Genetic Diversity in *Festuca* Species as Shown by AFLP

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Introduction Fescues (*Festuca* spp.) are widely occurring temperate grasses with more than 450 species that represent a vast resource for genetic improvement of turfgrass and forage cultivars. Fescues are normally outcrossing species and exhibit many ploidy levels(x=7). Much of the work in classification of *Festuca* is predominantly based on morphological and cytological features. Difficulties in morphological characterization, which are largely subjected to environmental influences, have resulted in many synonymous species and uncertainties in phylogenetic relationships. DNA fingerprinting is considered a more stable and reliable technique to explore genetic diversity and relationships.

Materials and methods To study the genetic diversity and relationships between fescue species, forty-six accessions from the USDA's germplasm collection representing thirty-seven species of *Festuca* from twenty countries were investigated using AFLP analysis. Selective amplifications for fescue were made using different primer combinations from the final products of EcoRI/Mse digestion, adapter ligation and pre-amplification. From the initial twenty selective primer combinations, nine combinations were chosen for clarity, repetitiveness in duplicated gel runs. Four hundred and forty-eight AFLP markers from 9 chosen primer combinations were used to differentiate between fescue accessions using a bulk of 25 genotypes per accession. Polymorphic bands were scored manually and analyzed using NTSYS v.2.1. Cluster analysis using the UPGMA was run to produce the dendrogram.

Results and discussion Initial testing of AFLP techniques on fescue species demonstrated that selective amplification with the *Eco*RI + 3 and *Mse*I + 3 primer combinations produced too many fragments (≈200) to be resolved on polyacrylamide gels, whereas *Eco*RI + 3 and *Mse*I + 4 primer combinations generally produced the desired number of fragments (40~100). a total of 448 AFLP (418 polymorphic and 30 monomorphic) fragments were scored from nine primer combinations. The E-AGC and M-CGCG primer combination produced the greatest number of polymorphic fragments (61), while the E-ACA and M-CTCG primer combination yielded the fewest polymorphic fragments (33). The majority of scored fragments were in the 80- to 350-bp range, however, fragments were found across the entire size range of 60 to 500 bp.

Genetic similarities between accessions ranged from 0.36 to 0.87 showing no duplication in the collection and a high level of diversity in *Festuca*. According to both principal component analysis and UPGMA dendrogram, all accessions can be clearly divideded into seven groups. Genetic relationships between fine-leaved species and broad-leaved species were revealed in the cluster groupings. Specific markers were found for *F. occidentalis, F. pallens, F. arizonica, F. pungens, F. novae-zelandiae, F. heterophylla, F. gigantea, F. altaica, F. ovina*. Our results support earlier observations based on morphological characters that broad-leaved taxa are distinct from fine-leaved species but closely related.