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A Search of Pollen Fertile Clones in the Iberian Garlic by RAPD Markers

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Introduction

Various types of markers, such as isozymes, RFLP, RAPD, AFLP, SCARs, and CAPSs (cleavage amplified polymorphic sites), have been used in a lot of plant science fields, including genetic mapping[7], identification, amplification or introduction of the favorable genes [2, 17], MAS in breeding[11, 13, 24, 22], and testing seed quality[20]. However, it has been limited still within the phylogenetic analyses in garlic[1, 2, 12, 16, 21]. In the previous report[9], it was found that the two RAPD markers, $OPJ12_{1300}$ and $OPJ12_{1700}$, were related to the pollen fertility. In this report, prior to the fertility examination, the analysis by these two markers was applied to 30 clones collected in Spain and Portugal, of which 29 were bolting and one was incomplete-bolting clones.

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Materials and Methods

Plant materials and total DNA isolation.

Thirty garlic clones (Table 1) collected in Spain and Portugal in the summer of 1996 were used in this study. Nos. 419 to 450 were offered friendly by Spain Gene Bank, and No. 452 to No. 457 were collected at the local markets in Spain. Nos. 460 to 470 were offered kindly by Braga Gene Bank of Portugal. They were grown at Kagoshima University from the autumn of 1996 to the summer of 1997.

Total DNA was extracted from fresh young leaves and was purified by the methods described in the previous report[9].

Primer and PCR conditions

A 10-base oligonucleotide primer, OPJ12 (5'-GTCCCGTGGT-3') from Operon Technologies (Alameda, USA), was used in this study to amplify the total DNAs. In the previous study, two RAPD markers, $OPJ12_{1300}$ and $OPJ12_{1700}$, were obtained by this primer[9]. They were considered to be related to the pollen fertility

The PCR protocol described by Williams[23] was employed for DNA amplification. The elements of cocktail and the temperature profile for PCR were described in the previous report[9]. The amplification products were separated by electrophoresis in the gel of 1.4 % agarose in TAE, including 1.0 μg/ml ethidium bromide. The electrophoretic patterns were analyzed, using Densitograph (ATTO Macintosh Version 1.0) to find the RAPD markers

Table 1. Thirty garlic clones collected in Spain and Portugal for this study

Clone No.	Origin or Acc. No.	Source	Bolting scars when collected
No. 419	410/86	Spanish Gene Bank	Bolting
No. 222	879/86	Spanish Gene Bank	Bolting
No. 423	A. Campo	Spanish Gene Bank	Bolting
No. 424	A. Ianez	Spanish Gene Bank	Bolting
No. 425	Alpujarras	Spanish Gene Bank	Bolting
No. 427	Cabra Monturque	Spanish Gene Bank	Bolting
No. 430	C. Rica	Spanish Gene Bank	Bolting
No. 432	D. Ramos	Spanish Gene Bank	Bolting
No. 433	E. Garcia	Spanish Gene Bank	Bolting
No. 434	Frio	Spanish Gene Bank	Bolting
No. 435	In vitro	Spanish Gene Bank	Bolting
No. 436	Isidoro Diaz	Spanish Gene Bank	Bolting
No. 437	J. Corral	Spanish Gene Bank	Bolting
No. 438	J. Garcia	Spanish Gene Bank	Bolting
No. 442	M. G. Victoria	Spanish Gene Bank	Incomplete-bolting
No. 444	Morado de Cordoba	Spanish Gene Bank	Bolting
No. 445	N. Mora	Spanish Gene Bank	Bolting
No. 447	Rojo de Cuenca	Spanish Gene Bank	Bolting
No. 448	Rojo de Falces	Spanish Gene Bank	Bolting
No. 450	S. Moreno	Spanish Gene Bank	Bolting
No. 452	Ajo Rojo	Spanish Gene Bank	Bolting
No. 453	Alcazar de San Juan Market-2	Spain	Bolting
No. 454	Alcazar de San Juan Market-3	Spain	Bolting
No. 457	Segovia market-1	Spain	Bolting
No. 460	20/96A	Braga Gene Bank	Bolting
No. 461	21/96A	Braga Gene Bank	Bolting
No. 462	22/96A	Braga Gene Bank	Bolting
No. 463	23/96A	Braga Gene Bank	Bolting
No. 468	36/96A	Braga Gene Bank	Bolting
No. 470	40//96A	Braga Gene Bank	Bolting

having relationship with pollen fertility. Reaction was repeated three times.

Fertility analysis by RAPD markers

In the previous study, two RAPD markers, $OPJ12_{1300}$ and $OPJ12_{1700}$, were amplified in all the fertile garlic clones[9]. And these were considered as the characteristic markers of pollen fertility. After the fragments derived from the total DNAs of garlic clones were amplified, the existence of these two characteristic markers was examined in the amplified products of all the materials.

Results

These garlic clones obtained from Gene Bank of Spain and Braga Gene Bank of Portugal did not show any morphological characteristics of fertility. Table 1 shows their bolting habits when they were collected. All the clones showed some sterile characters in morphology. They kept growing during winter, and their leaves did not resemble those of the fertile clones having horizontal leaves in winter[6]. In late June, their growth became suddenly weakened, and they could not mature normally. They did not bolt at all at Kagoshima in this growth cycle.

The total DNAs of garlic clones were amplified by the primer OPJ12, and the 11 discrete fragments of DNA whose sizes ranged from 0.5 to 2.0 Kb were obtained (Fig. 1). There were 4 common bands in all the 30 garlic clones, but none of the two markers was detected.

Discussion

The improvement of DNA technology has provided a lot of active and fresh approaches for the analyses in the plant researches, and the utilization of molecular markers is one of the most important approaches. A great number of works were carried out in various crops, including some crops in *Allium*. Hong et al.[8] reported phylogenetic relationship among several *Allium* species by RAPD-PCR technology. Shigyo[18] carried out a RAPD analysis to determine the chromosomal locations of RAPD markers in shallot. Masuelli and Galmarini[15] used several RAPD markers to distinguish cultivars in onion. In garlic, only a few studies on the intraspecific relationship using markers were reported[1, 12, 16].

The two RAPD markers, $OPJ12_{1300}$ and $OPJ12_{1700}$ related to pollen fertility, were obtained in the previous study. They were used in the present study to test pollen fertility in the clones from Spain and Portugal. According to the Spanish Gene Bank[14], these clones develop about 50–60 cm long scapes with smaller inflorescence, and generally the spathes do not open without producing seeds. Although no detailed informations about those collected in Portugal and

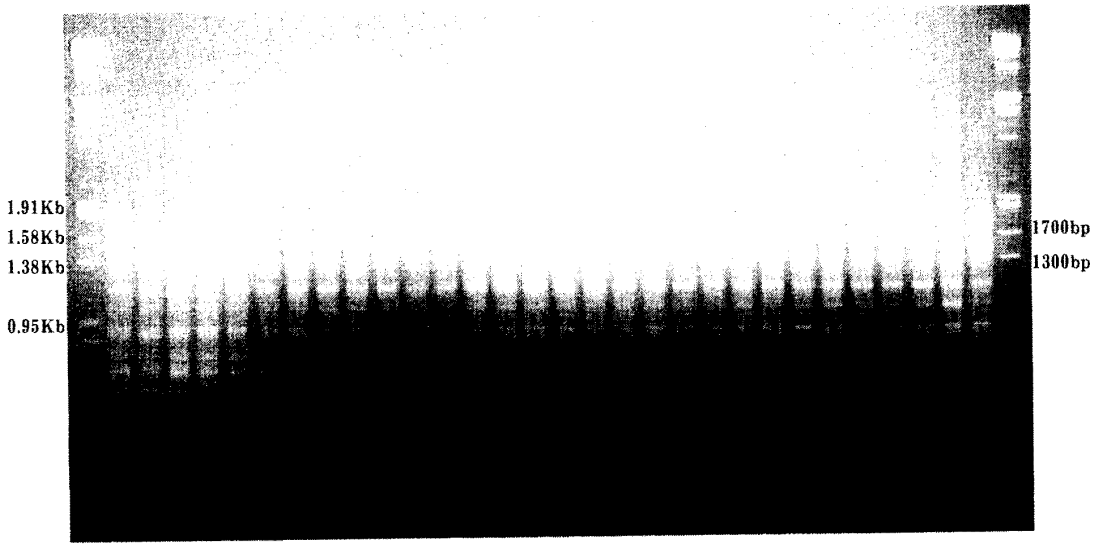

Fig. 1 Electrophoretic pattern of PCR-amplified DNA products from genomic DNAs of 30 garlic clones collected in the westernmost area of distribution for search of pollen fertile clones. Neither of the RAPD markers was amplified in all the examined clones (M; λ /*Hin*dIII · *Eco*RI double digest).

those purchased at Spanish markets were offered, there were scape scars left remaining in the bulbs when they were obtained. However, in Kagoshima they did not show any morphological characteristics as fertile clones[6], and they showed non-basic karyotypes different from the basic karyotype of the fertile clones[10]. Moreover, the fact that these Iberian clones develop bulbils in the flowers under the unsuitable climate conditions[14] suggests that they are sterile plants as Etoh and Ogura stated[4]. The existence of malformed flowers is regarded as an evolutional evidence that the reproductive organ turned into vegetative one. A slight possibility that some of the materials might be fertile could not be removed completely. However, probably all the materials from Spain and Portugal may be sterile. Generally a plant has its own genetic information, and the information does not change with the stages of growth. Therefore, the examination of the genetic markers needs more accuracy than the identification of morphological characteristics. In the present study, the two markers related to pollen fertility were not detected in all the examined clones collected in Iberian area. This probably proves the fact that no pollen fertile clone exists in the Mediterranean areas where no complete-bolting cultivars were previously reported except for the Iberian Peninsula. The result was in accordance with the examinations of the morphological characteristics[10]. Therefore, it becomes certain that these two RAPD markers can be used as characteristic markers to search pollen fertile clones in the practical utilization.

In the present study, DNA markers were used for the first time to identify the genetic character in garlic. In the previous studies, isozyme markers were used to compare the zymogram pattern of the fertile clone with those of the sterile ones[5]. Pooler and Simon[16] suggested that all the seed-derived progeny resulted from a sexual process rather than an agamospermic event by isozyme analysis. Although utilization of molecular markers has not been very popular in garlic research, the initial step has been taken. Using these markers, fertile clones containing the bands associated with the fertile genes may be readily detected, and the time for searching may be saved. Even though they are only slightly linked with pollen fertility, these two RAPD markers the authors obtained will be quite useful for the practical identification of the pollen fertile clones in garlic.

Summary

Two PAPD markers, $OPJ12_{1300}$ and $OPJ12_{1700}$, related to garlic pollen fertility were used to analyze the fertilities of 30 garlic clones collected in Iberian Peninsula, of which 29 were bolting and one was incomplete-bolting prior to fertility examination. These two RAPD markers were not amplified in the Iberian clones. All of the 30 clones did not bolt in Kagoshima, and they were identified to be sterile clones. This offered a genetic evidence for the fact that fertile clones do not exist in this area. At the same time, the effectiveness of these two markers was confirmed. These will be quite useful in garlic research and breeding.

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