Long-term Storage of Tea Cuttings at Low Temperature and Its Application to Breeding

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It is desirable to maintain many varieties and lines as genetic resources under a condition which makes it possible to utilize them whenever needed, for efficient breeding works. However, for perennial arbor crops like tea, large area of fields and much labor and expenses are required for their preservation and management.

As the tea is a self-incompatible crop, all varieties and lines are genetically heterozygote. Therefore, vegetative organs such as cuttings or roots, etc., have to be used for preserving given clonal varieties (cultivars). It has been made clear that cut roots can be preserved for 3–5 years when stored at low temperature.[1] However, storage of cuttings is more advantageous from the viewpoint of the utilization after storage. In the present paper, therefore, conditions of low temperature storage of cuttings, changes in chemical components during storage, multiplication of stored cuttings by grafting, and application to breeding will be described based on the results of recent studies.

Low temperature storage of cuttings

1) Season to get cuttings and preparation

To know the optimum season for getting cuttings, cut shoots sampled in the summer (June and July), in the autumn (October), or in the winter season (December, January and February) were stored at different temperatures (above or below 0°C). The result showed that the cuttings obtained in the winter season, especially in January and February, can be stored with the highest stability. This is because the winter shoots are filled with high content of carbohydrates (Fig. 1) and highly tolerant to freezing. Even with the summer

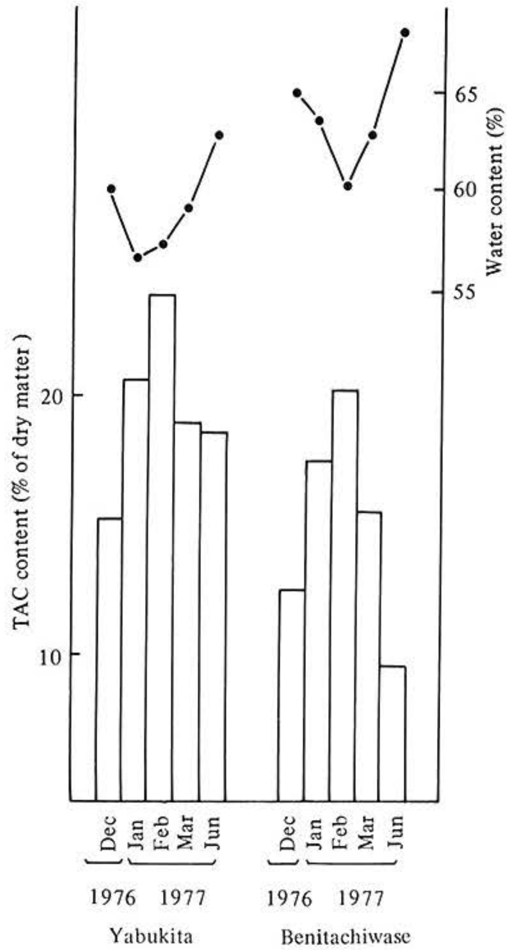

Fig. 1. Seasonal changes of total available carbohydrates (TAC) and water content in leaves of mature shoots of two cultivars

shoots, a considerably good result can be obtained, when the hardened portions of shoots are stored at about 2°C.

The autumn shoots showed the same tendency as the summer shoots.

As the cuttings not bearing mature leaves do not survive in either case of cuttage or grafting,[4] cut shoots of about 20 cm in length, bearing about 5 mature leaves were prepared.

2) Method of storage

It is convenient to employ water culture method with 3–4 cm of basal portion of the cut shoots being immersed in water. Although this method needs to supplement water for evapotranspiration loss once every 1–2 months, it gives better result as compared with polyethylene bag method, by which shoots are sealed tightly into polyethylene bags. Particularly, when the storage is made in the light, as will be described later, the water culture method is much advantageous.

3) Storage temperature

Within the range of temperature from −7° to +5°C, temperatures about +2°C gave the best result for shoots obtained in any season. The winter shoots could be stored at the temperature down to about −1°C, but they suffered from freezing damage at the temperature below that.

The summer shoots were all killed by two-month storage below 0°C, due to high water content and low freezing-tolerance.

4) Effect of light on storage

Illumination is very effective in keeping the shoots to be healthy in low temperature storage (Fig. 2). When winter shoots were stored at 2°C in darkness, the health rate was reduced to 50% (rooting rate: 20–70%), a critical limit for practical storage in 6–9 months. In this case, difference between the water culture method and polyethylene bag method was scarcely observed. On the other hand, in the light the storage period (with 50% of health rate) was extended to more than 10 months with the water culture method, but not so remarkable with the polyethylene bag method

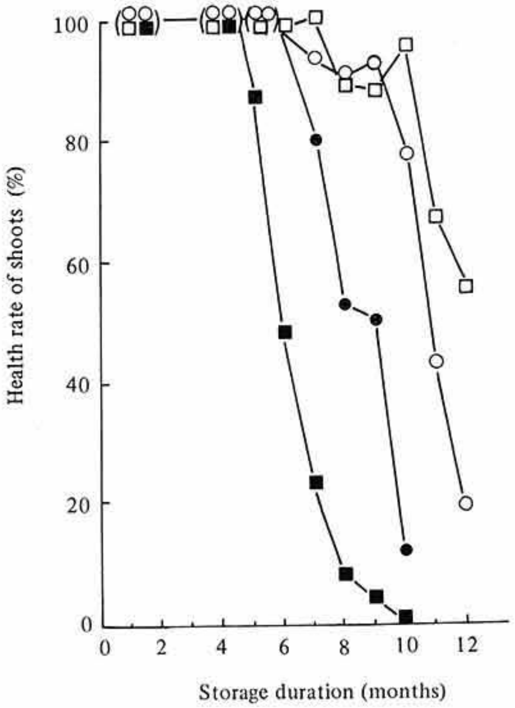

Fig. 2. Reduction of the health rate of shoots stored at 2°C (water culture) in darkness or in the light

Notes: 1) -○- *Yabukita*, light. -●- *Yabukita*, dark. -□- *Benitachiwase*, light. -■- *Benitachiwase*, dark.

2) Health rate (%) $= \frac{\sum d \cdot s}{3\,n} \times 100$

d: Health rating based on a scale of 0 to 3, with 3 indicating no damage.

s: Number of shoots showing each health rating.

n: Number of shoots examined.

(Table 1).

Effect of light in extending storage period was observed with weak light of about 100 lux.

5) Changes of carbohydrates in storage

It was pointed out that the amount of carbohydrates contained in shoots has a close relation with the storage ability of shoots[2,5,6]. Therefore, content of total available carbohydrates (TAC) in leaves was determined with shoots stored at 2°C under light or in darkness in the above-mentioned experiment. The result showed that the content of TAC decreased linearly with time after the start of storage in

Table 1. Comparison of health rate and TAC content of leaves between water culture method and polyethylen bag method (variety: Benitachiwase)

Storage method	Stored cuttings	Storage condition and duration (in month)							
		Light				Dark			
		0	5	9	11	0	5	9	11
Water culture	Health rate (%)	100	100	88	66	100	86	3	0
	TAC* (%)	12.9	17.2	7.9	17.2	12.9	4.9	3.2	—
Polyethylene bag	Health rate (%)	100	100	30	20	100	96	3	0
	TAC* (%)	12.9	11.8	4.2	6.1	12.9	6.6	2.0	—

* % of dry matter

darkness, whereas it showed periodical fluctuations under the light (Fig. 3). Such changes of TAC contents in darkness and in the light were observed in both water culture and polyethylene bag method. TAC content of stems also showed the same tendency. This result suggests that the shoots may possibly carry out photosynthesis under the condition of low temperature and weak light.

Thus, the carbohydrate content is closely related to storage ability. It was kept at a higher level in the light. This might have caused the tolerance of shoots in the light against the longer period of storage.

6) *Elongation of axillary buds during storage*
At 6–7 months of low temperature storage of cuttings in the light, it was observed that axillary buds sprouted to the length of about 1 cm. When such cuttings were used for planting in soil or for water culture, the axillary buds elongated rapidly before rooting occurred. On the contrary, neither sprouting of axillary buds during storage nor rapid development of new shoots after planting occurred with shoots stored in darkness. Such sprouting of axillary buds is related to the change of carbohydrate content shown in Fig. 3: the time when the sprouting occurred coincided with the time when the carbohydrate content decreased.

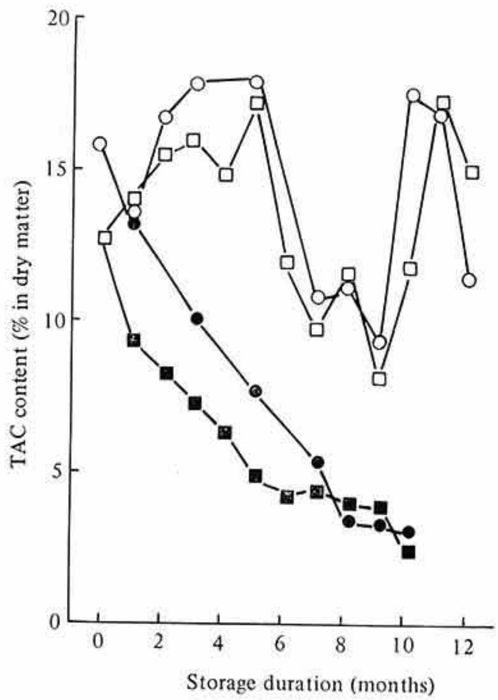

Fig. 3. Changes of TAC content in mature leaves of the shoots stored at 2°C (water culture).
Note: -○- *Yabukita*, light. -●- *Yabukita*, dark. -□- *Benitachiwase*, light. -■- *Benitachiwase*, dark.

Use of stored shoots for grafting

Stored shoots are used as materials for cuttage or grafting. At present, propagation of tea plants is made mostly by cuttage, so that technique of cuttage is well established. However, for the utilization of stored cuttings as breeding material, grafting is a more important means than cuttage. As there are many problems which are not yet made clear with the use of grafting, the least findings[4] will be presented below.

Although veneer-grafting or crown-grafting

Table 2. Grafting compatibility between varieties (success of grafting%)

Stock variety	Scion variety											
	Assam origin			Assam hybrids				Chinese origin	Japanese origin			
	Ai 2	Ai 16	Ai 103	Hatsu-momiji	Beni-kaori	Beni-hikari	Beni-homare	Yama-nami	Yabu-kita	Yamato-midori	Kanaya-midori	Average
Assam origin Ai 16	70.0	70.0	80.0	80.0	80.0	50.0	60.0	50.0	60.0	80.0	90.0	70.0
Assam hydrid Hatsumomiji	30.0	80.0	50.0	100	100	80.0	90.0	40.0	50.0	70.0	50.0	67.2
Japanese origin Yabukita	30.0	20.0	30.0	60.0	70.0	60.0	70.0	30.0	50.0	20.0	50.0	42.7
Average	43.3	56.6	53.3	80.0	83.3	63.3	73.3	40.0	46.6	46.6	63.3	60.0

Note: By Matsushita (1980), Success percentage determined 255 days after grafting.

(3–4 scions used per 1 stock) has generally been practiced for tea plants, top grafting (at 35–40 cm above ground) is also made successfully when protected with cheese-cloth cover or rush-mat cover. By this method, many lines are maintained on one stock, because 10–18 scions can be grafted on one stock. As in the case of rooting of cuttings, shoots without mature leaf were found to be quite difficult to survive in grafting. This point is different from the grafting of deciduous fruit trees and citrus trees.

Regarding the graft-incompatibility which often occurs with fruit trees, it was made clear with tea that there is no significant difference in growth of scions grafted by different combinations of scions to stocks, although a few difference occurs in percentage of success in grafting. Thus, incompatibility was not apparently observed (Table 2).

Application of stored cuttings to breeding

As storage period of tea cuttings is limited to only 1 year at present, an ideal system by which a large number of varieties and lines can be stored efficiently in a small space has not yet attained. However, even now the following applications are possible.

1) Storage of varieties and lines for several months

As tea plants have vigorous regrowth ability, it is possible to obtain cuttings almost all the year round provided plant growth is regulated, but good cuttings showing high rate of success in rooting and grafting can be obtained only in a relatively limited season. Accordingly, when good cuttings obtained at the optimum season are stored, they can be used at any time when needed for cuttage or grafting, with efficient use of fields for propagation, and efficient use of stocks, but without competition with other farm operations.

2) Screening materials for resistance to diseases and insect pests

One of the important problems in breeding is disease and insect resistance. At present, it is possible to use cut shoots in screening resistance to a few diseases. As mentioned earlier, cut shoots stored in the light show an uniform elongation of axillary buds, so that new leaves produced on them can be used for the screenings. This method is very useful, because in the screening for white scab (*Elsinoe leucospila*) it is essential to inoculate it to new leaves.

The screening for anthracnose (*Gloeosporium teae-sinensis*) is also possible by spraying conidiospores to new leaves expanded on stored shoots, and this method will become practical in future.

Although the test for insect resistance by the use of cut shoots has not been made yet, there is a possibility that this method will be employed with the progress of research.

The utilization of stored shoots has two great merits: the risk that the testing materials have already been infested by the diseases or insect pests is quite small, because newly developed shoots from axillary buds are used, and the tests can be done at any time throughout the year.

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