

Tall fescue expressed sequence tag and simple sequence repeats: important resources for grass species

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Introduction Expressed sequence tag (EST) databases have been growing exponentially. The simple sequence repeat (SSR) has become one of the most useful molecular marker systems in plant breeding and is widely used in cultivar fingerprinting, genetic diversity assessment, molecular mapping and marker-assisted selection. ESTs are a potential source for SSRs. The EST-SSR markers are of high quality and have versatile applications in molecular breeding (Bughrara *et al.*, 2003; Saha *et al.*, 2004 a, b, c). Here, we present an overview of our efforts to develop SSRs from tall fescue ESTs and their application for the genetic improvement of forage and turf grass species.

Materials and methods A total of 157 SSRs were developed from the first 20,000 tall fescue ESTs. Amplification, polymorphism and transferability across several grass species were evaluated. The EST-SSRs were mapped in a “two-way pseudo-testcross” tall fescue population, a three generation annual x perennial ryegrass population, and in creeping bentgrass. These have also been used for genetic diversity analysis in darnel ryegrass, fescue species, and Canada and Virginia wildrye populations. EST-SSRs were also used to track the *Festuca* genome introgression in *Festuca x Lolium* hybrids and backcross populations.

Results Among the tall fescue EST-SSRs, trinucleotide motifs were the most abundant (70%) type followed by dinucleotides (20%). A higher percent (92%) of the primers produced characteristic bands in at least one species (Saha *et al.*, 2004b). The polymorphism rate was fairly high in tall fescue and ryegrass (66%) and substantially lower in rice (43%) and wheat (38%). We developed a set of EST-SSR markers for 12 grass species covering eight genera, four tribes and two sub-families of the Poaceae family. A tall fescue linkage map was constructed with AFLP and SSR markers (Saha *et al.*, 2004c). Tall fescue EST-SSRs were present in all linkage groups and were the major source of SSR loci. In a ryegrass genetic map, one of the EST-SSR markers (TF21-230) was within 2 cM of the *SOD* locus, which discriminates the annual and perennial types (Warnke *et al.*, 2004). A creeping bentgrass population was also mapped with these loci (Zhao *et al.*, 2003). These EST-SSRs were successfully used to assess the genetic diversity within darnel ryegrass accessions and their relationship with *Festuca* species (Zwonitzer *et al.*, 2004), and variability within and among accessions of Canada and Virginia wildrye (Saha *et al.*, 2004a). Tall fescue EST-SSRs were successfully used to discriminate the *Festuca* and *Lolium* genomes and track the introgression in hybrids and subsequent generations which can reduce the number of backcross generations (Bughrara *et al.*, 2003).

Conclusions Tall fescue EST-SSR markers are valuable genetic markers for the *Festuca* and *Lolium* genera and can also be extended to other forage grass species. A set of markers have been developed for different grass species in which there is limited or no marker data available. The potential for using these markers in tracking genome introgression, genetic diversity, molecular mapping and trait association was established.

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