Genetic Diversity and Relationships in cocksfoot by Molecular Markers

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Introduction

Cocksfoot (*Dactylis glomerata* L.) originated from the Northern Hemisphere, including Eurasia and North Africa. It is widely distributed in temperate climates and has been widely used in the world more than 100 years, especially in North America, Europe and Japan. Genetic variability of cocksfoot had been detected by RAPD (Kölliker *et al*. 1999), and the correlation between genome size of natural populations of cocksfoot and location altitude has been analyzed by AFLP (Reeves *et al*. 1999). To date, information on the genetic diversity of cocksfoot at the molecular level is still scarce, This study is the first to combine the genetic diversity research of cocksfoot with SRAP (Sequence Related Amplified Polymorphism) and ISSR (inter-simple sequence repeat) markers evaluating genetic diversity among 45 populations of cocksfoot collected from eight different countries from four continents. The objectives of the research were: (1) to evaluate the levels and patterns of genetic diversity about cocksfoot; (2) to compare ISSR and SRAP markers for the molecular characterization of cocksfoot, including the evaluation of the degree of polymorphism generated from each technique as a pre-requisite for (3) their applicability to formulate appropriate strategies for the conservation and utilization of the wild cocksfoot genetic resources available and to discuss scientific breeding measures according to the status of cultivars.

Materials and methods

Plant samples and PCR analysis

Forty five materials (including 26 wild materials and 19 Cultivars) of cocksfoot plant were sown in the germplasm resources Garden of Sichuan Agricultural University. Nineteen were collected from China (including the three Chinese cultivars), eight from Denmark, seven from Sweden, four from America, two each from Holland, England and Germany, and one from Australia. Twelve ISSR primers and 21 pairs of SRAP primers were used.

Data analysis

Amplified fragments were scored for the presence (1) or absence (0) of homologous bands and two matrices of the different SRAP and ISSR phenotypes were assembled; these two matrices were then used for the following statistical analyses respectively. Genetic diversity was measured (Nei and Li 1979) by genetic similarity (GS) and the percentage of polymorphic bands (PPB). These coefficients were used as operational taxonomic units (OTUs) to construct dendrograms and PCA by the unweighted pair group method (UPGMA) in NTSYS software 2.1.

Results

Genetic polymorphism analysis of PCR product

The SRAP and ISSR Genetic polymorphism have been analyzed. On average, 19.3 SRAP bands per primer and 9.67 ISSR bands per primer were amplified. We could conclude that both of SRAP and ISSR are efficient measures for the polymorphism analysis of cocksfoot, but according to the number of polymorphic bands, PPB (%) and approximate size range of bands, we thought SRAP marker is better than ISSR.

The analysis of genetic comparability

The GS range by SRAP was 0.6248 to 0.9686, average of 0.7958, while GS range by ISSR was 0.6116 to 0.9231, average was 0.7790. For the Chinese 16 wild materials and 3 registered cultivars, the genetic similarity coefficient ranged from 0.7269 to 0.9686 in SRAP and 0.6880 to 0.9231 in ISSR. For the 4 American materials tested the GS ranges were 0.6613 to 0.8523 and 0.6563 to 0.8633, for SRAP and ISSR respectively. This indicates that cocksfoot from China and America have abundant genetic diversity. Both the SRAP matrix and ISSR matrix indicated the rich genetic diversity of cocksfoot. SRAP and ISSR could give approximately consistent results by genetic comparability analysis in cocksfoot, but more genetic variance could be reflected in the SRAP marker.

The polygenetic relationships of cocksfoot

Based on cluster and principal component analysis on the genetic characteristics, all collections could be divided into four groups and five groups for the two markers, respectively. The accessions from the same continent were classified into the same group, indicating the geographical distribution of genetic diversity of cocksfoot. The results suggested that the entire genetic basis of cultivars is narrow, especially in the Chinese cultivars. The collection from Australia was different from other collections in genetic diversity.

Discussions

Genetic diversity of natural populations results from the interaction of drift, migration, mutation, and selection. Generally, cocksfoot has a rich basis of genetic variation because it is distributed widely in the world and has diversification in chromosome number, chromosome form and chromosome behavior. By SRAP and ISSR analysis, we obtained similar results and proved the abundance of genetic diversity of cocksfoot by the method of molecular biology. As SRAP had a better clustering effect than ISSR, it could reflect the genetic relationship of cocksfoot more clearly. In the genetic similarity analysis, we found that the genetic diversity of cocksfoot from China and USA was richer, and cocksfoot from Australia was different from other places in genetic diversity. This may be caused by the extent of land, diversity of climates, and large differences of origin and evolution situation. This study has proved the abundance of genetic diversity about cocksfoot and suggested a higher requirement for the collection and conservation of cocksfoot germplasm. Because cocksfoot is an allogamous plant, efficient measures need to be taken to avoid cross-pollination among different populations and mixing of germplasm. According to this research, SRAP and ISSR can give a clear and coherent electrophoresis diagram with good repeatability and high polymorphism.

Thus, SRAP and ISSR are two good markers to distinguish the variety of cocksfoot for property right protection. SRAP marker could be a better marker to use because of the use of longer primer combinations and higher anneal temperature. Furthermore, SRAP and ISSR could also be applied to construct molecular genetic linkage map. This may be the next step of cocksfoot molecular marker research.

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