Application of SCoT markers for accessing of genetic diversity of gramineous forage grass species

Yu M Mavlyutov, A O Shamustakimova, I A Klimenko
Federal Williams Research Center of Forage Production & Agroecology
141055 Lobnya, Moscow region, Russia
Corresponding author’s e-mail: nastja_sham@mail.ru

Abstract. Using the SCoT marker system, 8 varieties of cereal grasses belonging to 5 species were analyzed: Festuca pratensis, Lolium perenne, Lolium multiflorum, Festuca rubra, Festulolium. Of the 10 tested SCoT markers, 7 informative markers were selected that reveal interspecies genetic polymorphism. According to the results of the analysis, DNA profiles characteristic of each studied species were obtained, and primers allowing to detect intervarietal differences for subsequent identification and molecular genetic passportization were selected.

1. Introduction
Cereal grasses are widely used in animal husbandry for obtaining cheap and complete fodder, as well as for creating lawns and protecting the soil from water and wind erosion, due to such valuable properties as winter hardness, longevity, plasticity and ability to vegetative regeneration.

One of the key tasks of cereal grasses breeding is the development of reliable methods for evaluating the initial material and selecting promising forms for use in the breeding process. In recent years, molecular genetic markers have been successfully used for this purpose, allowing to reduce the time and economic cost of creating a new variety and carry out its DNA-identification [1].

Among the numerous techniques of molecular genetic analysis based on the PCR method in studies on cereal grasses, the SCoT (Start Codon Targeted Polymorphism) marker system has well established itself [2, 3, 4, 5]. SCoT markers detect polymorphism by annealing a single 18-mer primer on the forward and reverse chain in the regions flanking the start codon ATG. The advantage of the system is the high reproducibility of the results obtained and the relatively low cost of analysis.

The aim of this work was to evaluate the possibility of using SCoT markers to study interspecific DNA polymorphism of cereal grass varieties selected by FWRC FPA.

Eight released varieties of fodder grasses of different species were the objects of the study (table 1).

| № | Type | Variety |
|---|------|---------|
| 1 | Meadow fescue (Festuca pratensis Huds.) | Quarter |
| 2 | Grassland ryegrass (Lolium perenne L.) | Quart |
Seedlings grown on wet filter paper in Petri dishes (30 genotypes from each cultivar) were used for DNA isolation. The method based on SDS lysis buffer was used [6].

The structure of the 10 CoT markers used for analysis is shown in table (table 2).

Table 2. Sequence of nucleotides of used SCoT markers

| №  | Title     | Sequence (5’-3’)               |
|----|-----------|-------------------------------|
| 1  | SCoT 1    | CAACAATGGCTACCACCA            |
| 2  | SCoT 2    | CAACAATGGCTACCACCC            |
| 3  | SCoT 20   | ACCATGGCTACCACCGC             |
| 4  | SCoT 23   | CACCATGGCTACCACAG             |
| 5  | SCoT 31   | CCATGGCTACCACCGCCT            |
| 6  | SCoT 6    | CAACAATGGCTACCACGC            |
| 7  | SCoT 13   | ACGACATGGGCGACCATCG           |
| 8  | SCoT 21   | ACGACATGGGCGACCCACA           |
| 9  | SCoT 32   | CCATGGCTACCACCGC              |
| 10 | SCoT 15   | ACGACATGGGCGACCCGGA           |

The total volume of 10 μL PCR mixture included: 1x Taq Turbo buffer, 1x dNTP mix (250 μM of each deoxynucleotide in the final volume), 0.5U Taq-DNA polymerase, and 0.8 μM primer and 30 ng DNA. The amplification mode in «a Bio-Rad T100» thermal cycler (USA) was as follows: 3 min at 94 °C; then 35 cycles of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C; and 5 min at 72 °C.

Electrophoresis was performed in 1.6% agarose gel at 50 V for 1 h 30 min. Detection of PCR products and determination of their size was performed on a « Gel Doc XR+» device using the program package «Image lab» relative to the molecular weight marker 1 kb DNA Ladder («Eurogen», Russia). A binary matrix was prepared based on the results obtained, where the presence of the product was designated as «1» and its absence as «0». Statistical processing and dendrogram preparation by the method of paired-weighted grouping with averaging (UPGMA) was performed in the PopGene program.

2. Results and discussion

Based on the analysis of 10 SCoT markers with cereal grass samples, we were able to identify 7 primers that allow obtaining a clear and reproducible result. The greatest number of products with the maximum percentage of polymorphism was obtained using primers SCoT 1, SCoT 20, and SCoT 13 (Table 3).

Table 3. SCoT-primers informativity indicators for the analysis of cereal grass species and varieties

| №  | Marker | Total number of PCR products | Amount of polymorphic products | % polymorphism | Size of PCR products, nn |
|----|--------|-----------------------------|--------------------------------|---------------|-------------------------|
| 1  | SCoT 1 | 33                          | 13                             | 39,3          | 822-1754                |
| 2  | SCoT 2 | 36                          | 2                              | 5,5           | 406-1254                |
| 3  | SCoT 20| 38                          | 13                             | 34,2          | 619-2202                |
| 4  | SCoT 23| 21                          | 4                              | 19            | 1229-2693               |
| 5  | SCoT 31| 31                          | 5                              | 16,1          | 558-1482                |
A total of 222 PCR products were identified using 7 informative markers, of which 55 were polymorphic. The greatest number of amplification fragments was obtained with the SCoT 20 primer (Figure 1). PCR products ranged in size from 406 to 2693 bp. The SCoT 1 and SCoT 13 markers showed the highest percentage of polymorphism at 39.3.

![Figure 1. Electrophoregram of DNA amplification products of cereal grass species and varieties using SCoT 20 marker. 1 - Meadow fescue «Kvarta»; 2 - Meadow fescue "VIK 5"; 3 - Grazing ryegrass "Karat"; 4 - Annual ryegrass "Rapid"; 5 - Red fescue «Diana»; 6 - Red fescue «Dipa»; 7 - Festulolium «Fest»; 8 - Festulolium «Allegro»; 9 - control (H2O); M - molecular weight marker (1 kb DNA Ladder, «Eurogen»).](image)

The electrophoregram shown in the figure shows that each species is characterized by a unique DNA profile. For example, clear differences were found between samples of meadow fescue and red fescue; ryegrasses, pasturegrass and annual ryegrass, differ in DNA profile. It should be particularly noted that the marker system used revealed similarities between ryegrass and Festulolium, which was of the ryegrass morphotype.

According to the results of statistical processing, the following indicators of variability of the analyzed breeding material were revealed (table 4).

| №  | Marker | Effective number of alleles (ne) | Shannon index | Primer informativity index |
|----|--------|---------------------------------|---------------|--------------------------|
| 1  | SCoT 1 | 1.3792                          | 0.4162        | 0.258                    |
| 2  | SCoT 2 | 1.6216                          | 0.5595        | 0.375                    |
| 3  | SCoT 20| 1.4620                          | 0.4742        | 0.302                    |
| 4  | SCoT 23| 1.4667                          | 0.4738        | 0.302                    |
| 5  | SCoT 31| 1.5294                          | 0.5155        | 0.336                    |
The dendrogram based on Ney's genetic distance data shows a division into 4 distinct clusters characterizing interspecific differences in the cereal crops studied (figure 2).

| 6 | SCoT 13 | 1.4366 | 0.4617 | 0.291 |
|---|---------|--------|--------|--------|
| 7 | SCoT 6  | 1.4735 | 0.4573 | 0.297 |

The dendrogram based on Ney's genetic distance data shows a division into 4 distinct clusters characterizing interspecific differences in the cereal crops studied (figure 2).

![Dendrogram](image)

**Figure 2.** UPGMA dendrogram based on the values of Nei genetic distances for the studied species and varieties of cereal grasses.

The first cluster includes meadow fescue, the second - ryegrass, the third - festuloliums, and the fourth - red fescue. Clusters 2 and 3 have a common branch, which confirms the hybrid nature of festuloliums.

Based on the results, we can conclude that SCoT markers are suitable for assessing genetic variability between cereal grass species. Using this marker system, we were also able to identify inter-varietal DNA-polymorphism of meadow fescue (SCoT markers 23 and 13) and festulolium (SCoT markers 1 and 20).

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