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Cross-amplification of EST-derived markers among 16 grass species

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ABSTRACT

The availability of a large number of expressed sequence tags (ESTs) has facilitated the development of molecular markers in members of the grass family. As these markers are derived from coding sequences, cross-species amplification and transferability is higher than for markers designed from genomic DNA sequences. In this study, 919 EST-based primers developed from seven grass species were assessed for their amplification across a diverse panel of 16 grass species including cereal, turf and forage crops. Out of the 919 primers tested, 89 successfully amplified DNA from one or more species and 340 primers generated PCR amplicons from at least half of the species in the panel. Only 5.2% of the primers tested produced clear amplicons in all 16 species. The majority of the primers (66.9%) were developed from tall fescue and rice and these two species showed amplification rate of 41.6% and 19.0% across the panel, respectively. The highest amplification rate was found for conserved-intron scanning primers (CISP) developed from pearl millet (91%) and sorghum (75%) EST sequences that aligned to rice sequences. The primers with successful amplification identified in this study showed promise in other grass species as demonstrated in differentiating a set of 13 clones of reed canary grass, a species for which very little genomic research has been done. Sequences from the amplified PCR fragments indicated the potential for the transferable CISP markers for comparative mapping purposes. These primer sets can be immediately used for within and across species mapping and will be especially useful for minor grass species with few or no available molecular markers.

1. Introduction

The grass family, *Poaceae*, is one of the largest families of flowering plants, with approximately 10,000 species in 700 genera. *Poaceae* surpasses all other botanical families in economic importance. Three grain crops, wheat (*Triticum aestivum*), rice (*Oryza sativa*) and corn (*Zea mays*), are the world's predominant food sources, but the family also includes several other less-researched crops. Tef (*Eragrostis tef*), for example, is a major staple food in Ethiopia, but almost unknown elsewhere. Turf and forage crops such as tall fescue (*Lolium arundinaceum*), Kentucky bluegrass (*Poa pratensis*) and bermudagrass (*Cynodon dactylon*), are vital to the rangeland management and lawn care industries generating millions of dollars in seed sales, but have limited genetic resources available. In addition, future biofuel crops such as switch grass (*Panicum virgatum*) and reed canary grass (*Phalaris arundinacea*) are also examples of crops that are definitely in need of more research to bring about the required improvements.

Genetic studies of these minor crops are hindered because of the scarcity of molecular markers available. Because marker development is laborious, time-consuming, and expensive, given the limited resources and researchers available for minor crops such as turf/forage species, it has lagged behind that of major and well-researched crops. Microsatellite markers (SSR) developed from genomic libraries (gSSR) have been widely used for mapping and population genetic analysis. This can be mainly attributed to their high level of polymorphism, abundance and dispersion throughout the genome, besides their codominant nature of inheritance and

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reproducibility (Gupta and Varshney, 2000; Squirrell et al., 2003). The disadvantage of gSSR markers is the high initial cost of development and their low transferability across genera and beyond (Roa et al., 2000; Kindiger, 2006).

A large amount of coding sequence information has been generated by EST (expressed sequence tag) projects for gene discovery in several crop species, and deposited in the National Center for Biotechnology Information (NCBI) database. EST-based markers are derived from transcribed regions of the genome, which are more constrained with respect to sequence diversity since they code for functional proteins. For this reason, EST-derived markers are more likely to produce amplicons in multiple species than those designed from non-coding sequences (Yu et al., 2004; Zhang et al., 2005; Parida et al., 2006). By December 21st, 2009, NCBI had more than six million readily accessible ESTs in members of the *Poaceae*, 83% of which were derived from rice, maize or wheat. The large amount of genetic information available on the major grain crops, including maize and sorghum (Zhu and Buell, 2007), and the full genome sequences for rice (Yu et al., 2005) and brachypodium (Opanowicz et al., 2008) are useful resources that can be extended to less well-funded grasses using comparative genomics tools (Varshney et al., 2005; Feltus et al., 2006). Based on this information, one can search for variation in EST sequences to develop markers flanking SSRs, insertions and deletions (INDEL), and single nucleotide polymorphisms (SNP).

It has become widely accepted to screen EST-based markers derived from one species with other species in the same genus and even across genera within the same family. Gupta et al. (2003) reported that 24 out of 59 wheat EST-SSR markers amplified fragments in five species including barley, maize, oat, rice, and rye. Similarly, Wang et al. (2005), demonstrated the transferability of EST-SSR markers from maize, sorghum, rice and wheat to minor grass species (finger millet, seashore paspalum and bermudagrass) and observed the correlation between the transferability rate of markers and the phylogenetic relationship of the species tested. Numerous studies, however, have used the term "transferability of markers", which implies amplification of orthologous loci, to describe amplification of an amplicon regardless of orthology. While many studies have suggested that EST-SSR are most interesting because of their amplification of conserved (orthologous) sequences across different grass species (Varshney et al., 2005; Feltus et al., 2006), others have observed loss of sequence homology when markers developed from one species were screened on distantly related species (Asp et al., 2007; Sim et al., 2009).

The conserved-intron scanning primers (CISP) designed by Feltus et al. (2006) to conserved exonic regions flanking introns from sorghum/pearl millet ESTs and aligned to the rice genome, successfully amplified in barley, maize, tef and wheat. Those markers and others, such as the PCR-based landmark unique genes (PLUG) described by Ishikawa et al. (2009), are much more conserved than EST-SSR markers and could provide better resources for comparative mapping studies, provided they amplify orthologous sequences that are polymorphic. Thus, more research is needed on the level of transferability of molecular markers from well-researched cereal crops to distantly related, minor grass species and also on the nature of the products of those markers. It is crucial to understand whether those markers will only add novel markers to less-researched crops or will also provide the basis for comparative mapping work.

The objectives of this research were: (i) to evaluate the cross-amplification of 919 primers developed using EST sequences derived from wheat, rice, tef, sorghum, pearl millet, tall fescue, and rye on a panel of 16 grass species, (ii) to evaluate the utility of some of those markers in discriminating reed canary grass accessions, and (iii) to evaluate the transferability of markers for comparative mapping work of less-researched grass species.

2. Materials and methods

2.1. Plant materials

Sixteen grass (*Poaceae*) species, including maize (*Z. mays*), sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), bermudagrass (*C. dactylon*), tef (*E. tef*), creeping bentgrass (*Agrostis stolonifera*), harding grass (*Phalaris aquatica*), oat (*Avena sativa*), brachypodium (*Brachypodium distachyon*), smooth bromegrass (*Bromus inermis*), barley (*Hordeum vulgare*), western wheatgrass (*Pascopyrum smithii*), wheat (*T. aestivum*), Kentucky bluegrass (*P. pratensis*), tall fescue (*Festuca arundinacea*) and rice (*O. sativa*), were selected to represent 10 tribes from four of the six subfamilies within the *Poaceae* family (Table 1). The subfamily *Pooideae* was represented by 11 species of grain, turf and forage crops in this panel. To evaluate the level of polymorphism transferable markers provide, and investigate the nature of the amplified fragments, 13 reed canary grass clones and Indian lovegrass (*Eragrostis pilosa* accession 30-5) were employed. Those clones represent cultivars and accessions from northeast and north central United States, Canada and one European cultivar (Table 2).

Table 1
A panel of 16 grass species used for evaluation of 919 primers developed from seven species (boldfaced).

Common name	Variety	Scientific name	Tribe	Subfamily
Maize	B73	*Zea mays*	*Andropogoneae*	*Panicoideae*
Sorghum	BTx623	*Sorghum bicolor*	*Andropogoneae*	*Panicoideae*
Pearl millet	Titft23A	*Pennisetum glaucum*	*Paniceae*	*Panicoideae*
Bermudagrass	Midland 99	*Cynodon dactylon*	*Cynodonteae*	*Chloridoideae*
Tef	Kaye Murri	*Eragrostis tef*	*Eragrostideae*	*Chloridoideae*
Creeping bentgrass	AA61	*Agrostis stolonifera*	*Aveneae*	*Pooideae*
Harding grass	Maru 20-2	*Phalaris aquatica*	*Aveneae*	*Pooideae*
Oat	Ogle	*Avena sativa*	*Aveneae*	*Pooideae*
Brachypodium	Bd3-1	*Brachypodium distachyon*	*Brachypodieae*	*Pooideae*
Smooth bromegrass	Lincoln 8-7	*Bromus inermis*	*Bromeae*	*Pooideae*
Barley	Morex	*Hordeum vulgare*	*Triticeae*	*Pooideae*
Western wheatgrass	Barton	*Pascopyrum smithii*	*Triticeae*	*Pooideae*
Wheat	Chinese Spring	*Triticum aestivum*	*Triticeae*	*Pooideae*
Rye[a]	–	*Secale cereale*	*Triticeae*	*Pooideae*
Kentucky bluegrass	SR2394	*Poa pratensis*	*Poeae*	*Pooideae*
Tall fescue	KY31	*Festuca arundinacea*	*Poeae*	*Pooideae*
Rice	IR64	*Oryza sativa*	*Oryzeae*	*Ehrhartoideae*

[a] Rye was only used as a source of markers in this study.