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Sustainable Use of Genetic Diversity in Forage and Turf Breeding

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Chapter 65
DArTFest – A Platform for High-Throughput Genome Profiling Within the *Festuca – Lolium* Complex

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Abstract With the aim to facilitate high-throughput genome profiling and genetic and physical mapping within the *Festuca-Lolium* complex, we have developed a Diversity Arrays Technology (DArT) array for five important species: *Festuca pratensis, Festuca arundinacea, Festuca glaucescens, Lolium perenne* and *Lolium multiflorum*. The DArTFest array contains 7,680 probes derived from methyl-filtered genomic representations. Of 3,884 polymorphic DArT markers identified in the first marker discovery experiment, over 1,000 markers detected a positive allele in each species.

We assigned DArT markers to individual chromosome regions of *F. pratensis* using a series of single chromosome substitution and recombinant lines of *F. pratensis* in *L. multiflorum*. Moreover, we enriched the existing genetic map of *F. pratensis* by over 200 DArT markers and existing genetic map of *L. multiflorum* by over 500 DArT markers. The resources developed in this project will facilitate development of genetic maps in *Festuca* and *Lolium*, the analysis of genomic constitution in *Festuca* × *Lolium* hybrids, as well as marker-assisted selection for multiple traits.

Keywords DArT · Fescue · Hybrids · Introgression · Mapping · Ryegrass

Introduction

Grasses are among the most important and widely cultivated plants on Earth, with a total area of grassland estimated to be twice that of cropland. Among the cultivated grasses, ryegrasses (*Lolium* spp.) and fescues (*Festuca* spp.) predominate, especially in temperate climate conditions (Jauhar, 1993). Decades of breeding resulted in superior ryegrass and fescue cultivars outperforming their wild progenitors. However, there is a risk that some desirable alleles of progenitors were lost during

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the breeding process. The risk of erosion of species' gene pools calls for characterization of the natural genetic variability and its conservation in gene banks. The banks with well described accessions will allow for more effective selection of materials for breeding improved cultivars. In order to characterize the existing genetic diversity in detail and to provide sufficient information on the genetic make-up of a particular accession, thousands of genomic loci need to be quickly interrogated. Thus, high-throughput and cost-effective genotyping platforms are needed.

In addition to the analysis of genetic diversity, a high-throughput genotyping system is also required to speed up the development of genetic linkage maps. Until now, several genetic maps were constructed for *Lolium* spp. (e.g. Hayward et al., 1998). Within the fescue species, genetic maps were generated for the two agronomically important species – *F. arundinacea* and *F. pratensis* (Saha et al., 2005; Alm et al., 2003). However, the number of markers is too low for routine identification of markers tightly linked to genes underlying traits of interest. Finally, high-throughput genotyping platforms are needed to speed up the development of new cultivars with desirable attributes using marker assisted selection.

The need for high-throughput genotyping led to the development of various DNA arrays and chips (reviewed in Gupta et al., 2008). Although they are based on different principles, all of them can be used to screen thousands or hundreds of thousands of genomic loci in a single pass. Diversity Array Technology (DArT) is a microarray hybridization based technique that permits simultaneous screening of thousands polymorphic loci without any prior sequence information (Jaccoud et al., 2001). DArT is high-throughput, low-cost, quick and reproducible.

In this study we demonstrate the utility of the approach for the estimation of intra- and interspecific genetic diversity and genetic and physical mapping.

Material and Methods

Plant Material

For the development of the DArT array and analysis of intra- and interspecific diversity, 40 accessions each of *L. perenne* L., *L. multiflorum* Lam., *F. pratensis* Huds. and *F. arundinacea* Schreb. were used, plus all seven available accessions of *F. glaucescens* Boiss. The choice of accessions (ecotypes, cultivars and parents of mapping populations) aimed at the discovery of the maximum genetic variability. In order to map DArT markers to individual chromosomes and chromosome bins of *F. pratensis*, we used single chromosome monosomic substitutions and recombinant lines with various lengths of *Festuca* chromatin present in tetraploid *L. multiflorum,* as described by Kopecký et al. (2008). Mapping population of *F. pratensis* with 138 genotypes was used for genetic mapping (Alm et al., 2003).

Development of the DArTFest Array

DarTFest array was developed as described in Kopecky et al. (2009).

Analysis of Genetic Diversity

The DArTsoft-generated 0–1 scores were used as input for the RESTDIST and NEIGHBOR programs of the PHYLIP 3.6 software package to construct a dendrogram based on the Unweighted Pair Group Method with Algorithmic Mean (UPGMA) and Felsenstein's modification of the Nei/Li restriction fragment distance (Felsenstein, 2004).

Genetic and Physical Mapping of DArT Markers in F. pratensis

To anchor markers to individual chromosomes of *F. pratensis*, DNA isolated from chromosome substitution and recombinant lines were hybridized to the DArT array. A marker present in *F. pratensis* and absent in *L. multiflorum* was assigned to a chromosome if it was present in at least one substitution line for a particular chromosome. The same approach was used to anchor markers allocated on bins of *F. pratensis* using the recombinant lines described above. DNA from a mapping population of *F. pratensis* and *L. multiflorum* already used for the development of genetic maps (Alm et al., 2003; Studer et al., 2006) was hybridized to the array.

Results and Discussion

Development of DArT Array

We developed a DArT array containing 7,680 probes derived from methyl-filtered (through the use of *Pst*I restriction enzyme) genomic representations. In the first marker discovery experiment performed with 40 genotypes from each of the species *L. perenne*, *L. multiflorum*, *F. pratensis* and *F. arundinacea*, and seven genotypes of *F. glaucescens*, we identified 3,884 polymorphic markers with standard DArTsoft settings.

Analysis of Genetic Diversity Using DArT Markers

Of the 3,884 polymorphic markers detected, 2,629 markers gave unequivocal scores in the five species tested. Using these markers, we compiled a dendrogram including all 167 tested accessions of fescue and ryegrass. This differentiated two major groups, representing the fescue and ryegrass genera (Fig. 65.1). Both ryegrass species analyzed (*L. perenne* and *L. multiflorum*) were closely related, but divergent enough to form separate groups. Fescue species formed two major groups. The first one included *F. pratensis* forming a tight group in the dendrogram. The second group included two subgroups, one representing *F. arundinacea* accessions and the second *F. glaucescens* accessions. One accession of *F. glaucescens* Fg-07 clustered with the subgroup of *F. arundinacea*. Another inconsistent accession *F. pratensis* Fp-40 was located outside of all other species in the dendrogram, probably due to
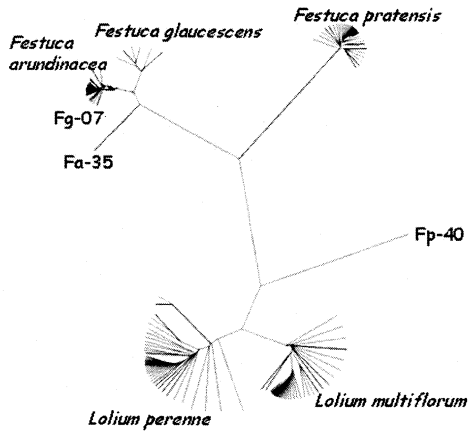
Fig. 65.1 UPGMA dendrogram (shown as radial cladogram) based on hybridization of 80 *Lolium* and 87 *Festuca* genotypes to 2,629 DArT markers and Felsenstein's modified Nei/Li restriction fragment distance. Two major groups representing the fescues and ryegrasses are clearly differentiated. Both ryegrass species display higher genetic diversity than fescue species. Note that the accession of *F. arundinacea* Fa-35 (Moroccan ecotype 599,533) was found distant of the major group. Similarly, one accession of *F. glaucescens* (Fg-07) clustered with the subgroup of *F. arundinacea*. Another inconsistent accession *F. pratensis* Fp-40 (cultivar Norild) was located separately outside of all other species

contamination of its DNA. Both accessions (Fg-07 and Fp-40) were excluded from further analyses.

Genus- and Species-Specificity of DArT Markers

Reliable discrimination of DNA markers from both grass genera tested here, and possibly also from individual species, would greatly expand the utility of the DArT array. As the DArTFest array contains markers derived from both genera and all five species tested, our subsequent analysis focused on identifying genus- and species-specific markers. Of 3,884 polymorphic DArT markers identified, over 1,000 markers detected a positive ("1") allele in each species. However, a large proportion of markers present in all five species tested reduced the numbers of species-specific markers (Table 65.1).

Physical and Genetic Mapping of DArT Markers in F. pratensis and L. multiflorum

We used a complete set of *Festuca-Lolium* single chromosome substitution lines of *F. pratensis* into *L. multiflorum* to assign DArT markers to individual chromosomes of *F. pratensis*. In total, 160 DArT markers were anchored using this approach with

Table 65.1 Number of species-specific DArT markers identified on a DArT array containing 7,680 probes of 40 accessions each of *L. perenne* L., *L. multiflorum* Lam., *F. pratensis* Huds., *F. arundinacea* Schreb. and seven accessions of *F. glaucescens* Boiss

Species	Scored markers[a]	Positive markers[b]	Polymorphic markers[c]	Species-specific markers[d]
Lolium perenne	2,638	1,725 (821–1127)	1,407	52
Lolium multiflorum	3,883	2,761 (1507–1852)	2,148	82
Festuca pratensis	3,884	2,257 (1619–1821)	1,078	123
Festuca glaucescens	2,630	1,346 (1059–1101)	387	9
Festuca arundinacea	2,638	1,572 (1000–1351)	512	34

[a]Note that some markers were not scored in all species, [b]Range of positive markers for individual accessions is in brackets, [c]Markers polymorphic among the accessions, [d]Identified after scoring 2,629 markers

between six and 34 DArT markers anchored to a particular chromosome. This represents 56% of all markers present in *F. pratensis* but absent in *L. multiflorum*. Up to date, we have dissected chromosomes 3 and 6 of *F. pratensis* into 7 and 9 bins, respectively, with between one and nine DArT markers anchored to each bin. The existing genetic map of *F. pratensis*, which includes AFLPs, RFLPs, isozyme and other markers (Alm et al., 2003) has been enriched for 204 DArT markers and its total linkage length increased by 170 cM to 775 cM. In case of *L. multiflorum*, existing genetic map was enriched for 531 DArT markers.

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