Rapid and Reliable Purity Identification of F1 Hybrids of Maize (Zea may L.) Using SSR Markers

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Abstract Using a rapid and cheap DNA extraction method, genomic DNA extracted from two maize F1 hybrids and their parental lines was subjected to SSR analysis with 10 pairs of primer. Four of them could detect polymorphism between the male parents and the female parents of the two hybrids, which could be used for purity assessment of the hybrids. Moreover, the purity of seed samples of the two F1 hybrids was identified using the SSR analysis with the four pairs of primer and isozyme analysis respectively. For one hybrid, the results obtained using the two methods are consistent, but for the other one, isozyme analysis failed to detect any polymorphism between the parental lines because they are closely related. These results clearly demonstrate that SSR marker should be useful for assessing purity of maize hybrid, even if the hybrid derived from two related parental lines.

Keywords Maize (Zea may L.), SSR marker, Purity identification, F1 hybrid

Background Hybrid maize technology is a feasible and readily available option for raising the field potential of it, and maintenance of high level of genetic purity of hybrid is essential to exploit the high level of heterosis. However, during hybrid seed production, incomplete detasseling will result in high degree of self-pollination between male (staminate inflorescence) and female (pistillate inflorescence) flowers of the same plant. Therefore one of the challenges is rapidly and accurately assessing the purity of the hybrid seeds before they are supplied to farmers.

The most widely used method for determination of sib (inbred seed, seed from self-pollination of parental lines) levels in the F1 hybrid is the grow out test (GOT) that involves growing a representative sample of the seed followed by analysis of several morphological and floral characteristics of the plant to determine the sib levels. This method is time-consuming and costly, and requires extensive use of land. Moreover, the grow out test are often affected by environment which making the determination difficult (Noli et al., 1999). Several alternative methods (such as isozyme analysis and isoelectric focusing) for the hybrid purity identification are also suggested. Whilst these methods have been proven to be effective, their uses are limited by their failure to detect polymorphism in some closely related lines. So there is a need for more sensitive methods to test purity of hybrid seed. Simple sequence repeats (SSR), also known as microsatellite, is a class of genetic marker that has proven to be abundant and well distributed throughout the genome of plants (Wu and Tanksley, 1993; Chin et al., 1996). Because they are codominant, detect high levels of allelic diversity, and are assayed efficiently by the polymerase chain reaction (PCR), they have been used for assessing purity of F1 hybrid seed in some crops (Smith and Register, 1998; Wu et al., 2001, Yashitola et al., 2002). In this study, coupled with a high-throughput DNA extraction based on 96-well microtitre plate, we also shown that SSR marker is a useful tool for purity identification in F1 hybrid of maize.

1 Materials and Methods
1.1 Plant materials
Maize seeds: two F1 hybrids, Nongda 108 (178 × Huang C) and Zhengdan 958 (Chang 7-2 ×Zhengdan
and their respective parents were analyzed in this work. The two hybrids are in commercial cultivation at various locations in China. Seeds were stored desiccated at 4°C until used.

1.2 DNA extraction
Embryos cut from seeds were placed into individual wells of a 96-well polypropylene microtitre plate. 200 μL 0.01 mol/L NaOH solution was added to each well used. The microtitre plate was covered using 96-well plastic plate covers and incubated in the boiling water for 4 min. Following heating, 200 μL neutralizing solution (HCl, pH 2.0) was added to each well used. After the plate being shaken slightly for 15s, the DNA extracts could be directly used for template of PCR amplification or stored in 4°C.

1.3 PCR amplification (SSR)
Ten pairs of primer (umc1165, umc1294, nc030, bnlg161, umc1165, phi001, phi034, phi057, phi080, phi119) chosen from Maize Database (http://www.maizegdb.org) were used in the present study. We performed PCR amplification in 10 μL reaction volume in each well of a 96-well plate. Each reaction well contained 1 μL DNA extract, 0.25 mmol/L dNTPs, 400 pmol/L forward and reverse primer, 1×PCR buffer without MgCl₂, 2.5 mmol/L MgCl₂, 1 unit (0.2 μL) Taq polymerase (Sangon, China). Thermocycling consisted of an initial 5 min denaturing step at 94°C followed by 35 cycles of denaturing (94°C) for 40s, primer annealing (60°C) for 35s, extension (72°C) for 45s, and a final extension of 5 min at 72°C. Polyacrylamide gel electrophoresis (PAGE) and fragment analysis of the amplified products were performed according to method of Creste et al. (2001).

1.4 Isozyme analysis
Proteins were extracted from individual parental and hybrid seeds by grinding in 50 μL of extraction buffer (0.1 mol/L Tris, 1.0% w/v reduced glutathione, pH 8.5). Whatman paper wicks were used to uptake the extracts and were loaded onto 12% starch (Sigma) gels. After 5 h of electrophoresis at 250 V at 4°C in Tris-citrate/borate buffer, the gels were stained for esterase (EST), peroxidase (PO), and malate dehydrogenase (MDH) (Stuber et al., 1988).

2 Results and Discussion
The major limitation to implementation of DNA molecular markers in purity identification is the lab involved in DNA extraction from hundreds of seeds. The current modified DNA extraction protocol (Steiner et al., 1995) relies on a multi-step procedure where samples are treated individually. This method allows one person to extract a maximum of 144 per day. To enable rapid identification, we have devised a sodium hydroxide DNA extraction method based on a 96-well microtitre plate from maize seeds, which reduces the extraction time while keeping the cost low and avoiding the use of hazardous chemicals. PCR detection of the DNA quality showed it was sufficient for SSR analysis. Using this procedure, one person can perform DNA extractions and initiate PCR amplifications at a rate of 4 plates per 3 h. Allowing 2 h for PCR amplification-382tion and an additional 2 h for PAGE electrophoresis, the genetic analysis of 384 samples can be carried out in approximately 7 h by one person.

Genomic DNA was extracted from two hybrids and their respective parents using the above method. 10 pairs of SSR primer (shown in material and method) were screened with the DNA for polymorphism between parental lines of each F1 hybrid. Because SSR markers are codominant, only one allele was detected in a hybrid when the parents were monomorphic for a particular microsatellite locus and two alleles (one allele per parent) were present in a hybrid when polymorphism was detected between the male parent and female parent. According to the principle, four (phi080, nc030, phi057 and phi034) of the ten microsatellite loci analyzed displayed polymorphism. For the parental combinations of Nongda 108, phi080, nc030, phi057 could be used for purity identification of the F1 hybrid (Figure 1A), and for the parental combination of Zhengdan 958, phi080 and phi034 could (Figure 1B).

In order to test the reliability of the SSR marker, all of the polymorphic primers screened above were used for purity analysis of 100 seeds of the two F1 hybrid samples respectively. The analysis result was shown in Table 1. For each F1 hybrid, the percentages of sib
obtained using different prime pairs are very similar to each other. Furthermore, except for one seed of the sample of Nongda 108 which was not detected by the primer pair of phi057, the seeds identified as sibs by one primer pair were confirmed to be sibs by the other primer pairs as well (Table 1). Those results indicate that SSR marker is a very reliable method for assessing hybrid seed purity.

Table 1 Genetic purity of maize F1 hybrids obtained using the SSR marker

<table>
<thead>
<tr>
<th>Variety</th>
<th>Parental combination</th>
<th>Total seeds tested</th>
<th>Primer pairs</th>
<th>Number of sibs</th>
<th>Sibs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nongda 108</td>
<td>178×Huang C</td>
<td>100</td>
<td>phi080</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>nc030</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>phi057</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Zhengdan 958</td>
<td>Chang 7-2×Zhengdan 58</td>
<td>100</td>
<td>Phi080</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>phi034</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Figure 1 Polymorphism of SSR markers between parental lines and their hybridsNotes: A: Polymorphism between the combination of Nongda108 at phi080 loci (lanes 2–4), nc030 loci (lanes 5–7) and phi057 loci (lanes 8–10); Lane1: DNA ladder marker, Lane 2: 178, Lane3: Nongda 108, Lane 4: Huang C, Lane5: 178, Lane 6: Nongda108, Lane 7: Huang C, Lane 8: 178, Lane 9: Nongda 108, Lane10: Huang C. B: Polymorphism between the combination of Zhengdan 958 at phi080 loci (lanes 2–4) and phi034 loci (lanes5–7); Lane 1: DNA ladder marker, Lane 2: Chang 7-2, Lane 3: Zhengdan 958, Lane 4: Zhengdan 58, Lane 5: Chang 7-2, Lane6: Zhengdan 958, Lane 7: Zhengdan 58

Isozyme analysis recommended by ISTA rule is commonly used for purity identification of maize hybrid seed (Orman et al., 1991; Smith and Wych, 1995). In this study, the purity of the two F1 hybrids sample was also assessed using the isozyme analysis. For Nongda 108, only esterase could detect the polymorphism between the parental lines. The percentage of sibs obtained using esterase is 6%, which is consistent with the result of SSR analysis. But for Zhengdan 958, the isozymes failed to detect any polymorphism between the parental lines. We think the main reason for this is that the parental lines of Zhengdan958 are closely related, and the standard isozyme method could not detect the slight difference between them. The result suggests that, compared with the isozyme analysis, SSR analysis is not only reliable, but especially useful when the hybrid derived from closely related parental lines.

The SSR marker is currently the preferred molecular marker for purity identification in some crops (Smith and Register, 1998; Yashitola et al., 2002) due to its highly desirable properties (high efficiency and simplicity). In this study, coupled with a high-throughput DNA extraction, we have shown that SSR marker is a useful tool for purity identification of maize hybrid. Furthermore, up to date, there are 2 300 pairs of SSR primer available in the Maize Database, which is sufficient for purity identification of all the maize hybrids. Thus, it is likely that SSR PCR identification of seed purity may supersede the current methods of grow out test and protein analysis.

Acknowledgements

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