

Harnessing Underutilized Gene Bank Diversity & Genomic & Phenomic Prediction to Improve Annual Ryegrass (*Lolium Multiflorum* Lamarck)

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Annual ryegrass (*Lolium multiflorum* Lamarck) is a cool-season forage grass broadly used for livestock feeding. It is an allogamous diploid species ($2n=2x=14$), and tetraploid ($2n=4x=28$) cultivars have been artificially created. Emphasizing dry matter yield, disease resistance, and climate resilience, the University of Florida's (UF) annual ryegrass breeding program, has released more than 40 cultivars since the 1980s. New efforts began in 2014 to introgress novel sources of genetic variability into existing advanced UF germplasm. Preliminary research revealed the ploidy level for 167 populations (139 accessions from GRIN, 15 breeding lines, and 13 commercial cultivars), and determined genetic parameters for 13 morphological and agronomic traits. Genetic diversity was assessed using 34 microsatellite markers (EST-SSR) in a total of 960 individual samples. Both STRUCTURE analysis and discriminant analysis of principle components revealed five distinct groups ($K=5$). The wild germplasm explained most of the genetic diversity, while cultivars, landraces, experimental lines, and unknown accessions clustered together. Later, 32 plants from 7 countries were visually selected in the field for disease resistance and plant vigor in Spring 2016. Plants were crossed to generate 54 full-sib families, and F1 full-sib families were grown in Fall 2017 to generate a polycross that resulted in 300 half-sib families. After seed threshing, 200 half-sib families were retained for field trials conducted in 2018 and 2023. An additional, 38 half-sib breeding lines from the UF program were included in field studies. The 238 half-sib families were grown in Citra, FL to phenotypically characterize the population for 14 traits, and families were genotyped using Capture-seq by bulking DNA from multiple individuals per family. 3,500 single nucleotide polymorphisms (SNPs) were used to build a genomic-relationship matrix (G) to develop genomic prediction (GP) models. Morphological and agronomic traits were measured in 2018 and 2023, and near-infrared spectroscopy (NIRS) was used to determine nutritive value in 2023. The spectra data from NIRS was used to develop a phenomic-relationship matrix (P) for phenomic prediction models (PP). BLUP-based models showed that GP provided greater predictive ability for dry matter yield (0.36 and heading date (0.73), and PP had greater predictive ability for canopy height (0.50) and tiller width (0.33). The introgression of novel germplasm into UF's breeding program and the application of advanced breeding methods will provide the basis for further genetic improvement in the species.