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Fertile Garlic Clones in the Gatersleben Collection

Takeomi ETOH, E. R. Joachim KELLER* and Angelika SENULA*
(*Laboratory of Vegetable Crops*)
*(*Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*)
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Introduction

Since Etoh's discovery of a fertile garlic clone in 1983[2], many fertile garlic clones have been found[3,7,15], and the recent progress in garlic breeding for seed production is remarkable[8,9]. It is necessary to collect far more amount of garlic clones in order to widen the genetic resources for the breeding. However, the garlic genetic resources for breeding are still limited probably because fertile garlic clones were found only in the limited areas.

Those fertile garlic clones were found mostly in Central Asia[3,7,15], and some of the fertile clones were found in the south and east Russia[1,10,11,12], Caucasus[4,6,11], Central and Eastern Europe[13,14]. However, no fertile clones were found in other areas of the world. Maaß and Klaas[16] proposed to group the old world garlic clones into four groups after isozyme and RAPD analyses, and they reported that only some accessions of *longicuspis* group, the most primitive group, from Central Asia still produce more-or-less fertile flowers. Moreover, they suggested that *ophioscorodon* group from Central and Eastern Europe probably originated from the *longicuspis* group in Transcaucasia and the region to the north of Black Sea, and that this group was sterile. Accordingly, there is a high possibility that those fertile clones found in Central and Eastern Europe were introduced from Central Asia or from Caucasus. Furthermore, they found some primitive and fertile accessions in Caucasus, but they thought those were introduced from Central Asia. However, judging from the reports by Gvaladze[6] and Etoh et al[4]., and Mathew[17], there is a possibility that there still exist some fertile clones in or around Caucasus besides those introduced from Central Asia.

From the reasons mentioned above, fertile garlic clones should be found more for breeding, and the distribution or spread of those fertile clones should be clarified concerning the original home of garlic or the fundamental grouping of garlic clones. A great number of garlic clones including both fertile and sterile accessions have been collected from all over the world by Institute of Plant Genetics and Crop Plant Research at Gatersleben, Germany. The present investigation was undertaken to find out fertile garlic clones in this Gatersleben collection with their collection sites.

Materials and Methods

Both the cultivated and wild forms of garlic, formerly separated as *Allium sativum* L. *and A. longicuspis* Regel, respectively, and today unified into *Allium sativum* L., of the Gatersleben collection, were surveyed. Bolting and flower-bud formation of all the accessions of those two species were observed during flower-bud stage. And then, most of the flowering

Table 1. Examined accessions, group of species, collection site of expedition, country of origin, and donor

Acc. No.	Group	Accession name	Origin (Collected material) [Donated material]	Donor Institute
All 129	CG		[China]	RIPP, CSSR
All 130	CG	Dunganskij Mestnyj	[former Soviet Union]	RBIV, CSSR
All 131	CG	Monsanskij	[USA]	RBIV, CSSR
All 132	CG	Americky Maly	[USA]	RBIV, CSSR
All 146	CG	Sirokolistnyj	[former Soviet Union]	RBIV, CSSR
All 766	CG		(Verchnjaja Mcara, Caucasus, Georgia)	
All 774	CG		(Narazeni, Caucasus, Georgia)	
All 780	CG		(village Skuri, Caucasus, Georgia)	
All 792	CG		(Cichisdzvari, Caucasus, Georgia)	
All 835	CG		(Arguni, Caucasus, Georgia)	
All 850	CG		(Moscow, Russia)	
All 877	CG		(Alma-Ata, Kazakhstan)	
All 889	CG		[former Soviet Union]	NGPS, USA
All 997	SAT		[Germany]	RICPO, CS
All 998	SAT		[France]	RICPO, CS
All 1009	SAT	Djambul 2	[Kazakhstan]	RICPO, CS
All 1012	SAT	Djamble 1	[Kazakhstan]	RICPO, CS
All 1016	SAT	Samarkand 2	[Uzbekistan]	RICPO, CS
All 1025	SAT	Frunze HB 1	[Kirgizstan]	RICPO, CS
All 1034	SAT	Dushanbijskij 125	[Tajikistan]	RICPO, CS
All 1035	SAT	Tashkent HB 3/171	[Uzbekistan]	RICPO, CS
All 1038	SAT	Osch 2/124	[Kirgizstan]	RICPO, CS
All 1039	SAT	Dushanbe 1/29	[Tajikistan]	RICPO, CS
All 1041	SAT	Zailijskij 45	[Kazakhstan]	RICPO, CS
All 1165	LO		[Netherlands]	RICPO, CS
All 1167	LO	Niori	(Rajon Sunchevi, Caucasus, Georgia)	
All 1175	LO		[Uzbekistan]	NGPS, USA
All 1176	LO	Moskwa-5	[Russia]	INRA, France
All 1177	LO	Alma-Ata-2	[Kazakhstan]	INRA, France
All 1279	CG		(Abakan, Khakassia, Siberia)	
All 1288	CG		(Katagan, Tajikistan)	
All 1320	CG		(Rzeszow, Poland)	
All 1471	CG		[Poland]	RIVC
All 1473	CG		[Siberia, Russia]	RIVC
All 1475	CG	Dubkowskij	[Moskow, Russia]	RIVC
All 1491	CG		(Bagnoli Irpino, Italy)	
K 10230	CG		[Svanetia, Georgia]	BE
TAX1125	LO		[Uzbekistan]	BG Minsk

Abbreviations: CG Common Garlic Group, LO *Longicuspis* Group, SAT *Allium sativum*;
RIPP Research Institute of Plant Production, Prague-Ruzyne , former CSSR
RBIV, Research and Plant Breeding Institute of Vegetables, Olomouc, former CSSR
RICPO Plant Breeding and Research Institute of Crop Production Prague-Ruzyne, Olomouc Station, Czech
NGPS National Plant Germplasm System, USA
INRA, Institut National de la Recherche Agronomique, Station Montpellier, France
RIVC Research Institute of Vegetable Crops, Skierniewice, Poland
BE Mrs. Dr. R. Beridze, Tbilisi, Georgia
BG Minsk Botanical Garden, Minsk, Belarus

accessions with the possibility of pollen fertility were examined concerning their meiosis or fertility, in other words, pollen mother cells (PMCs) or fertile pollen grains. However, some of the accessions were immature or overmature to examine pollen fertility within the limited observation period. Table 1 shows those accessions examined. *A. longicuspis* is classified as a group of *A. sativum* L. in the data base of the institute at Gatersleben, Institute of Plant Genetics and Crop Plant Research. Their PMCs and pollen grains were observed by smear-method with acetocarmine solution. The meioses, chromosome pairings, of PMCs were observed in a part of the examined accessions. The fertile pollen grains at or after pollen nuclear division were counted with empty or degenerating pollen grains, and the pollen fertility was calculated in each accession. About 500 pollen grains per one flower-bud were observed for calculation, and three flower-buds were used for each accession. The meiosis and pollen fertility were observed and examined from July 8 to 22, 1999, at Gatersleben.

Results

Table 2 shows the results of meiosis observation of the examined accessions. Meiosis, in other words, chromosome pairing at the first division, was observed only in five accessions because the observation period was restricted. The five accessions from Italy, Georgia of Caucasus, former Soviet Union, France and Tashkent (Uzbekistan) showed regular chromosome pairing, eight bivalent chromosomes (Fig. 1). All 998 from France was collected by RICPO of Czech, but it was originally collected by Botanic Garden of Paris. The true origin of this material is not clear. However, All 1491 was collected in Italy. It was confirmed that Southern Europe, besides Central Asia, Caucasus and Russia, has those garlic clones with regular meiosis. The pollen fertility of those two clones from Italy and France was not observed because their flower-buds withered so quickly after meiosis. Probably they did not produce fertile pollen at all.

Table 2 also shows pollen fertility of the examined accessions. Fifteen out of 35 accessions pollen of which has been counted showed pollen fertility to some extent. The highest pollen fertility was seen in All 1035, an accession from Tashkent, which now belongs to

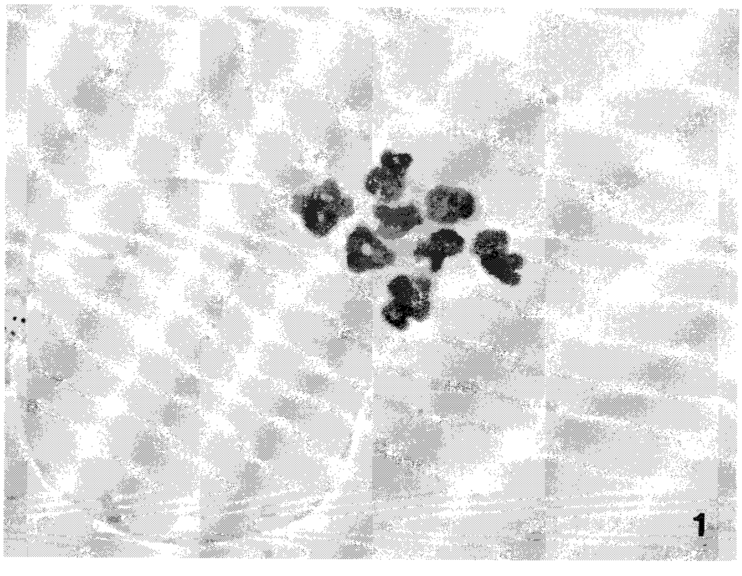

Fig. 1. Regular chromosome pairing (8II) at the meiosis (MI) of Accession All 1491.

today's Uzbekistan of Central Asia (Table 2). All 780 from Caucasus showed the second highest pollen fertility (77.6%), and All 1038 from Kirgizstan showed the third highest (66.8%). On the other hand, the lowest was seen in All 877, an accession from Alma-Ata, which is Almaty of today's Kazakhstan. The pollen fertility of this Alma-Ata clone was very low, and only a small number of fertile pollen grains were observed in one flower-bud. The rest of flower-buds did not show any fertile pollen. Hence, this may be regarded as a sterile clone. Except for the two highest pollen fertile clones, all the fertile clones did not show high pollen fertility, more than 70%. In an accession from China, All 129, fertile pollen was observed, and this was regarded as a fertile clone. However, the pollen fertility was not calculated because

Table 2. Meiosis, number of observed PMCs, pollen fertility, number of observed pollen grains, stage of pollen degeneration, number of observed flower-buds in the examined accessions

Accession No.	Meiosis	No. of observed PMCs	Pollen fertility	Number of observed pollen grains	Stage of pollen degeneration	Number of observed flower-buds
All 129			fertile	14		
All 130			41.5%	1,279		2
All 131			0		uninucleate stage	3
All 132			0		uninucleate stage	3
All 146			47.1	1,602		3
All 766	8II	4				
All 774			0		uninuleate stage	3
All 780			77.6	505		3
All 792			0		uninuleate stage	3
All 835			33.8	1,534		3
All 850			0		pollen tetrad	3
All 877			11.8	127	uninucleate stage	3
All 889	8II	22	0		pollen tetrad	3
All 997			0		uninucleate stage	1
All 998	8II	13				
All 1009			0 (0% in four but 59.9% in one flower-buds)			5
All 1012			0		uninucleate stage	1
All 1016			0		uninucleate stage	2
All 1025			61.1	1,123		3
All 1034			45.9	1,533		3
All 1035	8II	10	84.1	1,624		3
All 1038			66.8	1,088		2
All 1039			0		uninucleate stage	1
All 1041			62.1	1,676		3
All 1165			0 (only empty pollen)		unknown	3
All 1167			0		uninucleate stage	3
All 1175			55.4	1,594		3
All 1176			0		pollen tetrad	2
All 1177			39.5	1,664		3
All 1279			36.8	1,623		3
All 1288			0		uninucleate stage	3
All 1320			0		pollen tetrad	3
All 1471			0		uninucleate stage	2
All 1473			51.2	1,735		3
All 1475			0		pollen tetrad	3
All 1491	8II	53				
K 10230			0		pollen tetrad	2
Tax1125			0 (only empty pollen)		unknown	3

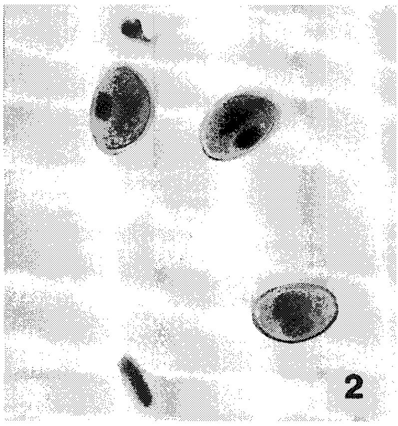

Fig. 2. Maturing pollen grains of Accession All 146.

Fig. 3. Inflorescence of Accession All 1175 with two hemispheres because of too many flower-buds and bulbils.

pollen grains were not counted.

Besides the two clones of high and low pollen fertilities, most of the fertile clones came from Central Asia, Caucasus and Russia. Those are All 130, All 146, All 780, All 835, All 1025, All 1034, All 1041, All 1175, All 1279, and All 1473 (Fig. 2). All the clones, except one, of the *longicuspis* group came from Central Asia or Caucasus, and the *longicuspis* group is supposed to be the most original group of the species *Allium sativum* from Central Asia. In total, 14 out of 15 fertile accessions came from Central Asia, Caucasus or Russia.

Discussion

A number of garlic clones from Central Asia and Caucasus already showed regular chromosome pairing, but regular chromosome pairing in European garlic clones has been rarely reported because of few bolting varieties or of few flower-developing varieties[5]. In the present examination, one clone from Italy showed regular chromosome pairing at meiosis. In the previous reports, Czechoslovakian, French, German, Polish and Romanian clones showed regular meiosis, eight bivalents (8II), besides irregular meioses[5]. Some of those European clones might have been introduced from Central Asia, Caucasus or Southern Russia, but Europe might originally have kept those clones with regular meiosis.

The other three accessions, All 766, All 889 and All 1035, also showed regular meiosis. They came from Caucasus, former Soviet Union and Central Asia. Almost all the clones of Central Asia, Caucasus and Russia showed regular meiosis as previously reported[5]. The present result coincides with that of those previous reports.

On the other hand, almost all the Asian clones, except Indian clones, showed only irregular meioses as seen in the previous reports[5]. Probably different clones of garlic might have been distributed in the east and in the west of its origin, Central Asia.

In the fifteen accessions, fertile pollen were observed, while 20 accessions showed pollen sterility. Fourteen of the 15 accessions came from Central Asia, Caucasus or Russia. One accession came from China. There is a possibility that this accession came from western China, that is, Central Asia, because fertile garlic clones were not found in China except for western China[5]. In the past, many fertile garlic clones were reported, but all of them originally came from Central Asia, Caucasus and Russia[3,4,7,15]. It is necessary to widen the genetic resources of garlic for breeding and to collect more fertile clones, and we recommend these three areas for the collection.

The 15 fertile accessions produced inflorescence with a great number of flower-buds. No accessions with a small number of flower-buds produced fertile pollen. The flower-buds were not counted in each plant, but probably all of the fertile plants produced more than two hundred flower-buds. Some of them probably produced more than four hundred flower-buds. A few accessions such as All 1175 developed two hemispheres of divided inflorescence because of too many flower-buds and bulbils (Fig. 3). Difference in scape length was seen among the fertile accessions. The scape of one accession, All 1175, was the longest, 110-130cm, and this was one of the longest scapes even among all of the Gatersleben collection. The shortest, 60-70cm, was All 129. Most of the fertile accessions showed 70-90cm long scapes. These morphological characters help us to find out other fertile clones.

In conclusion, 15 garlic accessions of Gatersleben collection showed fertile pollen. These accessions will be useful for breeding. The survey of fertile accession in the Gatersleben collection was insufficient because of some immature accessions. Therefore, there is a possibility that some more accessions may be pollen fertile. We will find more fertile accessions out of the Gatersleben collection in the future.

Summary

In the Gatersleben collection, fertile garlic accessions were surveyed. It was clarified that in total, 15 accessions were pollen fertile, and that 14 of them came from Central Asia, Caucasus or Russia. These accessions are useful for garlic breeding. Meiosis was examined in five accessions. All of them showed regular chromosome pairing at meiosis, eight bivalent chromosomes. Two of the five accessions came from Europe, Italy and France. It was confirmed that there exist some garlic clones with regular meiosis in Earope.

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