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REVIEW ON THE GENETIC AND BIOCHEMICAL CHARACTERIZATION OF *LOLIUM PERENNE* SPECIES

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Abstract: *Lolium perenne is a forage species still widespread as a natural population and is found in most of Europe, but also in part of the Mediterranean area and the Middle East. Due to the rapid growth and the ability to persist by asexual reproduction the species does not have to be sown every year, however frequent pruning makes it impossible to reproduce by seed. Genetic studies applied to the forage species L. perenne are needed to better understand the extent of intraspecific variation, how this diversity is divided in and between populations, and to help deduce phylogeographic patterns. The genus Lolium contains nine both inbred and inbred species, of which only Lolium perenne and Lolium multiflorum are the most important taxon from an agronomic point of view and are widely cultivated as forage species. In the family of the forage species Lolium perenne, polyploidy is predominant, being present both autopolyploids and allopolyploids. Productivity and persistence improvements have so far been achieved through hybridization and recurrent selection, or through the use of polyploidy. However, researchers consider it necessary to increase tolerance to limiting factors of production such as frost, drought and heat, resistance to pests and snow molds to expand the geographical area of the species. The genetic advances in the performance of Lolium perenne as a forage species, especially in pasture conditions, remain a constant challenge along with obtaining a good yield.*

Key words: *Lolium perenne, genetic advances, yield*

Importance and dynamics of the species *Lolium perenne*

In the last half century, the intensification of agriculture has led to the reduction of crop diversity in both field and landscape. A consequence of the reduction of diversity has been a decrease in regulation and support of agroecosystem services, such biological pest management and the nutrient cycle, which helps maintain crop productivity and yield stability (LOWRY, C.J., 2020). *Lolium perenne* is a forage species still widespread as a natural population and is found in most of Europe, but also in part of the Mediterranean area and the Middle East (BALFOURIER, F., 1998). It grows best on heavy, fertile, moist soils, but also on lighter soils with sufficient moisture, is often a dominant species in pastures and has a high potential for genetic diversity in the population (NIZAM, I., 2020).

The species, being over time the object of an intensive research, obtaining a considerable genetic progress. The cumulative dry matter yield of ryegrass varieties has increased between 0.3 and 0.9% per year over the last 40-50 years for summer and autumn dry matter production, but spring dry matter production has remained close unchanged. Feed-type ryegrass varieties with different maturity groups have been developed, from high-yielding early to late adapted to a long grazing season and with good growth in summer and autumn (BACHMANN-PFABE, S., 2018).

Elements of karyotypic characterization and genetic maps

The genus *Lolium* contains nine species, both inbred and inbred species, of which only *Lolium perenne* and *L. multiflorum* are the most important taxon from an agronomic point of view and are widely cultivated as forage species (GUAN, X., 2017). Both species are diploid (2n = 2x = 14), wind-pollinated with heterozygous individuals, and natural and synthetic populations are very heterogeneous in nature (WANG, J., 2014). *Lolium perenne* is thus a cross-pollinated species and the populations of varieties are formed from a background of heterozygous nuclear genome (DIEKMANN, K., 2009). The genetic relationships between adhesions and the biogeographical distribution of haplotypes were investigated using population genetic diversity measures and molecular variance analysis. The investigation concluded that much of the diversity of the European *L. perenne* ecotype (61%) could be attributed to population variation, finding evidence that also shows a probable migration path of *L. perenne* from the southern regions of Europe to the north (MCGRATH, SK., 2008).

Intraspecific genetic diversity is important for long-term population persistence for two reasons: genetically determined phenotypic variation is equivalent to population adaptive potential and neutral genetic variation of natural populations reflects inbreeding and genetic drift, which reduce population viability (KAHILAINEN, A., 2014). Many plants in the natural flora, including perennial ryegrass, lose their genetic variability over time, suffer from genetic pollution or disappear, research results have indicated that higher genotypes of perennial ryegrass could be used in future breeding programs (OZKOSE, A. & TAMKOC, A., 2014). Studies of the genetic diversity of ryegrass using morphological characteristics, initially focused on natural populations, to establish collections of valuable genetic bank materials and to help understand the processes of diversification in response to ecological factors. Following the development of molecular genetic marker systems, these technologies have been applied to studies on the diversity of both non-domesticated (spontaneous) ryegrass germplasm and those cultured in order to understand genetic relationships (WANG, J., 2016).

The implementation of molecular markers in breeding programs allows early and efficient selection of desired genotypes. Molecular markers can be used for DNA profiling to provide the information needed to identify the variety and certify seed purity (JONES, E.S., 2001), or provide useful information to help create improved populations adapted to future regional climates. Molecular markers have also been shown to be effective in characterizing the overall diversity of ryegrass (KEEP, T., 2020), the study of genetic variation requires knowledge of genomic sequences to develop new varieties. The genome of the *L. perenne* chloroplast was sequenced using plant material, thus detecting several polymorphisms with unique nucleotides during the assembly of the chloroplast genome. Such polymorphisms offer great potential in the development of markers for the study of genetic variation in and between populations of *L. perenne* and its close relatives (DIEKMANN, K., 2012). In recent years, more and more authors have advocated the use of markers that will improve the efficiency of breeding programs, (TURNER, L.B., 2010), the first map of the molecular genetic link of ryegrass was developed by crossing a perennial ryegrass with an interspecific hybrid of Italian ryegrass. The map had 106 loci, including a restriction fragment length polymorphism, randomly amplified DNA polymorph. It also contained a large number of isozymic markers and a length of 692 cm (SIM, S.,2005). In this case, genetic maps facilitate the study of genome structure, evolution, but also the identification of monogen, traits or Mendelian components of quantitative traits (QTL), necessary in breeding programs (BERT, P.F., 1999).

Genetic structure to support the productivity of *Lolium perenne* species

Genetic studies in *L. perenne* are needed to better understand the extent of infraspecific variation, how this diversity is divided into and between populations, and to help deduce phylogeographic patterns (MCGRATH, S.,2007).

The condition of plant polyploidy has adaptive importance in the evolution of species, ryegrass being the most desired forage species as a presence in the vegetation but also a key component of the most productive pastures (KATOVA, A., 2008). Although artificial polyploids have been created in the perennial ryegrass forage species, natural polyploidy has never been found, unlike the *Festuca* genus (TERRELL, E.E., 1966). Ryegrass is primarily diploid, but a tetraploid variety ($2n = 4x = 28$) has also been created to improve feed productivity and quality. As a self-incompatible species, the perennial ryegrass has a high degree of genetic diversity in the population, characterized by morphological features such as the vigor of the shoots, the width of the leaves that ensure a high forage yield (CUI, Y., 2015). In the *Gramineae* family, polyploidy is predominant, being present both autopolyploids and allopolyploids, there are even different reproductive systems, but the most common is the hermaphrodite flower. Cross-pollination in species with hermaphrodite flowers is imposed by a gametophytic genetic self-incompatibility controlled by two multiallelic and independent loci, S and Z.

The incompatibility phenotype of the pollen bean is determined by its haploid genome and the pollen bean is incompatible when the same S and Z alleles carried by pollen are present in the pistil (DO CANTO, J., 2017). The pollen grain is incompatible if its S and Z alleles are present in the female genotype. Where gametophytic genetic self-incompatibility is present, the development of varieties in synthetic or population varieties is limited, maintaining high levels of heterozygosity and heterogeneity and often segregation for alleles is harmful (DO CANTO, J., 2017). Self-incompatibility in plant populations ensures high levels of heterosis as well as genetic diversity that lead to greater resistance, adaptation to environmental changes. However, the existence of an effective self-incompatibility system in fodder crop species is problematic because the harvest to improved methods is limited (SLATTER, L.M., 2020).

To date, there are few published studies on genotypic differences in perennial ryegrass in growth responses, especially in cold climates. Cold climate stress occurs at sufficiently low temperatures (0 to 15 °C) and can cause damage to plant tissues, while freezing stress causes damage at temperatures below 0 °C, but plants in temperate regions are considered tolerant at varying degrees of cooling. Stress at low temperature also inhibits various metabolic reactions, which lead to changes in phenotypic characteristics (FÖRSTER, L., 2018). Improving winter hardiness is an important goal in breeding and marker-assisted selection has the potential to increase breeding efficiency for improved cold tolerance (SKØT, L., 2002).

The nature of the ryegrass reproductive system is the reason for the genetic progress made in breeding programs based on gene selection and sexual recombination. Continuous selection of whole clones or only from shoots contributes to the improvement of pastures, as the desired traits are evaluated after each generation; however, progress is quite slow

(ESQUEDA, M.K., 2017). There is, however, a gap in how the combination of plant species genotype and botanical composition would affect the performance of mixtures under different environmental conditions (HESHMATI, S., 2020).

Adaptations of *Lolium perenne* to climate change

To date, improvements in the species' productivity and persistence have been achieved through hybridization and recurrent selection processes, using both the polycross method and the polyploidy. It is considered that improvements in frost, drought and heat tolerance, pests and snow resistance will be needed in the future to achieve the expansion of the geographical area of the species (WILKINS, P.W., 1991). Studies show that drought will increase in frequency and severity in temperate regions due to climate change, and evidence suggests that highly productive grasslands will be more affected by drought than low-productivity grasslands. An understanding of drought-related mechanisms could serve to improve their performance in soil water deficit conditions through management and / or reproduction practices (HOFER, D., 2017). Also, pathogens significantly reduce the yield of green and dry matter as well as feed quality, researchers estimate that in the future will be needed varieties resistant to the alternation between dry and humid climate (BRAZAUSKAS, G., 2013). The germplasm from the natural population of *Lolium perenne* is useful for reproduction due to its adaptation to a wide range of climates. Climate-adaptive genes can be detected from associations between genotype, phenotype and climate, but an integrated framework for the analysis of the three sources of information is missing (BLANCO-PASTOR, J.L., 2021).

CONCLUSIONS

Studies in the field show that today, a complete range of perennial ryegrass varieties is available for the agricultural industry, from early flowering diploids to late flowering tetraploids. Genetic advances should be made from several germplasm using a combination of traditional breeding techniques with new technologies, such as marker-assisted selection. However, an overall performance of the forage species of *Lolium perenne* in pasture remains a challenge, as ryegrass varieties are used in mixture with white clover to obtain a good yield (STEWART, A., 2006). High-yielding genotypes of *Lolium perenne* can be improved by monitoring the efficient characteristics of the plant, which leads to the reproduction of new varieties, and the main goal in breeding forage plants is to develop and produce new superior genotypes. Only by breeding can a new variety of crops be cultivated, with high yield and resistance to pests and diseases (AYGÜN, C. & OLGUN, M., 2013).

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