Long-Term Storage of Germ Plasm of Tea (*Camellia sinensis* (L.) O.Kuntze)

By SHUN AMMA* and AKIRA WATANABE

Agronomy Division, National Research Institute of Tea
(Kanaya, Shizuoka, 428 Japan)

Preservation of plant materials required for tea breeding works must be done by planting mother plants or their clones in the field, because the tea plant perform mainly cross fertilization. However, this method of preserving breeding materials requires a large area of field, and a lot of both labor and operational expenses. As a matter of fact, it causes a heavy burden to tea breeding. Therefore, it is urgently desired to develop a highly efficient method of storing tea clones.

Furthermore, there are spontaneous varieties, called "*Yamacha*", and native varieties, called traditional local tea, in Japan. These varieties are rapidly disappearing due to afforestation or replanting of tea fields by new varieties. It is also a quite urgent task to collect these genetic resources and hand them over to our descendants. Otherwise, irrepalable loss may occur. Thus, the establishment of long-term storage methods for seeds and pollen is also urgently needed.

With such a background, a series of studies were carried out to develop efficient methods of long-term storage for genetic resources of tea.

Storage of nursery tea plants

1) *Material and method*

Young tea plants, which had grown for 20 months in a nursery by the usual method of raising rooted cuttings, were dug up in March, and subjected to different storage treatments shown in Table 1. A part of the plants in the storage was taken out in March every year. They were planted to unglazed pots, and were grown for 5 months in a greenhouse to examine their survival rate and growth of survived plants.

2) *Result and discussion*

Table 1 shows survival rate and plant height after 1, 2, 3 and 4 years of storage. The storage at room temperature caused the death of all plants, irrespective of different treatments, within 1 year of storage. At the storage temperature of 1 or 5°C, all plants survived completely for 1 year only by sealing them in airtight polyethylene bags. However, the survival rate decreased to 30–70% after 2 years of storage, and 10–30% after 3 years. The storage after the removal of leaves showed apparently increased rates of survival. When plants with roots wrapped with moist sphagnum or embedded in semi-dry sterilized soil were sealed in polyethylene bags, they survived well for 2 years, showing 10–70% survival even after 3 years. In this case too, the removal of leaves gave distinctly higher survival rates, i.e., 50–70% of survival rate after 3 years, and increased plant height. But, after 4 years of storage, survival was hardly observed, and regrowth of the survived plants was extremely poor.

Therefore, the limit of storage period for two-year-old nursery tea plants was regarded only 4 years. From the standpoint of long-term storage of useful germ plasm, the storage of nursery plants is not very promising.

Present address:
* Hokkaido National Agricultural Experiment Station
(Hitsujigaoka, Sapporo, 004 Japan)

Table 1. Survival and regrowth of nursery tea plants stored at low temperature for 1, 2, 3 and 4 years

Storage temperature	Condition in storage	Leaves	Survival ratio (%)				Plant height (cm)		
			1 year	2 years	3 years	4 years	2 years	3 years	4 years
Room temp.	P*	Retained	0	0					
		Removed	10	0					
	P+Sph**	Retained	0	0					
		Removed	0	0					
	P+Soil***	Retained	0	0					
		Removed	10	0					
1°C	P	Retained	90	30	20		16	12	
		Removed	100	70	30		36	19	
	P+Sph	Retained	90	90	10		24	14	
		Removed	100	100	60	0	27	19	
	P+Soil	Retained	100	90	40		27	16	
		Removed	100	100	70	10	37	27	8
5°C	P	Retained	100	40	10		17	6	
		Removed	100	70	30		27	22	
	P+Sph	Retained	100	70	10		30	8	
		Removed	100	100	50	0	36	25	
	P+Soil	Retained	100	80	20		31	9	
		Removed	100	90	70	10	35	27	10

* Nursery plants were sealed in polyethylene bags.
** Roots of nursery plants were wrapped with moist sphagnun and sealed in polyethylene bags.
*** Roots of nursery plants were buried in the sterilized semi-dry soil in the polyethylene bags, and the bags were sealed up.
Age of nursery plants was 20 months after cutting. Survival rate and plant height were measured after 5 months of pot culture subsequent to the storage.

Long-term storage of roots of tea plants

As tea plants easily produce adventitious buds and adventitious roots from their roots, the long-term storage of cut roots was attempted.

Preliminary experiments[1] revealed that (1) it is better to use roots dug up in early spring, because more reserve nutrients are contained in roots at that season, (2) thick and long roots are better used, and (3) airtight storage at 1 or 5°C offers good results. By taking these facts into account, an experiment on long-term storage of cut roots at low temperature was carried out.

1) Material and method

Thick roots taken from plants of 20 years of age (cv. *Yabukita*) in early spring of 1975 were treated with fungicide, and stored. Their survival rate was examined after 1, 3, 5 and 7 years of storage. The fungicide used to control fungi was an organo-mercury compound (EMP, Table 2), and 3 g of it was dissolved in 10 *l* of water. Before storage, cut roots were dipped in that solution for 30 min or wrapped with sphagnum pre-immersed in that solution. After the dipping treatment,

Table 2. Survival rate of tea roots stored at 1° or 5°C for 1, 3, 5 and 7 years

Fungicide*	Storage temperature	Condition of storage**	Diameter of stored roots	Adventitious and formation (%) after storage of			
				1	3	5	7 years
Applied	1°C	P + S	20—15	100	90	80	60
			14—10	100	80	80	40
		P + Sp	20—15	100	80	70	40
			14—10	100	90	70	30
	5°C	P + S	20—15	100	70	70	60
			14—10	100	70	60	40
		P + Sp	20—15	100	50	50	40
			14—10	90	50	40	30
Not applied	1°C	P + S	20—15	100	80	70	50
			14—10	100	80	70	40
		P + Sp	20—15	80	70	60	30
			14—10	70	60	50	20
	5°C	P + S	20—15	90	60	50	50
			14—10	80	50	50	40
		P + Sp	20—15	70	50	50	30
			14—10	60	40	40	30

* A mixture of methoxyethyl mercuric chloride and ethyl mercuric phosphate (EMP), Hg 4%.
** P+S: Roots were sterilized with 0.03% fungicide for 30 min and buried in the sterilized semi-dry soil in polyethylene bags. P+Sp: Roots wrapped with sterilized moist sphagnun were put into polyethylene bags.
Ten cut roots were used in each plot.

the cut roots were embedded in semi-dry sterilized soil contained in polyethylene bags. The cut roots wrapped with the sphagnum were sealed in polyethylene bags without further treatment.

In this experiment, cut roots with diameter of 10–14 mm and 15–20 mm were used, and storage temperature was 1 or 5°C. At the end of the storage period the cut roots were transferred into polyethylene bags containing semi-dry sterilized soil, and kept at 25°C for 5 months to examine sprouting of adventitious buds.

2) Results and discussion

Results obtained are shown in Table 2. Efficacy of fungicide application was not appreciable, though some effect was observed. As to storage temperature, no difference was observed between 1 and 5°C. The storage of sterilized cut roots in semi-dry sterilized soil in polyethylene bags gave the better result than cut roots wrapped with sterilized sphagnum. Apparently the thicker roots showed higher rates of adventitious bud formation as compared with slender roots.

It was made clear from these results that cut roots of 1.5–2 cm in diameter, which were sterilized and then embedded in sterilized, semi-dry soil contained in airtight polyethylene bags could be stored for a fairly long period at the temperature of 1 or 5°C. After 7 years of the storage, 70–90% of the total number of the cut roots were found to be alive, and 60% of the total were able to produce adventitious buds.

Sakai et al.[3] made an estimation that the critical length of storage life of tea roots is 16.9 years on the basis of the highest content of total available carbohydrate observed in

many experiments so far conducted.

The present authors took out cut roots stored for 6 years from several storage experiments on cut roots,[1] and tried to grow plants originated from the cut roots by pot culture. After 2 years, the plants reached the stage enough to be utilized as pollen parents, and after 3 years from the end of the storage as mother plants for crossing.

The method to diagnose vitality of cut roots[2] under storage by measuring the amount of K exuded from roots in water was adopted. It makes monitoring of vitality of cut roots possible during the storage period.

Thus, it was made clear that cut roots can be utilized for the long-term storage of germ plasm of tea.

Long-term storage of tea seeds

With an aim of preserving genetic resources of native varieties of tea in Japan, experiments on long-term storage of tea seeds were carried out.

1) Material and method

Open-pollinated seeds of cv. *Yabukita* collected in 1975 were sealed in polyethylene bags and stored at 1°C. Sampling of the seeds in storage was made in October every year to examine seed germinability. The sample seeds were soaked for 24 hr in water, sealed in polyethylene bags, and then placed in an incubator at 25°C for 30 days. For each experimental plot 100 seeds were used with 4 replications.

2) Result and discussion

As given in Table 3, the seeds stored in airtight containers at 1°C for 6 years showed as high as 73% of germination rate. A simple method of detecting seed germinability by measuring the amount of sugars exuded from seeds[1] was applied to the seeds in storage.

Preservation of genetic resources by means of seed storage will become extremely useful when seeds of Japanese native varieties are collected in a large scale covering each region, and are stored in airtight containers at 1°C, with periodic monitoring of seed viability.

Table 3. Viability of stored tea seeds*

Years stored	Percentage of germination	Coefficient of variation
	(%)	(%)
0	86	12.3
1	91	2.9
2	98	4.2
3	78	16.2
4	88	5.8
5	74	6.9
6	73	10.5

* Seeds collected in 1975 were sealed in polyethylene bags and kept at 1°C.

Long-term storage of tea pollen

As the pollen is minute in size and tolerant to various conditions, it is adapted to long-term preservation of a large amount of genes. Many studies have been done on the long-term storage of pollen for other crops, but no authentic study has been made with tea pollen. Therefore, the present authors carried out the long-term, low temperature storage of tea pollen.

1) Material and method

Pollen of cv. *Yabukita* and *Benifuji* were collected in a room and every definite amount of them was wrapped in a paraffin paper. Then, they were divided into the following 3 groups, and all the groups were stored at 1, −40 and −80°C.

1) Freeze-dried: Pollen were freezed at −35°C for 7 days and then vacuum-dried for 2 days.
2) N_2 gas package: Pollen were packed in plastic film containers which are used for packaging green tea, and inside air was replaced by N_2 gas.
3) Standard: Pollen are sealed in filmcases (lidded film case used for 35 mm film).

Sampling was made once a year to examine pollen germinability. Pollen sown on artificial germination beds (agar 2% + sucrose 10%) were placed in a wet chamber at 25°C to induce germination. Rate of germination and

Table 4. Germinability of tea pollen stored for 62 months at low temperature

Items	Clonal variety	Method of storage	Before storage	Stored temperature		
				1°C	−40°C	−80°C
Percentage of germination (%)	*Yabukita*	Freeze-drying*	80.2	0	67.8	54.8
		N_2 gas package**		0	79.6	77.2
		Standard***		0	62.8	73.2
	Benifuji	Freeze-drying	66.3	0	68.6	54.8
		N_2 gas package		0	77.2	68.3
		Standard		0	13.0	72.8
Lengh of the longest pollen tube (μ)	*Yabukita*	Freeze-drying	617		632	723
		N_2 gas package			898	842
		Standard			727	642
	Benifuji	Freeze-drying	492		661	661
		N_2 gas package			797	727
		Standard			187	655

Pollen were wrapped with paraffin papers and then;
* freezed at −35°C for days and vacuum-dried for 2 days.
** packed in gastight bags with N_2 gas.
*** sealed in film case.

length of the longest pollen-tube were measured. The germination test was done with 5 replications.

2) Result and discussion

Germination of the pollen stored for 62 months is shown in Table 4. The pollen stored at 1°C for 5 years lost their germinability, irrespective of the different treatments shown above. The storage at −40°C was effective to preserve pollen germinability for a variety, but not so effective for the pollen stored at the standard condition for other variety. The storage at −80°C was effective for both varieties. Thus, it was made clear that the storage at −80°C of either freeze-dried pollen or pollen packed in N_2 gas or film cases can preserve sufficiently their germinability even after 5 years of storage.

The pollen which had been stored at −80°C were used for crossings. The result showed that normal hybrid seeds with high germination percentage were obtained by the use of the stored pollen. It implies that preservation of genes by pollen storage is quite useful.

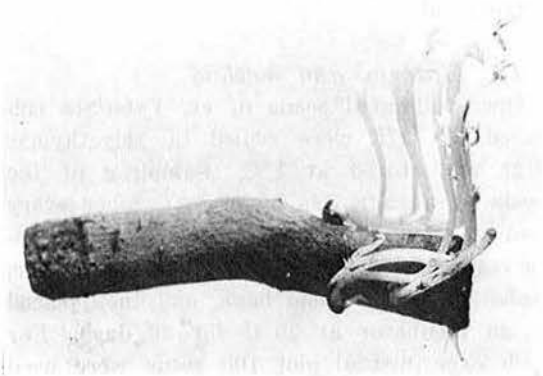

Plate 1. Adventitious buds sprouted from a thick tea root stored for 4 years at 1°C

Summary

A series of long-term storage experiments on rooted cuttings taken from the nursery, cut roots, seeds, and pollen were carried out to find out effective methods of long-term storage of germ plasm of tea plants. It can be concluded that in case of vegetative organs, the low temperature storage of cut roots taken from thick roots is most suitable for the long-

term storage, and in case of preservation of genes of certain plant populations, seeds or pollen can be used for long term storage.

References

1) Amma, S.: A rapid germinability test using sugars exuded from tea seeds. *JARQ*, 13, 247–249 (1979).
2) Amma, S. & Watanabe, A.: Long-term storage of germ plasm in tea (*Camellia sinensis* (L.) O. Kuntze. *Bull. Nat. Res. Inst. of Tea*, 19, 29–57 (1983) [In Japanese with English summary].
3) Sakai, S., Doi, Y. & Nakayama, A.: Changes in regenerative ability and carbohydrate reserve of tea root during a long-term storage for the maintenance of useful germ plasm. *Study of Tea*, 53, 5–12 (1977) [In Japanese with English summary].

(Received for publication, November 7, 1984)