Studies on the Varietal Differentiation of Frost Resistance in the Tea Plant

I. The seasonal change of frost resistance and its related physical and chemical properties of leaves

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Introduction

Recently studies on frost resistance have been developed in different ways on the plant such as wheat (LEVITT, 1957 ; HEBER, 1958), *Pinus tungeri* (PARKER, 1958), black locust (SIMINOVITCH and BRIGGS, 1953), *Hedera helix* (PARKER, 1962), cabbage and saxifraga (LEVITT, 1957), mulberry tree, white birch, willow, poplar, rose and other trees (SAKAI, 1960, 1962), tea plant (SIMURA, 1940, 1954, 1957 ; HARADA, *et al.* 1960).

In the physical aspect special attention has been paid to osmotic concentration (MÜLLER-THURGAU, 1882), permeability (SIMURA, 1940, 1957 ; TOMO, *et al.* 1955) and viscosity (KESSLER, 1935) of the plant cells, and in the chemical aspect to carbohydrates (LEVITT, 1957 ; SIMINOVITCH and BRIGGS, 1953 ; PARKER, 1958 ; SAKAI, 1960, 1962), sulfhydryls (LEVITT *et al.*, 1961, 1962) and thiouracil (JUNG, 1962).

The present study represents several experiments and observations in the physical and chemical analysis of frost resistance of the tea plant, especially in the seasonal change of frost resistance and its relation to osmotic concentration, water content, sugars, tannins and nitrogens with regards to varietal differentiation.

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Materials and Methods

The experiments were repeated at intervals of 7 to 15 days from September 1961 to January 1963. The varieties of the tea plant used were as follows : one group belongs to Japanese origin (Yabukita, Y-3, Y-6, U-22 and U-24), and the other group belongs to Indian origin (Benihomare, Y-1, X-10 and X-12). All of the 9 varieties used were grown in the experimental field of the Faculty of Agriculture, Nagoya University and were 6 or 7 years old. Fully grown leaves were collected from 5 plants in each variety, and were used for the following different investigations.

(1) Frost resistance. Leaves were dipped in water and then fully moistened with water to prevent them from overcooling by freezing. Five leaves of each variety was placed into the freezer kept at 0 °C for 3-6 hours, and then kept at -10 °C ~ -20 °C for

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1–2 hours. After freezing, the leaves were cultured in the flask containing water for 1–2 days at room temperature (20 °C – 25 °C). Frost injury was indicated as the grade from 0 to 10 by estimating dead surface area of leaves which was in proportion to frost injury.

(2) Total sugars and reducing sugars. 13 g of leaves was homogenized in 20 ml of phosphate buffer (pH 7.0) in a waring blender and reflexed with 80 %-ethanol. Total sugars and reducing sugars were spectrophotometrically determined by Somogyi's method.

(3) Tannins. 10 g of leaves was homogenized in 20 ml of phosphate buffer (pH 7.2) and reflexed with methanol. By the general process, the content of tannin was spectrophotometrically determined.

(4) Nitrogens. Total nitrogen were determined according to the usual Kjeldahl method. The content of total nitrogen and water soluble protein was determined spectrophotometrically according to the method of SIMINOVITCH *et al.* (1953 b).

(5) Osmotic concentration. A number of epidermis sections of leaves were stripped off, and by the usual plasmolytical method, the osmotic concentration of the epidermal cells was determined in balanced salts solution (NaCl : CaCl₂ = 9 : 1).

Experimental Results

1. The seasonal change of frost resistance.

From June 24 to September 9 the frost resistance showed no difference between varieties as shown in Table 1. All the varieties began to decrease their frost injury

Table 1. Seasonal change of frost resistance.

Variety			Yabukita	Y-3	Y-6	U-22	U-24	Y-1	Benihomare	X-10	X-12
Date	Origin of the variety		Japan	Japan	Japan	Japan triploid	Japan triploid	India	India	India	India
1962 Feb.	10		1	2	2	0	0	2	2	3	3
	27		0	2	2	0	0	2	3	3	3
Mar.	11		0	2	2	0	0	2	3	3	3
	23		1	2	2	1	1	2	3	3	3
Apr.	10		2	3	3	2	2	3	4	4	4
	19		4	4	4	4	4	5	6	6	6
May	10		5	6	6	6	5	6	6	7	7
	20		5	5	6	5	5	6	6	7	7
June	10		6	7	7	6	6	7	7	7	7
	20		6	7	7	6	6	7	7	7	7
July	10		7	7	7	7	7	7	7	8	8
	20		7	8	8	8	8	8	8	8	8
Aug.	2		10	10	10	10	10	10	10	10	10
	20		10	10	10	10	10	10	10	10	10
Sep.	7		9	9	9	9	10	10	10	10	10
	17		9	9	9	9	9	10	10	10	10
Oct.	1		5	7	7	7	6	7	5	7	7
	16		4	5	6	3	3	5	6	6	6
	25		3	5	4	3	3	4	5	6	6
Nov.	12		3	4	4	3	3	4	4	4	5
	20		3	4	3	2	2	3	4	4	4
Dec.	13		2	3	3	1	2	3	3	4	3
	20		1	2	3	1	1	2	3	3	3
1963 Jan.	8		0	2	2	0	0	2	1	3	3

Note : Leaves were kept at 0 °C for 3 hours, then at –10 °C for an hour and finally kept at room temperature for 2~3 days. Frost injury is graded from 0 to 10.

to different degree from September 17 on. U-22, U-24 and Yabukita began to get frost resistance from October 16 on—far earlier than the other varieties. Y-1, Y-3 and Y-6 were not so resistant as U-22, U-24 and Yabukita at this time but moderately resistant. Benihomare increased slowly resistance, and it was less resistant than U-22, U-24 and Yabukita on December 13 but similar in resistance to Y-1, Y-3 and Y-6. X-10 and X-12 increased resistance more slowly from October 16 to December 13. After a period from December 20 to January 8, all the varieties attained to the maximum resistance and kept it until March 23, but they decreased it abruptly on April 19. The result revealed that U-22, U-24 and Yabukita could be grouped in the very resistant varieties; Y-1, Y-3, Y-6 and Benihomare are in the moderately resistant varieties and X-10 and X-12 in the slightly resistant varieties. Among these varieties U-22 and U-24 are triploid and others are all diploid. Yabukita, U-22, U-24, Y-3 and Y-6 are of Japanese origin and Y-1, Benihomare, X-10 and X-12 are the hybrid between Assam and China.

2. The seasonal change of osmotic concentration and water content of leaves

As shown in Table 2 from August to October when the plant was not yet hardened, all the varieties tested showed low osmotic concentration and almost no varietal difference could be observed. From November, however, the osmotic concentration generally rose in each variety, and in January when it became very cold, it increased suddenly. At that time the most remarkable varietal difference of osmotic concentration could be observed, and the higher the osmotic concentration rose, the more the variety showed frost-resistance. Thus in January and February high resistant varieties such as Yabukita, Y-6, U-22 and U-24 showed the highest osmotic concentration, while low resistant varieties such as X-10 and X-12 showed the lowest osmotic concentration. Intermediate resistant varieties such as Y-1, Y-3, and Benihomare showed also intermediate osmotic concentration.

The seasonal change of water contained in leaves is shown in Fig. 1. In each

Table 2. Seasonal change of osmotic pressure in leaves

Date	Variety	Yabukita	Y-3	Y-6	U-22	U-24	Y-1	Benihomare	X-10	X-12
1962		M	M	M	M	M	M	M	M	M
Aug.	24	0.35						0.35		
Sept.	2	0.4						0.35		
	12	0.4						0.35		
	26	0.35						0.35		
Oct.	12	0.4						0.3		
	25	0.35						0.35		
Nov.	11	0.5						0.4		
	22	0.5						0.45		
	30	0.5						0.5		
Dec.	6	0.5						0.45		
1963										
Jan.	17	0.7	0.55	0.7	0.7	0.7	0.5	0.6	0.5	0.5
Feb.	17	0.7	0.55	0.7	0.7	0.7	0.5	0.6	0.5	0.5
March	17	0.55	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
April	17	0.45	0.45	0.4	0.45	0.4	0.4	0.4	0.4	0.4

Fig. 1. Seasonal change of water content in leaves coming from Yabukita, Y-3, Benihomare, and X-10.

variety the water content began to increase from April and a sudden rise could be observed in May. Then from May to August it maintained a maximum value. However, it began to decrease in September and it fell steeply from October to November. Then from December to April the water content kept the minimum value, although in March and April the osmotic concentration decreased gradually as shown in Table 2.

3. The seasonal change of some chemical constituents of leaves

(A) Total sugars and reducing sugars

The leaves generally contained rich sugars in winter and poor sugars in summer as shown in Figs. 2 and 3. The content of total sugars in leaves is high from December to March, and at this period varietal difference is apparent (Fig. 3). The high frost-resistant variety such as Yabukita showed higher content of total sugars than the lowest frost-resistant variety such as X-10. The intermediate varieties such as Benihomare and Y-3 showed the intermediate content. From the

Fig. 2. Seasonal change of total sugars content in leaves coming from Yabukita, Y-3, Benihomare, and X-10.

Fig. 3. Seasonal change of reducing sugars content in leaves coming from Yabukita, Y-3, Benihomare, and X-10.

Fig. 4. Seasonal change of tannins content in leaves coming from Yabukita, Y-3, Benihomare, and X-10.

after March it increased rapidly, and reached a maximum value in May or June. In summer it showed a high value, and from September it decreased again; reached a minimum value in December. The relation between tannins content and frost resistance is not always apparent and Benihomare showed always higher content than Yabukita as in Fig. 4.

(C) Nitrogens

Generally speaking total nitrogen and water soluble nitrogen in leaves were rich in summer and poor in winter (Fig. 5 and 6), while water soluble protein was poor in summer and rich in winter (Fig. 7). As shown in Fig. 5, total nitrogen began to increase from June and reached a maximum value from July to September, but in the end of September it decreased rapidly and reached a minimum value from October to January. There seemed to be no relation between total nitrogen in leaves and frost resistance.

Water soluble nitrogen, as shown in Fig. 6, decreased rapidly from October to December, and thereafter all the varieties kept a minimum value in equilibrium, when it was ranked in order of the content: Yabukita, Benihomare, Y-3 and X-10. It might have no relation to frost resistance.

The seasonal variation of water soluble protein contained in leaves is shown in Fig. 7. It kept the minimum value in all the varieties from May to September, and then it began to increase rapidly until it attained to the maximum value from December to January. At this maximum value there is an apparent relation between its content

end of March to the end of April total sugars decreased steeply and reached a minimum value around June. Then it kept the same low value during summer, but from September to January, when the plant got hardened, it increased rapidly. As for reducing sugars, they generally changed parallel to total sugars as in Fig. 3, but the relation to frost resistance with reducing sugars was not so apparent as with total sugars. In Fig. 3, it could be observed that in some time of winter Benihomare showed higher content than Yabukita which is high frost-resistant variety.

(B) Tannins

Contrary to sugars, tannins increased in summer and decreased in winter as shown in Fig. 4. In January and February, tannins content kept a minimum value;

Fig. 5. Seasonal change of total nitrogen content coming leaves from Yabukita, Y-3, Benihomare, and X-10.

Fig. 7. Seasonal change of water soluble protein content in leaves coming from Yabukita, Y-3, Benihomare, and X-10.

Fig. 6. Seasonal change of soluble nitrogen content in leaves coming from Yabukita, Y-3, Benihomare, and X-10.

in leaves of each variety and the degree of frost resistance, and the most frost resistant Yabukita contained the most water soluble protein, then followed by Benihomare, Y-3 X-10 in order of frost resistance.

Discussion

SIMURA (1940) already reported that the water content in leaves of the variety in severe winter had a negative correlation with frost resistance. The present result showed also the same relation between water contained in leaves and frost resistance, and varieties with leaves containing less water were more frost resistant. This relation agreed with the results of LEVITT (1957 a) in cabbage,

wheat and saxifraga, and SIMINOVITCH and BRIGGS (1953 a) in black locust trees. It is likely that the decrease of water content in leaves is one of the factors enhancing frost resistance with only an exception where the abrupt decrease of resistance found from the end of March to middle of April does not follow the increase of water content. A possible significance for this exception will be discussed later in relation to osmotic

concentration and sugars content of leaves.

As for osmotic concentration, SIMURA (1940) reported that in winter osmotic concentration of leaves among varieties had a positive correlation with frost resistance. The result also showed the same relation, and high osmotic concentration found from December to February corresponded with decreased water and increased frost resistance, but as mentioned above from the end of March to the middle of April during which time the abrupt decrease of resistance appeared, when the decrease of osmotic concentration occurred without the increase of water content.

In agreement with the view of LEVITT (1957a and b) who described both water and sugars to be the factors related to osmotic concentration; it seems likely that the decreased osmotic concentration observed at that period is associated with the decrease of sugars (Fig. 2).

The relation between frost resistance and total sugars is elucidated as follows: The increase of frost resistance observed from the end of October to the beginning of November and its maximum peak observed from January to February correspond completely respectively with the increase and its peak of the content of total sugars. It is obvious that the content of total sugars in leaves has an intimate relation to the frost resistance of varieties, and indeed it is shown that the more total sugars leaves contain, the more frost resistant the variety is in midwinter (Table 1 and Fig. 2).

The problem what sorts of sugars increase during frost resistance has not been elucidated in the present study. PARKER (1957, 1958, 1959/60) found that sucrose, raffinose and stachyose increase in leaves of white pines and *Hedera helix* L. during frost resistance, to which SAKAI (1960) opposed and said from his experiments with many species of woody plants that it might not be likely that raffinose and stachyose play any more important part in enhancing frost hardiness than the other sugars, especially sucrose, glucose and fructose.

The problem whether a definite relation exists between the increase of reducing sugars and frost resistance has remained unsettled. SIMINOVITCH and BRIGGS (1953 a, 1954) found it in the bark of black locust trees and SAKAI (1962) also in the bark of larch trees and roses.

SIMURA *et al.* (1954) also observed in the tea plant that there is a positive correlation between frost resistance and the content of reducing sugars in leaves among both seasons and varieties. HARADA *et al.* (1960), however, reported that the content of reducing sugars showed no correlation with frost resistance among varieties, but his result is too unreliable for any definite conclusions to determine correlation, for he discussed on their result with only four varieties. In the present study the content of reducing sugars in leaves increased with the increase of frost resistance from the end of October, reached a maximum value in midwinter. (Fig. 3 and Table 1). Its seasonal change is considered to be parallel to that of total sugars, but as regards to the variety, however, even in midwinter the most frost resistant variety did not always show the most content of reducing sugars in leaves, therefore it can be thought that the relation

between reducing sugars and frost resistance is not so close in degree as that found between total sugars and frost resistance.

The literature on the relation between tannins and frost resistance is still very scanty. The results of the present study showed no definite relation between them so far as in the varieties tested, although the previous work of SIMURA (1940) has revealed that tannins content of leaves has a negative correlation with frost resistance. Tannins contained in leaves decreased most rather in a period when frost resistance was the highest, and increased most in summer when the frost resistance was the lowest (Fig. 4).

As for the varietal difference of tannins content, from the present result and SIMURA's previous work, however, it can be said that the varieties which have abundant tannin in leaves are always less resistant, but the contrary is not always true; namely the varieties of which leaves are scanty in tannins are not always more resistant (Fig. 4 and Table 1).

As regards to nitrogen the seasonal variation of total nitrogen and water soluble nitrogen contained in leaves is similar to that of the tannins content. Both of nitrogen decrease with the increase of frost resistance, and no significant difference of both of them could be observed between varieties of different frost resistance. It seems likely that both forms of nitrogen would have no direct relation with frost resistance (Figs. 5 and 6).

It was demonstrated, however, in the present study that a positive correlation would exist between the content of water soluble protein and frost resistance. One month before the time of onset of frost resistance—from beginning of October on—it began to increase rapidly, reached a maximum value at the end of November and remained constant in coincidence with a maximum of frost resistance. The variety which has abundant water soluble proteins was very frost-resistant (Fig. 7).

In general agreement with the present results SIMINOVITCH and BRIGGS (1953b) found in black locust trees that water soluble protein increased from the end of July, reached a maximum peak about the middle of November and thereafter remained constant synchronically with the corresponding maximum of frost resistance. PARKER (1962) obtained somewhat different result in leaves of *Hedera helix* L. that water soluble protein increased from the end of August on and maintained an equilibrium with increased frost resistance, but finally reached a maximum value at the middle of April at which time frost resistance disappeared.

The mechanism of contribution of water soluble protein to the increase of frost resistance has been described by SIMINOVITCH and BRIGGS (1953 a and b). Water soluble protein inhibits plant cells from intracellular freezing prior to extracellular freezing lowering the freezing point of the cytoplasm and at the same time serves as a sort of mechanical buffers or as a kind of modifiers before shrinkage of plant cells due to dehydration by extracellular freezing to prevent the cell mechanism from irreversible disintegration.

LEVITT (1958), contrary to this interpretation, pointed out the increase of the water soluble protein in hardening treatment of potatoes which have no frost resistance. SAKAI (1960) stated using branches of mulberry trees that water soluble protein is prerequisite for inducing frost resistance increased by hardening but has no relation with the following increase of frost resistance.

HEBER and ULLICH (1958) and HEBER (1958 a and b) described that chloroplast of wheat contained some proteins sensitive to freezing disintegration and proteins contributed to the increase of frost resistance. In agreement with the results of HEBER and others, the results obtained in the present experiments reveal that water soluble protein in leaves of the tea plant is one of the important factors contributing to the increase of frost resistance.

As for the varietal difference, the varieties of Japanese origin were more frost resistant than the ones of Indian origin. SIMURA (1957) reported the triploid varieties were more frost resistant than the diploid, and in agreement with his previous results the present study also revealed that the triploid varieties, such as U-22 and U-24 were more frost resistant than the other diploid varieties.

Summary

Frost resistance of nine different varieties of the tea plant was studied in different ways mainly as regards to seasonal change. The following results were obtained.

Frost resistance of nine varieties tested is ranked in the following order ; Yabukita, U-22 (triploid) and U-24 (triploid) are very resistant ; Y-1, Y-3, Y-6 and Benihomare moderately resistant ; X-10 and X-12 are slightly resistant.

Frost resistance increased with the decrease of water content in leaves, and simultaneously with the increase of osmotic concentration in the leaf cell.

Frost resistance increased also with the increase of sugars content. Varieties with leaves containing more total sugars in midwinter were more resistant, although varieties with leaves containing more reducing sugars were not always more resistant.

Tannins content in leaves had no definite relation with frost resistance.

The increase of frost resistance has no direct relation with the content of total nitrogen and water soluble nitrogen, while water soluble protein increased with the increase of frost resistance, and varieties which contain more water soluble protein in leaves in midwinter are more frost-resistant.

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チャの耐凍性の品種間差異に関する研究

I 耐凍性と葉の物理化学的諸性質の季節的变化

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チャの耐凍性と葉の細胞の浸透濃度や水分その他の化学成分の季節的变化について調べた。実験材料として、次の9品種を用いた。日本種からヤブキタ, U-22, U-24, Y-3, Y-6, インド雑種からベニホマレ, Y-1, X-10, X-12, このうち U-22 と U-24 の2品種は3倍体 ($2n=45$) で、他はいずれも2倍体 ($2n=30$) である。

耐凍性の強さは、各品種とも春夏の候には弱く、品種間のちがいがほとんどみられないが、9月下旬から次第に増大し、1~2月に最高に達し、3月以降再び弱くなる。厳寒の1~2月に耐凍性の強さが品種間で大いに異なり、ヤブキタ, U-22, U-24 の3品種は最も強く、Y-3, Y-6, Y-1, ベニホマレは中位、X-10, X-12 の2品種は最も弱かった。

葉の水分含量は秋から冬にかけ減少し、1~2月に最低となり耐凍性の増大と平行するが、3月以降耐凍性が減るときに葉の水分は増さない。このときの耐凍性の低下は糖類の減少によるものである。

葉の細胞の浸透濃度は秋から冬にかけ増加し、耐凍性の増大と平行する。耐凍性の強い品種では冬期葉の水分が少く、浸透濃度は高い。葉の全糖及び還元糖は秋から冬にかけ、耐凍性が増大するにつれ、増加し、冬期耐凍性の強い品種では全糖含量が多いが、還元糖は必ずしも多くない。水溶性蛋白質含量も、全糖と同じく、耐凍性と平行して増減する。葉のタンニン、全窒素および水溶性窒素の含量は耐凍性が増大するとき減少する傾向あり、品種間にも明かな関係を示さなかつた。