DNA Markers in Soybean

Abstract
Plant molecular identity (ID) is necessary to describe molecular characteristics of plants, which should contain all of the required information. Using Inter-Simple Sequence Repeat (ISSR) and Random Amplified Polymorphic DNA (RAPD) primers, molecular ID can be described. Complete molecular ID system is described in this study for Soybean, which is important for the modern breeding and biotechnology point of view. Using five Soybean cultivars, we analysed the products of PCR with ISSR and RAPD primers and discussed the strategy for establishing their molecular ID. Using the segmented naming method, we designate the simple names and the full name systems of five Soybean cultivars.

Key words: Soybean; Molecular identity; Simple name; Full name; segmented naming method

Introduction
DNA markers have proven to be an efficient tool for molecular characterization in plant breeding, and are widely used in fingerprinting, diversity analyses, and gene mapping (1; 2). Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers, on the other hand, require only small amount of DNA sample without involving radioactivity tests and are simpler as well as faster. RAPD has proven to be quite efficient in detecting genetic variations (3), even in closely related organisms such as two near isogenic lines (NIL). ISSR technique is also very simple, fast, cost-effective, highly discriminative and reliable (4). At present, RAPD and ISSR markers have been successfully applied to detect the genetic similarities or dissimilarities in micro-propagated material in various plants (5; 6; 7; 8).

The inter simple sequence repeat (ISSR) technique is another PCR-based method, which involves amplification of the DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite directions. The technique uses microsatellites, usually 16-25-bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to mainly amplify ISSR sequences of different sizes. ISSR-PCR is a simple, quick, and efficient technique with high reproducibility. ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping, and evolutionary biology (9; 1). Random amplified polymorphic DNA (RAPD) is easy to perform and requires no information about the DNA sequence to be amplified. It has been used, therefore, to study the genetic diversity in various plant species (10; 11), authenticate the herbal medicinal materials (12), and detect adulterants (13; 14). These molecular IDs have proven very helpful in spice classification, identification, and germplasm protection. However, molecular IDs can vary a lot depending on the methods for naming the IDs as well as the construction system of the molecular IDs. It would be possible to establish
a plant ISSR and RAPD based ID system if a standard naming system were developed that contains enough information to reflect PCR conditions, such as primers used in the experiment for a certain plant, annealing temperatures, and PCR products. In general, the same crop should have the same molecular ID, and different plants should be distinguished with ISSR and RAPD markers. Using five Soybean cultivars, a complete ISSR and RAPD based molecular ID system is described in this study, which can be easily used and expanded with much more information. The objective of this study is an attempt to develop a plant molecular identity system in soybean using the DNA banding patterns from RAPD and ISSR marker systems. This can be useful in identity testing of plant varieties especially in case of disputes with respect to varietal identification.

Material and Methods

Five commonly used major Soybean (Glycine max) cultivars ASO-138, ASO-104, ASO-NRC37, ASO-JS9305 and ASO-JS335 were taken in this study (Table 1 and Fig. 1). All five cultivar showing different growth pattern in terms of Flower color, Pods hair, Days to 50% flowering and Plant height (cm.) and on the basis of above characters these cultivar were finalized. After election Genomic DNA was isolated by a modified cetyl-trimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1990). Genomic DNA was quantified on 0.8% and later NanoDrop Spectrophotometer. PCR was performed with 8 RAPD and 5 ISSR markers (Table 2). The PCR mixture contained: approximately 50 ng template DNA, 10 μL 2X Taq MasterMix, 1 μL 10 μM primer, and topped with distilled water to a total volume of 20 μL. The PCR program was run as follows: 5 min at 95°C for pre-denaturation; 36 cycles each at 94°C for 1 min, step down annealing temperature (Tm in°C,) for 1 min, and 72°C for 1 min; with a final extension at 72°C for 10 min. PCR products were electrophoresed on a 1.8% agarose gel. Clearly detectable amplified ISSR and RAPD bands were obtained ranging in size from 120 to 1200 bp for the five Soybean cultivars. Among the 14 primers, 5 were suitable ASO-138, ASO-104, ASO-NRC37, ASO-JS9305 and ASO-JS335. Out of five primers three RAPD (RIPC3, RIPC4 and OPB4) and two ISSR (B17899 and HB12) could be used for all five cultivars, and there were no PCR products observed for eight primers (RPIC 7, OPY 11, RPIC 9, OPB 4, B17898, A17898, HB10, and OPG 4).

The ISSR and RAPD fingerprinting profile for ASO-138, ASO-104, ASO-NRC37, ASO-JS9305 and ASO-JS335, based on the selected primers, are shown in Figure 2. A complete molecular ID was used to describe the ISSR and RAPD fingerprints for ASO-138, ASO-104, ASO-NRC37, ASO-JS9305 and ASO-JS335, based on the selected primers, are shown in Figure 2.

Table 2. List of markers used in this study

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Primers</th>
<th>Tm (in °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RIPC 3 (RAPD)</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>RIPC 7 (RAPD)</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>OPY 11 (RAPD)</td>
<td>32.8</td>
</tr>
<tr>
<td>4</td>
<td>OPB 4 (RAPD)</td>
<td>26.4</td>
</tr>
<tr>
<td>5</td>
<td>RIPC 9 (RAPD)</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>OPB 4 (RAPD)</td>
<td>26.4</td>
</tr>
<tr>
<td>7</td>
<td>B17899 (ISSR)</td>
<td>47.7</td>
</tr>
<tr>
<td>8</td>
<td>B17898 (ISSR)</td>
<td>44.7</td>
</tr>
<tr>
<td>9</td>
<td>A17898 (ISSR)</td>
<td>40.1</td>
</tr>
<tr>
<td>10</td>
<td>HB10 (ISSR)</td>
<td>37.4</td>
</tr>
<tr>
<td>11</td>
<td>HB12 (ISSR)</td>
<td>56.2</td>
</tr>
<tr>
<td>12</td>
<td>RM1 (R,F) (SSR)</td>
<td>66.3</td>
</tr>
<tr>
<td>13</td>
<td>OPG 4 (RAPD)</td>
<td>29.2</td>
</tr>
<tr>
<td>14</td>
<td>RIPC 4 (RAPD)</td>
<td>35</td>
</tr>
</tbody>
</table>

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Fig. 1. Seed Morphology of Five Soybean Varieties

Fig. 2. ISSR and RAPD based fingerprinting profile of five Soybean cultivars with different primers. (M: 100bp DNA ladder and 1 to 5: AS0-138, AS0-104, AS0-NRC37, AS0-JS9305 and AS0-JS335)

<table>
<thead>
<tr>
<th>Seed type Features</th>
<th>AS0-138 (1)</th>
<th>AS0-104 (2)</th>
<th>AS0-NRC37 (3)</th>
<th>AS0-JS9305 (4)</th>
<th>AS0-JS335 (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower color</td>
<td>PINK</td>
<td>WHITE</td>
<td>WHITE</td>
<td>PINK</td>
<td>PINK</td>
</tr>
<tr>
<td>Pods hair</td>
<td>SMOOTH</td>
<td>HAIRY</td>
<td>HAIRY</td>
<td>SMOOTH</td>
<td>SMOOTH</td>
</tr>
<tr>
<td>Days to 50% flowering</td>
<td>35</td>
<td>35</td>
<td>46</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td>Plant height (cm.)</td>
<td>60</td>
<td>80</td>
<td>50</td>
<td>40</td>
<td>50</td>
</tr>
</tbody>
</table>
RAPD fingerprinting features for a certain plant. The ID should contain the molecular ID naming part and the related explanatory part. The explanatory part should contain information related to the suitable primer sequences, optimized annealing temperature for the ISSR and RAPD-based PCR reaction, the PCR reaction system, and the electrophoresis image. Considering the informative characteristics and ease of use, we named the molecular ID with two forms: a simple name and a full-informative name. For the simple name, we designated the molecular ID with numbers reflecting the bands obtained from PCR. The complete name contains detailed information of the ISSR fingerprint profile.

The simple name was designed with six digital serial numbers (Figure 3). The 1st five letters (for RAPD primers and six latter in case of ISSR markers) digits represent the primer name (e.g., RPIC), and the 4th represents band numbers smaller than and including 500 bp, the 5th represents band numbers between 500 and 1000 bp, and the 6th represents band numbers larger than 1000 bp.

The full-informative name should contain all of the details of the ISSR fingerprinting profile. It is segregated into four parts, which include name of primer (A), total no of bands (B), PCR bands number (C), I, II, III, etc., to indicate the PCR fragment size (bp), and a final part, @abl which provides information about the institute (Figure 4).

![Figure 3. Demonstration of the simple name](image_url)

![Figure 4. Demonstration of the full name](image_url)
Simple name of ASO-138
RPIC3241-RPIC4021-B17899431-HB12440-OPB4730

Simple name of ASO-NRC37
OPB4730-RPIC3030-RPIC4022-B17899441-HB12430

Simple name of ASO-104
RPIC3030-RPIC4021-B17899241-HB12430-OPB4730

Simple name of ASO-JS9305
RPIC3331-RPIC4021-B17899441-HB12330-OPB510

Discussion

ISSR marker analysis is an easy, fast, inexpensive, accurate, and reliable technique for genetic analyses of various plant species (2). A complete molecular ID should contain all of the detailed information of target plants. To establish the representative molecular ID, it is critical to collect and reflect all of the information from the amplified ISSR profile. The molecular IDs currently used are relatively quite simple and

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incomplete. Most of the time, the PCR band is scored as 1 when it is present and as 0 when it absent, and serial numbers are assigned to molecular IDs based on PCR results (16; 17; 18; 19). In 2009, a molecular identity (ID) dataset, based on numeralized data from polyacrylamide gel electrophoresis (PAGE) bands, was established with ID Analysis software for 83 Soybean cultivars in Heilongjiang, China (20). Subsequently, more molecular IDs of more plants were established in China, including those of sweet sorghum (21) kenaf (22), flax (23), hybrid rice (19), peach (24), sugarcane (25), peanut (17), and Spiraea (16). Such molecular IDs are difficult to be widely used across plants due to their limitations and low information content. In the present study, we established naming systems, which fully reflect the plant characteristics of molecular ID. The names contain information related to primers, the PCR reaction system, bands obtained, and the information provider. The system is also easy to use. For example, primer RPIC3 can be used to distinguish ASO-138 and ASO-NRC37, ASO-JS9305 with their simple names RPIC3241, RPIC3030 and RPIC3331, it is easy to find the full name difference between ASO-138 (ARPIC3B07C1200II45 01II600IV700V8 00VI11000VI1200@ablr), ASO-NRC37 (ARPIC3B03C160 01II700II750@ablr) and ASO-JS9305 (ARPIC3B07C 1200II250III4 01IV600IV700VI8 00VII11000@ablr). Both simple and full names can be easily used or combined according to specific needs. As the 1st four letters represent the primer name, the molecular ID database can also be easily expanded when more primers are introduced into the experiment. For this system, more than 999 primers can be chosen for one plant.

In conclusion, we designated a complete naming system that includes descriptive information and molecular ID information. The descriptive part should contain the detailed information of ISSR and RAPD amplification conditions, and the molecular ID part should contain the simple name and full name system using the segmented naming method. Therefore, a new complete molecular ID system was established, which can be easily used and expanded as more information becomes available. Hopefully, this system will provide an improved solution for the characterization of ISSR and RAPD markers.

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References

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