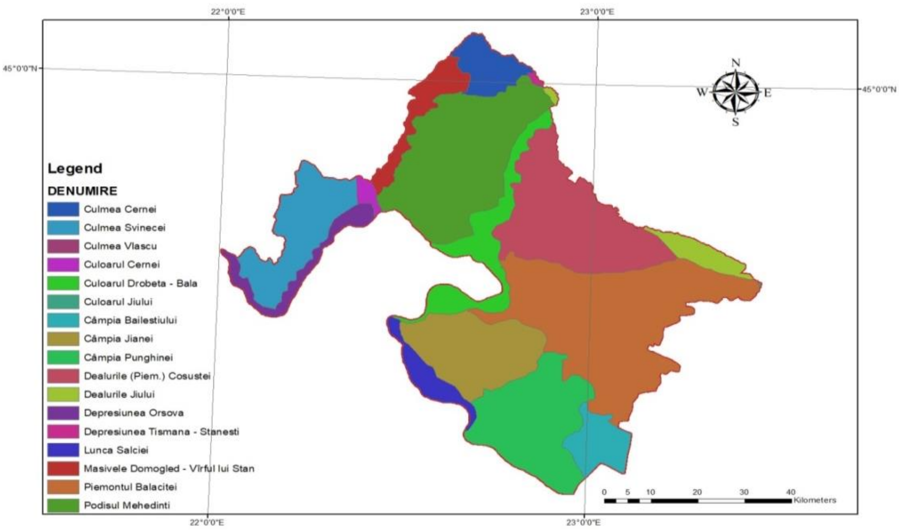

Figure 3. Satellite map of Mehedinți county

Location – Balta, Mehedinti County
Pasture exploited for grazing cows and horses
Predominant vegetation *Lolium perenne*, *Trifolium pratense* +, *Trifolium repens*
Present: *Taraxacum officinale*, *Achillea milefolium*.

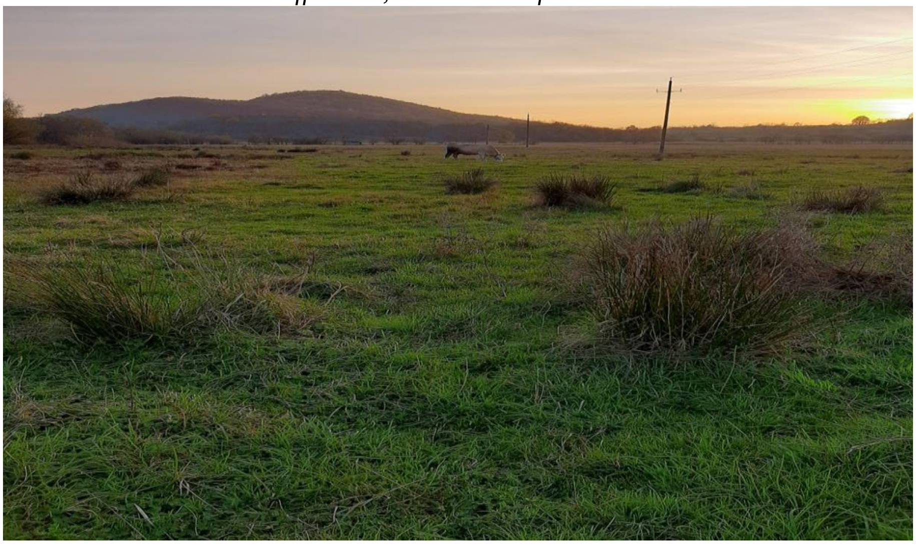

Figure 4. Meadow from Balta commune, Mehedinți county

Location 4 - Șișești
Meadow exploited for sheep grazing.
Vegetation dominated by *Lolium perenne, Trifolium repens*
Present: *Taraxacum officinale*, *Achillea millefolium, Ranunculus repens, Plantago lanceolata.*

Figure 5. Meadow from Șișești commune, Mehedinți county

Location - Bencecu de Jos, Timis County
Meadow used for grazing sheep.

Dominant vegetation: Perennial lolium

Present: *Trifolium repens, Cynodon dactylon, Polygonum multiflorum, Xanthium spinosum, Cichorium intybus, Juncus conglomeratus e*tc.

Figure 6. Meadow from Bencecu de Jos commune, Timiș county

Location - Vinga, Timis County

Vegetation: *Lolium perenne, Agropirum repens, Cynodon dactylon, Euphorbia, Trifolium repens, Daucus, Cicorium intibus, Plantago lanceolata*.

Figure 7. Meadow from Vinga commune, Timiș county.

The biological material of Lolium perenne collected is used in the breeding process by analysis and characterization "in situ" and "ex situ". The collection field for the study is made at the Lovrin Agricultural Research Station, where the biological development of the collected material is followed.

The study of the species of *Lolium perenne* is performed by determining the ecological and biodiversity indices, performing weekly measurements with the registration of temperatures and precipitations. A plant-soil quality analysis of the soils in the collected area is performed.

RESULTS AND DISCUSSIONS

The pratological study of the *Lolium perenne* ecotype is completed by collecting the seeds from the collection field to be multiplied. Discussions are taking place on the results obtained both on seed collection and on the data obtained from the analysis of the soil in the collected areas.

The germplasm of the natural population of *Lolium perenne* is important for reproduction due to its adaptation to a wide range of climates. Climate-adaptive genes can be detected by associations between genotype, phenotype and climate (BLANCO-PASTOR J. L. ET ALL., 2020). There are opportunities to increase the rate of genetic gain for perennial ryegrass through molecular reproduction approaches, such as genomic prediction and hybrid reproduction. (SI) in feed species (BYRNE S. L. ET ALL., 2015).

Leaving the excess perennial ryegrass leaf in situ causes severe damage (vaguely referred to as "apparent winter killing") and temporarily affects the productivity of the forage species the following spring. New germinated grass from the previous year's seed crop helps regenerate plants, compensating for yield losses (BUCKINGHAM D. L. ET ALL., 2011).

CONCLUSIONS

The motivation for this work is to discover productive varieties from a forage point of view that are resistant to biotic and abiotic stressors.

The scientific conclusions drawn from the practical studies carried out, allow us to recommend the maintenance of the studied meadows, in order to be valuable from a fodder point of view.

O'CONNOR T.G., 1991 argues that changes in temporal and spatial grazing patterns in human-influenced systems have created the possibility of local extinction. The catastrophic mortality of the established populations appears as a consequence of the droughts in combination with the sustained grazing, which ultimately leads to the elimination of the population. These species cannot form persistent seed banks due to their limited intrinsic ability (2-3 years) to survive when in a state of induced or forced rest.

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