

## ANALYSIS OF THE FREQUENCY AND DISTRIBUTION OF GENETIC VARIATION IN PERENNIAL RYEGRASS POPULATIONS

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**Abstract.** Grasslands play an important role in contributing to carbon sequestration, soil biogeochemistry and maintaining biodiversity. Genome analysis studies have suggested that species of the subfamily Pooideae have a similar chromosomal structure, being derived from a common ancestor with 7 pairs of chromosomes. Among the species of this family, *Lolium perenne* stands out due to its high agronomic productivity influenced by climate and soil conditions and hereditary traits such as flowering time. The natural variation at phenotypic and genotypic level is ensured by a reproduction system that requires cross-pollination, producing pronounced genetic variations in the morphological and molecular characteristics of and among its many varieties. Analysis of the frequency and distribution of genetic variation in natural perennial ryegrass populations has supported the view that its center of origin is the Middle East, and its distribution has expanded following a clinical geographic model, most of which is in Europe. At present it is considered that due to its excessive nature and recent domestication the genetic diversity of the varieties is very high. Reproduction programs generate extensive phenotypic data covering the genetic and phenotypic diversity of the germplasm used annually to select the best performing plants or populations. We consider in the light of these scientific results obtained globally that the species *Lolium perenne* through genetic diversity is a potential resource for the development of reference populations specific to genomic selection. The genetic improvement of perennial ryegrass provides an inexhaustible source of increasingly diverse genetic diversity in the world in terms of the challenges of environmental factors.

**Keywords:** genome analysis, genetic variation, perennial ryegrass

### INTRODUCTION

The *Poaceae* family is known taxonomically with over 10,000 species characterized and classified according to a variety of morphological characters related to inflorescence and spicule, but also the multitude of micromorphological and molecular characters (KELLOG, 2006). Genome analysis studies have suggested that species in the subfamily *Pooideae* have a similar chromosomal structure, being derived from a common ancestor with 7 pairs of chromosomes (SHINOZUKA ET.AL., 2012).

*Lolium perenne* is distinguished from the species of this family due to its high agronomic productivity influenced by climate and soil conditions and hereditary traits such as flowering time (GAGIC ET. AL., 2015). Taxonomically, *Lolium perenne*, or perennial ryegrass, is a diploid species ( $2n = 2x = 14$ ), classified in the subfamily *Pooideae* (PFEIFER ET. AL., 2013), the family *Poaceae* (grass and cereals).

The genome of *Lolium perenne* has a chloroplast genome (cp) of 135 282 bp, a typical quadripartite structure, with genes for 76 unique proteins, 30 tRNAs and 4 rRNAs. As with other forage plant species, the *accD*, *ycf1*, and *ycf2* genes are absent (Diekmann et. al., 2009). *Lolium perenne* is a wind-pollinated, self-incompatible breeding species, and Balfourier et al. (2000) reported significant patterns of geographical variation in terms of diversity indices and allele frequencies observed in perennial ryegrass (cited by FERREIRA ET. ALL., 2015).

In nature, *Lolium perenne* varies phenotypically and genotypically, probably due to a reproductive system that requires cross-pollination, in addition, it has pronounced genetic

variations in morphological and molecular characteristics of and among its many varieties (CHEPLICK, 2008).

### **The frequency and distribution of genetic variation in perennial ryegrass**

Analysis of the frequency and distribution of genetic variation in natural perennial ryegrass populations has supported the view that its center of origin is the Middle East, and its distribution has expanded following a clinical geographic model (SENDA ET AL. 2005), most of which are in Europe but also in part of the Mediterranean area (BALFOURIER ET. AL., 1998), and due to its excessive nature and recent domestication, a high degree of genetic diversity is expected among varieties (BRAZAUSKAS ET. AL. , 2011).

The level and distribution of genetic variation in the collections of ecotypes of the genetic bank are still largely unknown, but are of great interest for the planning of breeding programs (BOLARIC ET. AL., 2005). Reproductive programs generate extensive phenotypic data covering the genetic and phenotypic diversity of germplasm, but this information has been used in isolation each year or generation to select the best performing plants or populations.

However, data are a potential resource for the development of breeding populations specific to the breeding program for genomic selection. Genotypic data from biological materials, such as leaf tissue or seeds, can be combined with phenotypic data to establish a reference population from which marker effects can be estimated (PEMBLETON ET. AL., 2018).

Molecular markers are effective in revealing structural models of genetic diversity by creating baseline collections to identify the major effect of QTL through genome-wide association studies (GWAS) and genomic prediction (GP) models. In perennial raigrass, genomic prediction (GP) has been used to predict the phenotypes of individuals evaluated as distant plants, the insane performance of offspring families, including half-siblings, and the vegetation performance of synthetic varieties (KEEP ET. AL., 2020).

Guthridge et al. studied six populations of *L. perenne* with a bulk sampling strategy and AFLP markers, where they found that the mutual relationships revealed by bulk sampling were consistent with the results obtained by individual analysis. By applying bulk samples, Nestmann et al. investigated the influence of composition on different pastures on the differentiation of *L. perenne* populations with SNP markers developed by Sretenovic Rajicic et al, and Byrne et al. used Genotyping-by-Sequencing (GBS) to analyze populations and with their new approach to “Genome Allele Frequency Fingerprints” (GWAFF) they were able to distinguish eight varieties of *L. perenne* (LIU ET. AL., 2018).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) has been shown to be the best method available for determining gene expression changes due to its ability to quantify target genes rapidly and accurately, even with very low expression levels. (Lee et. al., 2010), and molecular markers (DNA) have recently become available and allow the study of various traits, including vernalization response, feed quality, and disease resistance, and QTL mapping has proven to be a successful method.

For dissecting the genetic basis of complex traits from several important cultures since the 1990s (XING ET. AL., 2007).

Permanent old meadows are a real reservoir of genetic diversity compared to managed, fertilized and sown meadows, and in order to preserve this diversity, an extensive ex situ seed collection and storage program was carried out between 1979 and 1983 by Teagasc (Authority for agriculture and food development), taking a total of 534 sites, which were then propagated under isolation. In 1994, a European *Lolium kernel* collection program was launched, including 163 different accessions from different genetic banks to assess genetic diversity within this species (DIEKMANN ET AL., 2012). Naturally, *Lolium perenne* is a diploid

forage species; however, tetraploids have also been developed to improve feed quality and productivity.

As breeders continue to improve germplasm, future use of perennial ryegrass may be complicated if the ploidy level of known germplasm is unclear. Mixed ploidy levels in the perennial ryegrass population could be a challenge for marker development and gene identification, ultimately influencing marker-assisted selection to improve desired traits (WANG ET. AL., 2009) as well. marker-assisted selection has the potential to increase reproductive efficiency (SKØT ET. AL., 2002).

Reproduction has led to adapted varieties more under rare than frequent pruning, and reproduction for managing higher productivity under defoliation frequency should be considered (GHESQUIÈRE ET. AL., 1994). The creation of a genotyping matrix for *Lolium perenne* with over 2,000 validated markers provided the opportunity to interrogate germplasm in new ways, and reproducible and comparable estimates of diversity and linkage imbalance will identify selected genomic regions and help guide judicious choice of genetic diversity in reproductive programs (BLACKMORE ET. AL., 2016).

### CONCLUSIONS

Researchers consider it important to increase the tolerance of the *Lolium perenne* forage species to frost, drought and heat, as well as resistance to pests and snow molds to expand the geographical area of the species, and genetic progress in achieving performance as a forage species, especially in conditions of grazing, remain a constant challenge along with obtaining a good yield (Toporan and Samfira, 2021).

In order to maintain and increase the use of the *Lolium perenne* fodder plant as fodder in livestock farming, there is a need for continuous improvement in biomass yield, quality, disease resistance and seed yield, but genetic gain for traits such as biomass yield was relatively modest (Grinberg et. al., 2016),).

Adequate seed production and resistance to a wide range of diseases and pests are also required. Productivity and persistence improvements have so far been achieved through hybridization and recurrent selection using the polycross and polyploidy method (Wilkins, 1991).

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