ISSN 1068-3674, Russian Agricultural Sciences, 2010, Vol. 36, No. 3, pp. 168–171. © Allerton Press, Inc., 2010.
Original Russian Text © P.P. Strel'chenko, O.I. Romanov, A.V. Konarev, K. Okuno, 2010, published in Doklady Rossiiskoi Akademii Sel'skokhozyaistvennykh Nauk, 2010, No. 3, pp.16–19.

PLANT INDUSTRY

Identification of Global Centers of Genetic Diversity of Grain Sorghum with the Use of Rice DNA Markers[1]

P. P. Strel'chenko[a], O. I. Romanov[a], A. V. Konarev[1], and K. Okuno[b]

[a] *Vavilov All-Russian Research Institute of the Plant Industry, St. Petersburg, 190000 Russia*

[b] *University of Tsukuba, Ibaraki, Japan*

e-mail: p.strelchenko@vir.nw.ru

Received September 22, 2009

Abstract—STS and InDel markers developed on the basis of DNA sequences of certain rice genes are used for a comparative study of grain sorghum accessions. The main sorghum genetic diversity centers are revealed, two in Africa and a third in regions of Central, East, and South Asia.

Key words: sorghum, genetic resources, DNA markers

DOI: 10.3103/S1068367410030055

The process of formation of cereal grains during their spread throughout regions with different soil and climatic conditions occurred under the effect of artificial and natural selection and brought about great ecogeographic and variety diversity, which is still difficult to classify. At the same time, information about genetic diversity of cultivated plants is of paramount importance both for perfecting the strategy of their effective preservation ex situ and purposeful expansion of genetic variability in collections and for determining ways of using collections in selection. A number of marker systems have been developed in molecular genetics which make it possible with a high accuracy to reveal polymorphism of plants in the structure of their DNA (PDRF, RAPD, AFLP, SSR, and SNP markers). Information recently obtained on the complete structure of the rice genome [1] opens broad possibilities for comparative genomics of cereals with consideration of the high level of synteny between their genomes [2] as well as the great similarity in primary gene sequences, even of such species that diverged long ago as rice and sorghum [3]. On the basis of these data, we assumed that rice DNA markers can be effective for investigating genetic diversity of other species of cereals and attempted to study the possibility of using them for developing an intraspecific genetic classification of sorghum, *Sorghum bicolor* (L.) Moench.

METHOD

Of the 48 studied sorghum accessions, 17 varieties originated from various African countries and 13, 11, and 7 from countries of respectively Central, South, and East Asia. Extraction of DNA (each accession was represented by one plant) and the polymerase chain reaction (PCR) were carried out as described earlier [4].

We used a set of 166 markers offered by Dr. S. Fukuoka from the National Institute of Agrobiological Sciences (NIAS, Tsukuba, Japan). It included 128 STS markers developed on the basis of rice cDNA sequences and 38 InDel markers obtained by identification of insertions/deletions in a comparative analysis of the genomic sequences of rice varieties. In the experimental design, each marker represents a component of the electrophoretic spectrum of DNA fragments obtained upon amplification with two oligonucleotide primers, which were identical to certain unique sequences of the rice genome. As a rule, these primers provide amplification with rice DNA of a 120–700 bp section of the identified gene, which differs in length in different varieties.

The products of PCR with sorghum DNA were separated in 3% agarose gel plates, stained with ethidium bromide, and documented in transmitted UV light. PCR products similar in size were analyzed by capillary electrophoresis in the HAD-GT12 system (eGene Inc.)in accordance with the producer's recommendations. The presence or absence of amplified DNA fragments when composing the binary matrix of initial data was encoded with the digits 1 or 0. On the basis of this matrix of the data, we constructed a matrix of simple matching similarity coefficients, which was used for clustering the varieties by the UPGMA method and for analysis by the principal axes method. All calculations and graphic constructions were done

[1] This work was supported by the Japan Society for Promotion of Science, JSPS, www.jsps.go.jp.

with the use of the NTSYS 2.0 statistical program package.

RESULTS AND DISCUSSION

Each of the 166 pairs of primers was used for PCR with DNA genotypes of six sorghum varieties originating from various regions of Asia and Africa. As a result of analysis, we selected 14 STS and 10 InDel markers which revealed differences between genotypes. These markers clearly revealed single loci localized in 10 of the 12 chromosomes of the rice genome (except chromosomes 6 and 10). They were all identified in the rice genome with the use of the database (RAP-DB, http://rapdb.dna.affrc.to.jp/) according to the sequences of both forward and reverse primers. Of the 24 selected primer pairs, 20 pairs provided synthesis of sections of 27 genes of proteins with a different category of their identification. The majority of proteins are a constituent of key enzyme complexes of cell metabolism and have sections binding ATP, FAD, NAD, zinc, and calcium. These complexes probably play an important role in adaptation and intraspecific differentiation of plants. It is interesting that among the identified genes there were also genes of stress-inducible proteins: heat shock and pathogenesis-induced.

We took into account 32 DNA fragments (components of the electrophoretic spectrum) in the analysis of genotypes of 48 sorghum varieties of various geographic provenances with all 24 primer pairs. One-component spectra were found for 20 markers; therefore, we can presume that the studied loci of the sorghum genome are orthologous to the corresponding loci of the rice genome. The distribution of 48 sorghum accessions (genotypes) on the phenogram constructed on the basis of the results of cluster analysis (Fig. 1) almost completely corresponded to their arrangement in the space of the first two principal axes (Fig. 2). In both cases, all accessions formed three main groups. Cluster A, as group A in Fig. 2, contained accessions mainly from different regions of Africa. Cluster B was the largest and had a complex structure: it united five subclusters. The majority of accessions of subclusters 1–3 originated from countries of South Asia (Bangladesh, India, Nepal, and Pakistan). Subcluster 4 contained accessions only from the region of Central Asia (Western China, Northern Pakistan, Turkmenia, and Uzbekistan), and subcluster 5 contained sorghum accessions originating mainly from East Asia (China, Korea, and Japan). On the whole, cluster B corresponded in composition of accessions to group B in Fig. 2. In this case, accessions both from Central and East Asia had a tendency toward a combined arrangement. Cluster C included accessions chiefly from East Africa (Kenya, Tanzania, and Uganda) and corresponded to group C, although

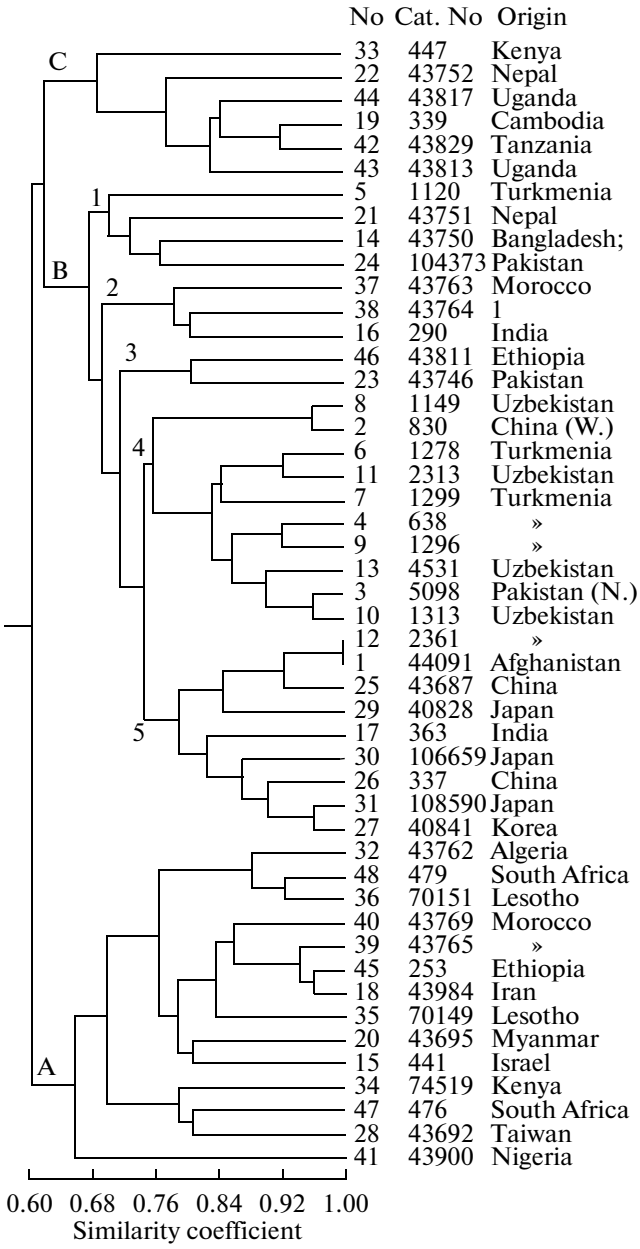

Fig. 1. Distribution of 48 sorghum accessions on a dendrogram constructed from the results of their analysis with the use of STS and InDel rice markers: Cat. No. is the catalog numbers of the NIAS Genebank (http://www.gene.affrc.go.jp/databases.plant_search_en/php); the catalog numbers of the VIR collection are underlined (http://www.vir.nw.ru/data/dbf.htm).

two accessions from Kenya occupied an intermediate position between groups A and C.

C. Kimber [5] suggested that sorghum was cultivated in East Africa about 3000–6000 years ago and then spread throughout the entire African continent and penetrated into Asia only in the first millennium of our era. As a consequence of the vast diversity and

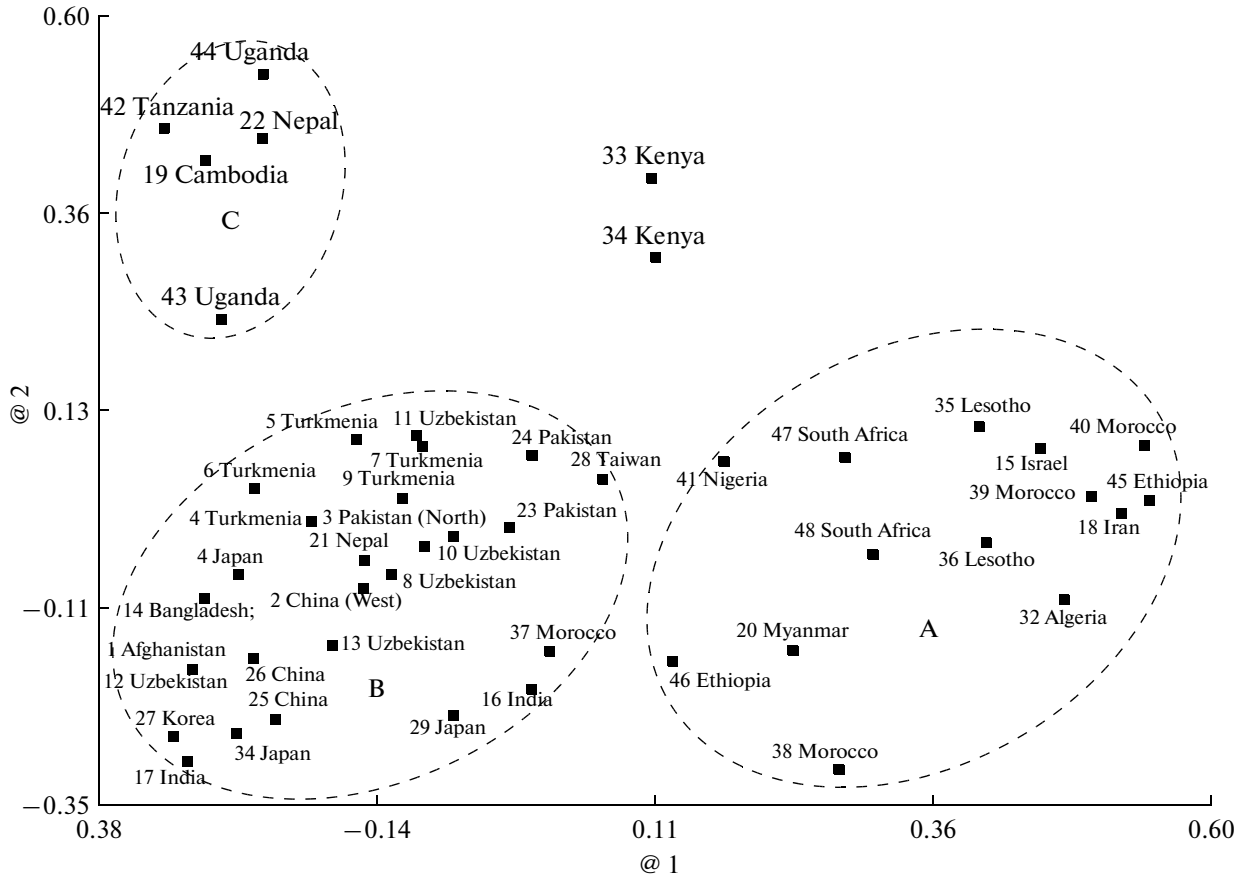

Fig. 2. Arrangement of 48 sorghum accession in the space of the first two principal axes according to the results of analyzing STS and InDel rice markers: the numbers of the accessions correspond to the numbers in Fig. 1.

large number of intermediate forms, there are various and rather complex botanical grain sorghum systems [6, 7]. In an investigation of a core sorghum collection with the use of PDRF markers [8], two main geographic pools were identified in the sorghum gene pool related to regions of Africa north or south of the equator. The majority of Asian sorghum accessions were united with African in different clusters. Such divergence in the evolution of African sorghum is possibly related to ethnic isolation of tribes that existed as far back as prehistorical time [9].

On the basis of an analysis of sorghum with the use of STS and InDel rice markers, we also showed the presence in the African sorghum gene pool of two main centers of genetic diversity, They corresponded to the two genetically most differing groups: C, which includes accessions from East Africa (Uganda and Tanzania), and A, containing accessions from other regions of that continent.

Since in our investigation Asian sorghum accessions formed an independent genetic group (B), we for the first time propose to single out Asian sorghum into an independent, third center of genetic diversity of this plant. According to our data, Asian sorghum evidently has an East African origin: the differences in arrangement of their varieties in Fig. 2 are determined only by the second principal axis. However, we represent it as an independent center of genetic diversity of sorghum, which has a long period of independent evolution. This is indicated also by our revealed differentiation of Asian sorghum into South Asian (subclusters 1–3), Central Asian (subcluster 4), and East Asian (subcluster 5). It should be noted that sorghum accessions from Central Asia were studied by us for the first time with the use of DNA markers and their genetic uniqueness is shown. They are represented most completely in the collection of the Vavilov All-Russian Research Institute of the Plant Industry (VIR) and are practically absent in other collections.

REFERENCES

1. International Rice Genome Sequencing Project. The Map-Based Sequence of the Rice Genome, *Nature*, 2005, vol. 436, no. 7052, pp. 793–800.
2. TIGR Syntenic Blocks between Rice and Sorghum, *Rice Genome Annotation*, 2007, http://www.tigr.org/tdb/synteny/sorghum/figureview_desc.shtml.

3. Campbell, M.A., Zhu, W., Jiang, N., et al., Identification and Characterization of Lineage-Specific Genes within the Poaceae, *Plant Physiol.*, 2007, vol. 145, issue 4, pp. 1313–1322.
4. Strel'chenko, P.O., Mitrofanova, O.P., and Konarev, A.V., Comparison of the Possibilities of RAPD, AFLP, and SSR Markers for Distinguishing Local Hexaploid Wheat Varieties, *Agrar. Rossiya*, 2004, no. 6, pp. 3–9.
5. Kimber, C.T., Origins of Domesticated Sorghum and Its Early Diffusion to India and China, *Sorghum: Origin, History, Technology, and Production*, Smith, C. and Federikson, E.C. (Eds.), New York: Wiley, 2000, pp. 3–98.
6. Yakushevskii, E.S., Species Composition of Sorghum and Its Selection Use, *Tr. Prikl. Bot., Genet. Selek.*, Leningrad: VIR, 1969, vol. 41, issue 2, pp. 148–178.
7. Harlan, J. and de Wet, J., A Simplified Classification of Cultivated Sorghum, *Crop Sci.*, 1972, vol. 12, issue 2, pp. 172–186.
8. Deu, M., Rattunde, F., and Chantercau, A Global View of Genetic Diversity in Cultivated Sorghums Using a Core Collection, *Genome*, 2006, vol. 49,no. 2, pp. 168–180.
9. Doggett, H., *Sorghum*, London: Longman Scientific and Technical, 1988.

SPELL: 1. synteny, 2. bicolor, 3. ethidium