Original Scientific paper
10.7251/AGRENG2203112K
UDC 582.542:575.2

DNA MARKERS AS A MEANS OF ASSESSING THE GENETIC DIVERSITY AND IDENTIFICATION OF GRASSES

Iryna KONDRATSKAYA*, Andrei YUKHIMUK, Olga CHIZHIK, Vladimir RESHETNIKOV

The State Scientific Institution «Central Botanical Garden of the National Academy of Sciences of Belarus» Minsk , Belarus
*Corresponding author: ikondratskaya@mail.ru

ABSTRACT

The effectiveness of breeding is largely depends on the presence of the gene variability in the initial breeding material. The genetic variability assessment helps to evaluate the source material during a fundamentally new forms with economically valuable traits creating. In this regard, the assessment of the genetic polymorphism of the cereal grasses genotypes which are of interest both as a source of breeding material and as a variety candidate was carried out by DNA markers. Seventy-three loci were identified for the Festulolium and Lolium L. genotypes using the SCoT marker system, 69 of markers were polymorphic. This marker system revealed a high level of polymorphism in the studied genotypes of Festulolium and Lolium L. – 94.52%. For the genotypes of intergeneric hybrids of the Agropyron L. genus and their parent forms 90 loci were identified – 46 SCoT markers and 44 SRAP markers. From the total pool of markers 73 markers were polymorphic. On average, the level of polymorphism was 81.1%. For the genotypes of the Alopecurus L. genus interspecific hybrids and their parent forms 157 loci were identified – 52 for RAPD-PCR and 105 – for ISSR-PCR. Of the total pool of markers, 104 were polymorphic According to the results of DNA genotyping the genetic passports of cereal grasses were compiled.

Keywords*: cereal grasses, genetic passport, genotype, SCoT markers, ISSR, RAPD, SRAP.*

INTRODUCTION

Scientific and practical achievements in the fields of genetics and breeding lead to the reduction of the erms for hybrids and varieties creation. So, today one can see a significant increasing in both the rate of new varieties creation and their number. At the same time the modern breeding is characterized by a tendency to reduce the genetic distances between newly created varieties. This is due to the fact that when a new varieties creating, the same genotypes with economically valuable properties are often involved in crossing. The genetic diversity of varieties reduction demands to identify a large number of varieties of the closely related origin (Kilchevski,

2014). At the present time such assessment can be carried out using DNA marker systems.

To solve the problem of pure undegraded DNA extraction from plant objects is very important to define the first stage of molecular genetic research. Different plant species, plants and organs at different stages of development, even different organs of the same species at the same stage of plant development, contain different amounts and classes of secondary metabolites and spare substances (Raybushkina, 2019). The component composition has a significant impact on the quality and quantity of extracted DNA. For DNA isolation it is better to use young plant organs and leaves, since they contain a smaller amount of spare substances and secondary metabolites. However, young leaves and plant organs are not always available. The methods of DNA isolation have to be modified to obtain the satisfactory results for the adult plant organs, herbarium material, etc. To isolate DNA both dry and sprouted seeds can be used or lyophilically dried or fixed in silica gel leaf tissue. To carry out DNA labeling and to create the DNA collection of perennial grasses we carried out the research work to optimize the method of DNA isolation, which provides a high yield of high-quality DNA preparations.

The DNA extraction procedure was specific for cereal grasses which are characterized by the high protein content and a high concentration of polysaccharides in leaves.

MATERIAL AND METHODS

Object of investigations: cereal grasses – the representatives of Festulolium, Lolium, Agropyron and Alopecurus families. To isolate DNA, we used: 1) dried seeds; 2) turgid seeds (12 hours of soaking); 3) fresh leaf material; 4) leaf tissue fixed at -200 C and -800 C; 5) leaf tissue dried from 370°C to 420°; 6) dried plant tissue with silica gel until complete dehydration. Preparations of high-quality DNA were obtained using a modified STAB method from silica-dehydrated leaf tissue of cereal grasses. (Dempster E.L.,1999). Total DNA labeling was carried out using PCR technique. To label the hybrid forms and varieties of perennial grasses included in the studies, the multilocus primers were selected: ISSR (inter simple sequence repeat); RAPD (randon amplification of polymorphic DNA); SCoT (start codon targeted); SRAP (Sequence-related amplified polymorphism) and microsatellite primer SSR (Simple Sequence Repeat).

RESULTS AND DISCUSSION

After optimizing of the DNA isolation process we obtained the high-quality DNA preparations of cereal grasses. Preparations with a DNA concentration of at least 50 ng/ml and ratios A260/A280 ≥ 1.7 and A260/A230 ≥ 1.5 were considered qualitative. Data on the quality preparations of total DNA of the *Lolium* L. genus and their hybrid forms are represented in the table 1.

Table 1. Qualitative and quantitative parameters of total DNA preparations

№	Name	A260/280	A260/230	c (DNA), ng/μL
1	Lolium perenne L. cv. Guslayr	1,8	2,1	602
2	Lolium multiflorum Lam. cv. Matador	1,8	2,1	906
3	Lolium perenne × multiflorum hybrid 22–7	1,8	2,1	931
4	Lolium perenne × multiflorum hybrid 22–7–21	1,8	2,1	700
5	Lolium perenne × multiflorum hybrid 22–7–23	1,9	2,3	817

The obtained DNA preparations of hybrid forms and varieties of perennial grasses have been included in the DNA collection of perennial grasses and placed for guaranteed long-term storage at a temperature of -80 ° C in the DNA BANK of the Department of Biochemistry and Biotechnology of plants of the State Scientific Institution "Central Botanical Garden of the National Academy of Sciences of Belarus"(Table2).

Table 2. Passport of the basic DNA collection on the example of representatives of the genus Lolium L, and hybrid forms. Curator of the DNA collection – a scientific researcher Yukhimuk A.N.

№	**Name**	**Source of DNA**	**Selection method**	**Quality indicators (A260/A280)/ (A260/A230)**	**Concentration**	**Volume**
1	Lolium perenne L. Cv Guslayr	leaf tissue	Demster, 1999	1,8/2,1	602	>100
2	Lolium multiflorum Lam. cv. Matador	leaf tissue	Demster, 1999	1,8/2,1	906	>100
3	Lolium perenne × multiflorum Bybor 22–7	leaf tissue	Demster, 1999	1,8/2,1	931	>100
4	Lolium perenne × multiflorum, hybrid 22–7–211	leaf tissue	Demster, 1999	1,8/2,1	700	>100
5	Lolium perenne × multiflorum, hybrid 22–7–25	leaf tissue	Demster, 1999	1,9/2,3	817	>100
					ng/μL	μL

Currently, the Department of Biochemistry and Biotechnology of Wildebeest Plants of the Central Botanical Garden of the National Academy of Sciences of Belarus has formed a collection of DNA varieties of representatives of genera:
- Lolium perenne L, Lolium multiflorum Lam.,
- Festuca arundinacea SCHREB.; - Agropyron cristatum L.;
- Festulolium; - Alopecurus pratensis L., Alopecurus arundinaceus Poir. and their:

- Lolium perenne L X Lolium multiflorum Lam.;
- Alopecurus pratensis L.X Alopecurus arundinaceus Poir.;
- Lolium perenne L, X Agropyron cristatum L.;
- Lolium perenne L, X Festuca arundinacea SCHREB.

39 samples in a total.

The obtained high-quality total DNA preparations of varieties and hybrid forms of perennial grasses were used for DNA labeling using marker systems.

Based on molecular markers, we have developed a system of DNA certification of varieties and hybrids of perennial grasses. For molecular genetic certification of varieties and hybrids of perennial grasses, based on the literature data (Paäakinskiene,2000, Arghavani,2010) a pool of primers was selected. All primers were tested for highly polymorphic, reproducible markers obtaining.

DNA labeling of all hybrid forms and varieties of perennial grasses using the marker systems, listed above, made it possible to differentiate all the genotypes studied, develop and compile unique profiles for each of them, calculate the genetic distances of kinship/remoteness. Based on the obtained DNA spectra the genetic passports were compiled for the studied samples (Table 3).

Table 3. Molecular genetic passports on the example of an interspecific hybrid of the genus *Lolium* L. and parental forms, compiled on the basis of the results of multilocus labeling of total DNA

Lolium multiflorum LAM. , Matador

Праймер	Маркер, bp
SCoT	
SCoT–01	1656, 1271, 828, 781, 715, 658, 607, 538, 427, 357, 248
SCoT–06	1003, 675, 604, 527, 382, 310, 269, 221, 176
SCoT–13	849, 690, 610, 475, 408, 356, 280, 202
SCoT–21	879, 693, 669, 481
SCoT–32	1093, 884, 534, 453, 386, 317, 264, 226
SRAP	
SRAP–Em06/Me02	970, 579, 449, 376, 298, 223
SRAP–Em06/Me09	1074, 907, 847, 686, 489, 453, 369, 213
SRAP–Em12/Me09	990, 896, 776, 533, 429, 349, 327
SRAP–Em13/Me05	1573, 1429, 1251, 544, 423, 365

Lolium perenne L., Guslyar

Праймер	Маркер, bp
SCoT	
SCoT–01	1656, 1271, 973, 828, 715, 607, 559, 510, 427, 357, 248, 201
SCoT–06	1003, 701, 527, 421, 221
SCoT–13	729, 690, 610, 488, 449, 408, 356, 280, 231, 202
SCoT–21	879, 693, 669, 550, 481, 304
SCoT–32	1525, 1093, 884, 613, 490, 352, 317, 264, 226
SRAP	
SRAP–06/Me02	579, 472, 376, 343, 258, 223
SRAP–06/Me09	1074, 847, 686, 630, 489, 453, 413, 249, 213
SRAP–12/Me09	896, 776, 459, 349
SRAP–13/Me05	1573, 887, 513, 423, 342, 288

Lolium L., hybrid 22-7, Vybor

Праймер	Маркер, bp
SCoT	
SCoT–01	1656, 1094, 866, 715, 658, 538, 486, 427, 357, 275, 201
SCoT–06	1119, 1003, 845, 742, 476, 310, 269, 221
SCoT–13	1002, 690, 610, 449, 408, 356, 280, 231, 202
SCoT–21	879, 812, 693, 669, 481, 432, 227
SCoT–32	1093, 884, 613, 490, 453, 431, 386, 317, 264
SRAP	
SRAP–m06/Me02	970, 895, 579, 472, 404, 376, 223
SRAP–m06/Me09	1074, 974, 907, 847, 630, 489, 453, 413, 369, 213
SRAP–m12/Me09	925, 842, 776, 459, 385, 349
SRAP–m13/Me05	1573, 730, 513, 423

CONCLUSIONS

The DNA passport presence for new highly competitive varieties of cereal grasses creation will allow:

- to check the compliance of new varieties with the criteria of the OOS test;
- to evaluate the genetic novelty of varieties, lines and hybrids;
- to assess the compliance of seed batches with the standard;
- to confirm the conditioning of seeds purchased abroad;
- eliminate the possibility of falsification of varieties and related economic losses.
- to improve the system of patenting new varieties, as well as to solve controversial issues of conformity and authorship of the variety according to the characteristics of the allel state of loci.

REFERENCES

Kilchevski A. (2014) Genetic foundations of plant breeding. Vol.4. Biotechnology in plant breeding. Genomics and genetic engineering. Minsk, Belarus, 653p.

Raybushkina N.(2019). The specifics of DNA extraction from plant objects.Biotechnology. Theory and Practice, No., pp. 9-24.

Dempster E.L., Pryor K.V., Francis D., Young J.E., Rogers H.J. (1999). Rapid DNA extraction from ferns for PCR-based analyses. Biotechniques.V. 27(1), pp.66–68.

Gorji, A.M., Poczai,P., Polgar,Z.,Taller,J.(2011) Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD)for diagnostic fingerprinting in tetraploid potato. Am.J. Potato Res.V88, pp.226-237.

Qing Hao, Zheng-An Liul, Qing-Yan Shu, Rui'eZhang, Jan De Rick, Liang-Sheng Wang.(2008) Studies on Paeonia cultivars and hybrids identification based on SRAP analysis. Hereditas 145, pp 38-47.