Morphological and phenological adaptation for convergent development of tillers in Widely spaced wheat sown on different dates

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ABSTRACT
This study analyzes the dynamics of leaf and tiller emergence and development of widely spaced wheat sown on different dates. We used the phyllochron (time interval between the emergence of successive leaves at the same stage), site filling (the relative rate of increase in the number of tillers per phyllochron), and indices associated with ‘the synchronous growth’. Number of productive tillers per plant (including the main shoot, MS) reached 45.7 in early sowing, and significantly decreased by the delay in sowing. The increase in tiller number with the elapse of leaf age (Haun stage) of MS (HS_{ms}) was almost the same as that expected from synchronous growth until HS_{ms} 8.6 and site filling before this HS_{ms} was close to 0.481. The phyllochron was shortest in MS and increased in axillary tillers with increasing tiller order and position, but the differences in phyllochron between MS and axillary tillers within a plant were significant only at higher order and positions of tillers. The time of emergence of tillers was always advanced in comparison with that expected from synchronous growth. A very close negative linear relationship was found between the number of days from emergence to heading of tillers and the time of their emergence expressed as day of year; this relationship was independent of the tiller order, position and sowing date. Although the phyllochron gradually became longer, a progressive decrease of the leaf and spikelet per spike with the increasing tiller order and position may contribute considerably to the convergence to heading time.

Introduction
Dry matter production of Poaceae crops is largely determined by the size of the leaf area, which is regulated by the kinetics of emergence, growth, and senescence of tillers and leaves. Detailed quantitative description of tiller and leaf dynamics may contribute to increasing crop yield potential and to improving the accuracy of crop simulation models. The development of Poaceae crops has been quantified based on the concept of building canopies by the formation, growth and senescence of phytomers, which is the unit comprised of the leaf, axillary bud, node, and internode (McMaster, 2005). Based on the phytomer concept, leaf and tiller number dynamics in wheat are frequently quantified by the phyllochron (time interval between the emergence of successive leaves at the same stage; Wilhelm & McMaster, 1995) and site filling (the relative rate of increase in the number of tillers per phyllochron; Bos & Neuteboom, 1998; Davies, 1974; Neuteboom & Lantinga, 1989).
The phyllochron and its reciprocal, the leaf appearance rate (LAR) are used to analyze the genetic and environmental effects on plant development (Alzueta et al., 2012; Ochagavia et al., 2017) and in growth simulation models (Abichou et al., 2018; Baumont et al., 2019; Evers et al., 2007). The phyllochron is affected by sowing date (Kirby et al., 1985; Miralles et al., 2001; Mulholland et al., 1997), temperature, day length (Cao & Moss, 1994), and N status of soil (Longnecker et al., 1993). Site filling, or tillering is controlled by genetical and environmental factors. Tiller number dynamics is the result from the number of tillers that grow to tillers among the total number of tiller buds, so that the outgrowth of tillers largely depends on the availability of resources such as nutrients (Dreccer et al., 2013) and light intensity (Bos & Neuteboom, 1998). In addition, light quality (red/far-red ratio, R:FR) is closely related to tiller production and abortion (Ballaré & Casal, 2000; Evers et al., 2006; Sparkes et al., 2006; Toyota et al., 2014).

Because tillering ceased earlier at higher population densities in wheat (Evers et al., 2006), the lower the planting density, the higher the number of tillers emerge from a plant. Katayama (1951) devised ‘the concept of uniform cohorts’ which illustrates the regularity of leaf and tiller dynamics of isolated rice and wheat that has many emerged tillers. Goto and Hoshikawa (1988) defined as ‘the synchronous growth’ on the basis of a putative growth pattern derived from Katayama’s concept. The synchronous growth assumes that the potential number of tillers per phyllochron follows the Fibonacci series (one tiller increase $e^{0.481}$ times per one phyllochron). In reality, however, the increase of leaf and tiller number in rice not always match with that expected from synchronous growth (Goto & Hoshikawa, 1988).

In the present study, we investigated the morphology and development of leaf and tiller in widely spaced wheat sown on three different dates to expand the number and variation of tillers. The result showed that the number of tiller emergence per phyllochron was almost the same as that expected from synchronous growth until a certain leaf age (Table 3). This result raises the question of whether the emergence of individual tillers coincides with the timing expected from synchronous growth, or varies by the tiller positions. And if it varies by its position, the regularity that enables the number of tiller emergence follows the Fibonacci series should be elucidated.

On the other hand, the mechanisms of convergent development of wheat with a wide range of sowing dates are not fully understood. For example, the time of flowering in winter wheat sown with a wide range of sowing dates from September to March (181 days) in southwestern Scotland narrowed to only 16–20 days from June to July (Hay, 1986). In Australia, a 113-day difference in sowing dates from May to July narrowed to 38–47 days in spring and winter type wheat, respectively (Manupeerapan & Pearson, 1993). Thus, cereal crops are able to converge on the most favorable period (Hay & Kirby, 1991), but the underlying mechanisms remain unclear.

In the present study, we found a very close negative linear relationship between the number of days from emergence to heading of tillers and the time of their emergence expressed as day of year regardless of the tiller order, position and sowing date (Figure 5(a)). The convergence of development within and between plants shown in Figure 5(a) should be accompanied by a progressive shortening of the phyllochron (acceleration of leaf appearance rate) or a decrease in the number of organs such as final leaf number and the number of spikelets per spike. Our second question is what morphological and phenological regulation exists that enables convergent development of heading time.

This study investigates the dynamics of leaf and tiller emergence and development of widely spaced wheat sown on three different dates. The objectives were to: (1) explore whether the emergence of individual tillers coincides with the timing expected from synchronous growth and if not, what regularity enables the number of tiller emergence to follow the Fibonacci series, and (2) elucidate the mechanisms that underlie the linear relationship between the number of days from emergence to heading of tillers and the time of their emergence expressed as day of year. For these purposes, we analyze the dynamics of leaf and tiller emergence and development using the phyllochron, site filling, and indices associated with ‘the synchronous growth’.

Materials and methods

Study site, plant material and treatments

A field experiment was conducted under natural climatic conditions in the experimental field of Kagawa University, Japan (E 134°7', N 34°16') during the 2002–2003 growing season. Spring wheat (Triticum aestivum L., ‘Sanukinoymte 2000’) were sown manually at a 2 cm depth with wide spaces (0.7 m × 0.8 m) on November 5 (early), November 19 (middle), and December 3 (late) in three replicated plots (1.4 m × 1.6 m) containing four plants each. Compound fertilizer (N, 0.10 g g⁻¹; P₂O₅, 0.08 g g⁻¹; K₂O, 0.10 g g⁻¹) was applied twice at the seedling emergence and spike formation stages, at a rate of 5.4 g m⁻² of N.

Air temperature, daily total solar radiation, and daily precipitation were measured at the meteorological
station of the Faculty of Agriculture, Kagawa University, which is located adjacent to the experimental field. The 15-year mean values from 2001 to 2015 at the meteorological station were compared with the values in 2002–2003 season.

**Plant measurement**

We measured 6 well growing plants (2 plants from each of the 3 replicated plots) selected from 12 plants (4 plants per plots) for each sowing date. The leaf blade length of the youngest and second youngest visible leaves for all visible tillers including the main shoot (MS) was recorded nondestructively twice a week. If the ligule of the youngest leaf had not appeared from the leaf sheath of its parent tiller, only the blade length of the youngest leaves was recorded. Leaf positions and the name of the tiller or MS were recorded using the naming system developed by Klepper et al. (1982, 1983). The combination of ‘L’ (leaf) or ‘T’ (tiller) and ‘#’ (acropetal order of organ within a plant) identifies a specific leaf or tiller as ‘L#’, ‘T#’ or ‘T##’. For example, ‘L1’ is the first foliar leaf of MS, ‘T1’ is the primary tiller derived from the axil of L1 in MS, and ‘T10’ is the secondary tiller emerged from the prophyll of T1. The leaves on tillers are expressed as L#.#: for example, L1.1 is the first leaf of T1, and L10.0 is the prophyll of T10. Tiller order (TO) is expressed as TO1, TO2, and TO3 for the primary, secondary and tertiary tiller, respectively. Coleoptile tiller (T0) were not included in this study because the rhizome separates morphologically between T0 and T1 or higher tillers (Percival, 1921), and T0 is more susceptible than other tillers to environmental conditions (Peterson et al., 1982; Rawson, 1971). In the present study, though T0 did not emerge in most plant, we thinned emerged T0 at the early vegetative stage. The final leaf number (FLN), the time of heading, and number of spikelets per spike for MS and productive tillers were recorded.

Haun stage (HS; Haun, 1975) is a numerical expression widely used to quantify the stages of Poaceae development. HS for MS (HSms) and any axillary tiller (HSt) were used to quantify the development of each tiller. HS was calculated as (Haun, 1975; McMaster, 2005):  

\[ H_{S_{ms}} = (n-1) + L_n/L_n - 1 \]  

(1)

where \( n \) is the number of visible leaves on the tiller, \( L_{n-1} \) is the blade length of the second youngest leaf on the tiller, and \( L_n \) is the blade length of the youngest leaf extending from the enclosing leaf sheath. The data used were recorded from the appearance of the first leaf ligule until the appearance of the last leaf (i.e. flag leaf) ligule of the tillers.

The ellipse of plant and shoot development was expressed as a calendar day or growing degree-days, the cumulative temperature above the base temperature (GDD, °Cd). GDD was calculated using the following equation:

\[ GDD = \sum \left( \frac{T_{max} + T_{min}}{2} - T_b \right) \]  

(2)

where \( T_{max} \) and \( T_{min} \) are daily maximum and minimum temperature, respectively, measured at 1.5 m above the soil surface, and \( T_b \) is the base temperature assumed to be 0°C (Baker et al., 1980; Kirby et al., 1985).

**Terminology of the synchronous growth**

On the basis of the phytomer concept (McMaster, 2005), we used indices associated with ‘the concept of uniform cohorts’ (Hanada, 1993; Katayama, 1951). Goto and Hoshikawa (1988) defined ‘the synchronous growth’ (putative growth pattern derived from Katayama’s concept) as follows: (1) the appearance of leaf N on a tiller is synchronized with that of the first leaf on the tiller, which is formed on the axil of leaf (N – 3), and (2) every tiller in the same plant has the same phyllochron interval. The definitions mean that two phyllochrons separate the emergence of a tiller and its first daughter tiller; the potential number of tillers per phyllochron follows the Fibonacci series (Bos & Neuteboom, 1998; Skinner & Nelson, 1992).

Goto and Hoshikawa (1988) also devised the relative tiller position (RTP), which indicates the acropetal tiller position arranged by the theoretical order of tiller emergence expected from synchronous growth:

\[ RTP = n_1 + n_2 + n_3 + n_4 \ldots + n_i + 2N = \Sigma n_i + 2N \]  

(3)

where \( n_i \) is the number of each digit from left to right of the tiller name, and \( N \) is the number of digit. For example, RTP of T1 is 3 (1 + 2), T10 is 5 (1 + 0 + 4), and T210 is 9 (2 + 1 + 0 + 6). Illustration of the calculation of RTP is shown in Figure 1. Synchronous growth assumes that tillers with the same RTP emerge simultaneously and grow at the same rate (same phyllochron intervals).

The relative tiller age (RA) is the sum of RTP and HS, of axillary tillers (Goto & Hoshikawa, 1988); this index enables an easy comparison of the progress of tiller age within the same RTP group. For example, RA for T1 (RTP 3) at HS1.1 is 4.1 (RA = RTP+HS = 3 + 1.1 = 4.1).

**Analysis of leaf and tiller dynamics**

We defined the time of individual tiller emergence as the day when we first recognized the appearance of
RTP = n₁ + n₂ + n₃ + n₄...ni + 2N = ∑ni + 2N

Number of digit (N=1)

\[ T \begin{array}{c}
\downarrow \\
n_1
\end{array} \quad \rightarrow \quad RTP = 1 + (2 \times 1) = 3 \]

Number of digit (N=2)

\[ T \begin{array}{c}
\downarrow \\
n_1 \downarrow n_2
\end{array} \quad \rightarrow \quad RTP = 1 + 0 + (2 \times 2) = 5 \]

Number of digit (N=3)

\[ T \begin{array}{c}
\downarrow \\
n_1 \downarrow n_2 \downarrow n_3
\end{array} \quad \rightarrow \quad RTP = 2 + 1 + 0 + (2 \times 3) = 9 \]

**Figure 1.** Illustration of the calculation of relative tiller position (RTP).

A new leaf tip of the tiller above the leaf sheath of its parent tiller. To calculate LAR (leaf ("Cd")⁻¹) and its reciprocal, the phyllochron ("Cd" leaf⁻¹) for every shoot, a simple linear regression between HS and GDD was used. The data of the tillers were excluded from the analysis if the significance level of a linear regression was higher than 0.05. If the data were available for three or fewer of six plants, they were excluded from the calculation of the mean value of each tiller for a particular sowing date.

The relationships between tiller number per plant and elapsed HSₘₐₛ were calculated from the plant measurements, but this was inconvenient because the HSₘₐₛ of plants measured was mostly limited to 10, when tiller emergence had not yet ceased. Therefore, a simple linear regression between HSₘₐₛ and GDD was applied to convert the relationship between tiller number per plant and GDD to that between tiller number per plant and elapsed pseudo HSₘₐₛ which assumes that new leaves appear with same phyllochron intervals even after the full appearance of the flag leaf of MS.

The number of tillers per plant plotted against pseudo HSₘₐₛ until the maximum number of tillers followed a sigmoidal curve, so that the Richards function (Richards, 1959) was applied to the relationship:

\[
\text{no. tillers plant}^{-1} = A \left[1 + e^{(b - kHS_{ms})}\right]^{-1/n} \quad (4)
\]

where A is the asymptotic maximum number of tillers per plant, e is the base of the natural logarithm, and n, b, and k are model parameters.

Wheat tillering dynamics can be quantified with the phyllochron and site filling, the relative rate of the increase in the number of tillers per phyllochron (Bos & Neuteboom, 1998; Davies, 1974; Neuteboom & Lantinga, 1989). Site filling (Fs) is generally calculated as:

\[
Fs = \frac{(\ln T_1 - \ln T_2)}{(t_2 - t_1)} \quad (5)
\]

where T₁ and T₂ are tiller numbers (including MS) at the time t₁ and t₂, expressed as HSₘₐₛ. In this study, site filling was calculated graphically from the relationship between tiller number expressed on a natural log scale and HSₘₐₛ (Gautier et al., 1999). Because two lines appeared to be jointed at a point, a piecewise regression model (Toms & Lesperance, 2003) was fitted:

\[
\ln(\text{no. tillers plant}^{-1}) = \begin{cases} 
  b + FS_1x, & \text{if } x \leq a \\
  b + FS_1a + FS_2(x - a), & \text{if } x > a
\end{cases} \quad (6)
\]

where x is pseudo HSₘₐₛ, b is the intercept of the model, and FS₁ and FS₂ are the slopes of the lines corresponding to site filling before and after the breakpoint (a), respectively.

The index to evaluate the synchrony of tiller emergence is HS-delay (the difference between the HS of a tiller and the HS of its parent tiller above the leaf from which the axil tiller emerges; Bos & Neuteboom, 1998) (Figure 2). HS-delay evaluates the synchrony between a tiller and its parent tiller. The HSₘₐₛ-RA index evaluates the synchrony of emergence of a tiller and its theoretical time of emergence in HSₘₐₛ assumed from synchronous growth. The timing of the calculation of HS-delay and HSₘₐₛ-RA was at the first measurement of leaf blade length after the appearance of the leaf ligule of the first leaf of the tiller. Synchronous growth assumes that tiller number increases with elapsed HSₘₐₛ according to the Fibonacci series, and in this case HS-delay becomes 2 (Bos & Neuteboom, 1998) and HSₘₐₛ-RA becomes 0. For example, HS-delay of T₁ (1.45) in Figure 2 means that HSₜ of T₁ is advanced 0.55 compared with the HSₜ expected from synchronous growth. For another example, if the HSₜ of T₁ is 1.6 at HSₘₐₛ 7.1, this means that HSₜ of T₁ is advanced 0.5 compared with the HSₜ expected from synchronous growth. Because the RA of T₁₁ at HSₜ 1.1 is 7.6 (RA = RTP+HSₜ = 6 + 1.6 = 7.6), therefore HSₘₐₛ-RA is −0.5 (i.e. 7.1 − 7.6 = −0.5).

**Statistical analysis**

Differences in morphological characteristics, phenological stage and duration, phyllochron, site filling, breakpoint, HS-delay and HSₘₐₛ-RA among sowing dates were determined using ANOVA. When ANOVA detected a significant difference, the means were compared using the Tukey’s HSD test. Differences were considered significant at p < 0.05. All statistical tests, linear regression and non-linear model fitting
were performed in JMP 13 statistical software (SAS Institute Japan, Tokyo, Japan).

**Results**

**Meteorological condition**

Figure 3 shows the changes in mean temperature, daily total solar radiation, and precipitation at 10-day intervals during the 2002–2003 experimental periods. Hereafter, all significant differences in Figure 3 are based on the comparison with the 95% confidence interval of the 15-year means (2001–2015). The mean temperature in November was significantly lower than the 15-year means, but there was no particular trend in mean temperature after December. Solar radiation in 2002–2003 season was significantly higher than the 15-year means for many periods. There was no rainfall in mid-November. Precipitation was significantly lower in late March and mid-April.

**The number of tiller, final leaf number, and duration from sowing to heading in MS**

The number of tillers per plant and the duration from sowing to heading of MS (in days and accumulated GDD) were significantly decreased by delay in sowing, whereas the final leaf number on MS was not affected by sowing date (Table 1). We attributed the decrease in the number of tillers to a decline in the percentage of

| Sowing   | Number of tillers per plant | FLN on MS | Duration |
|----------|-----------------------------|-----------|----------|
|          |                             |           | Days     | GDD (°Cd)   |
| Early    | 45.7 a†                     | 10.2      | 159 a    | 1122 a      |
| Middle   | 38.2 b                      | 10.2      | 148 b    | 1043 b      |
| Late     | 35.5 b                      | 10.2      | 137 c    | 962 c       |

†Means (n = 6) followed by the different letter within a column are significantly different between sowing date by Tukey’s HSD (p < 0.05).
tiller emergence at RTP 8 and above, whereas the percentage of emergence was not affected by sowing date below RTP 8 (Figure 4).

![Percentage of tiller emergence](image)

Figure 4. The percentage of emerged tillers relative to the potential number of tillers according to the synchronous growth theory. Values are the means and the error bars show standard error of the mean (n = 6). Numbers above bars are potential numbers of tillers for each relative tiller position (RTP). The percentages at RTP < 8 were 100%.

**Table 2. Duration of tillering (without MS) and heading (including MS) within a plant and the longest and shortest duration from emergence to heading of tillers (including MS) in wheat sown on different dates.**

| Sowing  | Duration of tillering within a plant/ (without MS) | Duration of heading within a plant/ (including MS) | Longest | Shortest |
|---------|---------------------------------------------------|--------------------------------------------------|--------|---------|
|         | Days  | GDD (°Cd) | Days  | GDD (°Cd) | Days  | GDD (°Cd) | Days  | GDD (°Cd) |
| Early   | 125.8 a | 830.2 a | 24.3  | 407.7     | 141.8 a | 832.4 a | 17.2 a | 273.6 |
| Middle  | 90.5 b  | 639.0 b  | 19.3  | 331.6     | 134.5 b | 714.4 b | 18.0 b | 292.2 |
| Late    | 77.7 c  | 721.4 b  | 22.0  | 384.3     | 123.3 c | 655.4 ab | 14.3 ab | 240.0 |

*Means (n = 6) followed by the different letter within a column are significantly different between sowing date by Tukey's HSD (p < 0.05).

**Duration of tillering and heading and from emergence to heading**

The duration of tillering (without MS) within a plant decreased significantly with the delay in sowing, whereas the duration of heading (including MS) was not differed (Table 2). The longest and shortest duration from emergence to heading of tillers was significantly decreased by the delay in sowing (Table 2). The difference in the duration of tillering within a plant (78–126 days) converged to a 19–24 days difference in the duration of heading. The longest duration from emergence to heading in tillers (123–142 days in MS) was shortened to 14–18 days for the tillers that had the shortest duration from emergence to heading (Table 2).

The number of days from emergence to heading of tillers was significantly associated with the day of year (DOY, from 2003/1/1) of tiller emergence regardless of the sowing date and tiller order (Figure 5(a)). On the other hand, the relationship between days from emergence to heading and the time of emergence in GDD,

![Relationship between days from emergence to heading](image)

Figure 5. Relationship between days from emergence to heading and (a) day of year (DOY) at tiller emergence and (b) GDD at tiller emergence. In (a), the strait line represents linear regression for the pooled data of all tillers and sowing dates; the coefficient of determination (R²) and the level of significance (p) are shown. In (b), the curve represents nonlinear model fitting for each sowing date; the data for main shoots were excluded. Early: Days = 169.748 x e^(-0.00261 x GDD) + 5.164, middle: Days = 224.172 x e^(-0.00242 x GDD) + 0.5083, late: Days = 253.766 x e^(-0.00155 x GDD) - 26.539. TO, tiller order; RMSE, root mean square error.
except for the MS, was well fitted by an exponential decay model for each sowing date (Figure 5(b)). The days from emergence to heading shortened exponentially with the delay in tiller emergence in GDD. The difference in days from emergence to heading was largest at around 300 °Cd and gradually converged as GDD increased.

**Tiller number dynamics and site filling**

The linear relationship between HS$_{ms}$ and GDD was highly significant regardless of the sowing date (Figure 6). The mean leaf appearance rate of MS increased significantly with delay in sowing (data not shown). Tiller number showed a sigmoidal increase with elapsed pseudo HS$_{ms}$.

![Figure 6. Relationships between HS$_{ms}$ and GDD for wheat. Lines represent linear regression for each sowing date. Data and fitted lines are shown for a representative plant for each sowing date. Leaf appearance rate ([LAR, leaf °Cd$^{-1}$]) and coefficient of determination ($R^2$) are also shown. *** $p < 0.001.$](image)

No clear differences among sowing dates were observed until pseudo HS$_{ms}$ 8, but then after the increase in tiller number was slower with later sowing dates.

The same relationships are shown in Figure 8, except that tiller number per plant is expressed on the natural log scale. For all plants, two lines were jointed at a point, resulting in ‘broken-stick’ shape. Thus, site fillings before and after the breakpoint was estimated by fitting a piecewise regression model [6]. The sowing date did not affect the F$_{S1}$ and the breakpoint, but the F$_{S2}$ of late sowing was significantly lower than that of middle sowing (Table 3, Figure 8). The F$_{S1}$ of every sowing date was very closed to the theoretical value (0.481) of the Fibonacci series assumed from synchronous growth.

**Phyllochron and the timing of tiller emergence**

The phyllochron of the tillers estimated by the linear regression model between HS$_t$ and GDD for every emerged tillers decreased significantly with the delay in sowing except in TO1 (RTPS and RTP7) and TO3 (Figure 9). A significant difference in phyllochron among tillers

![Figure 8. Relationships between the natural log of the number of tillers per plant (including the main shoot) and pseudo HS$_{ms}$. Broken-stick lines represent nonlinear model fitting (piecewise regression model [6]). Data and fitted lines are shown for a representative plant for each sowing date.](image)

![Table 3. Site filling before and after the breakpoint estimated by a piecewise regression model, breakpoint (HS$_{ms}$), and the intercept of the model.](table)

| Sowing  | F$_{S1}$ | F$_{S2}$ | Break-point (HS$_{ms}$) | Intercept |
|---------|---------|---------|------------------------|-----------|
| Early   | 0.481   | 0.107   | ab                     | -0.830    |
| Middle  | 0.480   | 0.146   | a                      | -0.836    |
| Late    | 0.479   | 0.050   | b                      | -0.894    |

*Site filling before (F$_{S1}$) and after (F$_{S2}$) the breakpoint.

*Mean (n = 6) followed by the different letter within a column are significantly different between sowing date by Tukey’s HSD ($p < 0.05$).
Figure 9. Phyllochron of tillers estimated by a simple linear regression model between the Haun stage of the tiller (HS) and GDD for every individual tiller. Phyllochrons were classified by the tiller order (TO) and relative tiller position (RTP). Data points are means and error bars represent standard error of the mean. Number of data points for the mean varied according to the order and position of tillers. Different letters above the symbols indicate significant differences among sowing dates by Tukey's HSD (p < 0.05). Letter order corresponds to the vertical position of the symbols within the same RTP. ** in (c) indicates significant difference between sowing dates by t-test (p < 0.01).

Within a plant was observed in early and middle sowing but not in late sowing (Table 4). The phyllochron of MS is the shortest or second shortest among tillers within a plant and it gradually became longer with increasing the order and position of tillers in early and middle sowing (Table 4, Figure 9).

The HS-delay was significantly larger in late sowing than in early and middle sowing in TO1 (RTP 3 – 5 and RTP 7) and TO2 (RTP 6 and RTP 7), indicating that the time of tiller emergence from its parent tiller in late sowing tended to be later than in early and middle sowing (Figure 10). However, the HS-delay were below 2 in all tillers except RTP 3 in TO1 (2.03) and RTP 8 in TO3(2.01) in late sowing, and it was the lowest at RTP 5 in TO1 in early sowing (1.25). The time of tiller emergence from its parent tiller was nearly equal or slightly shorter than that expected from synchronous growth, with a maximum of 0.75 phyllochron at RTP5 in TO1 (Figure 10). The pattern of fluctuation in HS-delay was similar in early and middle but not in late sowing.

Because HS_{ms}-RA measures the difference between HS_{ms} and HS_{t}, HS_{ms}-RA, and HS-delay in TO1 give the same results (i.e. Figures 10(a) and Figures 11(a) shows the same results except that there is a gap of 2 between HS_{ms}-RA and HS-delay). The HS_{ms}-RA values of most tillers were negative except TO1 at RTP 3 (0.03) and TO2 at RTP 5 (0.06) in late sowing, indicating that the time of tiller emergence was nearly equal to or earlier than the time expected from synchronous growth (Figure 11). HS_{ms}-RA tended to decrease with an increase in the order and position of tillers. The lowest HS_{ms}-RA was TO3 at RTP 10 in early sowing, indicating that this tiller emerged 2.18 phyllochron earlier in HS_{ms} than expected from synchronous growth (Figure 11). The patterns of fluctuation in HS_{ms}-RA were similar in early and middle but not in late sowing (Figure 11).

**Final leaf number and number of spikelets per spike**

The final leaf number of MS were same for all sowing dates, and they were the largest among the tillers within a plant (Figure 12). The final leaf number of tillers were progressively decreased with an increase of the order and position of tillers, and those in early sowing was
was significantly larger than in middle sowing. However, there were no clear differences on the final leaf number between different tiller order in RTP 7 or higher. Synchronous growth estimates the final leaf number of a tillers as the difference in final leaf number of MS and RTP of the tiller. The final leaf number of all tiller were larger than the final leaf number expected from synchronous growth except TO1 at RTP 3 and RTP 5 in middle sowing (Figure 12).

The number of spikelet per spike was significantly larger in early sowing than in middle and late sowing in most tillers (Figure 13). The number of spikelets per spike in MS was not the largest among the tillers within a plant. In contrast to the progressive decrease of final leaf number with increase of RTP and tiller order, the number of spikelet per spike was not differed in MS and the tillers in TO1 at RTP 3–7, TO2 at RTP 5–8.

**Discussion**

Although many studies have been published on leaf and tiller development using the phyllochron and site filling in wheat grown at commercial population densities, the mechanisms of synchronous and convergent development among a large number of productive tillers within a widely spaced planted wheat are scarcely documented. Our experiment differs from the experiment with the wide range of sowing dates (Hay, 1986; Manupeerapan & Pearson,
in that the range of sowing time was narrow and the number of tillers per plant is much large. However, there would be similar mechanism that underlie the convergent development between our study and previous studies.

Tiller number dynamics and site filling

In the present study, the number of tillers per plant was significantly decreased by the delay in sowing, whereas the final leaf number on MS was not affected (Table 1). We attributed the decrease in tiller number to a decline in the percentage of tiller emergence at RTP 8 and above (Figure 3). Using the Richards model [4], we revealed graphically that the increase in tiller number with pseudo HSms slowed and the difference among sowing dates became apparent at about pseudo HSms 8.6 (Figure 7). The piecewise regression model provided quantitative evidence that the tiller number increased at nearly the same rate as the theoretical rate expected from synchronous growth (site filling = 0.481) until HSms 8.6, on average, and after the breakpoint site filling was higher in the early and middle than in late sowing (Table 3, Figure 8).

Phyllochron

The relationship between the number of leaves and GDD may be either linear (Baker et al., 1980; Dreccer
et al., 2013) or bilinear (Hay & Delecolle, 1989; Miralles et al., 2001; Ochagavia et al., 2017). In our study, the relationships between HS and GDD was linear regardless of sowing time (Figure 6). This means the phyllochron may vary by sowing date, but it is constant from emergence to the appearance of final leaf. The phyllochron of MS is the shortest or second shortest among tillers within a plant and it gradually became longer with increasing the order and position of tillers in early and middle sowing (Table 4, Figure 9), but we did not find any regularity in the difference of phyllochron among tillers. Functional genomics studies largely expand knowledge of controlling flowering (Laurie et al., 2004) and QTL controlling phyllochron were identified in rice (Miyamoto et al., 2004) and in durum wheat (Sanna et al., 2014). Our results, however, suggested that precise estimation of the phyllochron under various environmental conditions is still difficult.

**Timing of tiller emergence and the difference from synchronous growth**

The first objective of this study was to explore whether the emergence of individual tillers coincides with the timing expected from synchronous growth and if not, what regularity enables the number of tiller emergence follows the Fibonacci series. For these purposes, we evaluate the synchrony of the time of tiller emergence using the HS-delay and HS<sub>ms</sub>-RA indices, which measure the difference between the HS of a tiller and the HS of its parent tiller or HS<sub>ms</sub>, respectively. The former index measures the difference in the time for every axillary node and the latter represents the time difference from a standard expected from synchronous growth. Both indices showed that the time of emergence of tillers was always earlier than the time that expected from synchronous growth (Figures 10 and Figures 11). Although the differences in HS-delay were small, but their accumulation resulted in a larger difference in HS<sub>ms</sub>-RA. The maximum difference of the time of emergence from the time expected from synchronous growth was 0.75 phyllochron in HS-delay (Figure 10) and 2.18 in HS<sub>ms</sub>-RA (Figure 11). Thus time of tiller emergence was always earlier than the time expected from synchronous growth, and phyllochron tended to be longer with increasing tiller order and RTP. The trade-off between early timing of tiller emergence and longer phyllochron would enable the number of tiller emergence to follow the Fibonacci series. The pattern of fluctuation in HS-delay and HS<sub>ms</sub>-RA was similar in early and middle but not in late sowing, however, there were no clear regularity in the differences of HS-delay and HS<sub>ms</sub>-RA among the tillers.

**Convergent development to heading**

The second objective of this study was to elucidate the mechanisms that underlie the linear relationship between the number of days from emergence to heading of tillers and the time of their emergence expressed as day of year (Figure 5a).

This convergence of development within and between plants should be accompanied by a progressive shortening of the phyllochron (acceleration of leaf appearance rate) or a decrease in the number of organs such as final leaf number and the number of spikelets per spike (Figures 12 and Figures 13). Stern and Kirby (1979) showed that the rate of spikelet initiation increased and the duration decreased with later sowing. Our previous study showed that the rate of initiation in leaf decreased, but that in spikelet increased, and the duration of spikelet initiation phase decreased by the delay of sowing (Toyota et al., 2004).

Our results, however, showed that the phyllochron of MS was the shortest or second shortest among tillers within a plant and it gradually became longer with increasing the order and position of tillers within a plant, although the differences were not significant except for some tillers higher than RTP 7 (Table 4, Figure 9). These results are consistent with those of Bos and Neuteboom (1998), who reported a longer phyllochron in T0, T1, T2 than in MS, and Li and Yamazaki (1994), who showed slower leaf appearance rate in tillers than in MS. In our study, the phyllochron seemed to have counteract effect to shortening of the number of days to heading. On the other hand, a progressive decrease in the final leaf number (Figure 12) and in the number of spikelets per spike (Figure 13) with the increase in the order and position of tillers within a plant may contribute considerably to the convergence to heading time.

**Conclusion**

 Widely spaced planting density produced many productive tillers per plant and different sowing date expand further the variation of the time of emergence and heading. We analyzed the dynamics of leaf and tiller emergence and development using phyllochron, site filling, and indices associated with ‘the synchronous growth’.

 The number of tillers per plant was significantly decreased by the delay in sowing, while the number of tiller emergence per phyllochron was almost consistent
with that expected from synchronous growth for all sowing dates until HS$_{50}$, 8.6, on average. The time of tiller emergence was always earlier than the time expected from synchronous growth, and phyllochron tended to be longer with increasing the order and position of tillers. The trade-off between the early timing of tiller emergence and the longer phyllochron would contribute to the number of tiller emergence follows the Fibonacci series. However, we did not find any regularity on the deviation in the time of tiller emergence or fluctuation of phyllochron in individual tillers.

A very close negative linear relationship was found between the number of days from emergence to heading of tillers and the time of their emergence expressed as day of year regardless of the tiller order, position, and sowing date. Although the phyllochron gradually became longer, a progressive decrease of the leaf and spikelet per spike with the increase in the order and position of tillers may contribute considerably to the convergence to heading time. Coordination between the phyllochron and the degree of reduction in the number of organs per tiller seemed to be strongly influenced by the time of their emergence.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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