Genetic Diversity in the USDA-NPGS Annual Ryegrass Collection

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Despite its importance for breeding and germplasm conservation, an assessment of the genetic diversity present in the USDA-NPGS annual ryegrass collection has not been reported. The objective of this study was to assess the genetic relationship among/within accessions from the USDA-NPGS annual ryegrass collection, 15 experimental lines from the UF Annual Ryegrass Breeding Program and 13 commercial cultivars using 48 microsatellite markers (EST-SSR) with 556 polymorphic bands approximately. Approximately 100 mg of leaf tissue was placed in a 2.0 ml micro-centrifuge tube and ground to fine powder, and DNA extractions were performed with the DNeasy Plant Mini kit (Qiagen Inc, Valencia, CA). PCR reactions were prepared in a reaction volume of 10 µl with 20 ng of template DNA, 10× colorless GoTaq® buffer (Promega, California, USA), 0.15 mM dNTPs, 1.0 pmol of each reverse and M13 universal primer, 0.25 pmol of the forward primer, and 0.5 U GoTaq® polymerase (Promega). The M13 universal primer was labeled either with blue (FAM), green (VIC), yellow (NED), or red (PET) fluorescent tags. PCR products (3 µl) with different fragment sizes and different fluorescent labels were pooled and combined with 10 µl deionized formamide and 0.5 µl GeneScan-250LIZ internal size standard and analyzed on an ABI PRISM® 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The reactions were visualized and scored with *Fragman* package in R. For each marker, individual fragments were scored for each of the 960 individuals. Results according to population structure was assessed using STRUCTURE and DAPC with Neighbor Joining (NJ) and Principal Component Analysis (PCA) which were used to confirmed the relatedness within and between annual ryegrass collection. The Bayesian Analysis revealed that the collection of 960 accessions formed five distinct groups (K=5) and the PCA explained only 5.842, 10.983 and 14.620% of population variance respectively. The wild germplasm explained most of the diversity genetic with the discriminant power, and also cultivars, cultivated, experimental lines and unknown accessions clustered together.