An Empirical Method for Diagnosing Premature Bolting Risk in Spring Cabbage by Estimating the Flower Bud Differentiation Period

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Spring cabbage (Brassica oleracea var. capitata L.) is a crop type in which sowing is performed in fall and harvesting in spring. The flower bud differentiation, explained as the phase transition from the vegetative phase to reproductive phase, is induced by chilling after a certain plant size, then the risk of premature bolting is triggered by long days and high temperatures. Farmers empirically avoid bolting by selecting suitable varieties and sowing days. However, climate change may increase the risk of premature bolting. The objectives of this study were to evaluate the relationship between the number of head leaves at flower bud differentiation and premature bolting, and to develop a model to predict flower bud differentiation and the number of head leaves using data on the daily cumulative temperature. Firstly, we found that the risk of premature bolting was high for the ‘Kinkei-201’ cabbage variety when the number of head leaves (> 1 g) was less than 6.5 leaves in the flower bud differentiation period. The number of head leaves (> 1 g) (y) was estimated by the daily cumulative temperature (x): y = 0.0248x − 24.485 to 28.613, depending on year. The flower bud differentiation period was estimated based on the concept of the developmental rate (DVR) and the developmental index (DVI), in which the value of DVI at sowing was defined as 0 and that at the flower bud differentiation period as 1. Each parameter’s response to the cold treatment stage (RS) and the response to chilling (C) was estimated based on the daily mean temperature. The DVR model predicted the flower bud differentiation period in 2010–2014 with a root mean squared error = 5.3 days (without outliers). Therefore, the risk of premature bolting is predictable by estimating the number of head leaves (> 1 g) at the flower bud differentiation period using data on sowing date and mean temperature.

Key Words: DVI (developmental index), DVR (developmental rate), flower bud differentiation, global warming, number of head leaves.

Introduction

Cabbage (Brassica oleracea var. capitata L.) is the most consumed and produced vegetable in Japan (Ministry of Agriculture, Forestry and Fisheries, 2017; Ministry of Internal Affairs and Communication, 2017). Thus, stable supply is extremely important. Cabbage is supplied throughout the year by relay-cropping and differentiated as spring cabbage, summer cabbage and winter cabbage based on the harvest period (Fukuchi, 1996). Spring cabbage shipped during April to June is produced mainly in the Atsumi Peninsula, Boso Peninsula, and Miura Peninsula. Due to its green-plant vernalization characteristics, flower bud differentiation, which is defined as the phase transition from the vegetative phase to the reproductive phase (Fujime and Kakibuchi, 1992), occurs after the exposure of seedlings to a low temperature for a certain period (Ito and Saito, 1961; Iwama, 1972; Ngwenya, 2016). Although timing of flower bud differentiation in response to cold treatment differs according to the variety (Eguchi, 1950), exposure to long-day conditions following flower bud differentiation induces bolting (Ito and Saito, 1961; Iwama, 1972). Furthermore, if growth and development progress rapidly as a result of early...
sowing or early transplanting, even cabbage varieties that are relatively insensitive to cold treatment will undergo flower bud differentiation earlier, resulting in bolting in combination with fewer leaves (Fukuchi, 1996).

With increased demand from the processing market, serious incidents have been reported such as mixtures of flower stalks and flower buds in cut cabbage, a phenomenon caused by premature bolting (Alic, 2019; Kobayashi, 2006). Thus, producers growing spring cabbage carefully select varieties that are best suited to the local weather conditions, as well as the cropping period to prevent premature bolting and ensure stable crop production.

Hiraishi (1966) found that the total number of differentiated leaves was 52.5 for bolted cabbages of the variety ‘Kinkei-201’, and that the leaf number must be 60 or more for normal head formation. Fukuchi (1996) reported that premature bolting was associated with an insufficient leaf number. Based on this information, the appropriate sowing period is determined. In the Miura Peninsula, Okamoto (2002) found that risk of premature bolting was high when sowing was done between September 11 to 20, and that the appropriate sowing period was around September 25. In addition, Okamoto (2002) pointed out that warmer winters affect the appropriate time for sowing. In Japan, the mean annual temperature has risen by 4.5°C over the last 100 years (Ministry of Agriculture, Forestry and Fisheries, 2017). Thus, the appropriate sowing period needs to be modified to adapt to warmer winters caused by global warming. However, information is lacking on the appropriate sowing period to prevent premature bolting.

Premature bolting occurs after flower bud differentiation by subsequent exposure to long-day conditions. Thus, it is important to estimate the flower bud differentiation period. Hiraishi (1966) reported that the flower bud differentiation occurs after > 750°C of cumulative temperatures above 7°C and after exposure to a daily average temperature < 14°C for 30 days. In contrast, several studies found the relationship between flower bud differentiation and plant size could be estimated by the number of leaves (Matsubara and Hidaka, 1940) or by the stem diameter (Ito and Saito, 1961). These studies indicate that flower bud differentiation is predictable by temperature and plant size. Indeed, previous studies have reported the relationship between flower bud differentiation and cold temperature or plant size (Eguchi, 1950; Ito and Saito, 1961; Matsubara and Hidaka, 1940) and Hiraishi (1996) proposed a theory regarding flower bud differentiation. However, no model has been reported that evaluates plant size based on the plant age or that predicts flower bud differentiation based on the plant age estimated by the cumulative temperature.

The objectives of this study were to evaluate effects of temperatures on flower bud differentiation and bolting, to analyze the relationship between the head leaf number at flower bud differentiation and premature bolting, and to develop a predictive model for head leaf number and flower bud differentiation. We also examined a method to avoid premature bolting. It is difficult to detect bolting based on external appearance and impossible to remove cabbages with bolting prior to shipment. Thus, it is important to predict the risk of premature bolting prior to sowing or during the growth period.

Materials and Methods

General cultivation conditions and weather data

Different varieties require different exposure periods to prolonged cold to induce flower bud differentiation (Eguchi, 1950; Iwama, 1972; Matsubara and Hidaka, 1940). In this study, to simplify this variety effect, we used the same variety ‘Kinkei-201’ as the study by Hiraishi (1966). ‘Kinkei-201’ (Sakata Seed Corporation, Yokohama, Japan) is a representative spring cabbage type in the major spring cabbage producing areas in Japan. The seeds were sown a total of 17 times from September to October. This experiment was repeated from 2010 to 2014. Sowing was performed a total of 22 times in an open field at the Kanagawa Agricultural Technology Center, Miura Peninsula District Office (Hasse-machi, Miura City: annual mean temperature 15.8°C and mean annual precipitation 1,557 mm, Table 1). Crop cultivation summaries for each year were almost the same among the years: 100 kg a⁻¹ of cattle manure; 10 kg a⁻¹ of calcium fertilizer (34% CaO); and 4 kg a⁻¹ of magnesium fertilizer (25% MgO). Basal fertilization (N-P-O₃-K₂O) was 1.0:2.6:1.0 kg a⁻¹ and top dressing (N-K₂O) was 1.4:1.4 kg a⁻¹. Seedlings were raised on a soil bed containing dazomet granule-treated soil. The planting seedling age was at the 4- to 5-leaf stage. Planting conditions were as follows: furrow planting depth 51 cm; intra-row spacing 33 cm; and plant density 594.1 plants a⁻¹. During the seedling period, soil was treated with a neonicotinoid class granular insecticide (active substance: 0.5% clothianidin or 0.5% thiamethoxam or 1.0% imidacloprid). After transplanting, fungicides and insecticides were sprayed three to six times in accordance with Kanagawa Prefecture’s Guidance on Prevention of Pests and Weeds. The weather data (temperature and precipitation) were collected from the Automated Meteorological Data Acquisition System (AMeDAS), which is installed in Miura Peninsula District Office. The quantity of solar radiation was obtained from the Agriculture, Forestry and Fisheries Research Information Technology Center, which is in Miura Peninsula District Office.

Sampling and leaf measurement

During the period from transplanting to harvest, the above ground sections of six plants were sampled six to 10 times to determine the leaf number, head weight,
Table 1. Growth and development of cabbage in the 17 datasets.

| Dataset name | Sowing day | Investigation day | Days from sowing to investigation | Number of head leaves (> 1 g) | Cumulative temperature°C | Rate of flower bud differentiation (%) | Flower bud differentiation period | Rate of bolting (%) | Harvest time | Head fresh weight (g) |
|--------------|------------|-------------------|-----------------------------------|------------------------------|--------------------------|-----------------------------------------|----------------------------------|------------------|-------------|----------------------|
| 1            | 2010/9/27  | 12/10             | 74                                 | (5.7)                        | 1,178                    | 0                                       | 12/21               | 0                | 3/8         | 1,275                |
|              | 2010/9/30  | 12/21             | 82                                 | (6.7)                        | 1,230                    | 0                                       | 1/2                 | 0                | 3/15       | 1,325                |
|              | 2010/10/4  | 1/14              | 102                                | (8.6)                        | 1,326                    | 0                                       | 2/1                 | 0                | 3/23       | 1,676                |
| 2            | 2011/9/9   | 11/30             | 82                                 | 11.3                         | 1,555                    | 0                                       | 12/27               | 0                | 2/16       | 1,114                |
|              | 2011/9/29  | 12/8              | 70                                 | 5.7                          | 1,152                    | 0                                       | 12/16               | 0                | 3/27       | 1,494                |
|              | 2011/10/3  | 12/14             | 72                                 | 4.7                          | 1,119                    | 17                                      | 12/25               | 100              | 3/27       | 1,464                |
| 3            | 2012/9/24  | 12/17             | 84                                 | 4.5                          | 1,269                    | 33                                      | 12/18               | 100              | 3/22       | 1,760                |
|              | 2012/9/28  | 12/25             | 92                                 | 7.5                          | 1,326                    | 100                                     | 12/25               | 100              | 3/27       | 1,464                |
| 4            | 2013/9/24  | 12/6              | 73                                 | 5.3                          | 1,201                    | 83                                      | no data             | 0                | 3/11       | 1,365                |
|              | 2013/9/27  | 12/6              | 70                                 | 2.2                          | 1,135                    | 0                                       | 12/10               | 83               | 3/31       | no data              |
|              | 2013/10/7  | 4/4               | 179                                | 22.3                         | 1,867                    | 50                                      | 4/4                 | 0                | 4/4        | 1,344                |
| 5            | 2014/9/24  | 12/9              | 76                                 | 3.2                          | 1,231                    | 17                                      | 12/15               | 37               | 3/19       | 1,459                |
|              | 2014/9/26  | 1/5               | 103                                | 8.2                          | 1,423                    | 100                                     | 12/22               | 39               | 3/27       | 1,441                |
| 6            | 2014/9/29  | 3/3                | 152                                | 12.2                         | 1,640                    | 17                                      | 3/16                | 3                | 4/2        | 1,548                |

* Cumulative temperature were calculated from sowing dates to one day before investigation dates.

The day of flower bud differentiation was defined as the day when 50% of plants differentiated.

The number of head leaves (> 1 g) in 2010 (dataset 1–3) was estimated by the number of head leaves (> 1 cm).

Head fresh weight was 1,558 g at 3/26.
degree of flower bud differentiation and degree of bolting. Flower bud differentiation is predictable based on the plant size estimated by the number of leaves (Matsubara and Hidaka, 1940) or by the stem diameter (Ito and Saito, 1961). In cabbage, the stem diameter is easily affected by the measurement position. To minimize measurement error, the number of leaves was determined in this study.

Leaves were divided into outer leaves and head leaves, and head leaves were further grouped according to whether the weight was $> 1\, \text{g}$ or $< 1\, \text{g}$. In this study, head leaves were defined as leaves consisting of a short, thick stalk and a large, compact head of edible green leaves, with one to two outer layers of leaves that did not form a head. Measurements of head leaves $< 1\, \text{g}$ were performed only in 2013 and 2014. Small leaves $< 1\, \text{g}$ were observed under a stereomicroscope and judged to be differentiated leaves if a swelling part was observed near a growing point. As one to two layers of outer leaves were left intact on cabbage heads for shipment, these outer leaves were counted as head leaves. Fallen leaves were not included in the total leaf count. At harvest, the degree of bolting was evaluated using 30 plants from each sowing date. Details of the cultivation calendar and measurement parameters are shown in Table 1.

The degree of flower bud differentiation was determined by examining the growth point using a stereoscopic microscope ($\times 7$–$30$). Using the differentiation criteria of Eguchi (1950), morphology was classified as follows: no differentiation (0); early stage differentiation (1); differentiation (2); mid-differentiation (3); and late stage differentiation (4). Of the six samples examined, the day of flower bud differentiation was defined as the day on which three (50%) or more plants were at stage two or greater. To determine the degree of bolting, a cabbage was split into two equal sections and the cross-sections were classified into five levels ranging from zero to four (Fig. 1) as follows: 0; core was not elongated, neither lateral buds nor flower buds were observed; 1; slight core elongation and lateral bud growth were observed; 2; core elongation and lateral buds were more noticeable than 1; 3; core was markedly elongated, lateral buds and flower buds were noticeable, and head formation was not completed (not eligible for shipment); 4; core was elongated to the top of the head, lateral buds and flower buds were noticeable, but head formation was not completed (not eligible for shipment). Plants classified as three or four were not eligible for shipment. Premature bolting was defined if one (17%) or more plants of the six samples examined were classified as level three or four.

To carefully examine the relationship between leaf number and premature bolting, pruning tests were conducted in 2013 and 2014. Of the 200 plants in the first to third crop seasons of 2013 (sowing dates: September 24, 27, and 30 corresponding to the dataset 10 to 12), five outer leaves of each plant were pruned prior to flower bud differentiation on November 12, 19, and 25, respectively. For the plants from the September 30 sowing, pruning was performed on two levels (five outer leaves on November 25 and all nine outer leaves on January 14 after bud differentiation). At harvest, the leaf number and degree of bolting were examined using 72 to 96 plants from each sowing date. For 75 to 250 plants sampled from the 1st to 4th sowing in 2014...
Using 5°C as the reference temperature in onion (Usuki et al., 2019) to estimate the age of cabbage leaves. In spinach, the speed of leaf elongation was affected by not only air and soil temperature, but also soil moisture (Hamamoto, 1992). In daikon, data on temperature, solar radiation, and precipitation were used to estimate the root weight (Hiraishi et al., 1976). Therefore, we evaluated the relations between the number of head leaves > 1 g and cumulative temperature, solar radiation, and precipitation using datasets 7 to 17. Cumulative temperature above a reference temperature was calculated by summing up from the sowing day to one day before measurement. In addition, since the leaf number was estimated based on the mean temperature using 5°C as the reference temperature in onion (USuki et al., 2019), we also evaluated different reference temperatures (from 0°C to 7°C) to estimate cumulative temperatures. The number of head leaves > 1 g was estimated using the following equation (1), in which x is cumulative temperature and a, b are parameters.

\[ y = ax + b \] (1)

Using data from 2012 (datasets 7 to 9), 2013 (datasets 10 to 13), and 2014 (datasets 14 to 17), the rate of leaf appearance was estimated for each year and the average value for three years was regarded as parameter “a” in (1). Then, using data showing average growth in 2012, 2013, and 2014, the number of head leaves and cumulative temperature were substituted in equation (1) and the parameter “b” was obtained.

### Estimation of head leaf number

Okada and Sasaki (2016) and Okada and Sugawara (2019) reported linear models based on daily average temperatures to estimate the age of cabbage leaves. In spinach, the speed of leaf elongation was affected by not only air and soil temperature, but also soil moisture (Hamamoto, 1992). In daikon, data on temperature, solar radiation, and precipitation were used to estimate the root weight (Hiraishi et al., 1976). Therefore, we evaluated the relations between the number of head leaves > 1 g and cumulative temperature, solar radiation, and precipitation using datasets 7 to 17. Cumulative temperature above a reference temperature was calculated by summing up from the sowing day to one day before measurement. In addition, since the leaf number was estimated based on the mean temperature using 5°C as the reference temperature in onion (Usuki et al., 2019), we also evaluated different reference temperatures (from 0°C to 7°C) to estimate cumulative temperatures. The number of head leaves > 1 g was estimated using the following equation (1), in which x is cumulative temperature and a, b are parameters.

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### Predictive model for flower bud differentiation

In predicting flower bud differentiation, we used the developmental rate (DVR) reported by de Wit et al. (1970) and the developmental index (DVI) which is used to predict the appearance of ears of rice based on the DVR (Horie and Nakagawa, 1990; Kawakata, 1998). In DVR, de Wit et al. (1970) used maize and defined the development stage (DVS) is 0 at germination and 1 at the heading stage, and then quantified DVR linearly in two periods. Horie and Nakagawa (1990) reported that qualitative changes occurred after quantitative changes reached a threshold level in young panicle differentiation, heading and maturation of rice. In addition, as Kawakata (1998) pointed out difficulties in estimating the heading stage of rice based on the effective cumulative temperature, so we need to pay attention to a possible non-linear relation between temperature and development. Based on these findings, we hypothesized that flower bud differentiation occurs when quantitative changes proceed and the sum of changes exceeds a certain level. Under this hypothesis, DVI at n days after sowing indicates the integration of DVR during that period. A DVI prediction model (2) was prepared to reflect DVR, in which the time of sowing was DVI = 0 and the time of flower bud differentiation was DVI = 1. In this model, the following indices were used: development rate at day i after sowing (DVRi) and day n after sowing (DVI).

\[ DVI = \int_0^a DVRdt \approx \sum_{i=0}^n DVRi \] (2)

In this model, we assumed that cabbage growth from sowing to flower bud differentiation was in a vegetative stage, showing a similar response to the environment. As described previously, spring cabbage undergoes flower bud differentiation when seedlings of a certain size encounter a period of cold temperature (Iwama, 1972). Therefore, the DVR was modified to model (3) with the following parameters: coefficient for seedlings of a certain size that respond to cold treatment (response to cold treatment stage parameter; RS); cold temperature coefficient (chilling parameter; C); exposure time to cold temperature (chilling hours; CH), coefficient for growth and development response to temperature (growth response parameter; GR).

For a predictive model, using the minimum number of parameters is desirable, so the effect of environmental factors other than daily mean air temperature (°C) was ignored in this study.

Furthermore, the period from flower bud differentiation in response to cold treatment to differentiation representing the chill hours (CH) was displayed in units of days.

\[ DVR = \frac{1}{RS \times C \times GR} \times \frac{1}{CH} \] (3)

Subsequently, we indicated the parameters of the functions. While seedling size was expressed as the leaf number or stem diameter, we used Hiraishi’s (1966) method of effective cumulative temperature after sowing to estimate the size of seedlings. Furthermore, using Okada and Sasaki’s (2016) daily solar radiation utilization index for cabbage, we assumed that a plant’s response to cold treatment occurs before or after development up to a certain size and that gradual changes take place up to that point. Based on these assumptions, we developed a model (4) based on a sigmoid function to determine the response stage coefficient (RS).

\[ RS = \frac{RS.top - RS.base}{1 + \exp\left(\frac{RS.mid - T}{RS.range}\right)} + RS.base \] (4)
To determine the temperature range for assessing response to cold treatment, Kitada (1993) tested a daikon variety resistant to disease under changing temperature conditions and determined that response to cold treatment was high at 2 to 5°C, with an upper limit of under or over 12.5°C. In many plants that undergo vernalization, exposure to 25°C or more after vernalization suppresses or delays flower bud formation leading to de-vernalization (Fujita, 1993).

In the spring cabbage cropping type used in this study, the possibility of de-vernalization was not taken into consideration, as exposure to high temperature conditions after flower bud differentiation does not occur in the winter season (December to February). Based on a sigmoid function that assumes that sensitivity to cold treatment will increase with colder temperature, a model (5) was used to determine the low temperature coefficient (C).

\[
C = \frac{C_{top} - C_{base}}{1 + \exp\left(\frac{C_{mid} - T}{C_{range}}\right)} + C_{base}
\]  

(5)

The DVR is high at the optimal temperature (15 to 20°C) and particularly affected by temperature during the period between head formation and harvest (Takeda and Kagawa, 1999). Therefore, while flower bud differentiation requires exposure to chilling, this decreases the speed of growth and development. For this reason, model (6) was used to show a growth and development (GR) coefficient that assumes a certain temperature as the inflection point that gradually decreases in the sigmoid function with decreases in temperature.

\[
GR = \frac{GR_{top} - GR_{base}}{1 + \exp\left(\frac{GR_{mid} - T}{GR_{range}}\right)} + GR_{base}
\]  

(6)

There are several ways to estimate the flower bud differentiation period. For example, Eguchi (1950) decided January 15 as the day of flower bud differentiation. In leaf lettuce, the flower bud differentiation day was estimated based on the growth and development stage around flower bud development and cultivation (Tone, 1988). In some leafy vegetables, the day of flower bud differentiation is determined by microscopic examinations at 10-day intervals (Iwami et al., 1983). For bunching onion, the day of flower bud differentiation is not specified, and the differentiation rate is used instead (Yamasaki et al., 2012). In this study, the day of flower bud differentiation was defined as the day when 50% of plants differentiated, which was evaluated by periodical sampling and microscopic examination.

Predictive model for premature bolting

Premature bolting is related to a lack of leaves (Fukuchi, 1996; Hiraishi, 1966). We established a predictive model for premature bolting using the number of head leaves at flower bud differentiation. We used only head leaves > 1 g, since it is difficult to count the number of head leaves < 1 g. Firstly, we input data on daily temperature and growth parameters obtained from 2010 to 2014 into equation (1), which resulted in y (the number of head leaves > 1 g) = ax (cumulative temperature) + b. Next, we estimated the number of head leaves > 1 g at flower bud differentiation. If the number was < 6.5, we judged this as high risk for premature bolting, while if it was > 6.5 we judged it low risk. Finally, we simulated sowing periods with high risk of bolting based on the number of head leaves (> 1 g).

**Statistical analysis**

All statistical analyses were performed using Excel Statistics ver2004.

**Results**

The relationship between leaf number and premature bolting

In datasets 10 to 17, bolting was observed in only 0.3% (1 out of 345 plants) when the number of total leaves (head leaves and outer leaves, excluding fallen leaves) was > 48 (Fig. 2). In contrast, bolting was observed in 54% (50 out of 92 plants) when it was < 47.

There were no correlations between head weight and the number of total leaves or the degree of bolting (r = 0.22, P < 0.001 or r = 0.35, P < 0.001) (Fig. 3).

Estimation of head leaf number

Highly significant correlations (P < 0.001) were observed between the number of head leaves and cumulative temperature, daily solar radiation and precipitation in all years (Table 2). Among these three meteorological parameters, cumulative temperature showed the highest value in all years. The correlations between the number of head leaves (> 1 g) and effective cumulative temperature above 0 to 7°C indicated a relatively stronger relationship at a lower reference temperature, although all the reference temperatures had highly significant correlation coefficients (Table 3).

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**Fig. 2.** Relationship between the number of total leaves at harvest and bolting level. Data derive from dataset 10 to 17 in Table 1 (n = 179 in pruning treatment, n = 258 in non-pruning treatment). Total leaves include leaves with < 1 g, but not fallen leaves. □: normal (bolting level: 0–2), ■: bolting (bolting level: 3, 4).
We then examined the relationship between cumulative temperature above 0°C and the number of head leaves (> 1 g). There was a positive correlation in each year and the correlation coefficients were 0.739 (2012), 0.996 (2013), 0.981 (2014), and 0.933 (P < 0.005, P < 0.001, P < 0.001, and P < 0.001, respectively) (Fig. 4). As the slopes of the single regression analysis of leaf emergence speed were equivalent (0.0196 in 2012, 0.0293 in 2013, and 0.0255 in 2014, 0.0248) in average, we developed a regression analysis formula (7) based on the mean of the slopes. In regression analysis formula (1), y represents the number of head leaves (> 1 g) and x represents cumulative temperature after sowing.

\[ y = 0.0248x + b \]  \hspace{1cm} (7)

Next, we randomly selected data from the early head formation stage to determine the y-intercept (b) for each year from the single regression analysis formula (7). The data used for cumulative temperature after sowing and the number of head leaves (> 1 g) were 1,106°C and 2.2 leaves (2012); 1,201°C and 5.3 leaves (2013); 1,186°C and 0 leaves (2014). The prediction models were obtained in (8) to (10) for each year.

\[ 2012 \ y = 0.0248x - 25.229 \] \hspace{1cm} (8)
\[ 2013 \ y = 0.0248x - 24.485 \] \hspace{1cm} (9)
\[ 2014 \ y = 0.0248x - 28.613 \] \hspace{1cm} (10)

To verify the accuracy of these prediction models (8) to (10), we input each year’s post-sowing cumulative temperatures to determine the relationship between the estimated values and actual values for the number of head leaves (> 1 g) until flower bud differentiation.
There was a significantly high correlation coefficient ($R^2 = 0.957, P < 0.001$) and 34 out of 37 data fell within the 95% confidence interval (Fig. 5).

**Predictive model for flower bud differentiation**

In an example using dataset 14, flower bud differentiation (%) increased with time and 50% differentiation was observed on December 15 (Fig. 6). Therefore, December 15 was determined as the day of flower bud differentiation. Using the same method, we estimated the time of flower bud differentiation in the 16 crop seasons (Table 1).

In order to minimize errors in the predicted flower bud differentiation values from models (2 to 6), we began analysis with the initial value for each parameter to minimize the residual sum of squares (Table 4) and showed the relationship between predicted values and measured values (Fig. 7). The upper inner boundary point was 30, which was calculated by interquartile range (IQR) and third quartile. Differences of 30 days or more between measured and predicted values were observed in 4 datasets 3, 13, 16, and 17 (Table 1). Excluding these 4 data sets, the root mean square error (RMSE) was 5.3 days.

**Predictive model for premature bolting risk**

Using datasets 4 to 17, we evaluated the relation between the number of head leaves > 1 g and bolting at harvest (Table 1). The percentages of premature bolting were 0–100% in 2011, 33–100% in 2012, 0–83% in 2013 and 0–39% in 2014. Regardless of the year, premature bolting occurred if sowing was performed between September 24 and October 3. Furthermore, bolting occurred only when the number of head leaves

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**Table 4.** Values of parameters used to determine growth response, response to cold treatment stage, chilling and chilling hour.

| Parameter | Value  |
|-----------|--------|
| GR: Growth response parameter | GR.top 1.0, GR.mid 4.0, GR.base 0.0, GR.range 0.5 |
| RS: Response to cold treatment stage parameter | RStop 1.0, RSmid 750, RSbase 0.0, RSrange 10.0 |
| C: Chilling parameter | C.top 1.0, Cmid 13.0, Cbase 0.0, Crange 0.2 |
| CH: Chilling hour | 25 |

*top: top value of sigmoid function, mid: infection point, base: base value of sigmoid function, range: gain of sigmoid function.*
(> 1 g) at flower bud differentiation was less than 6.5 leaves (Fig. 8).

For the sowing periods of September 24, 27, and 30, bolting was observed in seven out of eight plants (88%) with a head weight of 1.8 kg and over (Fig. 9A). On the other hand, when sowing was performed on October 7, no bolting was observed in any of four plants with a head weight of 1.8 kg and over (Fig. 9B).

To evaluate the relationship between the number of leaves and bolting, we used the sowing date of September 24, 2014 (dataset 14) and the flower bud differentiation period of December 15 (Fig. 10). At flower bud differentiation, the number of head leaves > 1 g was 3.5, indicating high bolting risk. Indeed, bolting at harvest (head weight: 1,594 g) was as high as 36.7% and the number of total leaves was 46. In contrast, sowing on September 29, 2014 (dataset 16) produced plants with 16 head leaves (> 1 g) at flower bud differentiation and 59 total leaves harvest, with no evidence of premature bolting, although the head weight (1,638 g) was comparable to that observed above.

The relation between flower bud differentiation and the number of head leaves > 1 g was simulated using meteorological data in 2019 and assuming that sowing was done between September 10 and October 10. The relation was expressed as $y = 0.0247x - 25.954$ ($R^2 = 0.9987, P < 0.001$) (Fig. 11).

#### Discussion

**The relationship between leaf number and premature bolting**

When the number of leaves in cabbage is counted,
Fig. 11. Relation between estimated number of head leaves (> 1 g) and cumulative temperature in the flower bud differentiation period. Numbers in figure indicate sowing day. Assuming that sowing is done seven times at 5 day intervals between September 10 and October 10, the number of head leaves > 1 g was estimated using the formula (y = 0.0248x - 26.109) in Figure 4 and the daily average temperature in 2019.

Estimation of head leaf number
There was a positive correlation between cumulative temperature above 0°C and the number of head leaves (> 1 g) in each year (Fig. 4). However, the correlation coefficient was lower when the three years’ data were combined (r = 0.933). In particular, yearly variations were large in the earlier stage, when the number of head leaves > 1 g was one to two, and after this stage, the rate of leaf appearance was almost the same across three years. As reported by Hamamoto (1992) and Milford et al. (1985), the development of leaves is dependent not only on temperature, but is also influenced by soil moisture and light intensity. These weather conditions also fluctuate yearly and influence cabbage from sowing to the initial head formation stage according to cumulative temperature. In this study, we considered that leaf emergence speed in relation to yearly cumulative temperatures was the same during from 2012 to 2014. Our model can predict the number of head leaves (> 1 g) with even greater accuracy by adjusting with data on the number of head leaves collected at an early stage.

Predictive model for flower bud differentiation
This study developed a model (DVI) to estimate the flower bud differentiation period by the integration of DVR. DVI uses daily average temperature, not maximum and minimum temperature, and is therefore DVI is compact. However, four out of 16 datasets showed more than 30 days difference in the estimated days and they were considered outliers in this study. As the flower bud differentiation period differed by a maximum of...
one month even if the sowing date was the same, it is difficult to estimate the flower bud differentiation period precisely. The sowing dates were after September 29 in all four datasets, but the exact reason why they were outliers is not clear. Recently, low temperature response of the cabbage FLOWERING LOCUS C (FLC) orthologs gene cluster on floral induction was reported (Itabashi et al., 2019). Further studies on the relationship between gene expression and plant age or cold treatment may help to clarify the causes of outliers and contribute to more accurate estimates of flower bud differentiation.

**Predictive model for premature bolting risk**

Figure 9A shows that the bolting level increased with time if cabbage was sown on high risk sowing dates. Thus, caution is needed in terms of harvest dates. To avoid bolting, this study presented a prediction model mainly based on daily temperature. This model was improved by sampling in the early head formation stage and determining the parameter “b” in equation (7) \( y = 0.0248x + b \). In addition, the present model helps to decide the sowing date with low bolting risk by estimating cumulative temperature based on weather forecasts.

Our data showed that the number of head leaves > 1 g continued to increase even after flower bud differentiation (Fig. 10). This can be explained by the growth of differentiated leaves < 1 g. In Figure 10A, the number of total leaves after flower bud differentiation reached a plateau, while in Figure 10B, flower bud differentiation was late and thus the number of total leaves continued to increase. Although the number of total leaves, including fallen leaves and small differentiated leaves, affects bolting, the present study enabled prediction of premature bolting from the number of head leaves > 1 g.

There was a highly significant correlation between cumulative temperature and the number of head leaves > 1 g (Fig. 11). Since the number of head leaves > 1 g at flower bud differentiation of 6.5 is considered an indicator of premature bolting, the cumulative temperature of 1,058°C corresponded to the head leaves number of 6.5. These results suggest that there is high bolting risk if cumulative temperature between sowing and harvest day is less than 1,058°C, which indicates sowing dates after September 30. Consequently, Figure 11 enabled estimation of bolting risk based on the cumulative temperature at flower bud differentiation. However, we used the actual cumulative temperature data in 2019 in Figure 11. In practice, we need to use the average year value before sowing and then predict premature bolting. In this case, the prediction model can be improved by substituting cumulative temperature data with actual data after sowing.

Another concern is that as each of the models used in this study is based on ‘Kinkei-201’, so it is necessary to perform additional studies on the relation between cumulative temperature and leaf number in order to apply it to other cabbage varieties.

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