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Seed Productivity and Germinability of Various Garlic Clones Collected in Soviet Central Asia

Takeomi ETOH, Yasuhiro NOMA, Yukiko NISHITARUMIZU, and Tomoko WAKAMOTO

(*Laboratory of Vegetable Crops*)

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Introduction

Garlic, *Allium sativum* L., has been known as a sterile species. Accordingly, there has been no seed-propagated variety. However, one Russian clone was found to be fertile[3], and it was presumed that the breeding system in garlic may have evolved from sexual reproduction to asexual reproduction[5]. Basing on this presumption, various garlic clones were collected in Soviet Central Asia[6], which is presumed to be the center of origin of garlic[10]. The collected clones were grown at Kagoshima, Japan, and 14 of these clones were found to be pollen fertile. These results were reported in the previous paper[6].

In the present work, the seed productivity of these fertile clones was examined to obtain the fundamental data for breeding the seed-propagated garlic varieties. Besides those clones collected in Soviet Central Asia, two clones from Moscow Central Botanical Garden were also examined in the present work. Seed germinability of these clones was also examined here, as the garlic seeds were difficult to germinate[4].

Materials and Methods

The garlic clones used here are shown in Table 1. In 1983, the bulbs of these clones were collected in Soviet Central Asia[6]. However, clone No. 130 and No. 176 had previously been supplied from Moscow Central Botanical Garden. The former was the clone discovered to be fertile for the first time[3], and the latter was recently ascertained to produce viable pollen. Clone No. 200 did not produce any viable pollen[6].

Table 1. Garlic clones used for seed production and their clone numbers

Clone No.	Source	Clone No.	Source	Clone No.	Source
130	Moscow	197	Dushanbe-4	203	Alma Ata-1
176	Gribovo 60	198	Frunze-1	204	Alma Ata-2
184	Tashkent-4	199	Frunze-2	205	Alma Ata-3
190	Samarkand-2	200	Frunze-3	209	Moscow-3
191	Samarkand-3	201	Frunze-4	211	Moscow-5
192	Samarkand-4	202	Frunze-5		

The seed productivity and the seed germinability were examined twice: the seeds harvested in 1984 and in 1985 were examined, respectively. However, those of clone No. 176 were examined only in the second year.

The first year

The bulbs were planted in the open field at Kagoshima in the autumn of 1983, just after the collection. The field was covered with a plastic sheet to keep out the rain from the spring to the summer of 1984, the harvest time. In July of 1984, all the flowers of those clones were open-pollinated after removal of the bulbils in the inflorescences, and in addition to the open-pollination they were artificially cross-pollinated without emasculation to obtain as many seeds as possible, owing to the difficulty in seed setting[4]. As all the garlic plants were blown down by a typhoon on July 29, 1984, they were harvested on August 1. Therefore, the seeds appeared to be somewhat immature.

All the harvested seeds, including small or shriveled ones, were put on the wet filter paper in the petri dishes on October 16, 1984, for germination. The seeds harvested from one mother plant were divided into three to compare the respective germinations at three different temperatures; 5°C, 12°C, and room temperature (mean 18.7°C). However, when only a few seeds were harvested from one plant, these were put only at 5°C, or at 5°C and at room temperature.

The second year

The bulbs were planted in 1984, and the seeds were obtained in 1985, in a manner similar to that of the previous year. After all the harvested seeds were counted, the small, flattened or shriveled seeds were removed. The procedure for the germination test mentioned below is summarized in Fig. 1. The selected seeds were germinated at 20°C for three weeks to find out mother plants of high germination rate. However, a large number of seeds did not germinate at 20°C within three weeks. Especially in clone No. 130 and No. 200, a number of seeds were left without germination. Therefore, the seeds of full mature appearance in No. 130 and No. 200 were selected out of those ungerminated ones for further tests, and the further selected seeds of No. 130 and No. 200 were divided into three and were submitted to dry storage, moist-chilling, and plant hormone treatments, respectively.

Germination test at 20°C for three weeks
(All the seeds of mature appearance were used in all the clones.)

↓

(Ungerminated seeds of clone No. 130 and No. 200 were used
for dry storage, moist-chilling and plant hormone treatments.)

↓

Dry storage of seeds for three and six months at −20°C, at 5°C and at room temperature ↓ Germination test at 20°C for three weeks	Moist-chilling at 5°C for three and six months (The seeds germinated during moist-chilling.)	Germination test of seeds treated with plant hormones for three weeks at 20°C

Fig. 1. Scheme of germination tests, dry storage, moist-chilling, and plant hormone treatments of the seeds harvested in 1985.

For dry storage test, the seeds were put at −20°C, 5°C, and room temperature as control. For moist-chilling the seeds were put at 5°C in moistened condition, namely, on the wet filter paper. All the seeds for dry storage were kept for three or six months, and then they were germinated at 20°C for three weeks. However, the moistened seeds germinated even at 5°C, and accordingly there was no necessity for germination test at 20°C after moist-chilling.

For plant hormone treatments, the seeds were put at 20°C on the filter paper moistened by plant hormone solutions as shown in Table 10 for three weeks, and the germinated seeds were counted.

Results

The first year

The number of the harvested seeds in the examined clones is shown in Table 2. Among the clones used, No. 130 produced the largest number of seeds, and among the clones collected in Soviet Central Asia the highest seed productivity was found in No. 200. As No. 200 did not produce any fertile pollen grains[6], it was interpreted to be male sterile. On the other hand, clone Nos. 192, 199, 201, 203, 204, and 205 could hardly produce seeds. Of these clones, Nos. 203, 204 and 205 developed considerably fewer flowers than others. This was probably one of the reasons for their low seed productivity. All of the three clones were collected at the same town, Alma Ata.

Table 2. Seed productivity in the garlic clones harvested in 1984

Clone No.	Number of seeds		Number of mother plants	Clone No.	Number of seeds		Number of mother plants
	Harvested	Per plant			Harvested	Per plant	
130	1,654	24.7	67	200	360	18.0	20
184	156	11.4	14	201	13	1.9	7
190	89	7.4	12	202	305	13.3	23
191	79	7.2	11	203	5	0.6	8
192	16	2.3	7	204	12	3.0	4
197	167	11.9	14	205	36	3.3	11
198	220	13.8	16	209	315	13.1	24
199	2	0.7	3	211	52	5.2	10
				Total	3,481	13.9	251

Some of the harvested seeds appeared to be viable because of their big sizes, but others appeared to be dead because of their small, flattened or shriveled appearances. However, according to Aoba[1,2], low temperatures facilitate seed germination of some *Allium* species. Therefore, the harvested seeds were used for the germination test at 5°C, along with at room temperature and at the intermediate temperature, 12°C.

Table 3 shows the results of the germination tests at three different temperatures. In total, 19.4% of the treated seeds germinated at 5°C while 7.7 % of the treated seeds germinated at room temperature. At 12°C, only 5.7 % of the treated seeds germinated in total. In most of the clones, the best germination was observed at 5°C, and the worst germination at 12°C. Among the treated clones, No. 200 showed the highest germination percentage at each examined

Table 3. Influence of temperature on the germination of the seeds harvested in 1984

Clone No.	Number of seeds (Germinated/Treated)				Clone No.	Number of seeds (Germinated/Treated)			
	Room temperature	5°C	12°C	Total		Room temperature	5°C	12°C	Total
130	16/570	96/612	8/427	120/1,609	200	24/127	75/143	28/89	127/359
184	6/56	10/67	0/27	16/150	201	0/3	4/9	—	4/12
190	2/39	2/49	—	4/88	202	18/128	23/137	1/38	42/303
191	1/27	2/44	0/5	3/76	203	—	1/5	—	1/5
192	0/3	1/13	—	1/16	204	1/2	1/10	—	2/12
197	3/63	20/76	1/33	24/172	205	6/14	8/22	—	14/36
198	9/78	12/90	2/34	23/202	209	8/124	21/146	1/45	30/315
199	—	0/2	—	0/2	211	3/19	5/27	0/6	8/52
					Total	97/1,253 (7.7%)	281/1,452 (19.4%)	40/704 (5.7%)	418/3,409 (12.3%)

temperature. This clone is no doubt male sterile because of its pollen abortion[6].

Of the treated seed lots,one lot from No. 209(209×202) had the largest number of harvested seeds, but it had only one germinated seed of 96 harvested seeds in total. On the other hand, one seed lot (Q152) from No. 200(200×203, 204) had the second largest number of harvested seeds, and 44.7 % of 85 harvested seeds germinated in total. Thus, No. 200 showed very high seed productivity and germinability not only among the clones but also among the individual seed lots. In clone No. 200, the seeds germinated well even at 12°C. This clone seemed to have high potential of seed germination.

Clone No. 130 produced the largest number of seeds per plant among the treated clones (Table 2), but the seed germination was comparatively low. Clone No. 205 showed high germination rate, but this clone was not estimated to be a good mother line for breeding the seed-propagated varieties because of the small number of produced seeds.

As mentioned before, low temperature of 5°C brought about much higher total germination rate than others, but the germination proceeded slowly. Fig. 2 shows the changes of seed germination at three different temperatures. The number of germinated seeds in Fig. 2 is shown as a total of all the treated seeds. The major peaks of germination at 12°C and at room temperature existed within the first three weeks after seed soaking, especially in the second and the third week. On the other hand, at 5°C no seed germinated in the first four weeks. Instead, the seeds at 5°C germinated well during 11 to 21 weeks after soaking. This delay of seed germination at 5°C was consistently found in almost all the treated clones.

As compared with those in the first trial[4], a large number of seeds germinated in the present examination. Though many of the germinated seeds did not grow well and eventually died without bulb formation, at least a part of them grew up and formed bulbs. Moreover, a small part of the grown-up plants did form flowers, opening them within one year after seed soaking.

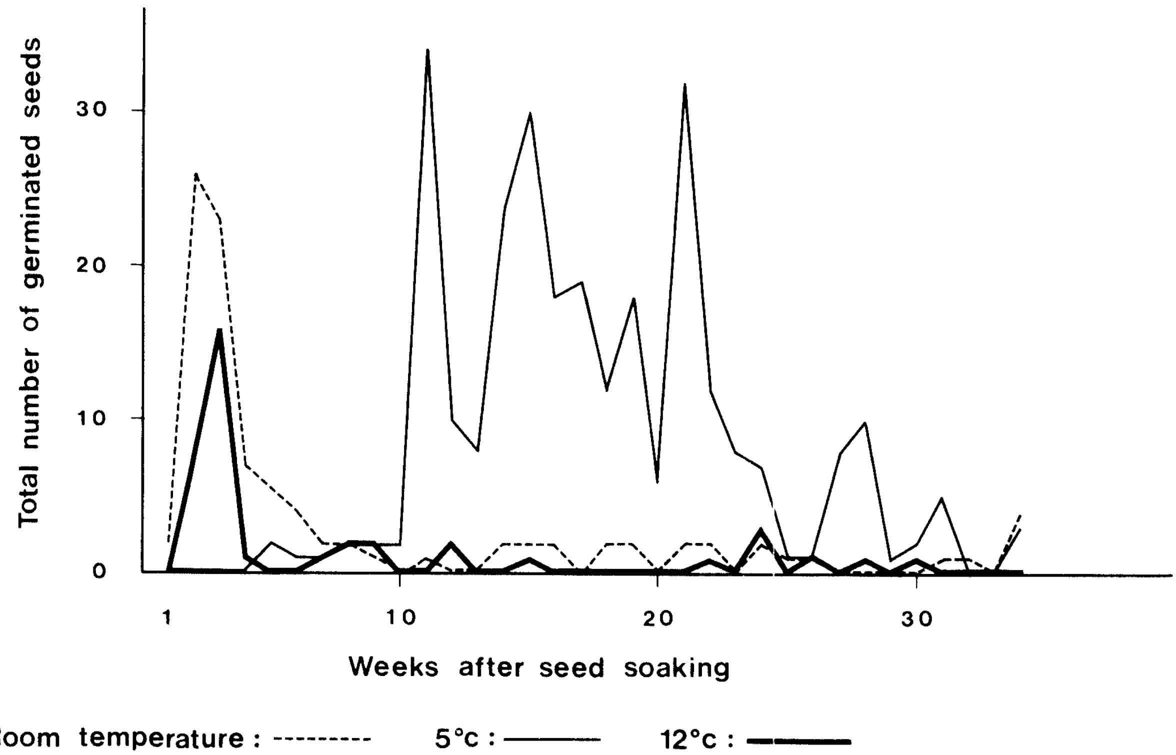

Fig. 2. Changes in the germination of the moistened seeds at different temperatures. The seeds harvested in 1984 were used.

Table 4. Plants of bulb formation and flower formation within one year after seed soaking*

Clone No.	Number of plants		Clone No.	Number of plants		Clone No.	Number of plants	
	Bulb formation	Flower formation		Bulb formation	Flower formation		Bulb formation	Flower formation
130	26	0	198	6	0	204	2	1
184	4	0	199	0	0	205	5	1
190	0	0	200	83	23	209	2	0
191	1	0	201	4	0	211	5	0
192	1	0	202	14	1			
197	6	0	203	1	0			
						Total	160	26

* The soaked seeds were harvested in 1984.

Table 4 shows the number of plants which formed bulbs or flowers within a year after seed soaking. Among the examined clones, No. 200 formed the largest number of bulbs: 83 seedlings from 15 seed lots formed bulbs. Though No.130 germinated as many seeds as No. 200 (Table 3), only 26 seedlings from 20 seed lots formed bulbs. No. 200 also formed the largest number of flowering plants, while none of the seedlings of No. 130 formed flowering plants. Among the individual seed lots, Q152 seed lot mentioned before produced the largest numbers of bulb-formed plants and flower-formed plants; 25 and 16, respectively.

The second year

Table 5 shows the number of seeds harvested in the second year, 1985. In total, 19,369 seeds were obtained, and the average of the examined clones was 16.8 seeds per plant. Clone Nos. 130, 184, 198 and 200 produced larger numbers of seeds per plant than the average. Of these four clones, No. 130 did not produce so many seeds as in the first year, while No.184 and No. 198 improved seed productivity in the second year and No. 200 yielded abundant seeds in both years. It is clear from these results that among all the collected clones No. 200 is the best mother line for breeding seed-propagated varieties.

Table 5. Seed productivity in the garlic clones harvested in 1985

Clone No.	Number of seeds		Number of mother plants	Clone No.	Number of seeds		Number of mother plants
	Harvested	Per plant			Harvested	Per plant	
130	9,157	18.1	506	200	3,823	29.2	112
176	97	2.7	36	201	476	8.5	56
184	1,285	25.7	50	202	252	9.0	28
190	409	12.4	33	203	41	5.9	7
191	974	14.3	58	204	17	2.1	8
192	188	8.6	22	205	52	3.3	16
197	1,175	13.5	87	209	631	9.3	68
198	104	26.0	4	211	688	11.7	59
199	0	0	1				
				Total	19,369	16.8	1,151

A part of the harvested seeds, however, were judged to be aborted, because all of the small seeds did not germinate in the first year. Therefore, these aborted seeds were removed. The germination was examined in every seed lot; in all the seeds of every mother plant. From the result of the first year, it was presumed that low temperature such as 5°C would be more favourable for germination than 20°C. The reason why germination test at 20°C was employed was to select the individuals of high seed germinability for practical use.

Table 6 shows the influence of mother line on the seed germination at 20°C. Among the

Table 6. Seed germination in various garlic clones harvested in 1985

Clone No.	Number of seeds		Germination rate (%)	Clone No.	Number of seeds		Germination rate (%)
	Soaked	Germinated			Soaked	Germinated	
130	6,708	1,406	21.0	200	2,161	848	39.2
176	57	11	19.3	201	373	49	13.1
184	329	59	17.9	202	213	29	13.6
190	371	56	15.1	203	40	9	22.5
191	715	137	19.2	204	16	2	12.5
192	150	20	13.3	205	29	6	20.7
197	829	185	22.3	209	488	35	7.2
198	60	2	3.3	211	495	69	13.9
				Total	13,034	2,923	22.4

examined clones, only No. 200 showed much higher germination rate than the average of the examined clones. Moreover, the rate of germinated seeds to harvested seeds was also very high in No. 200. It was very high also in No. 203, but this clone produced very few seeds per plant as compared with No. 200 (Table 5).

One of the purposes of the examination in the second year was to select the best individuals concerning seed productivity such as Q152 in the first year. All the harvested seeds were counted, and then the seeds of mature appearances were selected by removal of small seeds, as mentioned before. From the result of the first year, it was presumed that these of mature appearances would have the potentialities to germinate.

Of 1,151 plants harvested in August of 1985, 62 plants produced more than 40 seeds, and 31 of them belonged to No. 200. Three best plants of them produced more than 100 seeds, namely, 165, 129 and 102 seeds, respectively. Most of the seeds of these three plants, however, were too small to germinate, and only six seeds from the second plant germinated. There was a possibility that those small seeds might have had viable embryos, but they were virtually sterile. Therefore, individual mother plants of high yield were selected from the viewpoint of the number of fully matured seeds, not from that of the number of total harvested seeds. Table 7 shows five mother plants of the highest yield of fully matured seeds. These five plants appeared to have great potentiality of high yield of seeds. Especially the three plants, 200–765, 200–818 and 200–755, which had more than 30 germinated seeds appeared to become good breeding materials. Table 8 shows five seed lots of the highest germination rate among those having more than 20 harvested seeds. All of them showed more than 60% of germination rate, and two of them had more than 30 germinated seeds. One of the two, 200–755, also belonged to the five of the highest yield of fully matured seeds, as mentioned above.

Table 9 shows the effect of dry storage and moist-chilling on the germination. As mentioned before, the ungerminated seeds within three weeks were used here. From the result of the first

Table 7. Plants of the highest seed productivity in 1985

Clone No. Plant No.	Pollen parent clone	Number of seeds			Germination rate
		Harvested	Full matured	Germinated	
200 - 765	×130	99	96	32	33.3%
200 - 818	×130	77	77	38	49.4
200 - 858	×205	63	61	12	19.7
200 - 755	×190	55	53	35	66.0
200 - 860	×130	66	52	23	44.2

Table 8. Individual lots of the highest germinability of seeds harvested in 1985

Clone No. Plant No.	Pollen parent clone	Number of seeds			Germination rate
		Harvested	Full matured	Germinated	
200 - 815	×130	45	33	23	69.7%
200 - 762	×130	33	29	20	69.0
200 - 755	×190	55	53	35	66.0
200 - 834	×130	51	50	32	64.0
200 - 771	×191	29	27	17	63.0

Table 9. Effects of dry storage and moist-chilling on the germination of garlic seeds harvested in 1985

Clone No.	Months stored	Number of seeds (Germinated/Treated)			
		Dry storage			Moist-chilling
		Room temperature	5°C	−20°C	5°C
130	3	123/867 14.2%	5/227 1.8%	20/269 9.1%	76/550 13.8%
	6	82/874 9.4%	15/308 4.9%	17/256 6.6%	267/550 48.5%
200	3	9/210 4.3%	3/79 3.8%	2/78 2.6%	81/166 48.8%
	6	7/261 2.7%	2/82 2.4%	1/84 1.2%	137/166 82.5%

year, it was clear that moist-chilling could increase the germination rate of garlic seeds, but in moist-chilling the seed germination continued for a long time. Therefore, instead of moist-chilling, dry chilling was examined in order to facilitate the treatment of seeds. Moreover, freezing of seeds at −20°C was examined to shorten the duration of storage. However, dry chilling at 5°C did not increase the germination rate in the seeds from both clones (Table 9). Freezing storage did not increase germination rate, either. On the other hand, moist-chilling at 5°C remarkably increased germination rates in both clones. The seed germination rates of No. 200 were much higher than those of No. 130 during both three months and six months. In clone No. 200, 82.5% of the treated seeds germinated within six months after seed soaking. Though the seeds were selected after the first germination test at 20°C, this germination rate of 82.5% appeared to be very high. The selected seeds scarcely germinated just after the germination test at 20°C (Control in Table 10).

From these findings, it was disclosed that garlic seeds had great potentiality of germination and that their germinability could be improved by moist-chilling or stratification, especially by long-term stratification. However, prompt and uniform germination is essential to practical garlic growing by seeds. In this context, mechanical scarification was certainly effective in germinating the garlic seeds at the first trial in 1983[4]. According to Hartmann and Kester[7], scarification should not proceed to the point at which the seeds are injured. However, in garlic slightly injured

Table 10. Effects of plant hormones on the germination of garlic seeds harvested in 1985

Clone No.	Number of seeds (Germinated/Treated)									
	Control	Kinetin (ppm)			IAA (ppm)			Gibberellin (ppm)		
		1	10	50	1	10	50	1	10	100
130	15/224 (4.0%)	9/228 (3.9)	10/228 (4.4)	13/227 (5.7)	6/223 (2.7)	7/222 (3.2)	12/222 (5.4)	32/218 (14.7)	32/215 (14.9)	26/213 (12.2)
200	0/45 (0.0%)	1/46 (2.2)	0/46 (0.0)	2/45 (4.4)	0/46 (0.0)	1/46 (2.2)	1/44 (2.3)	0/51 (0.0)	0/51 (0.0)	11/51 (21.6)

seeds appeared to germinate well. Hence there is a possibility that garlic may have a certain mechanism which inhibits seed germination. Chemical stimulants promote germination in some kinds of dormant seeds[7]. Therefore, plant hormones were also examined in the present work.

Table 10 shows the effect of plant hormones on the germination of garlic seeds. Neither kinetin nor IAA increased seed germination at any concentrations, but gibberellin slightly increased seed germination rate of clone No. 130 at each of the concentrations. It increased the germination rate of No. 200 at 100 ppm as well. Gibberellin, however, did not improve germination rate so much as moist-chilling. Therefore, it would be worthwhile to examine the application of higher concentration of gibberellin.

Discussion

In the present work, it was clarified that garlic clones collected in Soviet Central Asia could produce viable seeds as well as viable pollen[6], and that their seed productivities varied with the clones. One clone, No. 199, did not produce viable seed in both years at all. In contrast with this, a male sterile clone produced the largest number of seeds per plant among the examined clones.

Pollen fertility as well as seed productivity varied with the clones. Is there any relationship between the pollen fertility and the seed productivity? It is possible that there may be a certain relationship between them. Both No. 184 and No. 198 showed high pollen fertility and high seed productivity. On the other hand, No. 199, No. 203, No. 204 and No. 205 showed low pollen fertility and low seed productivity. In the cross combinations between No. 200 of male sterility and others, 200×184 showed high seed productivity, 25.0 seeds per plant, and 200×203 and 200×204 showed low seed productivity, less than 10 seeds per plant. However, it may be difficult to discuss those mentioned above, because the data obtained in the present work were still insufficient.

Clone No. 200 showed considerably high seed productivity. No. 200 may have more functional ovules than other clones. Besides it, No.200 appears to have more reserve materials for developing the fertilized ovules than others, because No. 200 has long pedicels and extraordinarily long scapes[6] from which reserve materials of seeds may be translocated to the ripening seeds. At the seed ripening stage of garlic, the leaves and the roots are almost dead by maturing of bulbs. Accordingly, the deficiency of reserve materials may not be so serious in No. 200 as in other clones, though their ovules may be fertilized as well as those of No. 200. Besides, flower buds must compete with bulbils in the inflorescences for nutrition during their simultaneous developments[8]. Even in No. 200, the flower buds withered to death unless the bulbils were removed at their young stage, but if the bulbils were removed they developed fruits after artificial pollination.

One plant of No. 200, Q152, produced a number of seeds in the first year. The flowers of this plant were pollinated twice with different pollen (No. 203 and No. 204) by hand. Such a double pollination was presumed to be one of the causes of its good seed yield. In both years, a part of the plants were pollinated twice or more times with the pollen of the same pollen parents or of the different pollen parents. In the first year, double pollination produced 2,064 seeds in total and 20.2 seeds per plant, while single pollination produced 1,130 seeds and 10.9 seeds, respectively. A similar tendency was obtained in the second year.

No. 200 also showed high germination rate. One of the causes is probably that No. 200 shows earlier maturity than other clones; in other words, No.200 grows more rapidly than others.

No.200 did not have so strong rest in winter as other fertile clones[6]. This weak rest is an important character of the early varieties in garlic[5]. Therefore, earliness in No. 200 is presumed to be one of the causes for high germination rate even at 12°C in the first year.

On the other hand, the seed germination rate of No. 130 was not so high as that of No. 200, but it seemed that their positions were reversed after long storage of dry seeds at room temperature (Table 9). Perhaps a part of the seeds of No. 130 had stronger dormancy than those of No. 200. The plants of No. 130 have long rest of growth in winter[5] like dormancy, and they mature later than those of No. 200. Possibly after-ripening may be necessary for seed germination in No. 130. Garlic has never been selected for seed propagation, in other words, garlic still keeps wildness concerning seed germination. This must be a cause of low germination rate in garlic. As is well-known, the seeds of wild plants never show prompt and uniform germination, and neither does garlic. Perhaps the fertile garlic originated in Central Asia where winter was long and very cold. If garlic seeds germinated in autumn after seed formation in summer, the young and small seedlings could not survive the coldness of long winter. Accordingly, germination after cold winter should be beneficial to garlic seeds, and garlic must have kept this beneficial habit. The germination peak at 5°C existed from 11 to 21 weeks after seed soaking (Fig. 2). This means that presumably garlic seeds used to germinate after they had passed cold winter of at least two months in soil with moisture.

By moist-chilling at 5°C, more than 80% of the treated seeds germinated within six months in No.200 (Table 9). This germination rate was successful, but moist-chilling is not a convenient method for practical growing. Dry chilling at 5°C was not substituted for moist-chilling. Neither freezing storage nor plant hormone treatments produced such a high germination rate. However, there is a possibility that high germination rate may be induced by higher concentration of gibberellin such as 500 ppm or by a combination of ethylene and kinetin[9]. Some other chemical stimulants, for example, potassium nitrate, thiourea, or sodium hypochlorite, may also increase the germination rate[7].

In the first year, the seedlings developed from the germinated seeds, and some of them formed not only bulbs but also flowers within one year from the seed soaking (Table 4), that is, within one year from the seed harvest. This means that garlic can grow and develop from seeds to full mature bulbs with scapes within one year from seed harvest, namely, we shall be able to grow garlic from seeds in the same manner as that of bulbs. The seedlings were grown in the greenhouse at their juvenile stage, but the greenhouse was not heated. The seedlings which germinated after December of the seed-harvest year did not form flowers. The seedlings which passed the whole winter in greenhouse did not form flowers, either. It was estimated that those seedlings may have needed coldness of at least two months for flowering.

In conclusion, it was clarified that various garlic clones collected in Central Asia could produce a number of viable seeds. In the future, garlic growing from seeds, not from bulbs, will be realized by crossbreeding of these clones.

Summary

Garlic clones collected in Soviet Central Asia were grown at Kagoshima, Japan, and they produced seeds. Their seed productivity varied with the clones. One of the pollen-fertile clones could not produce any viable pollen, while a male-sterile clone, No. 200, could produce the largest number of seeds per plant. The seed germination was compared at three different

temperatures: the best germination was seen at 5°C; the second best at room temperature (mean 18.7°C); the worst at 12°C. However, the germination peak at 5°C existed from 11 to 21 weeks after seed soaking, while at 12°C and at room temperature it existed within three weeks after seed soaking. The seeds of the male-sterile clone, No. 200, showed the highest germination rate among those of the examined clones. Dry seed storage at 5°C, at -20°C, and at room temperature for three or six months did not increase germination rate so much as moist-chilling at 5°C. Plant hormone treatments did not increase it, either. Some seedlings from the germinated seeds formed bulbs and flowers within one year from the seed harvest.

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