Preservability and Carbohydrate Reserve of Tea Vegetative Organs in a Long Term Storage

By SHINSUKE SAKAI

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

For maintaining germplasm of the tea, an allogamous arbor crop, storage of vegetative organs seems to be very useful, particularly from the viewpoint of utilization after the storage. Detached roots, which are empirically known to survive in soil for long time, and cut shoots which can be propagated easily are considered to be suitable as materials for storage. It is desired to develop a storage method for these materials in facilities with controlled environment.

It is already known[1,2] that carbohydrate such as sucrose and starch is accumulated in stems and roots of tea, showing seasonal variations with high content in the winter season, and that it is used not only as substrate for respiration but also as a material for growth of new shoots. Thus, it may be reasonable to consider that vegetative organs with high content of carbohydrate which acts as energy source during storage and for regeneration, are suitable for long term storage. Therefore, physiological changes before and after the storage were examined mainly from the standpoint of carbohydrate metabolism, as a basis for developing storage technique. Information obtained as to the plant portions and collecting time suitable for storage, environmental condition and the maximum duration of storage, as well as promotion of bud sprouting after the storage will be presented in this paper.

Plant portions and collecting time most suitable for storage[3]

Seasonal variation and distribution in plant of carbohydrate reserve were examined with the purpose of knowing plant portions and time of collecting them most suitable for long term storage. Over the 1 year starting from April, 10-year old tea plants in plucking were dug up at the interval of 3 months, and total available carbohydrate (hereafter referred to TAC) of stems and of roots, classified by their diameter, was determined (Fig. 1). TAC content was highest in thick roots, followed by medium roots, stocks,* trunks (base portion of stem) and rootlets in that order. In each portion, TAC content was highest in the season from the winter to flushing in the next spring and lowest in the autumn. As to the changes in TAC content during March to May, a detailed study was carried out using 2-year old seedlings of 3 cultivars differing in bud opening time. The result showed that the increase of TAC content continued by the time of bud opening and then decreased with the expansion of new leaves.

To know the distribution of TAC in one root, roots with diameter more than 5 mm were sampled from the above mentioned 10-year old plants, and cut into 10 cm long root pieces, starting from the basal point of the roots. Diameter and contents of TAC and total nitrogen were measured with successive 10 cm long root pieces. The result showed that the closer the root pieces to the top of the plant, the higher was the TAC content. Therefore, a high correlation ($r=0.87$) was shown between diameter of root pieces and TAC content (Fig. 2), while a negative correlation ($r=-0.86$) was observed between TAC content and nitrogen

* Underground portion of cuttings where initial rooting occurs.

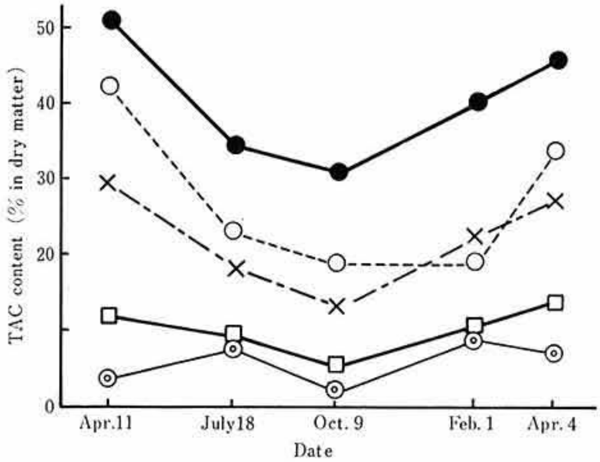

Fig. 1. Seasonal changes in TAC content in root and trunk of mature tea plants

●—● Thick root (> 10 mm in diameter).
○---○ Medium root (2 mm～10 mm in diameter)
×-·× Stock
□—□ Trunk (basal portion, 10 cm long, of stem)
◎—◎ Rootlet (< 2 mm in diameter)

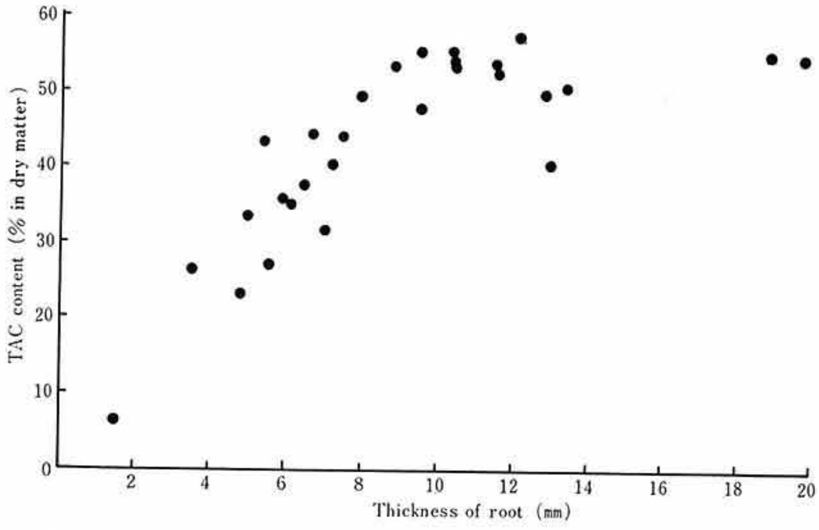

Fig. 2. Relation between thickness of tea root in diameter and TAC content

content. These results indicate that more TAC accumulates in the basal portion of roots, that is well thickened and low in nitrogen content: in other words, well lignified portion of roots.

Based on these results, it was made clear that in order to collect tea roots with high TAC content for long term storage, it is desirable to select the roots as thick as possible at the spring bud opening stage.

Respiratory ability of roots as related to storage condition[2]

Decrease of TAC in vegetative organs during the storage is considered to be caused mainly by respiratory consumption. As a basis for determining the optimum storage condition

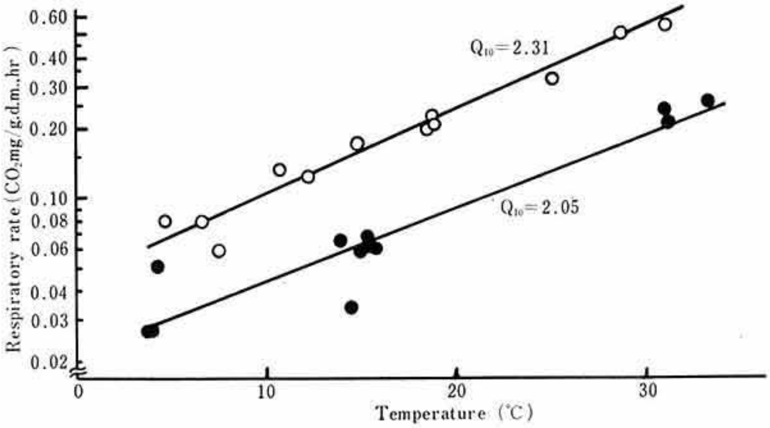

Fig. 3. Respiratory rate of tea roots at different storage temperatures
Note: ○ Prestored at 5°C
● Prestored at 20°C

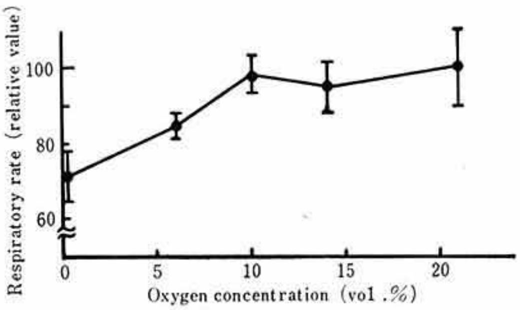

Fig. 4. Relation between oxygen concentration and respiratory rate of tea roots

of vegetative organs, relationship between respiratory rate and temperature was examined.

Using roots of 2-year old plants grown from cuttings, the respiratory rate of them was measured by the air-flow method at 4–35°C, after prestored at 5°C and 20°C for 1 month. As shown in Fig. 3, a close linear relationship was found between respiratory rate and temperature (temperature coefficient Q_{10}=2.31–2.05). Thus, it is apparent that low temperature reduces respiratory consumption during storage. The respiratory ability of roots prestored at high temperature was decreased and lower than that of roots stored at low temperature. This decrease may be caused by other factors such as root damage, etc., rather than reduced respiratory substrates occurred by accelerated respiration at high temperature.

Using roots of 1-year old plants grown from cuttings, relation between respiratory rate and oxygen concentration (21–0.1%) of air stream supplied to the roots was examined. As given in Fig. 4, the respiratory rate at the oxygen concentration higher than 10% was almost the same as that in air, but it decreased to about 70% of that in air at the oxygen concentration of 0.1%. When the oxygen concentration was changed as high→low (1%) →high, the respiratory rate was markedly reduced after the exposure to the low concentration, and it never recovered to the former level. For the application of reduced respiratory rate caused by low oxygen concentration to the long term storage, further studies will be needed as to the cause of, and countermeasures to such abnormality of respiration, and its effect on regeneration.

Changes of carbohydrate and regenerative ability during storage

1) Storage of roots[3)]

To know the physiological changes during storage and maximum duration of storage, roots taken from young plants and mature plants in April, i.e., bud opening stage, which is the most suitable time for collecting roots, were stored

Table 1. Changes of regenerative ability and TAC content during the storage of tea roots at 5°C in darkness

Roots[1)] taken from:	End of storage and planting	Storage period year, month	TAC content (%)	Rate of sprouting[2)] (%)
Young tea plants	Apr. 14, 1972	0, 0	30.8	100
	July 12, 1972	0, 3	23.6	100
	Oct. 23, 1972	0, 6	27.7	100
	Apr. 5, 1973	1, 0	22.0	100
	Apr. 15, 1974	2, 0	28.2	100
	Apr. 12, 1977	5, 0	9.7	100
Mature tea plants	Apr. 11, 1973	0, 0	42.2	100
	July 9, 1973	0, 3	44.7	100
	Oct. 9, 1973	0, 6	38.9	100
	Feb. 11, 1974	0, 10	34.6	100
	Apr. 15, 1974	1, 0	37.5	100
	Apr. 12, 1977	4, 0	27.1	100

Note 1) Roots were collected from young plants on Apr. 14, 1972 and from mature plants on Apr. 11, 1973. The collected roots were washed in water and packed into vinyl bags with wet sphagnum, and stored immediately at 5°C in dark room, while a part of roots were sampled for analysis.

2) No. of sprouted plants/total no. of plants $\times 100$.

Table 2. Changes in TAC content, respiratory rate, decreasing rate of TAC, and length of storage life of tea roots stored at 5 °C

		Material	
		4 years old young plant (Stored from Apr. 1972)	10 years old mature plant (Stored from Apr. 1973)
TAC content[1)]	At the beginning of storage	30.8 ± 4.03	42.2 ± 2.40
	At the end of storage (Apr. 1977)	9.7 ± 3.03	27.1 ± 7.78
Respiratory rate at 5 °C[2)]		0.00705 ± 0.00092	0.0057 ± 0.00201
Decreasing rate of TAC[3)]		42.20 (42.12*)	37.75 (34.05*)
Maximum length of storage life[4)] (years)		7.3 (7.3**)	11.2 (12.4**)

Note 1) % in dry matter ± standard deviation.

2) CO_2 mg/g, dry weight/hr ± standard deviation.

3) In terms of glucose mg/g of dry weight/year, estimated from TAC content as follows:

$$\text{Decreasing rate} = \frac{(G_2 - G_1) \times 1000}{t_2 - t_1}$$

* Figures in parenthesis indicate decreasing rate of TAC calculated by using respiratory rate as follows:

$$\text{Decreasing rate} = R \times 24 \times 0.682 \times 365$$

where

G_2, G_1: Glucose content at t_2, t_1

t_2, t_1: Measuring time

R = Respiratory rate (CO_2 mg/g, dry weight/hr)

0.682 = conversion rate from evolved CO_2 to glucose

4) $$\text{Length of storage life} = \frac{G(\text{start}) \times 1000}{\text{Decreasing rate}}$$

G(start) = Glucose content at the begining of storage

**: The values estimated from respiratory rate are shown in parenthesis at 5°C in a dark room. Since then, roots were sampled several times over a period of 5 years to measure TAC content and to plant them to field to examine their regeneration. The result is shown in Table 1.

With the elapse of storage time, TAC content in roots decreased gradually to 30% (roots of young plants) and 68% (roots of mature plants) of the initial content by the end of the experiment. During the storage, no mold was recognized, but a few new shoots and new roots emerged. After planting to field, 100% of regeneration occurred irrespective of the length of storage period, showing that the roots with TAC contents as such can be stored for 5 years or possibly more than 5 years.

Decreasing rate of TAC was calculated based on the data of respiratory rate and TAC content measured at the end of the experiment. As shown in Table 2, decreasing rate of TAC calculated by using respiratory rate coincided quite well with the value calculated with TAC, suggesting that it may be possible to estimate the storage period from the measurement of TAC content and respiratory rate at the beginning of the storage. Assuming that the stored roots can survive by the time when TAC content in the roots becomes to be nil, the calculation by the above method was made with the materials in Table 2, and was shown that the roots of young plants can survive for 7 years, and roots of mature plants for 11 years.

Furthermore, the possible longest survival period of tea roots was calculated similarly by assuming the highest TAC content of roots as 57.6%, the figure so far obtained as the highest in many studies. In this case, the longest survival period was shown as 16.9 years, and 3 more years can be added, if storage temperature is lowered to 1°C. However, by taking into account the need of carbohydrate for respiration and growth of new buds and new roots after planting in field, the limit of practical storage period may be about 15 years.

In addition, a high correlation ($r=0.70$) was observed between TAC content measured at the end of each storage period and percentage of regeneration in the field, with all the materials used in the present study. It indicates advantage of high TAC content for the regeneration.

2) *Storage of cuttings*

Apart from the long-term storage, the storage of cuttings for a short period of about 1 year is useful in relation to farm operations. With the purpose of establishing storage method for cuttings, changes of TAC content and regenerative ability of cuttings during the storage were examined (Table 3). The cuttings used were prepared by the customary method in June, and stored in a dark room at 5°C with their cut end immersed in water or sucrose solution (0.2M). In addition, some of the cuttings were grown in usual cuttings bed for 1 month before that storage.

By any method, TAC content and survival rate after planting were decreased with time, and in case of water immersion the storage of 2 months was the limit. However, the tentative planting to cutting bed increased TAC content and extended the storage period to 6 months. Application of sucrose to cuttings in storage was effective in keeping TAC content at a high level but it was difficult to extend storage period due to occurrence of molds.

From these results, it can be said that the storage longer than 6 months period is difficult with the cuttings prepared in June, and further studies using cuttings obtained in winter and early spring when TAC content in shoots is higher than that in June will be needed to extend the storage period.

Effect of removing tops at various times before root collection on the regeneration of stored roots[3]

To facilitate with safety rapid regeneration of stored roots, it would be useful to remove tops of plants from which roots are to be collected, with an aim of stimulating growth of bud primordia on roots before using for storage. To examine the effect of this method, tops of young plants were prunned at the height of 5 cm above ground in April, the time

Table 3. Changes in rooting ability and TAC content of cuttings stored at 5°C

Plot		Storage period (month)	Survived cuttings (%)	Rooted cuttings (%)	Weight of new shoots (g, D.W./10 plants)	Weight of roots (g, D.W./10 plants)	TAC content (% in dry matter)	
							Leaf	Stem
Control		0	100	100	9.3	4.2	15.2	15.7
I	W	1	90	100	1.8	2.5	7.0	10.3
		2	65	84	0.1	0.9	6.9	7.8
		4	10	0	—	—	3.7	3.4
		5	3	0	—	—	—	2.5
	S	1	90	100	1.3	2.4	8.7	15.0
		2	65	54	0.1	0.5	6.7	10.7
		4	35	0	—	—	6.2	6.8
		5	0	0	—	—	—	5.7
A	W	0	100	—	—	—	22.3	15.2
		1	100	100	0.5	1.8	14.2	13.0
		3	100	100	2.9	2.9	11.2	8.4
		4	90	75	2.9	2.9	8.5	6.5
	S	1	100	100	0.5	1.7	14.8	14.6
		3	80	80	3.1	3.2	11.3	10.3
		4	50	35	2.0	2.4	9.4	8.8

Note I : Cuttings were stored immediately after collecting.
A : Cuttings were planted in nursery bed, and stored a month after the planting.
W : Cultured with tap water.
S : Cultured with sugar solution.

Table 4. Effect of length of interval from top removal to root collection on regenerative ability and TAC content of tea roots stored at 5°C

Date of root collecting and the beginning of storage	TAC content (%)			Rate of sprouting at the end of storage		Rate of sprouting under field condition		Plant height (cm)	
	At the time of collecting	Apr. '73	Apr. '77	Apr. '73	Apr. '77	Dec. '73[1)]	Dec. '77[2)]	Dec. '73[1)]	Dec. '77[2)]
Apr. 13, 1972	40.8	30.1	14.2	7	20	75	33	20	3
Apr. 20, 1972	41.4	26.5	24.3	26	53	100	100	20	3
Apr. 27, 1972	41.9	34.9	20.3	21	80	88	100	16	16
May 4, 1972	38.1	33.6	17.0	38	50	100	80	15	11
May 13, 1972	38.0	25.5	22.8	84	71	100	80	19	16
May 20, 1972	33.8	30.6	15.8	88	100	88	100	15	5

Notes 1) Roots planted in Apr. 1973, and measured in Dec. 1973.
2) Roots planted in Apr. 1977, and measured in Dec. 1977.

of bud opening, and roots were sampled successively at the interval of 1 week. Thus, all the root samples were stored at 5°C in a dark room for 5 years.

Contents of TAC in the roots at the time of sampling, and rate of sprouting observed at the end of the storage and that occurred in field are shown in Table 4. It shows that the longer the time interval between top removal and root collection the higher was the rate of sprouting occurred during the storage. Rate of regeneration in field was higher with the roots collected after the time intervals than with the roots immediately collected after top removal, but differences by the length of time interval were not clear. TAC content was lower in roots collected later. During the storage, TAC content decreased to about 50% of the initial content in all plots showing no definite difference in decreasing trend by plots.

Thus, the treatment of keeping tea plants without tops in the field for some time before collecting their roots for storage causes a slight decrease in TAC content of roots at the start of the storage, but it exerts no clear effect on TAC content at the end of the storage, and it seems to stimulate regeneration after storage to some extent.

References

1) Nakayama, A.: Changes of carbohydrate and nitrogen compounds with growth in tea plant. *JARQ*, 6, 97–101 (1971).
2) Sakai, S.: Recent studies and problems of photosynthesis of tea plant. *JARQ*, 9, 101–106 (1975).
3) Sakai, S. et al.: Changes in regenerative ability and carbohydrate reserve of tea root during a long-term storage for maintenance of useful germ plasm, ed. by Akihama T. and Nakajima K., Long term preservation of favourable germ plasm in arboreal crops, Fruit Tree Research Station, 71–79 (1978).
4) Sakai, S.: Examination of a simple carbohydrate determination by the Weinmann method. *Study of Tea*, No. 36, 21–27 (1968) [In Japanese with English summary].
5) Sakai, S. et al.: Studies on the assimilation of carbon in tea plant. VI. On the apparatus for measuring gas-exchange by infrared gas analyzer, and the examination of some problems on measurement of gas-exchange with the apparatus. *Study of Tea*, No. 31, 10–22 (1965) [In Japanese with English summary].
6) Doi, Y. et al.: Changes in regenerative ability and carbohydrate reserve of tea cuttings during a long-term storage for the maintaninance of useful germ plasm. *Study of Tea*, No. 53, 13–16 (1977) [In Japanese with English summary].

(Received for publication, October 3, 1980)