Method for Evaluation of Chilling Requirement and Narrow-Sense Earliness of Wheat Cultivars

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Chilling requirement, i.e. the minimum duration of the chilling treatment necessary for full vernalization, of a wheat cultivar should be evaluated by using a measurement which involves not only the growth after the treatment but also the growth during the treatment. Based on this concept, two assumptions were proposed in this study for the growth during the chilling treatment. Subsequently an index 'Dof' and a plant-development model using the Dof (Fig.2) were developed on the basis of the assumptions. Through a series of experiments involving 15 wheat cultivars, the accuracy of the assumptions was verified, and the development model was found to be quite adequate for the evaluation of the chilling requirement and narrow-sense earliness.

The fifteen wheat cultivars examined in the present experiments were classified into two groups, i.e. spring and winter wheat cultivars by the pattern of flag-leaf unfolding in the absence of chilling treatment. This classification revealed a distinct difference in the chilling requirements between the two groups, and enabled to conclude that the wheat cultivars which required maximum of 30 days of chilling and those which required 40 or more days can be designated as spring wheat cultivars and winter wheat cultivars, respectively.

Among the spring wheat cultivars, three that had the \( Vrn \) 1 gene and two having the \( Vrn \) 3 gene required a chilling treatment of 0 and 30 days for full vernalization, respectively. This finding indicates that \( Vrn \) 1 makes wheat completely insensitive to the chilling treatment and that the chilling requirement controlled by \( Vrn \) 3 disappears by 30 days of chilling treatment.

The experimental results showed that the chilling requirement precisely reflects the demand of a wheat cultivar for low temperature for reproductive growth. Therefore, it is suggested that the chilling requirement is the supremely important trait for understanding the nature and genetics of vernalization.

KEY WORDS: Triticum aestivum, wheat cultivar, vernalization, development model, growth habit, chilling requirement, narrow-sense earliness.

Introduction

Early heading wheat cultivars can not easily become adapted to the areas with late frost, while late heading ones which are harvested in the rainy season in the wet monsoonal zone often experience wet-injury which impairs both grain yield and quality. Thus heading time is one of the most important characters controlling the adaptability of wheat cultivars, and no breeding program is valid unless enough attention is paid to this character.

However, breeding for heading time and for the traits linked with this character is difficult, because heading time of wheat is a complex character, which is determined by three internal factors, i.e. photoperiodic response, narrow-sense earliness and growth habit (Yasuda and Shimoyama 1965). Therefore, it is essential for effective breeding that the above internal factors are exactly evaluated in breeding materials especially in

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the plants that will be used as cross parents, as shown by Yoshida et al. (1983).

Of these three internal factors, the photoperiodic response refers to the retardation or acceleration of heading in response to day-length. It can be easily evaluated by the difference in the number of days from the fully vernalized stage to heading time between short-day and long-day conditions (Takahashi and Yasuda 1958). Narrow-sense earliness, which refers to the earliness of fully vernalized plants under optimum conditions for reproductive growth, can be expressed by the number of days from the fully vernalized stage to heading time under as high a temperature as to ensure optimum reproductive growth and a 24 h day-length regime (Takahashi and Yasuda 1958).

The third factor, i.e. growth habit, originally refers to the low temperature requirement necessary for the differentiation of the reproductive organs. According to Takahashi and Yasuda (1958), this factor can be evaluated without the chilling treatment, i.e. only by the duration from sowing to flag-leaf unfolding under a high temperature and 24 h day-length regime. However, the growth habit evaluated by this method involves not only the low temperature requirement but also the effect of narrow-sense earliness. In view of this fact, Gotoh (1976) suggested that the growth habit should be represented by the "vernalization requirement", i.e. by the minimum duration of exposure to the low temperature required for full vernalization, and he developed a method to evaluate the minimum duration. This concept is relevant, because the vernalization requirement is independent of the photoperiodic response and narrow-sense earliness. In his method, however, the minimum duration refers to the duration of the chilling treatment which shortens the period from the end of the treatment to the unfolding of the flag-leaf up to 34 days irrespective of the kind of variety examined. Therefore, the minimum duration estimated by this method is inevitably associated with some distortion resulting from the narrow-sense earliness of the variety tested.

Therefore, a method for the accurate evaluation of the low temperature requirement necessary for full vernalization was developed and evaluated in a series of experiments involving various chilling treatments in 15 wheat cultivars. In the present paper, the minimum duration of the low temperature required for full vernalization is referred to as "chilling requirement" to distinguish it from the "vernalization requirement" proposed by Gotoh (1976), which involves the effect of narrow-sense earliness.

**Basic concept for the evaluation of the chilling requirement**

As stated before, Gotoh (1976) attempted to evaluate the chilling requirement of wheat by the minimum duration of the chilling treatment (referred to as only "treatment" hereafter) necessary for full vernalization. However, he failed to evaluate accurately the minimum duration, i.e. the chilling requirement. This is because the number of days from the end of the treatment to flag-leaf unfolding (Dtff) was adopted as the index for determining the minimum duration of the treatment, as Dtff continuously decreases with the duration of the treatment and does not reach a plateau, as shown in Fig. 2 a). He assumed that the plant whose Dtff does not exceed a certain level has already been fully vernalized, and, based on some experimental results, he fixed the duration of the treatment which reduces Dtff to 34 days as the criterion for estimating the chilling
requirement. However, wheat plant grows also during the treatment. Therefore, in this criterion, which was selected irrespective of the duration of the treatment, the varietal difference in narrow-sense earliness is not considered. For instance, even if the chilling requirement of two cultivars is identical, in a cultivar with a larger narrow-sense earliness the estimation of the chilling requirement will be higher than in the other.

Therefore, for the accurate evaluation of the chilling requirement, the growth during the treatment as well as the growth after the treatment should be considered, and an index independent of narrow-sense earliness should be adopted. Therefore we attempted to evaluate the growth increment during the treatment, on the basis of the following two assumptions:

(1) The growth increment at the 1st leaf unfolding stage is constant irrespective of the duration of the treatment.

(2) The growth increment up to the end of the treatment is proportional to the duration of the treatment as shown in Fig. 1.

Based on these assumptions, the number of days from the end of the treatment to the 1st leaf unfolding (D_{t1}) decreases in proportion to the duration (days) of the treatment (X). This relationship is formulated as follows:

\[ D_{t1} = -BX + a \quad (a > 0, \ B > 0) \]  \hspace{1cm} (1)

In this equation, “a” represents the D_{t1} when X = 0, namely, the number of days from forced sprouting (start of treatment) to the time of the 1st leaf unfolding (D_{ol}) without chilling treatment, and “B” represents the relative growth rate during the treatment, namely, the ratio of the growth rate during the treatment to the growth rate after the treatment (=1). Therefore, the growth increment during X days under the treatment corresponds to the growth increment during BX days after the treatment.

As seen from Equation (1), D_{t1} changes depending upon the duration of the treatment, but this change is compensated by the corresponding change of BX, and thus the value of D_{ol} remains constant (=a) irrespective of the duration of the treatment. This means that the heading acceleration effects of various treatment durations can be compared by determining the number of days from the 1st leaf unfolding to flag-leaf unfolding (D_{lf}) or the days from forced sprouting to flag-leaf unfolding (D_{of}). Both indices, D_{lf} and D_{of}, are formulated as follows:

\[ D_{lf} = D_{tf} - D_{t1} = D_{tf} + BX - a \]  \hspace{1cm} (2)

\[ D_{of} = D_{ol} + D_{lf} = D_{tf} + BX \]  \hspace{1cm} (3)

Here, attention should be paid to the fact that both D_{lf} and D_{of} can be represented by the equation which involves BX, i.e. the growth increment during the treatment.
In a prolonged treatment exceeding the minimum duration necessary for full vernalization, no further acceleration of heading occurs, and hence, as shown in Fig.2, both Dlf and Dof exhibit the same respective values as those observed in the treatment with the minimum duration necessary for full vernalization. Thus, the minimum duration of the treatment for full vernalization can clearly be determined by the duration of the treatment which reduces Dlf or Dof to the constant value.

The values of Dol, Dlf and consequently Dof are all intrinsic to a cultivar, although the latter two depend on the degree of vernalization of the plants and the conditions under which the plants grow. Based on the concept of narrow-sense earliness (Takahashi and Yasuda 1958), the Dof of the plants which were fully vernalized and grown under both high temperature and 24 h day-length, represents also the narrow-sense earliness of the cultivars to which the plants belong.

Thus Dof may be the most suitable index for evaluating both the chilling requirement and the narrow-sense earliness of a wheat cultivar.

The notations for the number of days to leaf unfolding used in this study are presented in Table 1.

<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Days to leaf unfolding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dol</td>
<td>Days from forced sprouting to 1st leaf unfolding$^{1)}$</td>
</tr>
<tr>
<td>Dof</td>
<td>Days from forced sprouting to flag leaf unfolding$^{1)}$</td>
</tr>
<tr>
<td>Dlf</td>
<td>Days from 1st leaf unfolding to flag leaf unfolding$^{1)}$</td>
</tr>
<tr>
<td>Dt1</td>
<td>Days from the end of chilling treatment to 1st leaf unfolding</td>
</tr>
<tr>
<td>Dtf</td>
<td>Days from the end of chilling treatment to flag leaf unfolding</td>
</tr>
</tbody>
</table>

$^{1)}$ The number of days under the chilling treatment was adjusted to the number of days expected under post-treatment conditions as described in the text.
Materials and Methods

Using a total of 15 wheat cultivars covering a wide range of growth habit (KAKIZAKI and SUZUKI 1937), seven experiments involving various durations of chilling treatment were successively conducted, as shown in Table 2. In each experiment, seeds of the materials were soaked in water for 24 hours at 20°C so as to enable them to sprout uniformly. They were sown in small soil-filled containers at a spacing of 2 cm (between hills) × 3.5 cm (between rows), then subjected to chilling treatments (5°C) for various durations under a 24 h day-length regime. The time of seed soaking was adjusted to the duration of the treatment so that all the treatments would be completed simultaneously. Soon after the end of the treatment, the containers were transferred into a phytotron under a 20°C and 24 h day-length regime. Then the number of days to flag-leaf unfolding (Dtf) was scored to estimate Dof (= Dol + Dlf - Dol + Dtf - Dtl). Finally, the response of Dof to the duration of the treatment was examined to determine the suitability of Dof as the index for evaluating the chilling requirement.

A container consisted of 11 rows with 12 plants each. In each treatment, 12 plants (one row) were allotted to a cultivar except in the 6th and 7th experiments, where 6 plants were assigned to a cultivar. There were no replications, because a preliminary test had already shown no significant differences between the replications.

When the duration of the treatment was shorter than about 40 days, the 1st leaf unfolded after the treatment, but otherwise the 1st leaf unfolded within the period of the treatment, resulting in a negative value for Dtf. In such a case, the absolute value

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Date of exp.</th>
<th>Cultivar used</th>
<th>Heading date</th>
<th>Whole range of treatment durations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1981. 3. 26.</td>
<td>ASS Akasarishirazu-1</td>
<td>41.5</td>
<td>0 ~ 85 (days)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D-1 Dawson-1</td>
<td>42.9</td>
<td>0 ~ 85</td>
</tr>
<tr>
<td>2</td>
<td>1981. 5. 26.</td>
<td>ES Eshimashinriki</td>
<td>19.8</td>
<td>0 ~ 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-61 Norin-61</td>
<td>18.1</td>
<td>0 ~ 40</td>
</tr>
<tr>
<td>3</td>
<td>1981. 8. 6.</td>
<td>N-8 Norin-8</td>
<td>41.4</td>
<td>0 ~ 70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SB Shirobozu</td>
<td>27.4</td>
<td>0 ~ 70</td>
</tr>
<tr>
<td>4</td>
<td>1981. 11. 19.</td>
<td>AB Akabozu</td>
<td>24.4</td>
<td>0 ~ 65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-29 Saitama-29</td>
<td>22.4</td>
<td>0 ~ 65</td>
</tr>
<tr>
<td>5</td>
<td>1982. 5. 8.</td>
<td>AD Akadaruma</td>
<td>22.4</td>
<td>0 ~ 70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD Shirodaruma</td>
<td>20.7</td>
<td>0 ~ 70</td>
</tr>
<tr>
<td>6</td>
<td>1982. 12. 28.</td>
<td>B-1 Bezostaja-1</td>
<td>30.5</td>
<td>0 ~ 95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F-1 Fultz-1</td>
<td>42.0</td>
<td>0 ~ 95</td>
</tr>
<tr>
<td>7</td>
<td>1982. 9. 23.</td>
<td>HH Haruhikari</td>
<td>30.0</td>
<td>0 ~ 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K-25 Konosu-25</td>
<td>12.7</td>
<td>0 ~ 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MQ Marquis</td>
<td>38.9</td>
<td>0 ~ 40</td>
</tr>
</tbody>
</table>

1) Date when the plants were transferred into the phytotron
2) Heading date of the plants sown in the field in the fall of 1982, represented as the number of days from March 31, 1983, to heading (1983.4.1 = 1)
3) Minimum and maximum duration of chilling treatment applied
of Dtl did not represent the number of days at 20°C (temperature after the treatment) but the number of days at 5°C (temperature during the treatment), which was much greater than that expected at 20°C, as shown in Fig. 3. Hence, for the estimation of the Dof suitable for the evaluation of the chilling requirement, the observed Dtl should be adjusted to the number of days expected at 20°C. The "adjusted Dtl" is represented in the following equation,

\[
\text{Adjusted Dtl} = -B \times \text{Dtl},
\]

as B represents the ratio of the growth rate at 5°C to the growth rate at 20°C, as defined in Equation (1). There, B can be calculated using the data from the treatments in which all the 1st leaves unfolded after the treatment. For the treatments in which the 1st leaves unfolded within the period of the treatment, adjusted Dtl was calculated according to Equation (4), then using the adjusted Dtl, Dof was evaluated based on Equations (2) and (3). Thus, hereafter in this paper, Dof as well as Dlf will be expressed by the
values modified by the adjusted Dtl.

Chilling requirement, i.e. the minimum duration (number of days) of the treatment necessary for full vernalization, was examined for each cultivar by successively testing the differences of the DoF between the treatments. The procedure used was as follows:

First, in all the treatments, the total N, was set in the ascending order of the length of duration, the longest being the (N)th treatment. Next, the difference of DoF between the (N)th and (N-1)th treatments was examined by t-test. When the difference was not significant (P > .01), the plants of the (N-1)th treatment were considered to be as fully vernalized as those of the (N)th treatment. Then, the same t-test was again applied to the difference between the (N-2)th treatment and the mixed population of the (N)th and (N-1)th treatments. When no significant difference was observed, the plants of the (N-2)th treatment were also considered to be as fully vernalized as those of the mixed population. In this way, the difference between the (M)th treatment and the mixed population composed of (M+1)th to (N)th treatments was successively examined until a significant difference (P < .01) appeared. When a significant difference was detected, it was concluded that full vernalization was induced by (M+1 and more)th treatments, and that the chilling requirement of the relevant cultivar could be represented by the duration of the (M+1)th treatment.

Results

In all the experiments and cultivars, Dtl decreased linearly with the increase of the duration of the treatment, as reflected in the extremely high correlation coefficients (−.961 to −.998) shown in Table 3. Figure 4 illustrates this linear relationship observed in

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Cultivar</th>
<th>No. of treatments</th>
<th>b</th>
<th>a¹</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ASS</td>
<td>14</td>
<td>−.174</td>
<td>8.09</td>
<td>−.997**</td>
</tr>
<tr>
<td></td>
<td>D-1</td>
<td>14</td>
<td>−.186</td>
<td>8.23</td>
<td>−.997**</td>
</tr>
<tr>
<td>2</td>
<td>ES</td>
<td>9</td>
<td>−.106</td>
<td>6.19</td>
<td>−.995**</td>
</tr>
<tr>
<td></td>
<td>N-61</td>
<td>9</td>
<td>−.101</td>
<td>6.76</td>
<td>−.989**</td>
</tr>
<tr>
<td>3</td>
<td>N-8</td>
<td>13</td>
<td>−.098</td>
<td>6.00</td>
<td>−.980**</td>
</tr>
<tr>
<td></td>
<td>SB</td>
<td>13</td>
<td>−.105</td>
<td>6.07</td>
<td>−.980**</td>
</tr>
<tr>
<td>4</td>
<td>AB</td>
<td>13</td>
<td>−.142</td>
<td>8.11</td>
<td>−.994**</td>
</tr>
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<td></td>
<td>S-29</td>
<td>13</td>
<td>−.142</td>
<td>7.61</td>
<td>−.992**</td>
</tr>
<tr>
<td>5</td>
<td>AD</td>
<td>13</td>
<td>−.137</td>
<td>6.56</td>
<td>−.993**</td>
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<tr>
<td></td>
<td>SD</td>
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<td>−.124</td>
<td>6.06</td>
<td>−.995**</td>
</tr>
<tr>
<td>6</td>
<td>B-1</td>
<td>14</td>
<td>−.155</td>
<td>7.04</td>
<td>−.998**</td>
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<tr>
<td></td>
<td>F-1</td>
<td>14</td>
<td>−.157</td>
<td>7.38</td>
<td>−.997**</td>
</tr>
<tr>
<td>7</td>
<td>HH</td>
<td>9</td>
<td>−.219</td>
<td>6.95</td>
<td>−.986**</td>
</tr>
<tr>
<td></td>
<td>K-25</td>
<td>9</td>
<td>−.215</td>
<td>6.91</td>
<td>−.981**</td>
</tr>
<tr>
<td></td>
<td>MQ</td>
<td>9</td>
<td>−.215</td>
<td>6.86</td>
<td>−.984**</td>
</tr>
</tbody>
</table>

**; Significant at 1% level.

¹) Intercept of linear regression equation
the two winter wheat cultivars used in Experiment 1, Akasabishirazu 1 and Dawson 1. In this figure, D1t's in 45 or more day treatments are represented by their adjusted values, since in these treatments the 1st leaves unfolded within the period of the treatment. According to the results of the t-test, the regression coefficients exhibited a significant difference among the seven experiments, but no varietal difference was detected in any of the experiments. These results support the assumption that the growth increment during the treatment increases linearly with the increase of the duration of the treatment (Fig.1).

Table 4 and Fig.5 present the relationship between Dof and the duration of the treatment for all the cultivars examined and for the two cultivars Akasabishirazu 1 and Dawson 1, respectively. Figure 5 also shows the relationship between Dtf and the duration of the treatment for comparison. Except for the three cultivars Konosu 25, Marquis and Fultz 1 (Table 4), Dof's maintained or reached the respective constant values with the increase of the duration of the treatment, whereas Dtf's showed a continuous decrease until the end. These results confirm the suitability of the plant-development model shown in Fig.2.

In the two cultivars shown in Fig.5, for instance, the Dof values became constant after 65 days of treatment. According to the model, this finding implies that these cultivars were fully vernalized after treatments of 65 days and over, in other words, their chilling requirement is evaluated at 65 days. With the exception of the cultivars Konosu 25, Marquis and Fultz 1, whose Dof's finally increased with the increase of the duration of the treatment, the chilling requirement was evaluated in the treatments where the increase of Dof did not yet appear.

Data on the chilling requirement and narrow-sense earliness evaluated in the seven experiments are presented in Table 5, which shows that the chilling requirement tended to increase with the degree of the growth habit from grades I to VII. However, an inversion in the chilling requirement occurred between a spring wheat cultivar Saitama 29(III) and two winter wheat cultivars Akadaruma(IV) and Shirodaruma(IV). Also among the spring wheat cultivars, such an inversion was observed between a cultivar Akabozu(III) and two cultivars Eshimashinriki(II) and Norin 61(II). Moreover, the chilling requirement of the IIa cultivar, Marquis, was estimated at 0 days. Thus, the magnitude of the chilling requirement did not necessarily reflect the degree of growth habit.

As clearly seen in Table 4, all the cultivars could be classified into two groups by the
<table>
<thead>
<tr>
<th>Duration of treatment (days)</th>
<th>HH</th>
<th>K-25</th>
<th>MQ</th>
<th>ES</th>
<th>N-61</th>
<th>AB</th>
<th>S-29</th>
<th>AD</th>
<th>SD</th>
<th>N-8</th>
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<th>B-1</th>
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<th>D-1</th>
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<tbody>
<tr>
<td>0</td>
<td>29.1*</td>
<td>26.6*</td>
<td>31.9*</td>
<td>32.3</td>
<td>29.7</td>
<td>38.4</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
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<td>29.1</td>
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<td>30.5</td>
<td>29.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>×</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
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<td>10</td>
<td>29.9</td>
<td>26.3</td>
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<td>30.1</td>
<td>28.4</td>
<td>33.2</td>
<td>65.4</td>
<td>×</td>
<td>×</td>
<td>×</td>
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<td>—</td>
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<td>38.3</td>
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<td>34.9</td>
<td>39.9</td>
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</tr>
</tbody>
</table>

Lowest value** for Dof: 29.2 26.1 30.7 22.4 24.0 29.2 26.9 24.4 22.3 28.1 27.1 33.7 32.8 36.1 38.9

*; Indication of the treatment corresponding to chilling requirement (See the note on Table 5)

**; Mean Dof value among all the plants of the treatments which correspond to and longer than chilling requirement, except for K-25, MQ and F-1 as shown in text

—; No treatments were applied.

×; Treatment in which no flag-leaves unfolded within the period of the experiments
mode of flag-leaf unfolding in the absence of chilling treatment: the cultivars in which the unfolding occurred within 40 days, and those in which no unfolding occurred throughout the whole period of the experiments (ca. 70 days). The chilling requirement of the former ranged from 0 to 30 days, while that of the latter from 40 to 65 days. In the latter, it was also observed that the minimum duration of the treatment necessary for flag-leaf unfolding increased almost in parallel with the increase of the chilling requirement, suggesting that the chilling requirement corresponds well to the requirement of wheat cultivars for a low temperature.

Data on narrow-sense earliness, the lowest values of Dof shown in Table 4, are presented in Table 5. A statistically significant difference was observed between cultivars in each of the experiments except for the 6th one, indicating that there were varietal differences in narrow-sense earliness. The differences among the experiments, however, may not necessarily reflect substantial differences in the narrow-sense earliness, because the growing conditions, such as solar radiation, varied with the experiments.

**Discussion**

Evaluation of the chilling requirement, i.e. the minimum duration of the chilling treatment necessary for full vernalization, of a wheat cultivar requires a measurement which involves not only the growth after the treatment but also the growth during the treatment. Based on this concept, two assumptions for the growth during the treatment were proposed in the present study, then an index Dof and a plant-development model using the Dof (Fig.2) were developed on the basis of the assumptions. Throughout several experiments, the accuracy of the assumptions was clearly verified (Table 3 and Fig.4), and the development model proved to be suitable for the evaluation of the chilling requirement and narrow-sense earliness (Table 4 and Fig.5).

1) Relationship between the chilling requirement and other indices related to vernalization

The fifteen wheat cultivars examined in this study could be classified into two groups of 6 and 9 cultivars, based on whether flag-leaf unfolding occurred or not in the absence of chilling treatment (Table 4). This classification revealed a distinct difference in the
Table 5. Chilling requirement and narrow-sense earliness of 15 wheat cultivars and their relation to the indices formerly established

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Chilling(^{1}) requirement</th>
<th>Narrow(^{2}) sense earliness (Lowest value of Dof)</th>
<th>Growth(^{3}) habit</th>
<th>Vernalization(^{4}) requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH</td>
<td>0(days)</td>
<td>29.2(days)</td>
<td>—</td>
<td>0(days)</td>
</tr>
<tr>
<td>K-25</td>
<td>0</td>
<td>26.1</td>
<td>I</td>
<td>0</td>
</tr>
<tr>
<td>MQ</td>
<td>0</td>
<td>30.7</td>
<td>II(_{a})</td>
<td>—</td>
</tr>
<tr>
<td>ES</td>
<td>30</td>
<td>22.4</td>
<td>II</td>
<td>0</td>
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<td>N-61</td>
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<td>24.0</td>
<td>II</td>
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<tr>
<td>AB</td>
<td>20</td>
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<td>S-29</td>
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<td>SD</td>
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<tr>
<td>N-8</td>
<td>55</td>
<td>28.1</td>
<td>V</td>
<td>—</td>
</tr>
<tr>
<td>SB</td>
<td>60</td>
<td>27.1</td>
<td>V</td>
<td>—</td>
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<tr>
<td>B-1</td>
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<td>F-1</td>
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<td>ASS</td>
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</tr>
<tr>
<td>D-1</td>
<td>65</td>
<td>38.9</td>
<td>VI</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^{1}\) Minimum duration of chilling treatment necessary for full vernalization
\(^{2}\) Mean value of Dof of fully vernalized plants
\(^{3}\) Cited from KAKIZAKI and SUZUKI (1937).
\(^{4}\) Cited from GOTOH (1979).

The chilling requirement between the two groups, the former being estimated at 0 to 30 days and the latter 40 to 65 days (Table 5). Thus, the wheat cultivars with a maximum of 30 days for the chilling requirement and those with 40 or more days can be defined as spring wheat and winter wheat cultivars, respectively.

The chilling requirement of a wheat cultivar has so far been estimated indirectly by the following three indices:

a) “Growth habit” represented by the latest sowing time which allows the cultivar to develop up to the heading stage under field conditions (KAKIZAKI and SUZUKI 1937).

b) “Growth habit” represented by the duration from sowing to flag-leaf unfolding under a 24 h day-length regime without chilling treatment (YASUDA and SHIMOYAMA 1965, NAKAI and TSUENAWAKI 1967).

c) “Vernalization response” evaluated by the degree of the acceleration due to the chilling treatment of the flag-leaf unfolding under a 24h day-length regime (HAULSE and WEIR 1970, PUGSLEY 1971, RAHMAN 1980, HOOGENDORN 1984).

These three indices are useful for a rough classification of wheat cultivars into spring and winter wheat cultivars, since they can be evaluated more readily than the chilling requirement. Among them, the growth habit defined by KAKIZAKI and SUZUKI (1937) is a useful index for practical breeding, because it can be evaluated under field conditions.

However, each of these indices represents only one of the responses of wheat to natural or artificial vernalization. Besides, the growth habit defined by KAKIZAKI and SUZUKI (1937) is largely affected by the photoperiodic response as indicated by YASUDA and SHIMOYAMA (1965). These facts may account for the reasons why the chilling requirement
Evaluation of Chilling Requirement and Narrow-Sense Earliness

and the growth habit defined by Kakizaki and Suzuki (1937) did not correspond to each other in the three cultivars Marquis, Akabozu and Saitama 29 (Table 5). Saitama 29 was formerly considered as a spring wheat cultivar due to the lower score(III) of the growth habit (Kakizaki and Suzuki 1937), but it was considered here as a winter wheat cultivar because the chilling requirement exceeded 40 days (Table 5). Gotoh (1976) also reported that Saitama 29 was a winter wheat cultivar in terms of its "vernalization requirement". A similar discrepancy in the evaluation has been revealed also in the cultivar Hayakomugi, one of the parents of Saitama 29 (Kakizaki and Suzuki 1937, Kato and Yamagata unpublished). Thus, in some types of cultivars such as Saitama 29 and Hayakomugi, it is impossible to estimate the chilling requirement based on the growth habit only.

To estimate the low temperature requirement of wheat, Gotoh (1976) attempted to evaluate the vernalization requirement, an index expressed by the minimum duration of the treatment necessary for flag-leaf unfolding within 34 days after the end of the chilling treatment. The values of this index, however, are larger than those of the chilling requirement when cultivars have a large narrow-sense earliness, and vice versa, because it is determined without considering the varietal difference in narrow-sense earliness. For instance, in the two spring wheat cultivars with a small narrow-sense earliness, Eshimashinriki and Norin 61, the vernalization requirement (Gotoh 1976) was estimated at 0 days, while the chilling requirement was estimated at 30 days (Table 5).

2) Chilling requirement and the genes responsible for vernalization response

It is well known that the spring wheat cultivars having the Vrn I gene do not require a low temperature for flag-leaf unfolding. There are, however, discrepancies among the reports regarding the response of these cultivars to the chilling treatment, as some reports state that the cultivars do not respond appreciably to the chilling treatment (Pugsley 1971), while others state that they respond significantly to the chilling treatment when they are fully vernalized by a treatment lasting for a maximum of 35 days (Berry et al. 1980, Flood and Halloran 1984). In the present study, however, the three cultivars which had the Vrn I gene, Konosu 25, Haruhikari and Marquis (Table 6), showed each a constant Dof value irrespective of the application or duration of the treatment (Table 4), indicating that these cultivars did not respond to the chilling treatment at all. This finding, therefore, suggests that the slight or drastic reduction in the number of days to flag-leaf unfolding observed by Pugsley (1971), Berry et al. (1980) and Flood and Halloran (1984) is not due to vernalization by the treatment but to the growth during

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haruhikari (HH)</td>
<td>Vrn 1</td>
</tr>
<tr>
<td>Konosu 25 (K-25)</td>
<td>Vrn 2</td>
</tr>
<tr>
<td>Marquis (MQ)</td>
<td>Vrn 3</td>
</tr>
<tr>
<td>Eshimashinriki</td>
<td>vrn 1</td>
</tr>
<tr>
<td>Norin 61 (N-61)</td>
<td>vrn 2</td>
</tr>
</tbody>
</table>

Cited from Gotoh and McIntosh (1983).
the treatment, and that the \textit{Vrn 1} gene makes wheat completely insensitive to the chilling treatment.

It is well known that the \textit{Vrn 3} gene makes wheat slightly sensitive to the chilling treatment \cite{Gotoh1979}. In the present study, the chilling requirement of the two cultivars having the \textit{Vrn 3} gene alone, Eshimashiriki and Norin 61 (Table 6), was estimated at 30 days, as mentioned before. However, further analysis by using isogenic lines seems to be required for evaluating the magnitude of the action of the \textit{Vrn 3} gene.

3) \textbf{Narrow-sense earliness}

\textsc{Yasuda} and \textsc{Shimoyama} (1965) first succeeded in revealing the varietal difference of narrow-sense earliness in wheat based on the number of days from the end of the treatment to flag-leaf unfolding under the optimum conditions for reproductive growth. However, they could not evaluate the narrow-sense earliness itself, because the growth during the chilling treatment was not considered.

Recently, \textsc{Hoogendoorn} (1984) estimated the growth increment during the treatment in terms of the number of primordia differentiated, and she suggested that narrow-sense earliness, "earliness per se" according to her, can be evaluated by adopting the growth increment as a component factor of plant development. Her concept, however, does not allow for the exact evaluation of narrow-sense earliness, because the author considers that it is impossible to determine whether the wheat plant has reached a fully vernalized state.

In contrast with these reports, the present study actually succeeded in revealing the narrow-sense earliness of a given wheat cultivar. This may be exclusively due to the fact that the growth increment during the treatment and the fully vernalized state could be determined through the development model using the Dof index.

The accurate evaluation of the chilling requirement by the use of the Dof index may contribute significantly to the elucidation of the nature and genetics of vernalization, because the chilling requirement is not affected by narrow-sense earliness. Namely, the role of the various factors related to vernalization, such as genes, alien cytoplasms and chemical agents, could be successfully analyzed for their effect on the chilling requirement, and the results may contribute to the improvement of heading time \cite{Nagasawa1983} and to the shortening of the breeding cycle of wheat \cite{Barbas1978}.

\section*{Acknowledgment}

We would like to thank Professor K. \textsc{Hayashi}, Faculty of Agriculture, Kochi University, for his valuable suggestions.

\section*{Literature Cited}


コムギ品種の低温要求性及び純粋早熟性の評価法

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コムギの出穂期は日長反応性、低温要求性及ぶ純粋早熟性の3要因によって決定される複合形質である（Yasuda and Shimojama 1965）から、その育種に際して、これら3要因を個別に評価する必要がある（Yoshida et al. 1985）。しかしながら、3要因のうち評価が確立されているのは日長反応性のみであり（Yasuda and Shimojama 1965）、他の2要因について、従来播種や低温処理による出穂の促進度あるいは高湿長日下的生育日数などに基づく便宜的な推定方法に頼ってきた。このうち低温要求性については、後藤（1976）により完全春化に必要な最短の低温処理期間を基礎とすべきことが提唱され、その期間（春化要求度）の評価がいくつかの品種について試みられた。しかしながらその際、完全春化の判定基準が品種の純粋早熟性と無関係に設定されため、得られた春化要求度は必ずしも低温要求性を反映するものとはならなかった。そこで本研究では、純粋早熟性の大小に影響されず低温要求性を評価しうる方法を確立しようとした。このため、コムギの生育は低温処理中も処理期間（日数、X）に比例して進行すると仮定し、これに基づいて処理終了後第1葉展開前日数（D1）は、D1＝−BX+a（ただし B は処理中の生長速度と処理後の生長速度との比）によって表されることを示した（Fig. 1）。これに従えば、低温処理期間を、この期間中に進行した生育量と同等の生育量を与える処理後の日数（低温処理期間相当日数、BX）に換算することが可能。従って、低温処理後純粋展開前日数（D2）にこの日数（BX）を加えれば、春化（処理開始）後純粋展開前日数（Das）を求めることができる。Das は、低温処理により春化が進行している間中は処理期間が増すにつれて減少するが、完全に春化した状態になる低温処理期間の有無・短長にかかわらず品種固有の一定値を示すはずである（Fig. 2）。従ってこの一定値をもたらす低温処理期間を知ることにより、完全春化に必要な最短の低温処理期間
を明らかにすることができる。また、完全に春化された状態での Dof はそのまま品種の純粋早熟性を表すことになる。以上の仮説を検証するため、播性を異にするコムギ 15 品種を供試して、低温要求性及び純粋早熟性の評価を試みた。すなわち、15 品種の種子を 5℃ で 0〜85 日間低温処理した後 20℃、全日長条件のファイトロンで栽培し、第 1 菓及び止業の展開日を調べて Dof を求め、Dof を一定値にする最短の低温処理日数によって低温要求性を、また一定値に達した Dof によって純粋早熟性を評価した。本実験の結果、まず処理後第 1 菓展開分日数が低温処理期間に比例して減少した（Fig. 4, Table 3）ことから、低温処理期間中の生育に関する前記仮定の妥当性が確認できた。そこで、Dof の低温処理期間の増加に伴う変化を見たところ、低温要求性のある品種では、Dof は処理期間が増加するにつれて次第に小さくなり、やがて品種固有の一定値に達した（Fig. 5, Table 4）。一方、低温要求性のない品種では、Dof は低温処理期間の有無・長短にかかわらず一部品種ごとに一定値を示した（Table 4）。これらの結果から、Dof を春化の判定指標に用いればコムギの低温要求性及び純粄早熟性は正確に評価できることが確認された。なお、供試材料のうち、低温処理をしなくても容易に止業を展開する春播型コムギ品種群では低温要求性が 0〜30 日であったのに対して、低温処理をしないと止業展開が極端に遅れる冬播型コムギ品種群では 40〜65 日であり、両品種群間で低温要求性は明瞭に異なった。また、春播型品種のうち Vrn 1 遺伝子を持つものは 0 日、Vrn 2 遺伝子を持つものは 30 日の低温要求性を示し、春播性に関する遺伝子型とよく対応した。これらの結果は、本方法により低温要求性が極めて正確に評価されることを示している。