Genetic Diversity of Tall Fescue (Lolium arundinaceum (Schreb.) Darbysh.) Cultivars and Collections Using Chloroplast Microsatellite (cpSSR) Markers

Vincenzo Averello, Christine Kubik, Jennifer Vaicunias, William Meyer, Stacy Bonos, and Josh Honig

Department of Plant Biology and Pathology, Rutgers, the State University of New Jersey, New Brunswick, NJ.

Introduction

Tall fescue (Lolium arundinaceum (Schreb.) Darbysh.) is a hexaploid C3-cool season grass used for forage and turf. It is generally a bunch type grass but some genotypes produce rhizomes. Tall fescue has three recognized types: Continental (from Northern Europe, summer active, and the source of most cultivars), rhizomatous (related to Continental tall fescue, from Northern Portugal and Galicia region of Spain), and Mediterranean (incomplete summer dormancy, from North Africa, and does not produce fertile offspring with the other 2 types) (Borriol et al., 1971).

Continental tall fescue includes the genomes of both tetraploid Lolium arundinaceum subsp. [fanes (synonym of var. glaucensceus)] and diploid Lolium pratense (meadow fescue). It is less clear which species are the progenitors of Mediterranean tall fescue but Festuca maior is a candidate (Dierking et al., 2015).

The tall fescue plastome was sequenced (NC_011713.2) and compared to other species in the poaceae family with sequenced plastomes at the time. This comparison showed that L. arundinaceum closely aligned with L. perenne; however, there was a long branch length, suggesting some time since divergence. (Cahoon et al., 2010)

This tall fescue dataset has recently been genotyped using nuclear SSR markers (Averello et al., 2015). This research found that most of these populations (all except Rhambler and Sixpoint) were distinguished by AMOVA and generally matched known breeding histories.

Materials and Methods

Plant Material

179 Populations - each composed of 16 individuals

– Tall Fescue Populations

• 9 Turf-type cultivars
• 5 Forage cultivars
• 16 unproven collections (Fig. 1)

– Populations of Other Species

• Meadow fescue (Lolium pratense), 3 tetraploid tall fescue (Lolium arundinaceum subsp. [fanas], perennial ryegrass (Lolium perenne), and annual ryegrass (Lolium multiflorum))

The plant material for cultivars and collections came from turf plots at the research farm in Adelphia, NJ. The outgroups were grown out from seeds obtained from USDA-ARS.

cpSSR Markers

The 18 cpSSR markers were designed from the complete tall fescue chloroplast genome (GenBank accession NC_011713.2) using Softberry 3.4 to identify more, dr., and trinucleotide repeats. Primer designs were performed with Primer3.

Analysis of molecular variation (AMOVA)

Pairwise genetic distance matrices were calculated for binary haplodi data with GenEx 6.502 (Peakall and Smouse, 2006, 2012). Sabove was then used to perform AMOVA on the distances, partition variance into within population variance and among population variance. Additionally, ANOVA was used to calculate pairwise PhilP values. Statistical significance of AMOVA results and pairwise population PhilP values was based on 999 independent data permutations.

UPGMA Clustering

The pairwise PhilP values were imported into NTSyspc.2.2tig (Rohlf, 2008) as a dissimilarity matrix to create dendrograms. The cpSSR module was used to generate a UPGMA dendrogram. Goodness of fit between the UPGMA dendrogram and the original inter population pairwise genetic distance matrix was tested using the Coph (cosinehethetic) value and MultiCoph (Mantel test) modules, with the number of test permutations set to 999.

Results

Primer Products

The 18 polymorphic loci produced a total of 85 alleles with an average of 4.7 alleles per locus with a minimum of 3 alleles and a maximum of 8 alleles.

Haplotype Summary Statistics

145 different haplotypes were detected, with 103 of these being private haplotypes (a haplotype limited to 1 population). Populations ranged from having 1 haplotype present to a maximum of 9 haplotypes, with a mean of 2.4 haplotypes per population and a median of 2 haplotypes.

Interpopulation Relationships (AMOVA and PhilP) (Data not shown)

• AMOVA results for the cpSSR markers showed that 58% of the total cpSSR marker variation was due to among-population variance and 42% was due to within-population variance.
• 0f the 19931 between population pairwise comparisons, 2873 were not significantly different.
• In a previous study with the same data set using genomic SSR markers all individual populations (except Rhambler and Sixpoint) were distinguished from each other.

Chloroplast SSR Dendrogram and Discussion

Since a large number of pairwise comparisons were not able to distinguish the populations as statistically different, especially when compared to the nuclear genomic SSRs cpSSRs are not suggested to be used for cultivar or population identification in tall fescue.

nuSSR (presented previously) and cpSSR dendrograms did not result in similar separation of cultivars and accessions.

In general, the nuSSR dendrogram clearly separated turf-type cultivars, forage-type cultivars, and the majority of collections but in the cpSSR dendrogram these groups were not clearly separated (Figure 2).

However, species relatedness is similar in both dendrograms. Two of the tetraploid tall fescue (Lolium arundinaceum spp. [fanes] populations (PI289651 and PI289654, both collected from Spain) clustered with the collections from Albani, Turkey, Italy, Latvia, and Romania. The third population (PI99035, collected from France) clustered with the cultivars Fawn, Kentucky 31, and JesupW4x (forage types) and Firecracker and Jamboree (turf-types).

The majority of the outgroup species clustered independently from the Turf-type tall fescue. Nine collections from Morocco and Italy, and the perennial ryegrass (Lolium perenne) (Derby Extreems), annual ryegrass (Lolium multiflorum), and meadow fescue (Lolium pratense) form a sister group at the bottom of the tree. These 10 collections form a distinct group, potentially representing the Mediterranean morphotype, from the outgroups that are not Lolium arundinaceum. Within that outgroup cluster the perennial ryegrass is sister to the other 4 members of this group. Both of the annual ryegrass populations and the meadow fescue form separate sister groupings.

Chloroplast SSR Dendrogram and Discussion – continued

While the forage cultivars are closely grouped in the nuSSR dendrogram tree, there were 2 separate groups in the cpSSR dendrogram. Teton grouped with a cluster of turf-type cultivars at the top of the tree while Atlas formed its own group altogether, sister to the group that included Teton, Fawn, Kentucky 31 and JesupW4x, form their own distinct grouping. PI995548, a tetraploid tall fescue population, is also in this group.

While the nuSSR tree resembled the breeding history of the tall fescue cultivars, the cpSSRs do not as closely resemble the breeding histories. Notably there was very little clustering of collections and turf-type cultivars in the nuSSR dendrogram, while this is not uncommon in the cpSSR dendrogram. Homoplasy (two markers being the same but having different evolutionary history) has been shown in other SSRs in other species (including humans and chimpanzees (Garza and Dyer, 1999) and citrus (Barley et al., 2009), this could be one possible explanation for the difference between the nuSSR and cpSSR dendrograms and the deviation from breeding histories.

In conclusion, cpSSRs alone are not sufficient to understand the diversity and population structure of tall fescue and related species but could be used for morphotype and species identification.

References


