Studies on Physico-Chemical Properties of Expressed Plant Juice in Wintering Naked Barley and Wheat, and Some Effects of Stamping on These Properties of Wheat

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(Tables 1–7; Text-figs. 1–13)

CONTENTS

(I) INTRODUCTION

There are many studies with regard to both cold and drought resistance of plants investigated from the agronomical, physiological and ecological points of view. Earnest efforts have been concentrated on clearing up the internal factors of plant hardiness against both cold and drought injuries. To clarify such internal factors, many investigators tried to examine physico-chemical properties of plant cell sap, such as osmotic pressure, cell sap viscosity and so on. An examination of changes of these factors in plants under natural and artificial conditions, for example, by freezing plants by either natural low temperature or artificial refrigeration, may give some suggestions to the problem concerning what kind of materials in plants participate in these factors. With observation in farms, these suggestions may become an approach to the real causes of both cold and drought resistance. The author, limiting the problem to the cold resistance of wheat and naked barley plants, experimented on these internal factors from 1955 to 1956 in the Laboratory of Crop Science, Faculty of Fisheries and Animal Husbandry, Hiroshima University.

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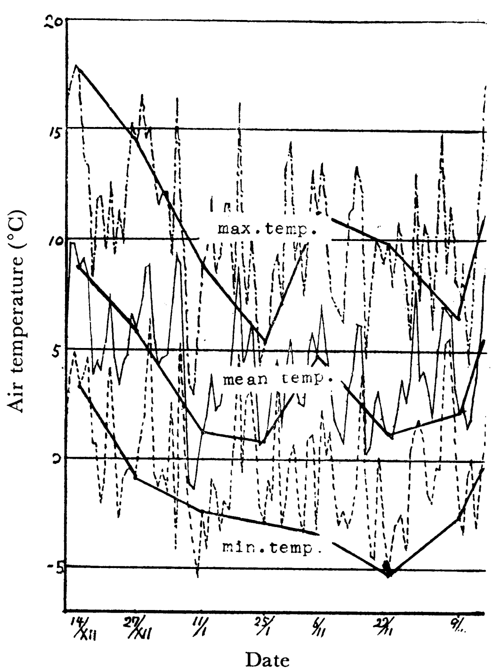
Agricultural College, Prof. M. IKEDA and Assistant A. MATSUI of the Faculty of Fisheries and Animal Husbandry, Hiroshima University, all of whom gave valuable advice and useful suggestions, and afforded many facilities to perform this study.

(II) MATERIALS AND METHODS

Plants used were a local variety of naked barley, named *Shirochinko* and that of wheat named *Shikoku no. 65*. They were sown in two lines a row, 3 *shaku* (approximately 0.9090 m.) apart from each other, at the rate of 4 *sho* (approximately 7.2156 lits.) per *tan* (approximately 991. 736 m^2.), in the attached farm of Hiroshima University on Dec. 16, 1955. Fertilizers were applied at the rate of $(NH_4)_2 SO_4$: 17 *kan* (approximately 63.75 kg.), KCl: 6 *kan* (approximately 22.50 kg.) and $Ca(H_2PO_4)_2$: 15 *kan* (approximately 56.25 kg.) per *tan*. Materials were carefully sampled at 10 a. m. each 10 days by means of thinning out of individuals planted. All leaves of plants used for tests were sampled from the early stage of growth to the jointing stage. Sampling was somewhat delayed when the rainfall occurred. Stamping treatments were performed only in wheat plots twice in winter, that is, the first, on January 11 and the second, on February 23. Fine cut samples were put into hard glass-medicine bottles, 500 c.c. by volume. These bottles were respectively placed in tin-cans for heating by boiling water for 2½ hours. After heating and subsequent cooling, the plant sap was squeezed out from materials under the pressure of 250 kg/cm^2, and centrifuged for ten minutes which the speed of 3000r/min. Upper cleared portion of the sap was used for tests of the following physico-chemical properties: (1) relative viscosity (by Ostwald's viscosimeter), (2) specific gravity (by Ostwald's pycnometer), (3) cell-sap concentration (by hand refractometer), (4) osmotic pressure (by Beckmann's apparatus for freezing points depression) and (5) relative electric conductivity (by Wheatston's bridge).



Text-fig. 1. Daily changes of atmospheric temperatures from Dec. 10 to March 9.

For the calculation of the osmotic pressure by the freezing points depression, Harris & Gortner's table was used.

The relative viscosity, specific gravity and relative electric conductivity were estimated with solutions kept at 25°C. Water contents were determined by drying the fresh samples placed in an oven kept at 105° C.

The author used the formula by TAGUCHI (1954) for the purpose of calculating the corrected indices which are useful for eliminating the effects of water contents upon those factors mentioned above.

$$n(T) = \frac{n' \times W(T)}{10}$$

$n(T)$ ······ the index of the respective factors measured, corrected by the water contents based on $W(T)$.

$W(T)$······the water contents based on dry matter.
n' ·········the respective factors measured.

Daily changes of air temperatures, including mean, maximum and minimum, were measured in order to find their effects upon plant growth and to interpret the relationship between those factors and the growth.

(III) RESULTS AND DISCUSSION

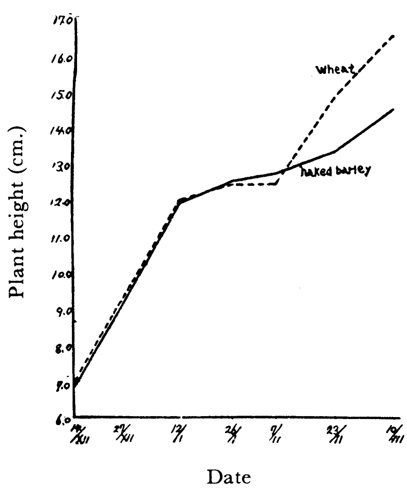
i) TOP GROWTH

Top growth of plants, especially plant height and number of tillers, during the course of the growth was shown in Table 1 and Text-fig. 2.

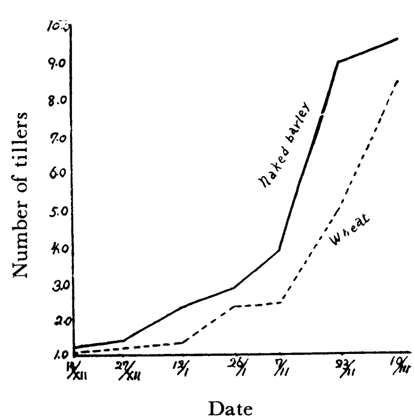
Table 1. Plant height and tillers of both naked barley and wheat plants.

Date	*Shirochinko*		*Shikoku, no. 65*	
	Plant height	Number of tillers	Plant height	Number of tillers
14/XII	6.8cm.	1.2	7.0cm.	1.1
27/XII	9.1	1.4	9.2	1.2
12/I	11.9	2.3	12.0	1.3
26/I	12.5	2.8	12.4	2.3
7/II	12.7	3.8	12.4	2.4
23/II	13.3	8.8	14.7	4.9
10/III	14.4	9.4	16.4	8.3

As shown in Table 1 and Text-figs. 2 and 3, the increase of plant height was very slow during the winter season in both naked barley and wheat plants, but with the rise of temperature in early March, they elongated remarkably. Number of tillers of both plants increased gradually as well as plant height in winter, but in early February they increased



Text-fig. 2. Plant height of naked barley and wheat plants.



Text-fig. 3. Number of tillers of naked barley and wheat plants.

strikingly.

But the naked barley plants were more flourishing in tillering than the wheat plants during the cold season. Even though some symptoms of cold injuries were shown at the tips of leaves in naked barley plants on January 11, when the air temperature dropped remarkably (min. temp.–7°C), while wheat plants were not damaged at all.

The observation made by YAMAZAKI (1931) was that hardy wheat varieties grew very slowly in contrast to less hardy ones during the cold period.

This fact which exhibits the difference of hardiness between the two varieties corresponds to that between wheat plants and less hardy naked barley plants.

ii) WATER CONTENTS

Water contents of plant tissues may be regarded as an index of their vitality. Therefore a large amount of water is usually found when plants grow very vigorously.

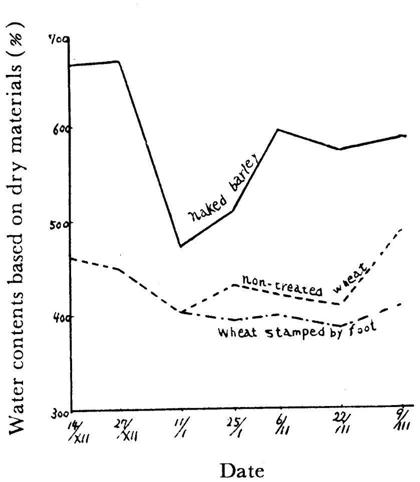
ALEKSEEV & GUSEV (1950) reported that the intensity of transpiration was directly related to the amount of free water ($r=0.76$). According to KUMAR (1952) daily changes of water contents of leaves run in parallel with the intensity of photoperiodic functions. DEMIDENKO & PORUTSKII (1953) said that the rest period in the plant growth generally corresponded to a period of a sharp decrease of free water contents in the root tissues, and of the lowered activity of root auxins. For all that it was different among species, varieties and environmental factors, the overwintering crops decline their vegetative growth remarkably during the period of cold weather, that is, it may be possible to consider that they are in a state of dormancy. Water contents of plants during this season decrease usually to a high degree. This fact is reported by many other investigators on miscellaneous plants, such as ISHIKAWA & SATO (1941) in medic, YAMAMOTO & OIZUMI (1951) in barley, TAGUCHI & MIYAZAKI (1953) in onion, and MIYAZAKI (1955) in chive.

Lower contents of water in plant tissues make plant more tolerant to cold. ANDO (1919) attributed the difference in the cold resistance among wheat varieties to the lesser amount of water contained. MARTIN (1927) reported that hardy wheat varieties contained less water than less hardy ones, and water contents of winter wheat decreased by hardening. SILKET *et al.* (1937) said that alfalfa varieties containing a large amount of water in the root tissues were less hardy. According to LAUDE (1937), there was a negative correlation between the cold resistance of winter wheat, rye and oats, and the water contents of these plants.

Such a relation was observed also in the leaves of several fruit trees by KOMA *et al.* (1955). Seasonal changes of water contents of both naked barley and wheat are shown in Table 2 and Text-fig. 4. The author did not use the water contents which were based upon the volume of tissue powder, because of the difficulty of obtaining uniform powder from fibrous tissues such as leaves of wheat. Naked barley plant (*Shirochinko*) contained a large amount of water at the early stage of the growth when the air temperature was still fairly high, but its water content dropped remarkably with the approach of the cold weather. Soon after the season the water contents recovered again and increased gradually up to the peak which was found on March 22 with the rise of temperature.

Table 2. The percentage of water contents based on raw and dried materials, and the relative values calculated regarding the first measurement as 100 (1955).

Date	Water contents based on raw materials						Water contents based on dried materials					
	Shirochinko		*Shikoku, no. 65*		*Shikoku, no. 65* stamped		*Shirochinko*		*Shikoku, no. 65*		*Shikoku, no. 65* stamped	
	%	rel. val.	%	rel. val.	%	rel. val.	%	rel. val.	%	rel. val.	%	rel. val.
14/XII	86.9	100.0	82.2	100.0	——	——	666	100.0	462	100.0	——	——
27/XII	87.0	100.0	81.8	99.5	——	——	670	100.6	449	97.2	——	——
11/I	82.5	94.9	80.1	97.4	——	——	473	71.0	403	87.2	——	——
25/I	83.0	96.2	81.1	98.7	79.7	97.0	509	76.4	431	93.3	393	85.1
6/II	85.6	98.5	80.8	98.3	80.0	97.3	596	89.5	420	90.9	399	86.4
22/II	85.2	98.0	80.3	97.7	79.4	96.6	574	86.2	408	88.3	386	83.5
9/III	85.4	98.3	83.0	101.0	80.3	97.7	586	88.0	488	105.6	408	88.3



Text-fig. 4. The percentage of water contents based on dried materials of both naked barley and wheat plants.

Wheat contained, in general, a smaller amount of water than naked barley from the early stage of the growth to the early spring. Though the water contents of wheat decreased as in naked barley during cold season, the degree of the decrease in wheat was smaller than that in naked barley, as shown in Text-fig. 4.

Water contents of wheat were lowered by stamping as reported by OTANI (1950). As mentioned above, the higher amount of water of naked barley at the early stage of growth and during the period of cold weather and those which changed more widely than in wheat, might be caused by tissues with active functions in naked barley. In consequence, naked barley might be more susceptible to low temperature than wheat. It was supposed that the smaller water-retaining power of naked barley might display lower resistance at those times. On the contrary, wheat existed in the state of dormancy, in which its water contents were so small and tissues were not so active that the plant could endure the cold weather. Such a presumption might be drawn from the fact already mentioned by YAMAZAKI (1931) that less hardy wheat varieties showed more vigorous growth during the cold period as compared with hardy varieties.

iii) CONCENTRATION OF EXPRESSED JUICE

The concentration of the expressed juice meaured by means of the hand refractometer shows the total amount of soluble substances existing in the plant juice. According to many reports, the concentration of cell sap increases in general when plants are exposed to low temperature. The cell sap concentration of hardy plants and varieties is therefore higher than that of less hardy ones.

WALTER (1925, 1926) said that the higher the cell sap concentration was in cold winter, the larger the resisting power was against sudden changes of environmental factors.

MARTIN (1927) reported that hardy wheat varieties showed high refractive indices. According to CONSTANTINESCU (1933), the total amount of dry matter in the expressed juice of barley increased when exposed to low temperature.

DEXTER (1933) recognized a close relation between total amounts of nutrients assimilated in plant and the cold resistance. EBIKO & WATANABE (1934) observed that when cultivated at low temperature, the refractive index of the expressed juice in young wheat plants belonging to the spring-type was smaller than that of the winter-type, and that the refractive index increased with sharp drop of air temperature. TAGUCHI & MIYAZAKI (1953) reported that the cell sap concentration of the bulbs of onion remarkably increased in cold winter. They presumed that such a phenomenon meant changes of physiological conditions of the plant for resisting against low temperature. MIYAZAKI (1954) recognized the remarkably increased concentration of the expressed bulb juice of chive was caused not only by the decrease of water contents but also by accumulation of soluble substances in bulbs.

The results obtained by the author on both naked barley and wheat are shown in Table 3 and Text-fig. 5.

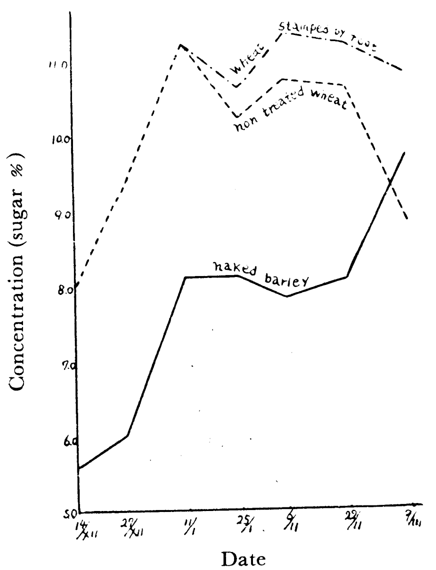
The concentration of the expressed juice in naked barley was low at the early stage of the growth, and with the sharp drop of air temperature in January it increased remarkably and kept high values during the cold season. This fact can be considered to show the difference of hardy characters between wheat and naked barley. The concentration of the expressed juice depends not only upon total amounts of soluble substances in it, but also on the water contents of the plant. Therefore, the presumption of the net amounts of soluble substances in the juice needs to eliminate the effects of water contents on the concentration of the juice. The author calculated the corrected indices using the formula proposed by TAGUCHI (1954).

Table 3. The concentration of the expressed juice (sugar %) and the corrected indices of both naked barley and wheat.

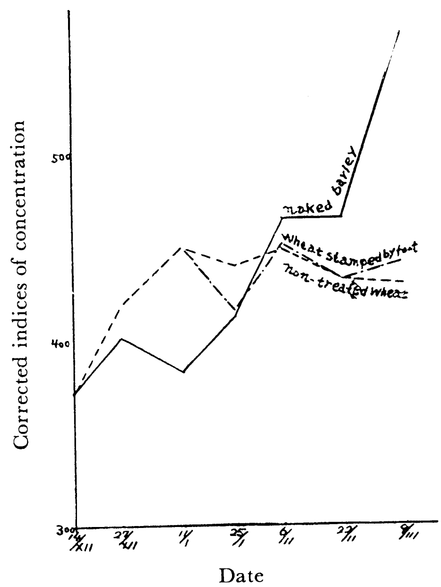
Date	Concentration of expressed juice measured (sugar %)						Corrected indices					
	Shirochinko		*Shikoku, no. 65*		*Shikoku, no. 55* stamped		*Shirochinko*		*Shikoku, no. 65*		*Shikoku, no. 65* stamped	
	conc.	rel. val.	conc.	rel. val.	conc.	rel. val.	conc.	rel. val.	conc.	rel. val.	conc.	rel. val.
14/XII	5.6	100.0	8.0	100.0	——	——	372	100.0	370	100.0	——	——
27/XII	6.0	107.1	9.3	116.3	——	——	402	108.1	418	129.7	——	——
11/I	8.1	144.6	11.2	140.0	——	——	383	103.0	450	121.6	——	——
25/I	8.1	144.6	10.2	127.5	10.0	132.5	412	110.8	440	118.9	417	112.7
6/II	7.8	139.3	10.7	133.8	11.3	141.3	465	125.0	449	121.4	451	121.9
22/II	8.1	144.6	10.6	132.5	11.2	140.0	465	125.0	432	116.8	432	116.8
9/III	9.7	173.2	8.8	110.0	10.8	135.0	568	152.7	429	115.9	441	119.2

The corrected indices are shown in Table 3 and Text-fig. 6.

The lower corrected indices in naked barley at the early stage of the growth explained that the total amount of soluble substances in the expressed juice did not increase at that period. It increased by degrees since early February and reached its peak from the latter part of that month to early March. In the case of wheat, the total amount of soluble substances was not so large as in naked barley at the early stage of the growth.



Text-fig. 5. The concentration of the expressed juice (sugar %) of both naked barley and wheat plants.



Text-fig. 6. The corrected indices of the concentration of both naked barley and wheat plants.

With the approach of cold season, it increased remarkably and kept large values till about early March. From the data of both the corrected indices and water contents, the marked increase of concentration of the expressed juice in naked barley was fairly affected by the decrease of water contents in early winter, but it was associated with both the decrease of water contents and the increase of the total soluble substances in wheat.

For all that naked barley showed larger amount of total soluble substances (corrected indices) than wheat on February 6 and 22, inverse relationship held for actual concentration measured in both plants at those times. This might be due to lower content of water in wheat, judging from the data of water contents.

Changes of the concentration of the expressed juice surveyed in the both plants during cold season, suggest a close relationship between the concentration of cell sap and the cold resistance, as considered in relation to their hardiness under field conditions.

The concentration in wheat which was stamped by foot was usually larger than that of the non-treated.

The total soluble substances in the juice scarcely increased by means of stamping during cold weather season as shown in Text-fig. 4. Therefore the larger concentration of the expressed juice in stamped wheat was presumed to be chiefly caused by the marked decrease of water contents, referring to the data of both the corrected indices and the water contents.

iv) OSMOTIC PRESSURE

Many investigations on the osmotic pressure were also conducted with regard to both cold and drought resistance. GAIL (1926) and JANSEN (1929) reported that with the approach of cold weather, the increased osmotic pressure of plant-cell sap, caused by the transformation from starch to sugars, made plants more hardy.

ONODERA & TAKASAKI (1930) said that the osmotic pressure of winter crops increased from autumn to winter in general, but causes of the cold resistance could not be explained merely by the osmotic pressure. According to CONSTANTINESCU (1933), the osmotic pressure of barley increased when exposed to low temperature. KAKIZAKI (1936) reported that the rise of the osmotic pressure of the leaf sap in wheat was found with the approach of snowing season. According to SHIMURA (1940), higher osmotic concentration of tea leaves went hand in hand with the decrease of water contents, and the osmotic pressure of hardy varieties was superior to that of less hardy ones in severe cold winter, but such a relationship went out of order under warmer temperature condition. ISHIKAWA & SATO (1941) reported that the osmotic pressure of both medicago and oil rape increased as the season proceeded, and there was a negative correlation between the water contents and the osmotic pressure. YAMADA (1948) observed that wintering wheat plants increased the cell osmotic values. KOZIMA & MURAKAMI (1950) said that the tetraploid plant of *pak-choi* (*Brassica sinensis* L.) was apparently hardier than the diploid one, partly because of the higher osmotic pressure of the former plants. According to MIYAZAKI (1954), the increased osmotic pressure of bulbs of chive made in general an adverse change in contrast to water contents during winter season. This increase of the osmotic pressure depended not only on the decrease of water contents, but also on osmotically active substances accumulated. On the other hand, in relation to drought resistance, GREATHOUSE (1932) observed that soy-bean plants grown on the soil with adequate moisture displayed striking xerophytic characters by the repetition of wilting and recovery with high osmotic pressure in consequence.

ONODERA (1940) reported that both upland and lowland rice plants increased their osmotic concentration with the decrease of the moisture contents in soil, but hardy varieties did not always show the higher osmotic pressure. ILJIN (1953) found a close correlation between the osmotic concentration of cell sap and the drought resistance. AVETISYAN (1954) said that the resistance against wilting was closely related to high concentration of the osmotically active substances, and a large amount of osmotically active substances in stem and leaves made plants comparatively stable for wilting.

According to the reports cited above, it is clear that the osmotic concentration of cell sap is closely related to both cold and drought resistance of plants, and hardier plants against cold and drought injuries have in general higher osmotic pressure than less hardy ones. Moreover, the osmotic pressure increase either when the season proceeds from autumn to winter, or when the hardening is given to plants by the repetition of wilting and its recovery. The actual values of osmotic pressure measured in both naked barley and wheat are shown in Table 4 and Text-fig. 7.

From these it was found that osmotic pressure of these plants increased remarkably with the marked drop of air temperature (in early January).

After that period, even when low temperature came frequently, it scarcely increased and tended to fall till the end of the experiment.

The osmotic pressure in wheat was always greater than in naked barley during the whole course of the growth. Especially it was conspicuous during cold weather season.

The osmotic pressure in wheat stamped by foot was much higher than any others including the non-treated plant. This fact was quite similar to the results obtained by OTANI (1950).

The osmotic pressure of plant-cell sap is affected not only by the total amount of osmotically active substances, i. e. osmotic concentration, but also by its water contents. For no other reasons but this, high values estimated do not suggest high amount of osmotically active substances.

Effects of water contained must be now eliminated, so that the real osmotic concentration will be found.

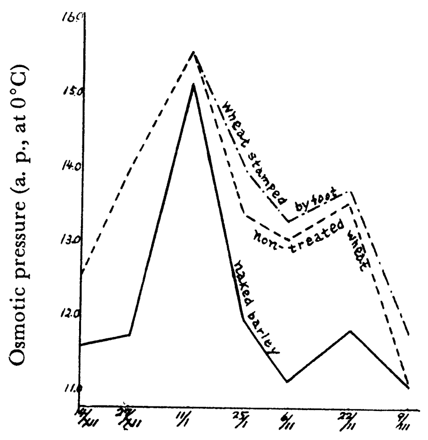
The corrected indices were also used here for the elimination of the influence of water contents.

Table 4. The osmotic pressure measured and the corrected indices on both naked barley and wheat.

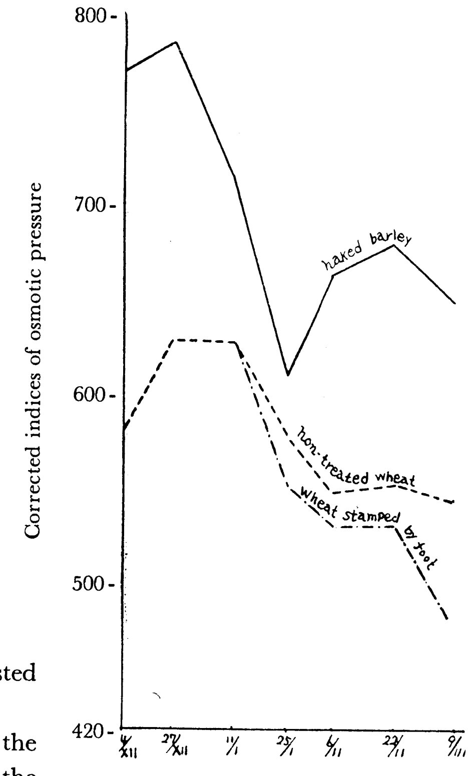
Date	Osmotic pressure measured (a. p. at 0°C)						Corrected indices					
	Shirochinko		*Shikoku, no. 65*		*Shikoku, no. 65* stamped		*Shirochinko*		*Shikoku, no. 65*		*Shikoku, no. 65* stamped	
	val. meas.	rel. val.	val. meas.	rel. val.	val. meas.	rel. val.	cor. ind.	rel. val.	cor. ind.	rel. val.	cor. ind.	rel. val.
14/XII	11.56	100.0	12.52	100.0	——	——	769	100.0	579	100.0	——	——
27/XII	11.68	101.0	13.96	111.5	——	——	784	102.0	626	108.1	——	——
11/I	15.04	130.1	15.52	124.0	——	——	711	92.5	625	107.9	——	——
25/I	11.92	103.1	13.36	106.7	13.96	111.5	607	78.9	576	99.5	549	94.8
6/II	11.08	95.8	13.00	103.8	13.24	105.8	660	88.8	546	94.3	528	91.2
22/II	11.80	102.1	13.48	107.7	13.66	109.1	677	88.0	550	95.0	528	91.2
9/III	11.02	95.3	11.08	88.5	11.74	93.8	646	84.0	541	93.4	479	82.7

Such indices are shown in Table 4 and Text-fig. 8. In both naked barley and wheat the largest amount of osmotically active substances was suggested to exist at the latter part of December. The former plant dropped its values succeedingly in general. But the latter kept the maximum value for a short period, namely from the end of December to the early January, and then gradually decreased. As shown in Text-fig. 8, both of the plants ran in a similar way, but naked barley plants were always superior to wheat during the whole course of the growth. Thus higher amount of osmotically active substances presumed and lower osmotic pressure found in naked barley, might be due to its higher water contents in contrast to wheat plants. In the case of naked barley the suggested amount of substances decreased markedly on January 25, but the reason why such a drop happened was never clarified as far as this experiment went.

The stamped wheat showed always higher osmotic pressure than the non-treated, but the supposed amount of osmotically active substances was smaller in the stamped wheat than non-treated one.



Text-fig. 7. The osmotic pressure of both naked barley and wheat plants.



Text-fig. 8. The corrected indices of the osmotic pressure of both naked barley and wheat plants.

Osmotically active substances in cells consisted chiefly of electrolytes and sugars.

GORTNER & LAURENCE (1921) proposed the ratio of the relative electric conductivity (λ) to the freezing point depression (Δ). This ratio (λ/Δ) means the comparison of the participation degree between electrolytes and organic substances such as sugars in the osmotic concentration.

Values (λ/Δ) obtained in this investigation are shown in Table 5 and Text-fig. 9. The values of both plants decreased remarkably from the early stage of the growth to the severe cold period, and then increased markedly in early March.

Wheat plants were always inferior to naked barley from the beginning of the experiment to the period when the values reached to the maximum.

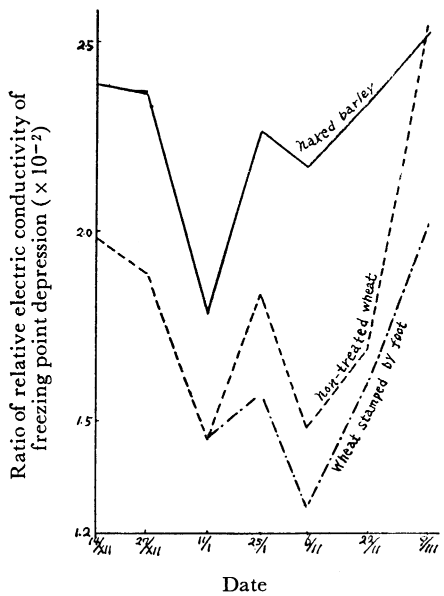
At the early stage of growth, when the temperature was fairly high, the participating degree of electrolytes in the osmotic pressure might be relatively high: organic substances as sugars, however, might influence much more during cold season. The regulation power of the former became larger again with the rise of air temperature.

The values (λ/Δ) in naked barley was superior to those in wheat represented higher participating power of electrolytes in the osmotic pressure, in the case of naked barley plants.

The value of wheat which was stamped by foot was always smaller than that of the non-treated. Namely in wheat, the participating degree of electrolytes in the osmotic pressure

Table 5. The ratio of the relative electric conductivity to the freezing point depression (λ/Δ).

Date	*Shirochinko*		*Shikoku, no. 65*		*Shikoku, no. 65* stamped	
	val. meas.	rel. val.	val. meas.	rel. val.	val. meas.	rel. val.
	$\times 10^{-2}$		$\times 10^{-2}$		$\times 10^{-2}$	
14/XII	2.39	100.0	1.98	100.0	—	—
27/XII	2.36	99.0	1.89	95.7	—	—
10/I	1.78	74.4	1.45	73.1	—	—
25/I	2.26	94.7	1.83	92.3	1.57	79.5
6/II	2.67	91.0	1.48	74.6	1.27	64.2
22/II	2.34	98.0	1.69	85.5	1.60	80.7
9/III	2.53	106.1	2.58	130.2	2.02	102.2



Text-fig. 9. The ratio of relative electric conductivity to the freezing points depression ($\times 10^{-2}$) of both naked barley and wheat plants.

decreased by the operation.

Having considered the facts above mentioned, the following conclusions have been drawn:

(a). The osmotic pressure in both plants increased markedly during cold winter and then decreased with the rise of temperature as the growth proceeded.

(b). Such an increase of the osmotic pressure may exert some influence on the resistance against the withdrawal of water from cells. Hardy wheat plants with high osmotic concentration showed much more tolerance than less hardy naked barley with low values, under severe field conditions.

As already described, the high concentration found in wheat was associated with high amounts of osmotically active substances.

v) RELATIVE VISCOSITY

The increase of viscosity of plant juice due to some reasons lowers down the physiological functions of plants such as transpiration, carbon assimilation and so on. The viscosity is affected by the amount of both bound water and hydrophilic colloids in plant cells. Plants exposed to low temperature and drought are said to show higher viscosity in the plant juice.

Therefore it is very important to investigate the viscosity for analysing the internal factors of the resisting power of plants against low temperature and drought.

According to SHREVE (1916, 1923, 1924) and SPOEHR (1919), the cactus plant containing a large amount of hydrophilic colloids than ordinary plants showed a high viscosity. Low transpiration of the cactus grown in the deserts was closely related to the high viscosity of the plant juice.

ALEKSEEV & GUSEV (1950) reported that the intensity of transpiration was correlated positively with the amount of free water, and negatively with the colloidally bound water ($r = -0.78$) and the hydration capacity of the protoplasm colloids ($r = -0.75$). They pointed out from these reasons that the process of transpiration should be studied from the colloid-chemical point of view. DEMIDENKO & PORUTSKII (1953) recognized a sharp decrease of free water contents in the root tissues in the rest period of the plant growth, and moreover a lowered activity of auxins in plants.

As mentioned in the item of the relative electric conductivity, the decrease of velocity of ion movement in solutions is caused by the increase of their viscosity. Such a phenomenon in cell lowers the vitality of cells or tissues, that is, their physiological functions.

MAXIMOV (1929) reported that a greater endurance to drought was attained by an increase of hydrophilic colloid amount. TUMANOV (1927) observed that the plant tissues recovered from drought injury contained in cells more hydrophilic colloid than those which were not damaged. Having investigated the cold and drought resistance on soy-bean, millet and cabbage plants, GREATHOUSE (1932) reported that leaves abundant in hydrophilic colloids and large resisting power against the withdrawal of water and these plants were protected by higher contents of hydrophilic colloids against excessive desiccation caused by heavy transpiration and freezing. DUNN (1933) recognized a close positive correlation between cold resistance of several plants and amounts of dye absorbed, using dye-absorption method. CHANDLER (1941) found that there was a close correlation between frost hardiness and amounts of bound water in wheat and alfalfa.

According to STOCKER (1954), the viscosity of the protoplasm was higher in drought resistant plants. MIKALOVSKII & BORJAKOVSKAYA (1955) reported that hardy clover varieties were characterized by high contents of hydrophilic colloids. MIYAZAKI (1955) reported that hardy fruit trees containing large amounts of water had large amounts of hydrophilic colloids.

TSUTSUMI *et al.* (1956) considered that the increase of cold resistance of tea plants was partly caused by the increase of hydrophilic colloids suggested by the dye absorption test. Because such a basic dye was absorbed only by negative colloids in solution, the existence of such colloids might be effective in increasing the cold resistance.

Changes of the relative viscosity observed in this investigation on both naked barley and wheat are shown in Table 6 and Text-fig. 10.

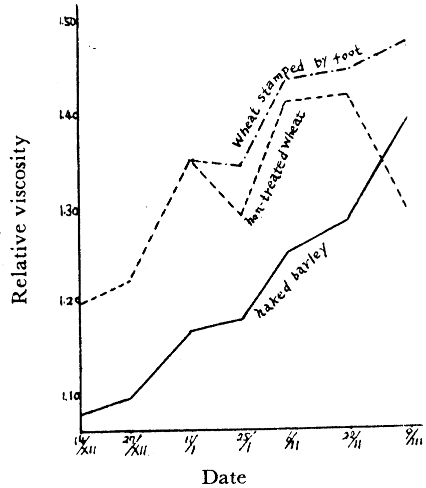
The relative viscosity of the expressed juice in naked barley was the lowest at the early stage of the growth, but after that it increased straight up to early March. In the case of wheat it was also the lowest at the early stage of the growth, and showed a similar tendency as in naked barley and reached the maximum value in late February.

The author supposed that such an increase of relative viscosity in both plants may be connected with the increase of resisting power against low temperature during the cold season.

The higher relative viscosity of wheat as compared with naked barley during the cold weather season, might imply that the former would have larger percentage of bound water in total water than the latter. Such a presumption is reasonable by the fact recognized by OKUNTSOV & TARASOVA (1952) that wheat plants showed the greatest hygroscopic power among the plants experimented, such as wheat, barley and begonia.

Table 6. The relative viscosity and the corrected indices of both naked barley and wheat.

Date	Relative viscosity measured (at 25°C)						Corrected indices					
	Shirochinko		*Shikoku, no. 65*		*Shikoku, no. 65* stamped		*Shirochinko*		*Shikoku, no. 65*		*Shikoku, no. 65* stamped	
	rel. vis.	rel. val.	rel. vis.	rel. val.	rel. vis.	rel. val.	cor. ind.	rel. val.	cor. ind.	rel. val.	cor. ind.	rel. val.
14/XII	1.079	100.0	1.197	100.0	—	—	719	100.0	553	100.0	—	—
27/XII	1.096	101.6	1.220	101.9	—	—	734	102.1	548	99.1	—	—
11/I	1.165	108.0	1.348	112.6	—	—	550	76.5	543	98.2	—	—
25/I	1.178	109.2	1.288	107.6	1.343	112.2	600	83.4	555	100.4	528	95.5
6/II	1.247	115.6	1.408	117.6	1.433	119.7	743	103.3	591	106.9	572	103.4
22/II	1.283	118.9	1.417	118.4	1.443	120.5	736	102.4	578	104.5	557	100.7
9/III	1.391	128.9	1.295	108.2	1.465	123.2	815	113.4	632	114.3	601	108.7



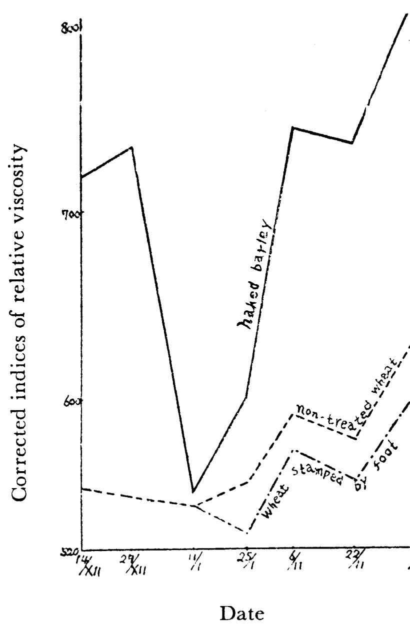
Text-fig. 10. The relative viscosity of both naked barley and wheat plants.

Since the relative viscosity depends not only on amounts of hydrophilic colloids and viscous substances, but also on water contents in cell, it is impossible to presume amounts of hydrophilic colloids and viscous substances contained in the juice merely by the values of relative viscosity measured.

In order to eliminate the influence of water contents upon the relative viscosity, the corrected indices of the viscosity were used as usual and shown in Table 6 and Text-fig. 11.

Corrected indices of the viscosity in naked barley were fairly high at the early stage of the growth and then showed a marked drop running parallel to the remarkable decline of air temperature in early winter.

Those of wheat were markedly low from the early stage of the growth up to late January, and after that period they increased slightly up



Text-fig. 11. The corrected indices of relative viscosity of both naked barley and wheat plants.

to late February being followed by the remarkable increase in early March. It seemed to be chiefly caused by the remarkably lower contents of water in wheat which showed the markedly higher relative viscosity as compared with naked barley (refer to Table 2) notwithstanding the small amounts of active substances of the former.

The relative viscosity in wheat stamped by foot was usually larger than that in the non-treated. An operation like stamping might make the plant more resistant against low temperature. Such an increase of relative viscosity in wheat is not presumed to be dependent on the increase of active substances associated (i. e. hydrophilic colloids and viscous substances), but on the decrease of water contents (refer to Table 2).

vi) RELATIVE ELECTRIC CONDUCTIVITY

DEXTER (1932) measured the relative electric conductivity of the solutions containing electrolytes exuded from frozen plant tissues. In the plants cultivated at high temperature, the electric conductivity of their tissues increased when frozen rapidly. Moreover, the plants hardened at fairly low temperature before frozen, showed the large resisting power against freezing, and the electric conductivity of the frozen tissues was small.

Thereforet he conductivity of the frozen tissues was affected either by the plant growth or by the season in which plants grew. The conductivity in roots of young alfalfa was low, when they were in dormancy, but in the plants growing vigorously it was high. According to CONSTANTINESCU (1933), the conductivity of the exuded solutions was related to the cold resistance of naked barley.

DEXTER (1934) reported that the total amounts of soluble mineral substances in the expressed plant sap of wheat presumed by measuring of the conductivity, were decreased in the plants hardened with the progress of the season. By this reason a negative correlation was found between the hardiness of wheat and the amounts of soluble salts in the expressed sap. He said, therefore, that the hardiness of wheat was concerned with the low concentration of soluble salts in the cell sap. According to TAGUCHI & MIYAZAKI (1953), notwithstanding that the relative electric conductivity went hand in hand with the refractive index of plants growing vigorously, the decrease of the former was not so remarkable as compared with the marked increase of the latter, in the case of the weak plant growth in winter. From these facts, they supposed that an increase of ions happened in the cell sap during the winter season. MIYAZAKI (1954) also observed that the increase of the conductivity was caused by increased amounts of ions in the cell sap in winter. In addition to these, many researchers, such as DEXTER (1933), VAN DOREN (1937), SILKETT *et al.* (1937), BULA (1954), KOMA *et al.* (1955), reported the relationship between plant hardiness and the relative electric conductivity of the exuded solutions of plant tissues.

The relative electric conductivity of the expressed sap corresponds to the concentration of ions produced by the dissociation of electrolytes containing in it. According to YAMASHITA (1948), the conductivity of the cell sap depends not only upon the amounts of electrolytes in the sap, but also on the state of the combination and dissociation. Thus the increase of dissociated ions in cell must render plants active in physiological and chemical functions. However, if the relative electric conductivity increases beyond limit, plants catch disease.

The relative electric conductivity measured in this investigation is shown in Table 7 and Text-fig. 12.

In both plants, the conductivity was similarly decreased from the early stage of the growth to the cold season, but in wheat it was remarkable in contrast to naked barley. As described above, the relative electric conductivity depends not only on the amount of ions, but also on water contents and moreover on the relative viscosity affecting the velocity of ion-movement. Presumption of total amounts of ions from the conductivity needs to eliminate the effects of both water contents and the relative viscosity.

Hence the author calculated the corrected indices as usual for the elimination of those effects.

These corrected indices of conductivity are shown in Table 7 and Text-fig. 13.

In naked barley, the amount of ions in the sap decreased remarkably with the marked drop of the temperature in early winter, and increased straight to early March. In wheat, ion amounts changed in parallel with the conductivity measured, and the decrease of the amount was small as compared with that of naked barley in winter. Moreover, as shown in Text-fig. 13, ion amounts in naked barley were always greater than wheat from the beginning to the end of the experiment.

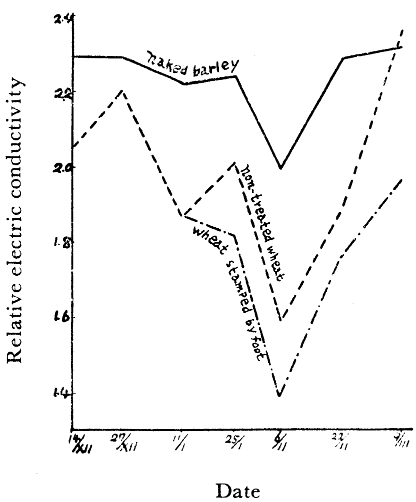
In wheat stamped by foot, both the conductivity and the amount of ions were usually smaller than those of the non-treated. Therefore it is presumed that the amount of ions in wheat cell sap decreased by the operation.

Having referred to YAMASHITA's opinion (1948), the author presumed that the high conductivity in naked barley during the cold winter meant its active physiological functions at those times, and the low conductivity in wheat was due to its low activities. Such a presumption coincides with the fact of the superior hardiness of wheat to naked barley under field conditions.

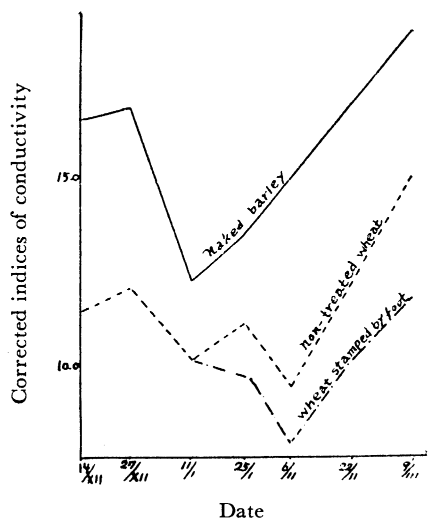
In addition to the fact above mentioned, the stamping method in growing wheat was presumed to be very effective on the hardening of the plant during the cold winter by decreasing the conductivity.

Table 7. The relative electric conductivity and the indices corrected by the corrected values of viscosity of both naked barley and wheat.

Date	Relative electric conductivity measured (mho, at 25°C)						Corrected indices					
	Shirochinko		*Shikoku, no. 65*		*Shikoku, no. 65* stamped		*Shirochinko*		*Shikoku, no. 65*		*Shikoku, no. 65* stamped	
	val. meas.	rel. val.	val. meas.	rel. val.	val. meas.	rel. val.	cor. ind.	rel. val.	cor. ind.	rel. val.	cor. ind.	rel. val.
	$\times 10^{-2}$		$\times 10^{-2}$		$\times 10^{-2}$							
14/XII	2.29	100.0	2.06	100.0	—	—	16.5	100.0	11.4	100.0	—	—
27/XII	2.29	100.0	2.20	106.8	—	—	16.8	102.1	12.0	105.8	—	—
11/I	2.22	96.8	1.87	90.7	—	—	12.2	74.1	10.1	89.1	—	—
25/I	2.24	97.7	2.01	97.6	1.82	88.7	13.4	81.5	11.1	98.0	9.6	84.7
6/II	2.00	87.2	1.59	77.5	1.39	67.9	14.9	90.1	9.4	82.8	7.9	69.0
22/II	2.29	100.0	1.89	92.1	1.81	88.0	16.9	102.4	12.1	106.3	10.1	88.6
9/III	2.32	101.1	2.37	115.2	1.97	95.8	18.9	114.6	15.0	131.6	11.8	104.1



Text-fig. 12. The relative electric conductivity of both naked barley and wheat plants.



Text-fig. 13. The corrected indices of the conductivity of both naked barley and wheat plants.

(IV) SUMMARY

The author studied the physico-chemical properties of the expressed cell sap in both naked barley (*Shirochinko*) and wheat plants (*Shikoku, no. 65*) during the cold winter, in relation to winter hardiness of these plants.

The main results obtained in this investigation were as follows:

(1) Naked barley plant showed the symptoms of cold injuries at the marked drop of air temperature in early winter, but wheat plant did not at all.

(2) Tillering of less hardy naked barley plant was more vigorous as compared with wheat plant.

(3) The changes of water contents based on dry materials of these two kinds of plants were generally similar, but naked barley plant showed larger amounts of water than wheat plant. Water contents were more changeable with change of air temperature in the case of naked barley plant.

(4) The concentration of the expressed cell sap in both plants increased remarkably, and the concentration in wheat plant was markedly larger as compared with that in naked barley plant. The total amount of soluble substances in cell sap presumed by the corrected indices of the concentration was large in wheat plant and kept nearly constant values, but in naked barley plant an increase of those substances did not happen.

(5) The osmotic pressure of these two plants increased remarkably with the marked drop of air temperature in early winter, but scarcely increased at low temperature which occurred afterwards. Wheat plant showed usually higher osmotic pressure as compared with naked barley plant. The total amount of osmotically active substances suggested from the corrected indices of the osmotic pressure declined with the progress of season in these plants, but in wheat plant it increased somewhat in early winter. Naked

barley plant showed usually the larger amounts of osmotically active substances as compared with wheat plant.

(6) Participating degree of electrolytes in osmotic pressure in contrast to organic substances was small in these plants during the winter season. However, the degree in naked barley plant was usually larger as compared with that in wheat plant.

(7) The relative viscosity increased linearly, in general, with the progress of season in both plants, but in wheat plants it was markedly larger as compared with that in naked barley plant. On the other hand, the total amounts of hydrophilic colloids and viscous substances presumed by the corrected indices of the viscosity were markedly large in naked barley plant, but they declined remarkably in early winter as compared with that in wheat plant.

(8) The relative electric conductivity in naked barley was larger as compared with that in wheat plant and the former plant kept almost constant values. But in wheat plant it decreased with the drop of air temperature. The total amounts of ions decreased remarkably in these plants with the drop of air temperature in early winter. In naked barley plant, the ion amounts increased linearly after the drop of temperature in early winter, but wheat plant kept the low amounts of ions till the dead of winter.

(9) Having summed up the results mentioned above, the author presumed that wheat plant was more tolerant against cold injuries than naked barley plant, by means of its smaller contents of water, higher values of the concentration (caued by both its smaller contents of water and the increase of total amount of soluble substances in cell sap), higher osmotic pressure (caused by both its smaller contents of water and the increase of total amount of osmotically active substances in cell sap), higher relative viscosity (chiefly caused by its small contents of water) and lower relative electric conductivity (chiefly caused by its small ion amounts) of the expressed cell sap.

(10) Wheat plant stamped by foot was characterised by its smaller contents of water, higher values of the concentration, osmotic pressure and relative viscosity and lower relative electric conductivity of the expressed cell sap as compared with non-treated one. But such an increase of its concentration, osmotic pressure and relative viscosity was not caused by the increase of respective substances participating in these properties, but chiefly by the decrease of water contents in the plant tissues caused by the treatments of stamping.

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