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Research Article

Application of the Restriction Landmark Genome Scanning (RLGS) Method for Analysis of Genetic Diversity between Asian and African Sorghum

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Abstract

Restriction Landmark Genome Scanning (RLGS) used to detect large numbers of restriction landmarks in a single experiment and applied to analyze the genetic diversity of Asian and African sorghum accessions. This method is one of the genome analysis tools based on the concept that restriction enzyme sites can serve as landmarks throughout a genome. RLGS uses direct end-labeling of the genomic DNA digested with a rare-cutting restriction enzyme and high-resolution two-dimensional electrophoresis. It has an advantage of providing precise information on a spot intensity that reflects the copy number of restriction landmarks and to visualize differences in methylation levels across the genome. RLGS becomes very useful for doing whole genome scans that equals the work of thousands of polymerase chain reactions. A study was carried out using Sorghum accessions collected from countries *viz.,* Morocco, Nigeria, Sudan, South Africa, Japan, South Korea, and China. One representative sample was chosen from a country for analysis carried out at National Institute of Agrobiological Sciences (NIAS). Two dimensional spot images for seven accessions obtained and spot intensities were scanned. Totally, 119 spots were detected of which 95 spots observed as polymorphic and 24 as non polymorphic. Unique presence and null spots were specifically detected in all accessions taken for study. A total of 37 unique spots and 12 null spots, detected in this experiment. Principal Coordinate Analysis indicated, four African accessions scattered in the diagram were diverse and three Asian accessions closely distributed with narrow diversity. The phylogenetic tree showed that Sudan and Nigerian accessions were distant while Chinna, Japan and Korea accessions had close proximity.

Key words:

Restriction Landmark Genome Scanning (RLGS), DNA polymorphism, genetic diversity, sorghum, Genetic resources

Introduction

Sorghum is fifth most important food crop in the world used for food and fodder and industrial applications such as ethanol productio. It is warm weather annual crop, grown in arid and semi arid regions across the African and other countries. It has ability to withstand even severe drought to a prolonged time before rainfall and assures minimum grain and fodder yield. It is originated from northern part of Africa to other countries including Asia many thousand years ago. Sorghum has its own local names in many countries. It is called “Yanavala” in Sanskrit, (followed Barley) later Jowar, in India while it is called “Morokoshi” in Japan. India stands first among world countries in total area of cultivation with 12 % world production while America leads with highest productivity and shared 20 % world production. Nigeria comes second in production next to America with 16%. The other countries have major share in world production are Mexico 10%, Sudan 7%, Ethiopia 5%, Argentina 5%, Australia 4%, Brazil 3 % and China 3%. The International Crop Research Institute of Semi-Arid Tropics (ICRISAT) has the largest Sorghum germplasm collection of 38,000 accessions (http://www.icrisat.org/ sorghum/ Project1/ pfirst.asp). In Japan, Sorghum grows well in summer seasons like other temperate countries and research on sorghum started long back ago at National Institute of Agrobiological Sciences (NIAS) NIAS Genebank (http: //www.gene.affrc .go.jp/index_j. php) preserves the native cultivars of Sorghum and collects and introduces sorghums from

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40 countries in Africa and Asia and preserves more than 4,000 accessions.

Marker technologies like Restriction Fragment Length Polymorphism (RFLP), Amplified Fragments Lengths Polymorphism (AFLP), and simple sequence repeat (SSR) were applied for detecting polymorphisms (1-8) for various biological studies. However, of late, single nucleotide polymorphisam (SNP) gained momentum because of its high specificity and accuracy are used for detecting even mutation at one base level but it was a cumbersome process to analyze large number collections when analysis focused at worldwide or world collection with large number of accessions. A new approach has been employed to study genetic diversity of biological materials of large collections at a time through combinations of restriction enzymes makes rare cutting across the genome and involve the process of running 2D gel electrophoresis for yielding high-resolution results (9-11). called "Restriction Landmark Genome Scanning (RLGS)". This method has many advantages like an informative scanning capacity that allows detection of thousands of landmarks in a single profile, using different landmark enzymes extends the scanning field, the intensity of spots or restriction land marks reflects the copy number and also useful in detection of DNA methylation level based on spot intensity by using methylation-sensitive enzymes. In this study, Sorghum accessions collected from countries *viz.,* Morocco, Nigeria, Sudan, South Africa, Japan, South Korea, and China and one representative sample was chosen from a country for analysis carried out at National Institute of Agrobiological Sciences (NIAS). It revealed Nigerian and Sudan accessions had diverse and one of the source of orgin for distribution to other countries. Asian accessions revealed close proximity among them in this study.

Materials and Methods

Genome DNA was extracted from the flesh leaf tissues, grown in the field by the CTAB method (11-12). Leaf tissues (0.1g) were frozen in the liquid nitrogen and powdered with mortar and pestle, added with 20mg Polyvinylpyrrolidone (PVP) and 20mg Sodium Deodecyl Sulfate (SDS). A volume of 0.8ml of Cetyl Trimethyl Ammonium Bromide (CTAB) 1%, 0.1M Tris-HC(pH8.0), 50mM Ethylene Diamine Tetraacetic Acid, 1.4M Sodium Chloride, 0.1% β-Mercaptoethanol) added and mixed throughly. Subsequently, 40μg Proteinase K (MERCK) was added to the mixture and then, it was incubated in 56 C for 30 minutes, the supernatant was collected using Phenol/Chloroform/Isoamylalchohol (25:24:1) extraction solution and again by Chloroform/Isoamylalchohol (24:1). Finally, genome DNA was precipitated using iso-propyl alcohol and air dried.

The RLGS method employed the combination of restriction enzymes of *Not*I-*Eco*RV-*Bam*HI and two dimensional electrophoresis. Genome DNA digested with the *Not*I restriction enzyme and end labeled with radioisotope (α-32P dCTP and α-32P dGTP) and again digested with *Eco*RV. The digested Genome DNA subjected to electrophoresis with 0.8% SeaKemGTG Agarose gel, at 100V for 1 hour at 230V for 23 hours. The gel tube was changed to replace the buffer and the above subjected DNA was again digested in gel with *Bam*HI (2000U) and subjected to electrophoresis with 5% polyacrylamide gel, at 100V for 1 hour and then at 150V for 23 hours as per the protocol (13). Resolved gel was dried and auto-radiograph was performed with Fuji BAS2500 system. Two dimension spot images analyzed using high capacity scanning systems and polymorphic spots were detected. For a genetic diversity analysis, GenAlEx6 (http://www.anu.edu.au/BoZo/GenAlEx/) and MEGA4 (http://www.megasoftware.net/) were applied.

Results and Discussions

Restriction Landmark Genomic Scanning is a genome analysis method that allows for rapid simultaneous visualization of thousands of landmarks, or restriction sites. Using a combination of restriction enzymes some of which are specific to DNA modifications, the technique can be used to visualize differences in methylations levels across the genime of a given organism. RLGS employs direct labeling of DNA, which is first cut by a specific series of restriction enzymes, and then labeled by a radoactive isotope (usually phosphorous 32). A two dimensional electrophoresis process is then employed, yielding high-resolution results. The radioactive second-dimension gel is then allowed to expose a large sheet of flim. The radiation produced by the radioactive labeling will cause the film to be exposed wherever the restriction fragments have migrated during electrophoresis. The film is then developed, yielding a visual representation of the results in the form of an autoradiograph(9-11). This system identifies different landmark restriction enzymes (LE) that produce a well-focused and informative spot pattern.

Sorghum accessions of seven representative samples collected from Asian and African countries (1) subjected to RLGS to assess the polymorphism and diversity. The clearest patterns were generated in the *Not*I-*Eco*RV-*Bam*HI restriction enzyme combinations and the numbers of spots detected in the profiles in this study were 118 and of which 24 non polymorphic spots shown in the Table (2). Unique presence or null spots analyzed using high capacity automated scanning systems and 49 spots in total detected from above seven samples of which 37 spots (31%) were unique presence and 12 spots (10%) were null. The unique polymorphic spots obtained country wise were shown (Table 2). Principal coordinates analysis performed using this RLGS polymorphic data with the analysis software, “GenAlEx6.” The X-Y plot was drawn to locate the similarities and dissimilarities among the representative samples. The contribution rate of the first coordinates was 27% and the accumulation contribution rate of the first and second coordinates was 51 % (Fig.2). The plot revealed four African samples were diverse, scattered and three Asian samples China, Japan and Korea were in close proximity to South Africa. The genetic distance analysis was worked out using the RLGS spot data using the software “MEGA4”. The phylogenetic tree diagram (Fig. 3) revealed Sudan and Nigeria accessions were diverse and might be the genetic sources served for subsequent distribution to other countries. The analysis supported the geographical distribution of sorghum from northern Africa to other African and Asian countries. The diversity among the Asian sorghum namely China, Korea and Japan was low because of its possibility of selection and adaptation.

Compared with the conventional gene-detection technologies, such as Southern blot analysis and PCR, RLGS has the advantages even though it needs specially designed instruments such as high-efficiency scanning capacity instruments. The process can be extended to generate specific and accurate data using alternate restriction enzyme combinations that can be applicable to any genome/ organism. The spot intensity reflects the copy number from the restriction landmarks. The level of methylation among the genomes can be analyzed using methylation-sensitive enzymes and this approach is useful for methylation studies in various biological organisms in large cillections. The RLGS protocol can be accomplished in 5 days to 2 weeks.

RLGS becomes very useful when doing whole genome scans, and can effectively do the work of thousands of polymerase chain reactions at a time. It readily detects alterations deviating from normal, and thus is exceptionally effective in identifying hyper/hypermethylation in tumors, deletions or amplications of genes, or simply changes in gene expressions throughout the development of an organism.

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