Physiological Study on the High and Low Temperature Tolerance of Crop Plant

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Introduction

The low temperature injury of crop plant can apparently be classified into two types; the freezing injury which occurs at the temperature below 0°C and the chilling injury which happens above 0°C.

As for the former, many studies have been carried out and its detailed general descriptions are not rare[11),12)]. The study on the latter started from the necessity of the countermeasure against the injury caused by the low temperature storage of fruit and vegetable originated in the tropics or in the subtropics. But the study on the physiological mechanism of the chilling injury is not yet advanced so much as that of the freezing injury.

Regarding the high temperature injury of plant, it is reported that the aquatic plants suffer visible injury at the temperature more than 40°C, and the xerophytes, at 50°C[11)].

It is well known that the physiological functions such as respiration, photosynthesis and nutrient absorption suffer even from the temperature below 40°C.

On the other hand, the thermophilic bacterium can survive at the temperature from 70 to 90°C because the protein and nucleic acid of its cell do not suffer from thermal denaturation.

But, if the physiological functions of crop plant are generally affected by the temperature even below 40°C, it could be easily concluded that the high temperature resistance of crop plant is due to the tolerance of protein and nucleic acid against thermal denaturation.

Effect of temperature on respiration and respiratory enzyme

Respiration is an important metabolic function to maintain the life of plant. So the author investigated the effect of high temperature on the respiration of the roots of rice originated in the tropics and of the roots of orchardgrass and Italian ryegrass originated in the temperate zone.

Table 1 shows the results. The respiration of rice increased linearly in accordance with the rise of temperature until 32°C, and at the temperature more than 32°C, the increasing rate of respiration began to decline. As for the respiration of orchardgrass and Italian ryegrass, the increasing rate of respiration

Table 1. Effect of temperature pretreatment on the cytochrome oxidase activity of rice and orchardgrass roots

	Temperature of pretreatment	Cyt. oxidase activity O_2 uptake, mm^3/f.w./hr
orchardgrass	32°C	31.2
	cont.*	51.2
rice plant	32°C	48.1
	cont.**	45.4
	38°C	35.1
	cont.**	40.7

* Left stand at room temp. (about 16°C)
** Ditto (about 22°C)

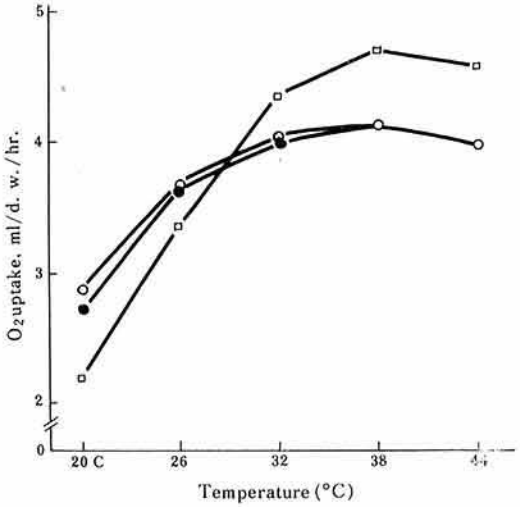

Fig. 1. Effect of temperature on the root respiration of rice plant. Italian ryegrass, and orchardgrass

Notes: —□—□— rice plant, —○—○— Italian ryegrass, —●—●— orchardgrass

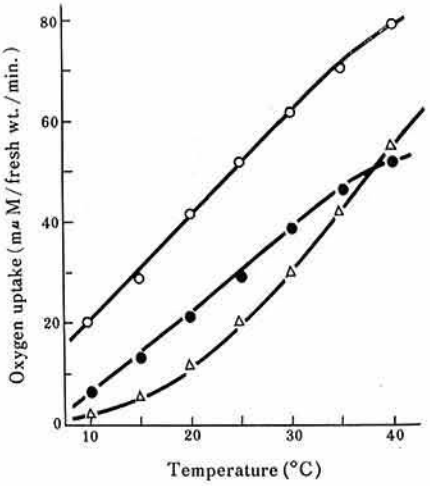

Fig. 2. Cytochrome oxidase activity at different temperatures

Notes: —●— Italian ryegrass
—○— Orchardgrass
—△— Rice

declined at the temperature even above 26°C, and at more than 32°C, the increase of respiration was retarded or respiration rather decreased in spite of the rise of temperature.

To examine the effect of temperature on the cytochrome oxidase which is one of the enzymes related to respiration, the activity of the cytochrome oxidase prepared from the roots exposed to high temperature for three hours was measured.

The cytochrome oxidase activity of the rice roots exposed to 38°C decreased but that exposed to 32°C showed no decline.

On the contrary, the cytochrome oxidase activity of the orchardgrass exposed to 32°C clearly declined (Table 1).

Since the enzyme activity can, thus, be affected by the temperature lower than the body heat of warm-blooded animal, the cause of the decline of the enzyme activity cannot be attributed to the thermal denaturation of enzyme protein.

The effect of low temperature on the activity of the cytochrome oxidase prepared from the leaves of the crop plants described above was investigated to find the relation between the cytochrome oxidase activity and temperature. Table 2 shows the results.

The enzyme activity of rice plant made gradual increase according to the rise of the temperature below 20°C but at the temperature more than 20°C, it increased rapidly.

As to the Italian ryegrass and orchardgrass, the cytochrome oxidase activity increased linearly in accordance with the rise of temperature between 10°C and 35°C.

The decrease of enzyme activity was not recognized in this experiment because the enzyme was not exposed to high temperature except for the very short time during the measurement. But when the material plant was exposed to high temperature for more than a certain time before the preparation of cytochrome oxidase from leaves, the decline of cytochrome oxidase activity was recognized as in the case of roots.

Effect of surfactant on the change of cytochrome oxidase activity by temperature

In cell, the enzyme protein of cytochrome oxidase is combined with phospholipid; and the phospholipid has an important role on

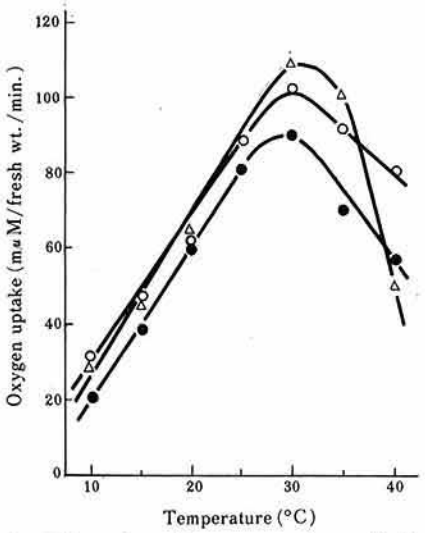

Fig. 3. Effect of surfactant on the activity of cytochrome oxidase at different temperatures

Notes: —●— Italian ryegrass
—○— Orchardgrass
—△— Rice

the activity of cytochrome oxidase[14].

Since the cause of the decline of the cytochrome oxidase activity by the temperature below 32°C cannot be attributed to the thermal denaturation of enzyme protein, the cytochrome oxidase activity is likely to change by the phospholipid affected by high temperature.

The relation between the cytochrome oxidase activity and temperature was then investigated changing the bond structure between enzyme protein and phospholipid by adding the surfactant (Polyoxyethyleneglycol alkylphenyl ether) which can solubilize the cytochrome oxidase.

And no significant difference in the change of the cytochrome oxidase activity caused by different temperatures was recognized even at the temperatures below 20°C among rice, Italian ryegrass and orchardgrass in Fig. 3, but at the temperature above 30°C, the cytochrome oxidase activity of every plant declined.

On the other hand, the activity of cytochrome oxidase prepared with rice leaves, adding or not adding the surfactant, was measured after high temperature treatment.

Table 2. Effect of surfactant on the thermostability of cytochrome oxidase from rice leaves

Pretreatment by surfactant	High temp. treatment	Cytochrome oxidase activity
Cont.	Cont.	30.2 (100)
	Treat.	30.0 (99.4)
Treat.	Cont.	123.6 (100)
	Treat.	3.2 (2.6)

Note: The enzyme was exposed to 40°C for 30 min. The unit of enzyme activity is oxygen uptake in mμM/fresh wt./min.

The cytochrome oxidase untreated by the surfactant did not decline in activity by high temperature treatment, whereas that treated by the surfactant was completely deprived of activity by high temperature (Table 2).

From these results, it may be presumed that the temperature reaction of cytochrome oxidase activity is caused by the phospholipid bonded with enzyme protein rather than by the enzyme protein itself.

Difference of fatty acid composition in phospholipid among some crop plants

The interaction between phospholipid and protein possibly influences upon the activity of cellular or whole plant metabolism as well as on that of cytochrome oxidase[5),6)].

It is suggested by a hypothesis that the unit membrane which consists of protein and lipid (mostly phospholipid) is the base of the fine structure of protoplasm.

In fact, the principal chemical components of cell membrane or mitochondria are protein and phospholipid.

More than 40 years ago, it was assumed that one of the causes which induce the high temperature damage of cell is the liquefaction protoplasm lipid, and a relation might exist between the maximum temperature tolerable for the organism and the melting point of protoplasm lipid[15]. Then the difference of the fatty acid composition of

phospholipid was investigated with some crop plants.

Table 3 shows the fatty acid composition of phospholipid in the leaf of rice, maize, Italian ryegrass and orchardgrass.

The ratio of unsaturated acid to saturated one was smaller in the phospholipid of rice or maize which is originated in the tropics than that of orchardgrass or Italian ryegrass originated in the temperate zone. As to the

Table 3. Specific difference in fatty acid composition of lipids from the crops grown under the same temperature condition

	R. R. T. *	1.00	1.13	1.27	1.46	1.63	1.90	2.00	2.41	2.79	3.81	Unsat. acid/Sat. acid
	Fat. acid**	$C_{16=0}$	$C_{16=1}$			$C_{18=0}$	$C_{18=1}$	$C_{18=2}$	$C_{18=3}$			
		%	%	%	%	%	%	%	%	%	%	
Phospho-lipid	Rice	34.2	4.9	0.8	—	2.3	4.5	22.1	29.8	0.8	0.1	1.68
	Maize	35.6	3.9	0.8	—	2.9	5.0	29.0	22.7	—	0.1	1.57
	Italian ryegrass	25.5	4.7	—	0.8	0.8	2.0	20.1	46.0	—	0.1	2.77
	Orchardgrass	26.7	3.7	—	0.9	0.8	1.9	16.7	49.4	—	0.1	2.61
Sulfolipid	Rice	13.1	—	0.7	3.6	1.7	1.5	7.6	71.2	—	0.6	5.43
	Maize	20.5	—	0.1	1.1	1.4	2.0	14.4	60.4	—	0.1	3.51
	Italian ryegrass	13.5	0.1	—	0.2	0.9	1.0	8.9	75.5	—	0.1	5.92
	Orchardgrass	12.1	—	0.1	0.5	0.4	1.5	5.6	79.6	—	0.1	6.94
Galacto-lipid	Rice	6.8	—	0.3	—	0.4	1.0	8.1	83.1	0.2	0.1	12.80
	Maize	8.2	—	—	—	0.5	2.3	9.8	78.8	—	0.4	10.45
	Italian ryegrass	7.0	0.2	—	—	0.6	1.6	10.0	80.4	—	0.3	12.11
	Orchardgrass	6.8	—	—	0.1	0.4	1.3	5.8	85.6	—	0.1	12.88

Notes: * ..... Relative retention time
** ..... In $C_{m=n}$, m and n represent the numbers of carbon atoms and double bonds respectively

Table 4. Alteration of the fatty acid composition of lipids due to the difference in growing temperature

		R. R. T. *	1.00	1.13	1.27	1.46	1.63	1.90	2.00	2.41	2.79	3.91	Unsat. acid/Sat. acid
		Fat.	$C_{16=0}$	$C_{16=1}$			$C_{18=0}$	$C_{18=1}$	$C_{18=2}$	$C_{18=3}$			
			%	%	%	%	%	%	%	%	%	%	
Phospholipid	30–20° C	Rice	41.9	2.8	1.5	1.2	1.2	4.2	24.3	22.8	0.1	0.1	1.26
		Italian ryegrass	32.3	2.8	—	1.0	0.6	2.5	24.7	34.6	0.1	0.1	1.96
	17° C	Rice	36.8	2.3	0.9	0.7	0.8	2.0	22.5	23.4	0.5	0.2	1.60
		Italian ryegrass	25.8	6.9	—	1.2	0.2	1.2	18.6	45.8	0.3	0.1	2.79
Sulfolipid	30–20° C	Rice	19.0	—	1.1	0.3	1.9	2.0	14.7	60.5	—	1.3	3.96
		Italian ryegrass	14.1	—	0.1	1.0	0.3	1.1	9.3	74.0	—	0.1	5.86
	17° C	Rice	14.5	—	0.6	0.5	1.2	1.7	9.1	71.0	—	1.4	5.21
		Italian ryegrass	9.8	—	—	—	0.1	1.5	4.7	83.8	—	0.1	9.09
Galactolipid	30–20° C	Rice	10.3	—	0.4	0.4	0.4	1.9	11.6	75.0	—	0.1	8.27
		Italian ryegrass	9.7	—	—	—	0.3	1.0	9.7	79.2	—	0.1	8.99
	17° C	Rice	9.1	—	0.9	0.1	0.9	0.7	5.4	82.7	—	0.1	8.83
		Italian ryegrass	8.1	—	—	—	0.5	0.8	4.6	86.0	—	0.1	10.62

Note: See notes in Table 3

sulpholipid and galactolipid, no such difference was recognized.

Though the fatty acid composition is variable according to the variation of temperature during growth of crop plant, this difference of fatty acid composition in phospholipid could be clearly recognized irrespective of the temperature variation during growth (Table 4).

The melting point of unsaturated acid is generally far lower than that of saturated acid. (Table 5).

Table 5. Melting point of fatty acids

Fatty acid	Melting point
Palmitic acid ($C_{16=0}$)	63—64°C
Palmitoleic acid ($C_{16=1}$)	−1°C
Stearic acid ($C_{18=0}$)	70—72°C
Oleic acid ($C_{18=1}$)	14—16°C
Linolic acid ($C_{18=2}$)	−5°C
Linolenic acid ($C_{18=3}$)	−11°C
Arachidic acid ($C_{20=0}$)	76—77°C

Note: See notes in Table 3

The above described results seem to give a certain basis to understand the difference of the optimum growing temperature of crop plants.

Jurtshuk et al.[8] reported, on the β-hydroxybutyric dehydrogenase, which is one of the enzymes of mitochondria, that the higher the unsaturation degree of the fatty acid in phospholipid which composes the complex with the protein of that enzyme, the higher the enzyme activity. And also, the higher the unsaturation degree of the fatty acid in the lipid of the cell membrane of *Mycoplasma laidlawii*, the higher the permeability of cell mebrance[13].

Consequently, it seems that the high unsaturation degree of fatty acid in the phospholipid of the crop plants originated in the temperate zone is related to the ability of these plants to maintain high metabolic activity even under low temperature, in other words, their optimum temperature for growth are low.

Effect of cholesterol on chilling injury

Though there may exist some relation between the unsaturation degree of the fatty acid in phospholipid and the optimum temperature for the growth of crop plant as described above, it has not been proved that the phospholipid of protoplasm is affected by high or low temperature injury.

Cholesterol, a kind of lipid, composing a complex with phospholipid, seems to have some relation to the thermal stability of biological membrane[9]. And the permeability of erythrocyte membrane is variable according to the difference of cholesterol contents[2),10]: the larger the cholesterol contents of membrane, the less the variability of permeability by temperature[3),4)].

Considering these effects of cholesterol on the physicochemical property of the membrane which is composed of phospholipid, the effect of cholesterol on the chilling injury was investigated.

Sorghum seedlings cultivated on the soil added with cholesterol were exposed to 6°C for six days when the third leaf spread, and were, then, kept at 15°C afterward.

Table 6 shows the results, that is, the plant grown without cholesterol showed the appearance of complete withering, but the one grown with cholesterol did not wither except its very few leaves.

Table 6. Effect of cholesterol on the low temperature damage of sorghum seedlings

Cholesterol (g/pot)	Percentage of damage plant	
	Stem breakdown	Softened and discolored leaves
0	70.6%	85.3%
0.2	0	15.0
0.5	0	0
1.0	0	0

Note: The pots used were 150 mm long, 55 mm wide and 100 mm deep. The plants were exposed to 6°C for 15 days

Accordingly, cholesterol seemed to be effective to restrict the chilling injury but it appeared to be quite ineffective to resume the growth of plant which had been stopped by low temperature.

By the way, the seedlings of rye and oat, which are the crop plants originated in the temperate zone, can evidently grow even at the low temperature of 6°C.

It can be concluded, from these results, that the phospholipid which composes the fine structure of protoplasm must be affected by the outbreak of the chilling injury.

Though cholesterol controlled the chilling injury, it failed to resume the growth retarded by the low temperature. Accordingly, the physiological mode of the tolerance to chilling injury seems a little unlike that of the growth under the low temperature.

Relation between chilling injury and protein denaturation

Protein is generally denatured by high temperature, and the denaturation of protein by low temperature is also reported recently.

As the structure of water which is mainly constructed by the polarization of water molecule and hydrogen bond becomes more ordered structure by low temperature treatment, it is presumable that the hydrophobic bond among the side chains of protein molecule is loosened, and consequently, the conformation of protein molecule changes resulting in the denaturation of protein.

On the other hand, alcohol, like ethanol or glycerol, and dimethyl sulfoxide, affecting water structure, are effective to restrict the denaturation of protein[1].

When the seedling of sorghum cultivated with pot in which alcohol or dimethyl sulfoxide added to the soil was exposed to 6°C after being kept in room temperature during one night, no chilling injury was recognized. (Table 7)

But when this seedling was replaced into the normal room temperature from 6°C, withering advanced from the tip of leaves.

Table 7. Effect of alcohol and dimethyl sulfoxide on the low temperature damage of sorghum seedlings

Reagents		Percentage of damaged plant	
		Stem breakdown	Softened and discolored leaves
Cont.		93.8%	87.5%
GL	0.2%	5.0	10.0
	1.0	0	0
	5.0	0	0
EG	0.2	0	50.0
	1.0	0	40.0
	5.0	0	26.3
PG	0.2	5.3	36.0
	1.0	5.3	10.5
	5.0	11.1	44.4
DEG	0.2	72.9	85.7
	1.0	85.7	76.2
	5.0	5.0	10.0
ET	0.2	5.3	10.5
	1.0	0	4.8
	5.0	0	14.3
DMSO	0 %	100 %	94.7%
	0.2	66.5	49.0
	1.0	28.0	21.3
	5.0	0	10.0
	10.0	6.2	33.3

Notes: GL...Glycerol, EG...Ethyleneglycol, PG...Propyleneglycol, DEG...Diethyleneglycol, ET...Ethanol, DMSO...Dimethyl sulfoxide
The seedlings were exposed to 6°C for 15 days

This might be caused by the alcohol accumulated in the leaf tips accompanied with the transpiration of water, because concentrated alcohol affects protein denaturation in normal temperature.

Anyway, it may be concluded that protein denaturation has relation to chilling injury from the fact that alcohol or dimethyl sulfoxide which was effective to restrict protein denaturation at low temperature could control the outbreak of chilling injury.

References

1) Brandts, J. F.: Thermobiology. (ed. Rose, A. H.) Academic Press, London and New York, 25–72 (1967).
2) Bruckorfer, K. R. et al.: The effect of partial replacements of membrane cholesterol by other steroids on the osmotic fragility and glycerol

permeability of erythrocyte. *Biochim. Biophys. Acta*, **183**, 334–345 (1969).
3) DeGier, J., Mandersloot, J. G. & Van Deenen, L. L. M.: The role of cholesterol in lipid membrane. *Biochim. Biophys. Acta*, **173**, 143–145 (1969).
4) Demel, R. A. et al.: Effects of temperature and cholesterol on the glucose permeability of liposomes prepared with natural and synthetic lecithins. *Biochim. Biophys. Acta*, **150**, 655–665 (1968).
5) Duncan, C. J.: The Molecular Properties and Evolution of Excitable Cells. Pergamon Press, Oxford, 50–76 (1967).
6) Green, D. E. & Fleisher, S.: The role of lipids in mitochondrial electron transfer and oxidative phosphorylation. *Biochim. Biophys. Acta*, **70**, 554–582 (1963).
7) Haskins, R. H.: Sterols and temperature tolerance in the fungus *Pythium*. *Science*, **150**, 1615–1616 (1965).
8) Jurtshuk, P. Jr., Sekuzu, I. & Green, D. E.: Studies on the electron transfer system. LVI. On the formation of an active complex between the apo-D-(-)-β-hydroxybutyric dehydrogenase and micellar lecithin. *J. Biol. Chem.*, **238**, 3595–3605 (1963).
9) Kavanau, J. L.: Structure and Function in Biological Membranes. **1**, Holden-Day, Inc., San Francisco, London and Amsterdam, 119–124 (1965).
10) Kroes, J. & Ostwald, R.: Erythrocyte membranes: Effect of increased cholesterol content on permeability. *Biochim. Biophys. Acta*, **249**, 647–650 (1971).
11) Levitt, J.: Frost, drought and heat resistance. *Ann. Rev. Plant. Physiol.*, **2**, 245–268 (1951).
12) Levitt, J.: Encyclopedia of Plant Physiology (ed. Ruhland, W.). II. Springer-Verlag, Berlin, 632–638 (1965).
13) McElhaney, R. N., DeGier, J. & Van Deenen, L. L. M.: The effect of alterations in fatty acid composition and cholesterol content on the permeability of *Mycoplasma laidlawii* B. cell and derived liposomes. *Biochim. Biophys. Acta*, **219**, 245–247 (1970).
14) Reich, M. & Wainio, W.W.: Role of phospholipid in cytochrome oxidase activity. *J. Biol. Chem.*, **236**, 3062–3065 (1961).
15) Yamaha, G.: General Cytology. Shokabo, Tokyo, 223–225 (1933). [In Japanese.]