Identification of Genetic Relationship of Hybrid Rice Varieties to Their Parents with PCR Products

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Abstract [**Objectives**] To establish a simple, rapid and accurate method for identifying the genetic relationship of hybrid rice varieties to their parents. [**Methods**] Taking F_1 hybrids Liangyou 336, Deliangyou Huazhan, and the parents of Liangyou 336, *i.e.*, C815S (♀) and R336 (♂), as experimental materials, the genetic relationship of the hybrid rice varieties to the parental materials was identified by way of PCR amplification with the 48 pairs of SSR primers of *Protocol for Identification of Rice Varieties: SSR Marker Method* (NY/T 1433-2014). [**Results**] The genetic relationship of the hybrid rice varieties could be determined by comparing the PCR amplification products of the mixed DNA of the parents and the DNA of the F_1 hybrids. [**Conclusions**] This method not only reduced the number of samples required but also had a good visual effect and high accuracy.

Key words Hybrid rice, Parent, SSR primer, PCR, mixed DNA, Genetic relationship

1 Introduction

With the continuous development of biotechnology, molecular marking based on PCR technology have been widely used to analyze the genetic relationship among hybrid seeds. The authenticity testing of hybrid rice seeds has always been a big problem that plagues the seed industry. After counterfeit seeds take the opportunity to enter the market, due to technical limitations, the seed management department cannot provide accurate and comprehensive evaluations of commodity seeds in time during law enforcement inspections, which often causes irreparable losses to farmers. The storage life of hybrid rice seeds at room temperature is generally 8 - 12 months[1]. Therefore, it is necessary to carry out parent seed propagation every year. Only after obtaining accurate authenticity and purity identification results can seeds be flowed into the market for agricultural production. How to identify the authenticity of hybrid seeds through the preserved parental seeds is of great significance for variety protection and enterprise rights protection. SSR markers have been widely used, for its stability, repeatability and good polymorphism, to test the purity and authenticity of hybrid rice seeds[2]. The currently used method to determine whether the hybrid combination promoted on the market comes from a pair of parents is to compare and analyze the PCR products of the test sample and its parents using 48 pairs of SSR primers of the agricultural industry standard of the People's Republic of China. According to whether the F_1 hybrid has a complementary pattern of male and female parents, it is determined whether the test sample is related to the father and mother, but the intuitiveness of this method is poor. To establish a rapid, simple and accurate method for identifying the genetic relationship among hybrid rice varieties is of great significance for the parental reproduction of hybrid rice varieties and the production of hybrid rice seeds.

2 Materials and methods

2.1 Experimental materials The experimental materials used in this study were F_1 hybrids Liangyou 336, Deliangyou Huazhan, and the parents of Liangyou 336, *i.e.*, C815S (♀) and R336 (♂).

2.2 Extraction of DNA from parental materials and samples The rapid extraction of seedling DNA referred to the method of Zhan Qingcai[3]. DNA of old leaves was extracted with CTAB method. A certain amount (0.5) g of leaf sample was added with an appropriate amount of liquid nitrogen, ground into powder, quickly transferred to a 2-mL centrifuge tube containing 0.5 mL of 2× CTAB extraction buffer, mixed thoroughly, added with 0.5 mL of 1×CTAB extraction buffer, placed in a constant-temperature water bath at 55 - 65 ℃ for 30 min, added with chloroform: isoamyl alcohol (24∶1) until the tube is full, shaken thoroughly for 5 min, and centrifuged at 10 000 g for 2 min at room temperature in success. The supernatant collected was added with 0.6 time the volume of isopropanol, mixed well, let stand at room temperature for 30 min, and centrifuged at 10 000 g for 5 min at 4 ℃ in success, and the precipitate was collected. The precipitate was fully dissolved in 0.8 mL of high-salt buffer solution and centrifuged at 15 000 g for 2 min. The supernatant collected was added

Received: March 3, 2020 Accepted: April 27, 2020
Supported by Agricultural Science and Technology Innovation Fund of Hunan Province (2019LS06).
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with two times the volume of 95% cold ethanol, let stand in a −20 ℃ refrigerator for more than 2 h, centrifuge at 15 000 g for 10 min, washed once with 70% cold ethanol, dried naturally, and dissolved in 200 μL of 0.1 × TE buffer or ultra-pure water. Although the CTAB method has more steps and requires more time, the purity of DNA extracted is high and the amplification effect is good. In order to ensure high-quality amplification effect and reliable repeatability, it is recommended to use the CTAB method[4].

PCR amplification was performed using the 10-μL reaction system and program optimized by Liu Zhixi *et al*[5]. The PCR reaction was carried out in a reaction volume of 10 μL, containing 1 μmol primer, 1 μmol dNTP, 0.2 unit Taq Gold DNA polymerase, 1 μL 10 × PCR reaction solution (100 mmol Tris-HCl, pH 8.3, 15 mmol $MgCl_2$, 500 mmol KCl, 0.01% gelatin) and 10 ng template DNA. The amplification program was as follows: denaturation at 94 ℃ for 4 min, 94 ℃ for 30 s, 55 ℃ for 30 s, 72 ℃ for 1 min, 35 cycles, and extension at 72 ℃ for 7 min (9700, PE Corporation). The amplified products were separated by electrophoresis on a 4% Nusieve 3:1 agarose gel, stained with ethidium bromide and observed and photographed under ultraviolet light.

3 Results and analysis

3.1 Identification of genetic relationship by comparing PCR products of DNA of male parent, female parent and sample Taking Liangyou 336, Deliangyou Huazhan, and the parents of Liangyou 336, *i.e.*, C815S (♀) and R336 (♂) as materials, authenticity testing was performed based on the 48 pairs of SSR primers of the agricultural industry standard of People's Republic of China (*Protocol for Identification of Rice Varieties: SSR Marker Method*, NY/T 1433-2014). The results show that there were differences in a total of 12 pairs of SSR primers between Liangyou 336 and Deliangyou Huazhan, and the similarity was 75%. The national standard stipulates that if F_1 hybrid does not have a complementary band pattern of parents at more than two pairs of primers in the 48 pairs of SSR primers, it is defined as a variety that does not come from the parents. As can be seen from the electrophoregram in Fig. 1, for the primers RM71, RM471, RM336, RM219, RM493 and RM289, Deliangyou Huazhan did not show complementary band patterns of C815S and R336, indicating that it is not a descendant of C815S and R336. In comparison, for the 48 pairs of SSR primers, Liangyou 336 all showed complementary band patterns of C815S and R336, indicating that C815S and R336 are the parents of the F_1 hybrid Liangyou 336 (Fig. 1).

3.2 Identification of genetic relationship by comparing PCR products of mixed DNA of parents and DNA of sample Using the 48 pairs of SSR primers of the agricultural industry standard of People's Republic of China, the mixed DNA of C815S and R336 and the DNA of Liangyou 336 and Deliangyou Huazhan was amplified by PCR. The PCR products were analyzed by electrophoresis. The results show that the electrophoregram of the amplification product of parental mixed DNA (C815S and R336) was identical with that of the amplification product of DNA of Liangyou 336, indicating that the F_1 hybrid Liangyou 336 is a descendant of C815S and R336. As shown in Fig. 2, the electrophoregram of DNA amplification product of Deliangyou Huazhan was completely different from that of the PCR product of the parental mixed DNA at primers RM71, RM471, RM336, RM219, RM493 and RM289, indicating that the F_1 hybrid Deliangyou Huazhan is not a descendant of C815S and R336.

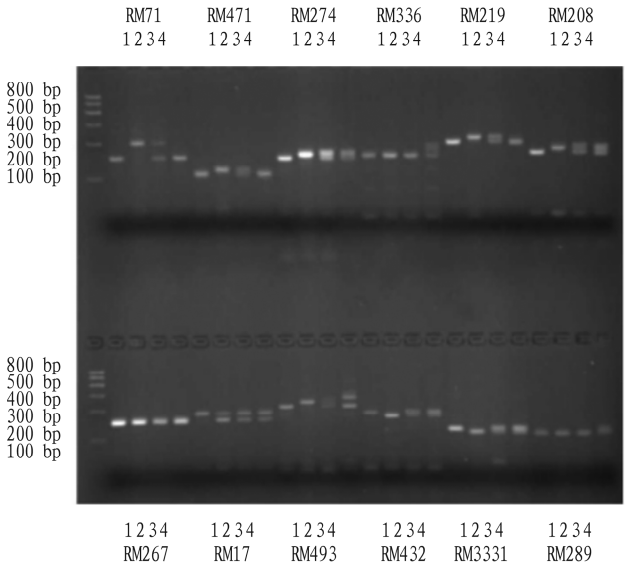

Fig. 1 Electrophoretogram of PCR products of the DNA of parents and F_1 hybrids based on 12 pairs of SSR primers

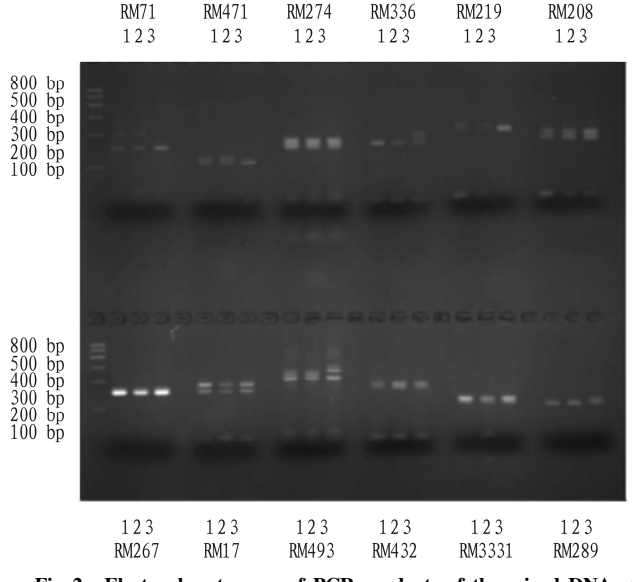

Fig. 2 Electrophoretogram of PCR products of the mixed DNA of the parents and the DNA of F_1 hybrids based on 12 pairs of SSR primers

4 Discussion

By comparing the DNA amplification products between parents and F_1 generation, the genetic relationship of combination

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platform.

5 Conclusion

To sum up, considering the existing problems of communication between alumni and students, the integration of learning resources and stimulating students' interest in the learning platform, the fully-participatory extracurricular learning platform with multiple linkage effect is constructed, relying on the alumni management system, with students as the main body, adding three major contents: interactive section, resource section and personality section. While enriching the functions of the platform, it can not only fully integrate alumni resources and give full play to alumni advantages, but also achieve effective interaction inside and outside the school, reduce school running costs and improve students' learning efficiency, so to provide reference for the establishment of extracurricular learning platforms for other majors inside and outside the school.

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with distant relationship between female and male parents and many polymorphic differences in the F_1 hybrid can even be identified accurately. Fig. 1 shows that sample 3 is a descendant of samples 1 and 2, that is, the F_1 hybrid Liangyou 336 is a descendant of C815S (♀) and R336 (♂). But for combination with close relationship and small molecular weight difference between male and female parents and small polymorphism difference with F_1 hybrid, a strong molecular biology foundation and experience are required to make accurate judgments. When identifying genetic relationship by comparing the DNA amplification products between sample and its parents, only the consistency of PCR products of several samples is compared, and the complementary band pattern of male and female parents does not need to be observed. Regardless of the genetic relationship between male and female parents and the difference in polymorphism of F_1 hybrids, the genetic relationship is judged accurately, and the intuitiveness is greatly enhanced. In addition, the sample size is reduced from 4 × 48 to 3 × 48. As shown in Fig. 2, samples 1 and 2 are the same variety, that is, the F_1 hybrid Liangyou 336 is a descendant of C815S and R336. Both the methods used in this study can accurately identify the kinship of the materials, but the method of comparing the PCR product of the parental mixed DNA with that of the DNA of control sample not only reduces the sample size but also has the advantages of clear band pattern and accurate, reliable and intuitive result. At the same time, it can be used for the genetic relationship identification of other crops and animals.

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