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Purity Test of Radish Hybrid Seeds Using Randomly amplified Polymorphic DNA Marker

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Abstract In order to develop a rapid and simple method for testing the purity of radish hybrid seeds using a procedure based on the PCR(Polymerase chain reaction), eighty random primers were screened with the genomic DNA extracted from five day old seedlings of inbred parent lines and their F1 hybrids. Two primers, HRM-02 (5'-GAGACC-AGAC-3') and HRM-19 (5'-TGAGGCGTGT-3'), generate reproducible unique PCR patterns which can identify each parent lines as well as their hybrids. In actual test of randomly selected hybrid seeds using the two marker primers, the purity tested by one primer was exactly same as that of other primer. It suggests that one marker primer selected in this experiment is enough for the purity test of radish hybrid seeds. We demonstrates the use of RAPD(randomly amplified polymorphic DNAs) markers to identify each of inbred parent lines and hybrids by rapid and simple method.

Key words: *inbred, hybrid, RAPD marker, purity test*

Introduction

Commercial seeds of the radish (*Raphanus sativus* L.) are F1 hybrids which are produced by crossing of self-incompatible parent lines[1, 2]. Sexual incompatibility is a widespread phenomenon in flowering plant which restricts inbreeding within populations and also seems to contribute to the reinforcement of species differentiation[3]. The self-incompatibility in *Cruciferae* has been well known phenomenon and contributed to effective breeding strategy for production of high quality and yield of hybrid radish roots [2, 3]. By crossing two genetically distant parent lines, hybrids are produced possessing the attributes of a high level of uniformity and increased vigor in terms of yield, quality, shape and texture as well as resistance to various environmental stresses [3-5]. The success for producing commercial F1 hybrid seed depends on maintenance of uniform parent lines and the reliable production of the hybrid seeds resulted from a direct cross between the two selected parents, normally self-incompatible [2,4,5].

In practice, the mechanism of self-incompatibility may not be entirely successful and a low level of self-pollination may occur resulting in the production of a small percentage of sibling or selfing production [4,5]. During the cultivation of commercial seed radish, the resulting 'sibs' or 'selfs' are readily identifiable because of their weakness in vigour as compare to hybrids. However, in commercial point of view, hybrid seeds contaminated with selfing or sibling seeds results in the reduction of uniformity and quality of the seeds and derogate their commercial value [2,3].

In case of autumn cropping radish, hybrid seeds are harvested on around June 15 and marketed on July 15 by seed companies, and then seeded by farmers on August 15 in Korea. The period of time between hybrid seed harvesting and marketing, the seed company must go through the process of drying, threshing, cleaning, coating, grading, packing and transporting. Due to the time restriction, the seed company cannot scrutinize the purity of hybrid seeds before marketing. To solve this problem, we tried to develope rapid and simple method for testing the purity of hybrid seeds using selected RAPD markers.

Materials and Methods

Inbreds and hybrids used

Radish inbred lines used were ER-101 and ER-450. These inbreds were maintained as pure stocks by subjecting to continuous selfing for about fifteen years and used as parent lines for producing hybrid seeds by crossing method of self-incompatibility.

DNA isolation and PCR

Seeds were germinated on water moistened filter paper in 9 cm petridish at 28℃. Five day old single seedling was collected and frozen in liquid N_2 and then ground with a mortar and pestle. Ground tissue was combined with CTAB buffer, and DNA was extracted as described previously [6].

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PCR reaction mix was 5 $\mu\ell$ 10×buffer, 0.1 mM each of dNTPs, 0.25 μM random primer, 10 ng template DNA and 0.3 units *Taq* DNA polymerase in 50$\mu\ell$ total volume. This mixture was exposed to 45 cycles of 1 min. at 95℃, 1 min. at 38℃ and 1 min. at 72℃ using the DNA Cycler from MJ Research. PCR products were electrophoresised in 1.2% agarose gel and visualized UV light by EtBr staining.

Primer selection and purity test

In order to select marker primers for the purity test of hybrid seeds, genomic DNA of parent lines and their hybrids were PCR analyzed using eighty primers. Two primers, HRM-02 and HRM-19, were selected based on the clarity and specificity of PCR products to parents and hybrids.

A randomly selected set of 100 hybrid seeds were germinated in 15 cm petridish at 25℃ for 3-5 days. Each single seedling was collected and extracted the genomic DNA for PCR analysis with selected marker primers. Based on PCR patterns of the hybrids, seeds were separated the crossing (hybrid) lines from the selfing (inbreeding) lines. The purity of hybrid seeds is calculated as number of hybrid seeds per those of total seeds tested.

Results and Discussion

Screening of primers for marker DNA

The PCR patterns of the radish genomic DNA amplified by random primers showed various profiles depending on the radish genomes as well as primers tested (Fig. 1). Eighty primers screened were classified into four groups based on the banding patterns of PCR products; First group is the primers showing no band in any of the parent lines, ER-101 and ER-450, second group for those showing same banding pattern between two parent lines (Fig. 1, Type I), third is different banding pattern among individuals within populations of the same parent line as well as different patterns between two parent lines (Fig. 1, Type II), and forth for same patterns among individuals within populations of the same line but clearly different between two parent lines (Fig. 1, Type III). For the purpose of purity test, only the forth group can be used as marker primer because the banding patterns of the PCR products are specific to each of parent lines as well as their hybrids.

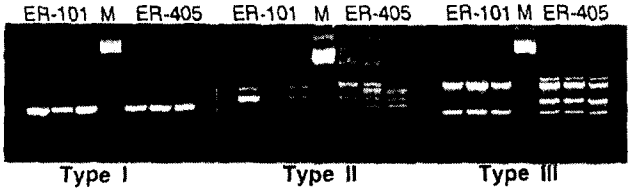

Fig. 1. Classification of RAPD primers by PCR amplication patterns with radish genomic DNA. Three individual seedling from each of inbred parents, ER-101 and ER-405, were analyzed by RAPD primers. M: marker DNA 123-ladder.

For purity test of the radish hybrid seeds, marker primer must possesses another requirement in addition to specificity between parent lines. The PCR patterns amplified by marker primer not only show no fluctuations among individuals within population of the same parent line but also among individuals in hybrids. Each parent line must possess at least one unique PCR band distinguishable to another parent line. Only two primers, so called HRM-02 (5'-GAGACCAGAC-3') and HRM-19 (5'-TGAGGCGTGT-3') out of eighty primers screened satisfied the requirements as marker primers for the purity test of radish hybrid seeds.

Purity test with marker primers

Fig. 2 show the PCR patterns amplified by hybrid specific primers, HRM-02 and HRM-19, with the genomic DNA of each parent lines and eighty randomly collected hybrid seeds from seed production farm. As indicated in the PCR profiles amplified by two marker primers, six inbred-lines, such as four ER-101 inbreds (marked with A) and two ER-405 inbreds (marked with B), were clearly identified in a group of eighty hybrid seeds (Fig. 2). Six inbred-lines which identified by HRM-02 primer could also be exactly identified by HRM-19 primer. It suggests that one marker primer selected in this experiment is enough for the purity test of radish hybrid seeds. We could calculate the purity of hybrid seeds by counting number of inbred individuals from total number of seeds tested. In case of Fig. 2, the purity of the hybrid seeds is 92.5%, six inbreds out of eighty seeds tested.

For the purity of hybrid seeds produced in recent three years, eighty to ninety-six hybrid seeds were tested with selected two marker primers, HRM-02 and HRM-19 (Table 1). The purity of hybrid seeds produced in the year 1997, 1998 and

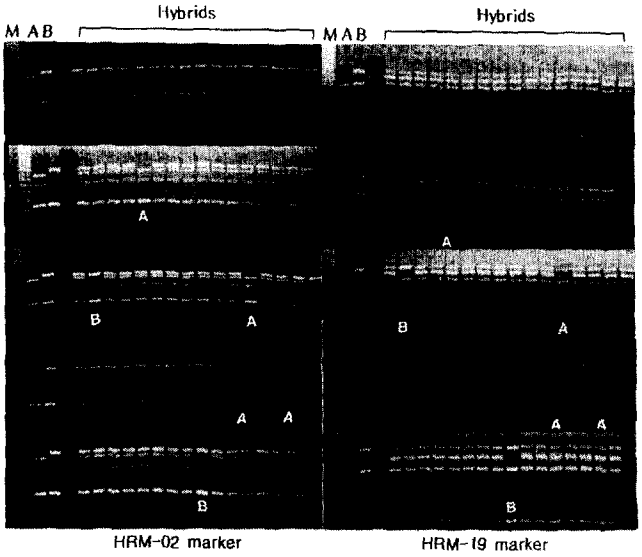

Fig. 2. Purity test on hybrid using RAPD markers, HRM-02 and HRM-19. M: marker DNA ladder, A: inbred parent ER-101, B: inbred parent ER-405. Selfed lines contaminated in commercial hybrid seeds are marked with A or B.

Table 1. RAPD purity test with HRM-02 and HRM-19 marker primers on the hybrid seeds produced by open pollination between ER-101 and ER-450 radish parent lines

Year of seed production	No. of seeds tested	Marker primers used	No good PCR	Ratio of inbreds and hybrids(%)		
				Inbreds		Hybrids
				ER-101	ER-450	
1997	96	HRM-02	6	2.2	3.3	94.4
1998	80	HRM-02	3	3.9	1.3	94.8
1999	80	HRM-02	0	5.0	2.5	92.5
1999	80	HRM-19	0	5.0	2.5	92.5

1999 showed 94.4, 94.8 and 92.5%, respectively. In this test, seeds showing no good PCR bands were excluded in calculating the purity of hybrid seeds. Our results demonstrate that RAPD markers are useful for testing the purity of F1-hybrid seeds.

Recently, techniques based on PCR have been used to detect polymorphisms in various plants, animal, and bacterial species [7-10]. RAPD markers can generate fingerprints of DNA product, some of which are polymorphisms that are present in one parent but not in the other. Polymorphisms detected by RAPD are inherited in a Mendelian manner and can be generated for any species without DNA sequence analysis [7, 10]. Detection of polymorphisms by using RAPD technology is faster and less laborious than by RFLP [11-13]. The main advantages of RAPD over traditional RFLP technology are increased speed of analysis and dramatic reduction in the amount of DNA required for analysis. Reduction in the amount of DNA required for analysis is particularly important to speed up early screening.

In case of radish, RAPD analysis could be done 3-5 days after germination instead of 2-3 weeks in RFLP or protein analysis. Using one 96-well PCR machine, a single person can complete the analysis of approximately 250 individuals with a primer in ten days period, from the beginning of seed germination to final results; similar results with RFLP analysis would take at least 3-4 weeks, and most likely longer. However, the main disadvantage of RAPD method in relation to the purity test of hybrid is that most of polymorphisms detected are considered as dominant marker, for example, presence versus absence in each of the parents, and therefore heterozygotes can not be distinguished accurately. To solve this problem, RAPD marker which can be used for purity test should be tested with many individuals of population in the same parent line to get reproducible result.

This experiment also suggest that it is the most important to select specific RAPD markers out of many random primers for rapid identification of each inbred parent lines and their hybrids, and for the purity test of hybrids.

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