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Introduction

The center of origin of garlic, *Allium sativum* L., has been considered to be Central Asia[3,11]. From the center of origin, garlic spread to west, south and east[2]. Maaß and Klaas[9] classified garlic species into four groups and one subgroup; longicuspis group, ophioscorodon group, sativum group, subtropical group and subgroup pekinense. The longicuspis group is distributed in Central Asia, and this group is considered to be the most primitive group. The rest three groups and one subgroup were supposed to be derived from the longicuspis group. The ophioscorodon is distributed from Central and Eastern Europe to the Caucasus, the sativum group in Mediterranean area, the subtropical group in South and South East Asia, and the subgroup pekinense in East Asia.

In the Central Asia, center of origin, some fertile garlic clones were discovered[3], and the garlic breeding for seed production began. In order to widen the genetic resources of fertile garlic, the garlic clones in Mediterranean area were collected and surveyed[4] because the Mediterranean area was thought to be the secondary center of origin[8]. No fertile clone was found in the Mediterranean area, except for Spain and Portugal, because most of those Mediterranean garlic clones were of non-bolting or incomplete-bolting[4]. In 1996, the present author collected garlic clones of bolting type in Spain and Portugal with the corporation of Spanish Gene Bank and Portuguese Gene Bank because some bolting clones had been known in this area. However, no fertile clones were found among the materials collected in this area[7]. There is a high possibility that no fertile garlic clone had been spread to the Mediterranean area.

Iberian Peninsula, namely Spain and Portugal, is situated in the westernmost area of the old continent, in other words, the westernmost area of garlic distribution in the old continent. The relationship between those garlic clones in the westernmost area of distribution and those in the center of origin should be clarified. Morphology and karyotype of the Iberian garlic clones were already compared with those of the Central Asian clones. In the past, there was no report of DNA markers which compared one local group of garlic with that of the primary center of garlic or which showed a dendrogram among Iberian clones and the Central Asian clones. Maaß and Klaas[9] made a wide survey of garlic by isozyme analysis and RAPD analysis, but no Iberian clones were used for RAPD analysis. Al-Zahim et al.[1] also revealed genetic variation of garlic by RAPD, using 27 cultivars, but no Iberian garlic cultivars were used. In

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this study, RAPD variation of the Iberian garlic clones was compared with that of the Central Asian clones.

Materials and Methods

A part of garlic clones collected in the Iberian Peninsula in 1996 (Table 1) were examined in this study. Clones from No. 419 to No. 450 were offered by Mr. F. Mansilla of Spanish Gene Bank in Cordoba, and Nos. 453, 454 and 457 were purchased in the markets. Those from No. 460 to No. 470 were offered by Dr. R. Farias of Portuguese Gene Bank in Braga. According to these Gene Bank researchers in charge of garlic, all of the bolting-type garlic clones in this area are included in the present collection. All the collected Iberian clones had dry flower-stalks when they were collected. This fact means that the collected materials were of bolting-type. Only No.442 clone had short, thin flower-stalks, showing that it was of incomplete-bolting type.

Table 1. Thirty examined Iberian garlic clones collected in Spain and Portugal

Clone No.	Origin or Accession No.	Source	Bolting
No. 419	410/86	Spanish Gene Bank	Incomplete
No. 422	879/86	Spanish Gene Bank	Incomplete
No. 423	A. Campo	Spanish Gene Bank	Incomplete
No. 424	A. Ianez	Spanish Gene Bank	Incomplete
No. 425	Alpujarras	Spanish Gene Bank	Incomplete
No. 427	Cabra Monturque	Spanish Gene Bank	Incomplete
No. 430	C. Rica	Spanish Gene Bank	Incomplete
No. 432	D. Ramos	Spanish Gene Bank	Incomplete
No. 433	E. Garcia	Spanish Gene Bank	Incomplete
No. 434	Frio	Spanish Gene Bank	Incomplete
No. 435	In vitro	Spanish Gene Bank	Incomplete
No. 436	Isidoro Diaz	Spanish Gene Bank	Incomplete
No. 437	J. Corral	Spanish Gene Bank	Incomplete
No. 438	J. Garcia	Spanish Gene Bank	Incomplete
No. 442	M. G. Victoria	Spanish Gene Bank	Incomplete
No. 444	Morado de Cordora	Spanish Gene Bank	Incomplete
No. 445	N. Mora	Spanish Gene Bank	Incomplete
No. 447	Rojo de Cuenca	Spanish Gene Bank	Incomplete
No. 448	Rojo de Falces	Spanish Gene Bank	Incomplete
No. 450	S. Moreno	Spanish Gene Bank	Incomplete
No. 452	Ajo Rojo	Spanish Gene Bank	Incomplete
No. 453	Alcazar de San Juan market-2	Spain	Incomplete
No. 454	Alcazar de San Juan market-3	Spain	Incomplete
No. 457	Segovia market-1	Spain	Incomplete
No. 460	20/96A	Portuguese Gene Bank	Incomplete
No. 461	21/96A	Portuguese Gene Bank	Incomplete
No. 462	22/96A	Portuguese Gene Bank	Incomplete
No. 463	23/96A	Portuguese Gene Bank	Incomplete
No. 468	36/96A	Portuguese Gene Bank	Incomplete
No. 470	40/96A	Portuguese Gene Bank	Incomplete

However, all of those Iberian garlic clones showed incomplete-bolting when they were grown in Kagoshima (Table 1). Those Iberian garlic clones grew vigorously with leaves erected during winter, offering a good example of adaptation to the warm area[3, 7].

A part of the garlic clones collected in the Central Asia in 1994[5] were also examined in this study (Table 2). These Central Asian materials were all collected in the markets. Those Central Asian garlic clones almost stopped their growth with leaves creeping on the ground during winter, offering a good example of adaptation to the very cold area[3, 7].

Total DNA was extracted from fresh young leaves and was purified by the method described in the previous report[6]. The PCR protocol described by Williams et al.[12] was employed for DNA amplification. The elements of cocktail and temperature profile for PCR were described in the previous report[6]. Primers for PCR (Table 3) were 10-base oligonucleotides from Operon Technologies Inc. (Alameda, USA). The amplification products were separated by elecrophoresis in the gel of 1.4% agarose in TAE, including 1.0 μg/ml ethidium bromide.

Table 2. Thirty examined garlic clones collected in Central Asia

Clone No.	Origin	Collection site	Bolting
No. 362	hina	Urumchi	Bolting
No. 365	China	Qingshuihe(Ili)	Non-bolting
No. 366	China	Ining(Ili)	Bolting
No. 367	Kazakhstan	Almaty-1	Bolting
No. 370	Kazakhstan	Almaty-4	Bolting
No. 371	Kazakhstan	Almaty-5	Bolting
No. 372	Kazakhstan	Almaty-6	Bolting
No. 373	Kazakhstan	Almaty-7	Bolting
No. 375-1	Kazakhstan	Almaty-9	Bolting
No. 375-2	Kazakhstan	Almaty-9	Bolting
No. 378	Kirghizstan	Bishkek-1	Bolting
No. 379	Kirghizstan	Bishkek-2	Bolting
No. 381	Kirghizstan	Bishkek-4	Bolting
No. 384	Kirghizstan	Bishkek-7	Bolting
No. 385	Kirghizstan	Bishkek-8	Bolting
No. 387	Kirghizstan	Bishkek-10	Bolting
No. 388	Kirghizstan	Bishkek-11	Bolting
No. 389	Kirghizstan	Bishkek-12	Bolting
No. 390-1	Kirghizstan	Bishkek-13	Bolting
No. 390-2	Kirghizstan	Bishkek-13	Bolting
No. 391-1	Kirghizstan	Bishkek-14	Bolting
No. 391-2	Kirghizstan	Bishkek-14	Bolting
No. 392	Kirghizstan	Kant	Bolting
No. 393-1	Kirghizstan	Cholpon-Ata-1	Bolting
No. 393-2	Kirghizstan	Cholpon-Ata-1	Bolting
No. 394	Kirghizstan	Cholpon-Ata-2	Bolting
No. 395-1	Kirghizstan	Cholpon-Ata-3	Bolting
No. 395-2	Kirghizstan	Cholpon-Ata-3	Bolting
No. 397	China	Kashgar-2	Bolting
No. 398	China	Kashgal-3	Bolting

Table 3. Nucleotide sequences of random primers (Operon Technologies Inc.) for RAPD analysis

Primer	Sequence	Primer	Sequence
K-01	5'-CATTCGAGCC-3'	L-01	5'-GGCATGACCT-3'
K-02	5'-GTCTCCGCAA-3'	L-02	5'-TGGGCGTCAA-3'
K-03	5'-CCAGCTTAGG-3'	L-03	5'-CCAGCAGCTT-3'
K-04	5'-CCGCCCAAAC-3'	L-04	5'-GACTGCACAC-3'
K-05	5'-TCTGTCGAGG-3'	L-05	5'-ACGCAGGCAC-3'
K-06	5'-CACCTTTCCC-3'	L-06	5'-GAGGGAAGAG-3'
K-07	5'-AGCGAGCAAG-3'	L-07	5'-AGGCGGGAAC-3'
K-08	5'-GAACACTGGG-3'	L-08	5'-AGCAGGTGGA-3'
K-09	5'-CCCTACCGAC-3'	L-09	5'-TGCGAGAGTC-3'
K-10	5'-GTGCAACGTG-3'	L-10	5'-TGGGAGATGG-3'
K-11	5'-AATGCCCCAG-3'	L-11	5'-ACGATGAGCC-3'
K-12	5'-TGGCCCTCAC-3'	L-12	5'-GGGCGGTACT-3'
K-13	5'-GGTTGTACCC-3'	L-13	5'-ACCGCCTGCT-3'
K-14	5'-CCCGCTACAC-3'	L-14	5'-GTGACAGGCT-3'
K-15	5'-CTCCTGCCAA-3'	L-15	5'-AAGAGAGGGG-3'
K-16	5'-GAGCGTCGAA-3'	L-16	5'-AGGTTGCAGG-3'
K-17	5'-CCCAGCTGTG-3'	L-17	5'-AGCCTGAGCC-3'
K-18	5'-CCTAGTCGAG-3'	L-18	5'-ACCACCCACC-3'
K-19	5'-CACAGGCGGA-3'	L-19	5'-GAGTGGTGAC-3'
K-20	5'-GTGTCGCGAG-3'	L-20	5'-TGGTGGACCA-3'

The electrophoretic patterns were analyzed, using Densitograph (ATTO Macintosh Version 1.0). The bands were named by the used primers, followed by the size of the amplified DNA fragments in base pairs.

All the bands were scored as 'present' or 'absent'. Common bands among the examined clones were analyzed to determine the genetic similarity between the respective pair of clones. The genetic similarities of RAPD fragments were calculated, employing the equation by Nei and Li[10].

Similarity $= 2N_{ab}/(N_a + N_b)$

N_{ab}; number of shared fragments between the clones 'a' and 'b'

N_a; number of scored fragments of clone 'a'

N_b; number of scored fragments of clone 'b'

The values for genetic similarity were then used for cluster analysis to generate a dendrogram.

Results and Discussion

The result is shown in Fig. 1. All the examined clones of both Iberian Peninsula and Central Asia are included in one dendrogram.

There are two big clone groups in the dendrogram. One group of the upper half contains 30 Iberian clones from clone No. 424 to No. 468. The other group of the bottom half contains

30 Central Asian clones from clone No. 392 to No. 390-2. The genetic similarity among the Iberian garlic clones was high, and the similarity was distributed between 0.90 and 0.98 (Fig. 1). On the other hand, the genetic similarity among the Central Asian clones was lower than that among the Iberian clones.

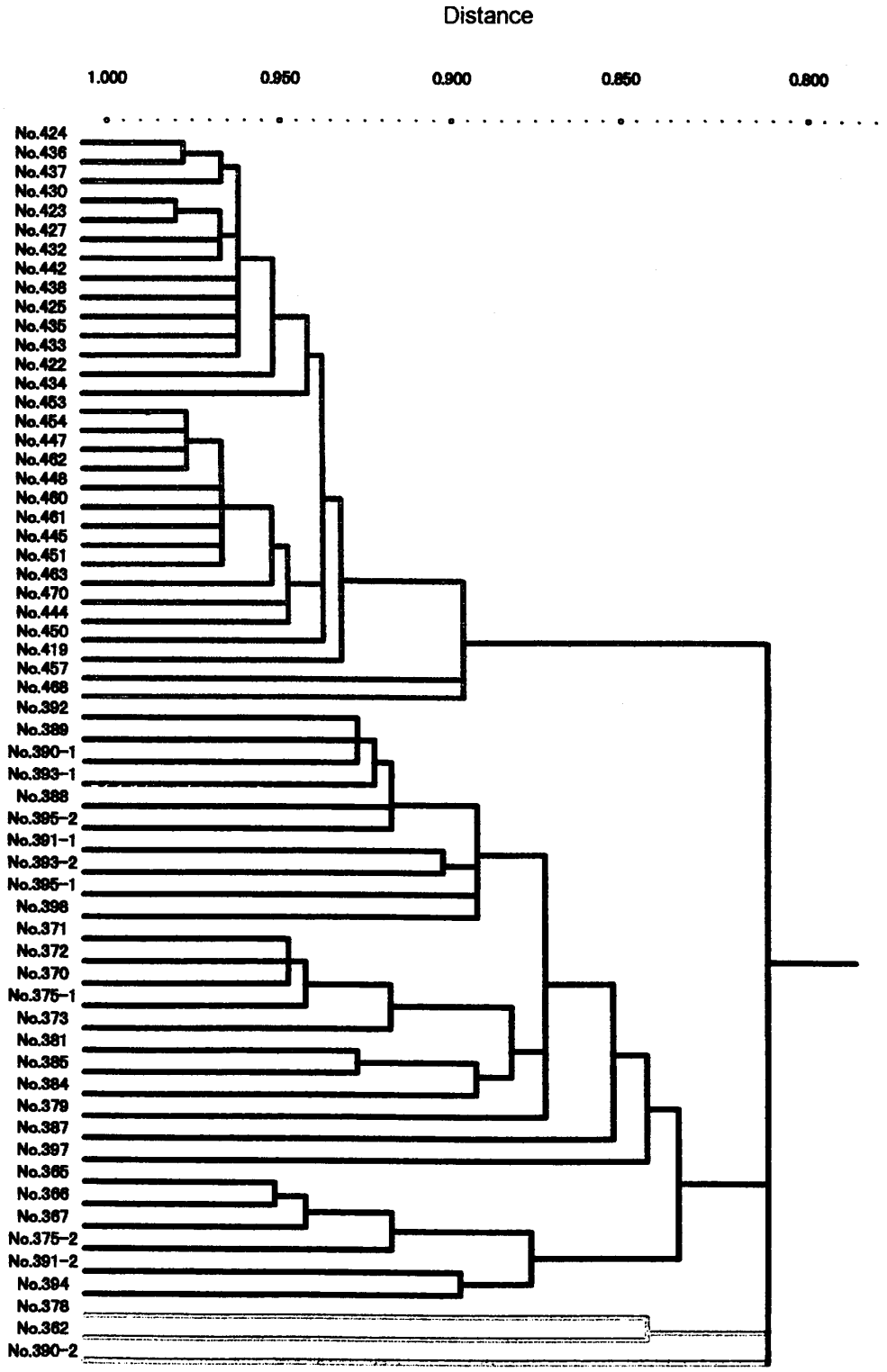

Fig. 1. Dendrogram of 30 Iberian garlic clones and 30 Central Asian garlic clones, based on RAPD markers by 20 primers of 10-base oligonucleotides (Operon Technologies Inc.).

It is clear that the genetic diversity among the Iberian garlic clones is very narrow. One of the reasons is that garlic is propagated only by vegetative organ such as bulbs or bulbils. This situation is the same both in Iberian Peninsula and in Central Asia. In Spain and Portugal, there were many non-bolting clones. However, only bolting or incomplete bolting clones were collected. This might be another reason why the genetic diversity among the Iberian garlic clones is narrow. If non-bolting clones were analyzed together, the Iberian garlic group would show wider genetic diversity. Garlic clones were collected differently in Portugal and Spain, but neither Portuguese nor Spanish clones formed its own group.

It is also clear that the genetic diversity among the Central Asian clones is much wider than that among the Iberian clones. However, there was no Central Asian clone close to Iberian clones. There is no doubt that garlic, *Allium sativum* L., has the center of origin in Central Asia. In this context, the fact of wide genetic diversity in Central Asia is very acceptable, and a distant relationship between the Central Asian clones and the Iberian clones suggests that the Iberian garlic was isolated or evolved far before from the Central Asian group.

Among the Central Asian clones, there existed several fertile clones as previously reported[7]. Those were Clone No. 375-1, No. 385, No. 389, No. 390-1, No. 391-1, No. 392, No. 393-1, and No. 395-1. They belonged to different groups though No. 392, 389, 390-1 and 393-1 may belong to one group. This fact suggests that different fertile types have still survived in Central Asia though the collection area was restricted around the Tien Shan Mountains[5].

Central Asia includes also western China, and the materials were collected on the north and the south sides of the Tien Shan Mountains. Clone No. 362 was collected on the northeast side, and No. 365 and 366 were collected on the north side, and clone No. 397 and 398 were collected on the south side. Those western Chinese garlic clones may be classified into two or three groups according to the distribution areas.

None of the previous reports have ever compared one local group of garlic with that of the primary center of garlic by DNA markers or showed a dendrogram among the Iberian clones and the Central Asian clones by DNA markers. Maaß and Klaas[9] made a wide survey of garlic by isozyme analysis and RAPD analysis, but only two Iberian garlic accessions were used for the isozyme analysis. Moreover, no Iberian clones was used for RAPD analysis. Therefore, they only showed that those two Spanish accessions were not so close to each other, and that those two Spanish accessions of sativum group were far from the Central Asian accessions according to isozyme analysis. In the present study, one garlic group in the westernmost area of distribution was compared with the most primitive[9] garlic group in the primary center by RAPD. The garlic group from the primary center showed much greater genetic diversity than that from the westernmost area of distribution. This may be a typical example of the distribution in cultivated plants.

Summary

RAPD variation of 30 garlic clones collected in the primary center of origin, Central Asia, was compared with that of 30 garlic clones collected in the westernmost area of distribution, the Iberian Peninsula. Central Asian garlic clones were complete-bolting type, and some of them were fertile clones. On the other hand, Iberian garlic clones showed incomplete-bolting type, and all of them were sterile clones.

Basing on the genetic similarity, a dendrogram among those garlic clones by RAPD was

made up. The genetic similarity among the Iberian garlic clones was high, and poor genetic diversity was estimated among the clones from Spain and Portugal, while the genetic similarity among the Central Asian clones was comparatively low, and greater genetic diversity was estimated among those Central Asian clones.

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