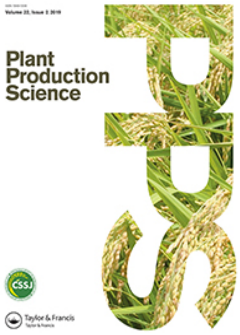

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Evaluation of dry matter production and yield in early-sown wheat using near-isogenic lines for the vernalization locus *Vrn-D1*

Hiroko Sawada[a], Hiromi Matsuyama[a], Hitoshi Matsunaka[b], Masaya Fujita[c], Natsumi Okamura[a], Masako Seki[a], Hisayo Kojima[c], Chikako Kiribuchi-Otobe[c], Toshiyuki Takayama[d], Shunsuke Oda[e], Kazuhiro Nakamura[b], Tetsufumi Sakai[b], Morio Matsuzaki[a] and Kenji Kato[f]

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ABSTRACT

Wheat (*Triticum aestivum* L.) grain yield is predicted to decrease in the future because of an increase in air temperature globally. To clarify the effects of the vernalization response gene in wheat to warmer winters, we compared dry matter production and grain yield between spring wheat 'Asakazekomugi' and its winter-type near-isogenic line (NIL) carrying different alleles of the vernalization response gene *Vrn-D1* under early-, standard-, and late-sowing conditions. Under early-sowing conditions, dry matter production of the NIL carrying the winter allele of *Vrn-D1*, named Asa (Vrn-D1b), exceeded that of 'Asakazekomugi' from mid-March (after stem elongation in Asa (Vrn-D1b)) when the temperatures rose. Tiller number and leaf area index under early-sowing conditions were consistently higher in Asa (Vrn-D1b) than in 'Asakazekomugi' from mid-March onward. It was suggested that the early-sown 'Asakazekomugi' could not effectively absorb solar radiation to produce dry matter because of the acceleration of stem elongation caused by the *Vrn-D1* gene during the cold season. The grain yield of Asa (Vrn-D1b) with early sowing was higher than with standard sowing. In contrast, the grain yield of 'Asakazekomugi' was lower in the early-sown crop than in the crop sown at the standard date. These results suggested that the higher grain yield of Asa (Vrn-D1b) than that of 'Asakazekomugi' under early-sown conditions could be due to Asa (Vrn-D1b) maintaining high dry matter production after the jointing stage by suppressing acceleration of growth caused by warm conditions after sowing.

Abbreviations: CGR: crop growth rate; HI: harvest index; LAI: leaf area index; NIL: near-isogenic line; SNP: single-nucleotide polymorphism

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CLASSIFICATION
Crop physiology

1. Introduction

Climate change is expected to seriously affect wheat (*Triticum aestivum* L.) production around the world in the future. Global wheat production is estimated to fall by 6% for each 1°C further temperature increase (Asseng et al., 2015). In Japan, according to an analysis of data collected over 45 years, the yield of wheat variety 'Norin 61' decreased with increasing mean air temperature before heading (Minoda, 2010). It is expected that rising temperatures will accelerate physiological development in wheat, such as time to jointing (stem elongation) and heading, resulting in an increased risk of frost injury to young spikes and a decrease in dry matter production (Nakazono, 2010).

Winter wheat requires a period of low temperature ('vernalization') to trigger the change from the vegetative phase to reproductive development. Kakizaki and Suzuki (1937) classified Japanese wheats into seven grades (degree of growth habit: I–VII) according to their heading behavior upon successive sowing from early spring to early summer. Grade I corresponds to the extreme spring habit and grade VII to the extreme winter habit. In the south-western and central parts of Japan, early-maturing cultivars mostly with spring-growth habit (grade I–III) have been developed, to avoid the disease and preharvest sprouting caused by monsoon rain. However, spring-type cultivars are prone to suffer frost injury, since the stem elongates actively on the occasional warm days during or before winter under early-sowing condition. It has been suggested that winter-type wheat can avoid frost injury to young spikes through delayed ear primordia initiation and stem elongation in warmer winters (Fujita, 1997; Fujita Taniguchi et al., 1995;

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Seki et al., 2007). In contrast, although winter-type wheat is known to produce substantial numbers of tillers, dry matter production and grain yield of winter-type wheat 'Iwainodaichi' were just slightly higher than or similar to those of spring-type wheat 'Chikugoizumi' when both cultivars were sown early in southwestern Japan (Fukushima, Kusuda, & Furuhata, 2001). Thus, it is not clear how higher temperatures, encountered under early-sowing conditions or in warm winters, affect dry matter production and grain yield in winter-type wheat.

The vernalization requirement is known to be controlled by five genes (*Vrn-A1, Vrn-B1, Vrn-D1, Vrn-D5*, and *Vrn-B3*) in wheat (Law & Wolfe, 1966; Pugsley, 1971, 1972; Yan et al., 2006). Winter-type wheats carry recessive alleles at all these loci. The *Vrn* genotype differs between areas, and *Vrn-D1* has been identified as the main spring-habit gene in Japanese wheat cultivars (Gotoh, 1979; Iwaki et al., 2000). Moreover, the *Vrn-D1* allele alone showed highest frequency among combinations of dominant *Vrn* alleles (*Vrn-A1, -B1, -D1*, and *-B3*) in major Chinese wheat cultivars released since the 1960s (Zhang et al., 2008). Recently, Zhang et al. (2012) have found a new winter allele which differs from the dominant spring allele of *Vrn-D1* by just a single-nucleotide polymorphism (SNP) in the promoter region. The authors designated the new allele as *Vrn-D1b* and showed the lower expression of *Vrn-D1* gene in the homozygous genotype (*Vrn-D1b/Vrn-D1b*) compared with the genotype (*Vrn-D1a/Vrn-D1a*, previously *Vrn-D1*). In Japanese winter-type cultivars, the same SNP in the promoter region of *Vrn-D1* has been identified (Takahashi et al., 2013), and winter-type near-isogenic lines (NILs) without dominant *Vrn* genes in previous studies by Fujita et al. (1995) and Seki et al. (2007) have been observed as NILs with homozygous *Vrn-D1b*.

Zhang et al. (2014) reported that the winter alleles (*vrn-A1vrn-B1vrn-D1*) caused the latest flowering and the highest spikelet number per spike as compared to the other genotypes such as *vrn-A1vrn-B1Vrn-D1* in diverse environments, and high kernel number per spike and grain weight in well-watered environments. Moreover, Meng et al. (2016) reported that the homozygous *Vrn-D1b* genotypes had significantly higher grain yield than the homozygous *vrn-D1* genotypes in northern China. Thus, vernalization response genes could be involved in the genetic control of yield and yield components in wheat.

In a previous study, we showed that spike initiation in spring-habit wheat cultivars with *Vrn-D1a/Vrn-D1a* occurred much earlier than in its NILs (*Vrn-D1b/Vrn-D1b*), under early-sowing conditions, where the average temperature after sowing was higher than that under standard-sowing conditions (Matsuyama et al., 2017). We therefore hypothesized that winter-type NILs carrying *Vrn-D1b* could prevent the shortening of the vegetative growth period caused by early spike development, and *Vrn-D1a* allele could negatively affect dry matter production in wheat under higher temperature conditions after sowing. In this study, to clarify the effect of *Vrn-D1* on dry matter production and grain yield in wheat experiencing higher temperature conditions during the early growth stages, we compared those traits between the spring wheat 'Asakazekomugi' carrying *Vrn-D1a* and its winter-type NIL with *Vrn-D1b*, named Asa (Vrn-D1b), under early-, standard-, and late-sowing conditions.

2. Materials and methods

2.1. Plant materials and field management

The early-maturing spring-habit 'Asakazekomugi' (growth habit: II) carrying *Vrn-D1a* and its NIL with *Vrn-D1b* (previously *vrn*) (Asa (Vrn-D1b); growth habit: IV) developed by backcrossing five times 'Asakazekomugi' as the recurrent parent and a winter-habit cultivar 'Ebisukomugi' as nonrecurrent parent (Fujita et al., 1995) were used in this study.

Field experiments were conducted in the 2015/2016 and 2016/2017 crop seasons in an upland field (light-colored Andosol). The site was NARO Agricultural Research Center located in central Japan (Ibaraki, 36°0′ N latitude, 140°0′ E longitude, 24 m altitude). The seeds of 'Asakazekomugi' and Asa (Vrn-D1b) were sown on 15 October and 14 October 2015/2016 (early sowing); 17 November and 14 November 2015/2016 (standard sowing); and 15 December and 13 December 2015/2016 (late sowing), respectively. A randomized complete block design with three replications was used in all experiments. The plots were approximately 9.3 m^2, each consisting of eight rows 15 cm apart. The sowing rate was 180 seeds m^{-2}. A chemical fertilizer (N:PO:KO = 14:14:14) was applied at a rate of 4.0 g m^{-2} prior to sowing, and ammonium sulfate was applied as a topdressing at a rate of 4.0 g m^{-2} at the jointing stage in both years. The jointing stage was defined as the stage at which the length of the main stem reached 20 mm.

Daily maximum and minimum temperatures (°C) and daily total solar radiation (MJ m^{-2}) during the wheat growing season were obtained from the Weather Data Acquisition System of the Institute for Agro-Environmental Sciences, NARO. We calculated the cumulative solar radiation from the daily total solar radiation.

2.2. Measurements of dry weight, leaf area index (LAI), and yield component

Plants were harvested every 27–33 days starting at tillering stage from each plot at randomly selected sites (0.3 × 0.45 m), and leaves, stems, and spikes were oven dried at 80°C to constant weight, and dry weight was determined. LAI was measured using a LI-COR LAI-2200C Plant Canopy Analyzer (LI-COR) on 12 April and 18 May 2016, and on 17 and 29 March, 14 April, and 17 May 2017. Crop growth rate (CGR, g m^{-2} day^{-1}) was calculated from the difference in dry weights of the aboveground parts between two adjacent sampling dates.

At the mature stage, grain yield m^{-2}, spike number m^{-2}, and 1000-grain weight were determined from two randomly selected 1.0 × 0.45 m subsamples. Grain number per spike was calculated from the spike number, 1000-grain weight, and grain yield. The harvest index (HI) was calculated using the equation: HI = grain yield/aboveground dry weight. The grain yield, 1000-grain weight, and aboveground dry weight were corrected to 12.5% moisture.

2.3. Statistical analysis

IBM SPSS Statistics version 22 software (IBM, Armonk, NY, USA) was used for statistical analyses. Data were analyzed using a two-way analysis of variance. The model was defined as a split-plot design, with the two cropping years as the main plot, three sowing dates as the subplots, the two NILs as sub-subplots, and the three replicates as blocks. Tukey's test or *t*-test was used for pairwise comparison of means ($P < 0.05$).

3. Results

3.1. Effect of sowing date on temperature and crop development rate between 'Asakazekomugi' and Asa (Vrn-D1b)

The daily minimum and maximum temperatures in 2015/2016 during the wheat growing season tended to be higher than the temperatures in the 2016/2017 cropping season in autumn and winter (Figure 1). The differences in the daily mean temperature from sowing to the jointing stage between early and standard sowings for the spring-habit 'Asakazekomugi' with *Vrn-D1a* were +4.5°C and +3.2°C in 2015/2016 and 2016/2017, respectively, whereas the corresponding differences for the NIL with *Vrn-D1b* were +2.3°C and +1.5°C in 2015/2016 and 2016/2017, respectively. The differences between standard and late sowing were in the range −0.2°C to +0.5°C for both wheat lines and both years.

The early-sown 'Asakazekomugi' began stem elongation during January, when the lowest mean temperatures were observed (Table 1, Figure 1). The number of days from sowing to jointing stage in 'Asakazekomugi' was 32–39 days fewer in the early sowing than in the standard sowing, whereas no such shortening of the period was observed between the two sowing dates in Asa (Vrn-D1b) (Table 1). In contrast, there was little difference in heading and maturity dates between

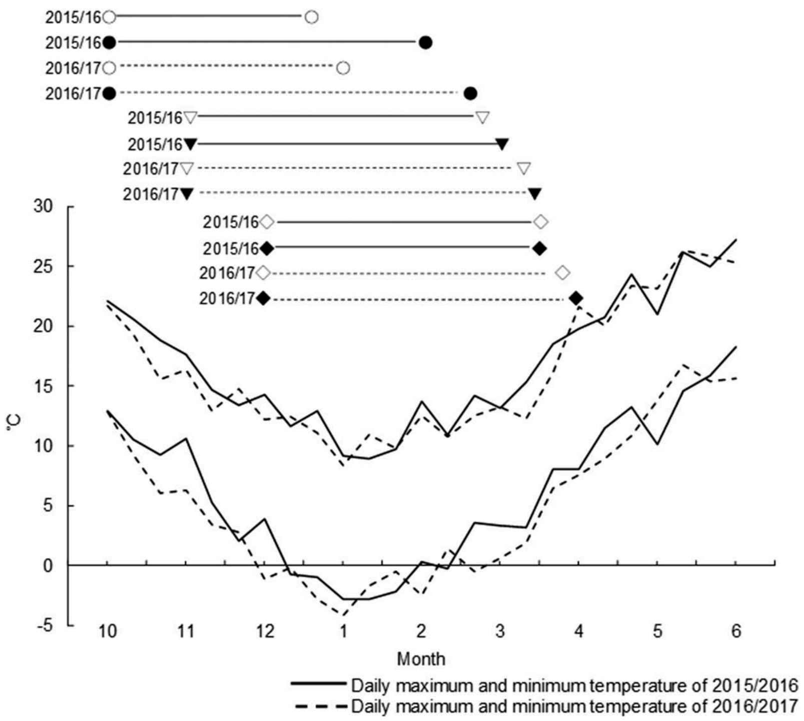

Figure 1. Daily maximum and minimum temperatures during wheat growth in Ibaraki. Ten-day averages are plotted.

○ and ● show the sowing and jointing dates of 'Asakazekomugi' and Asa (Vrn-D1b), respectively, in early sowing. ▽ and ▼ show the sowing and jointing dates of 'Asakazekomugi' and Asa (Vrn-D1b), respectively, in standard sowing. ◇ and ◆ show the sowing and jointing dates of 'Asakazekomugi' and Asa (Vrn-D1b), respectively, in late sowing.

Table 1. Phenological development of 'Asakazekomugi' and Asa (Vrn-D1b) under different sowing dates.

Crop year	Line	Sowing date		Jointing[a] (month/day)	Heading[b]	Maturity	Sowing–jointing (days)
2015/2016	Asakazekomugi	Early	10/15	1/4	4/10	5/25	81
		Standard	11/17	3/9	4/13	5/31	113
		Late	12/15	4/1	4/22	6/7	108
	Asa (Vrn-D1b)	Early	10/15	2/16	4/10	5/31	124
		Standard	11/17	3/16	4/15	5/31	120
		Late	12/15	4/1	4/22	6/7	108
2016/2017	Asakazekomugi	Early	10/14	1/13	4/16	6/1	91
		Standard	11/14	3/24	4/22	6/5	130
		Late	12/13	4/9	5/2	6/11	117
	Asa (Vrn-D1b)	Early	10/14	3/3	4/16	5/31	140
		Standard	11/14	3/28	4/21	6/6	135
		Late	12/13	4/12	5/1	6/10	120

[a] Jointing stage was when length of main stem = 20 mm.

[b] Heading stage was when 40–50% of all stem spikes had emerged from the boot.

'Asakazekomugi' and Asa (Vrn-D1b) with either early or standard sowing, whereas the effect of sowing date on heading and maturity dates were in the order (earliest first) early-, standard-, and late-sowing date.

3.2. Yield and yield components

At maturity, there were significant sowing date × wheat line interactions for grain yield, grain number per spike, and aboveground dry weight (Table 2). The grain yield of 'Asakazekomugi' was lower by 71% in the early-sown crop than in the standard-sown crop in 2015/2016, although such a decrease was not observed in 2016/2017. In contrast, the grain yield of Asa (Vrn-D1b) was higher in the early-sown crop than in the standard-sown crop by 107% and 138% in 2015/2016 and 2016/2017, respectively. Following late sowing, the grain yields of both wheat lines were lower than in the standard sowing. Total aboveground dry matter weight showed a response similar to grain yield, being lower in 'Asakazekomugi' sown early and higher in Asa (Vrn-D1b) sown early compared with the corresponding lines sown at the standard date. Grain number per spike of 'Asakazekomugi' was lower in the early sowing compared with that in the standard sowing in both crop years, whereas that of Asa (Vrn-D1b) in the early-sowing date was similar to that in the standard-sowing date.

HIs in early-sown crops were lower in both wheat lines than those from standard and late sowings. There were no significant differences between 'Asakazekomugi' and Asa

Table 2. Grain yield, yield components, and dry weight of aboveground parts in 'Asakazekomugi' and Asa (Vrn-D1b) under three different sowing dates.

Crop year	Line	Sowing date	Grain yield (g m^{-2})	Spike number m^{-2}	Grain number per spike	1000-grain weight (g)	Dry matter (g m^{-2})	Harvest index	Percentage of productive stems (%)
2015/2016	Asakaze-komugi	Early	405 ab	538 a	22.8 b	33.0 ab	1150 ab	0.33 b	68.8 a
		Standard	569 a	505 a	31.8 a	35.5 a	1306 a	0.43 a	82.4 a
		Late	384 b	421 a	28.0 a	32.6 b	796 b	0.43 a	98.3 a
	Asa (Vrn-D1b)	Early	627 a	581 a	31.4 a	34.4 a	1440 a	0.37 a	55.8 b
		Standard	586 a	543 ab	31.9 a	33.8 a	1135 a	0.42 a	106.9 ab
		Late	355 b	460 b	25.1 b	30.3 b	690 b	0.40 a	137.2 a
2016/2017	Asakaze-komugi	Early	586 a	647 a	27.9 a	32.5 a	1492 a	0.40 b	—
		Standard	552 a	497 b	33.0 a	33.7 a	1342 a	0.46 a	—
		Late	289 b	348 c	27.2 a	30.1 b	733 b	0.42 b	—
	Asa (Vrn-D1b)	Early	723 a	620 a	36.2 a	32.2 a	1943 a	0.42 a	—
		Standard	524 b	439 b	35.2 ab	33.9 a	1155 b	0.46 a	—
		Late	276 c	305 c	28.8 b	31.3 a	769 c	0.43 a	—
	Average	2015/2016	488	508	28.5	33.3	1086	0.40	91.6
		2016/2017	492	476	31.4	32.3	1239	0.43	—
		Early	585 a	596 a	29.6 b	33.0 a	1506 a	0.38 c	62.3 b
		Standard	558 a	496 b	33.0 a	34.2 a	1234 b	0.44 a	94.7 ab
		Late	326 b	383 c	27.3 c	31.1 b	747 c	0.42 b	117.8 a
		Asakazekomugi	464	493	28.5	32.9	1136	0.41	83.1
		Asa (Vrn-D1b)	515	491	31.4	32.6	1189	0.42	100.0
	ANOVA	Year	ns	ns	**	**	ns	*	—
		Sowing date	*	*	**	***	**	*	*
		Line	ns	ns	*	ns	ns	ns	ns
		Sowing date × Line	**	ns	**	ns	***	ns	ns

—: Not recorded. A two-way analysis of variance (ANOVA) was used for data analysis according to a split-plot design. ***, **, and * indicate significance at $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively. Different letters within a column indicate a significant difference among sowing date at $P < 0.05$ (Tukey's test).

(Vrn-D1b) with respect to spike number per m^2 and 1000-grain weight, although there was a significantly greater spike number per m^2 from early than from standard sowing. Studied in only the 2015/2016 season, the percentage of productive stems from the early sowing was lower in both wheat lines than from the standard or late sowings.

3.3. Dry matter production and CGR

Aboveground dry matter production by 'Asakazekomugi' and Asa (Vrn-D1b) sown at the three different dates was compared over the growing season in both years (Figure 2). In the early sowing of 2015/2016, dry matter production by 'Asakazekomugi' was higher than that in Asa (Vrn-D1b) until February, after which dry matter production by Asa (Vrn-D1b) exceeded that by 'Asakazekomugi.' We observed a similar result in 2016/2017, where dry matter production by Asa (Vrn-D1b) was higher than that in 'Asakazekomugi' after April in the early sowing, although there was no significant difference between two wheat lines until March. In the standard and late sowings, the dry matter production by 'Asakazekomugi' was similar to that by Asa (Vrn-D1b) (Figure 2).

Interline differences in CGR occurred over the growing season in both years for both the early- and standard-sowing dates. As with dry matter production in the early sowing of 2015/2016, CGR in 'Asakazekomugi' was higher than that in Asa (Vrn-D1b) until February, after which 'Asakazekomugi' exhibited slower growth and Asa (Vrn-D1b) faster growth (Figure 3). In the early sowing of 2016/2017, CGR in 'Asakazekomugi' was similar to that in Asa (Vrn-D1b) until February, after which CGR in Asa (Vrn-D1b) was higher than that in 'Asakazekomugi.' In the standard-sown crops of both years, CGRs in both wheat lines were similar until April, from which point to maturity 'Asakazekomugi' had the higher CGR.

Tiller number per m^2 in the early-sown 'Asakazekomugi' was significantly lower than that in Asa (Vrn-D1b) sown at the same time (Figure 4). In contrast, tiller number in the standard-sown 'Asakazekomugi' was higher than that in Asa (Vrn-D1b).

3.4. Relationship between solar radiation and dry matter production

LAIs in early-sown crops were consistently higher in Asa (Vrn-D1b) than in 'Asakazekomugi' from mid-March (after the jointing stage in Asa (Vrn-D1b)), though the differences were not statistically significant from mid-March to late-March (Table 3). In standard-sown crops, the LAI in Asa (Vrn-D1b) was not significantly different from that in 'Asakazekomugi' at any measurement date.

To identify which growth stage contributed most to the higher dry matter production in Asa (Vrn-D1b), we analyzed the effect of solar radiation on dry matter production for both lines at each growth stage (Figure 5). No significant correlation was detected between cumulative solar radiation and dry matter production for both lines for any individual stage during the period from sowing to jointing

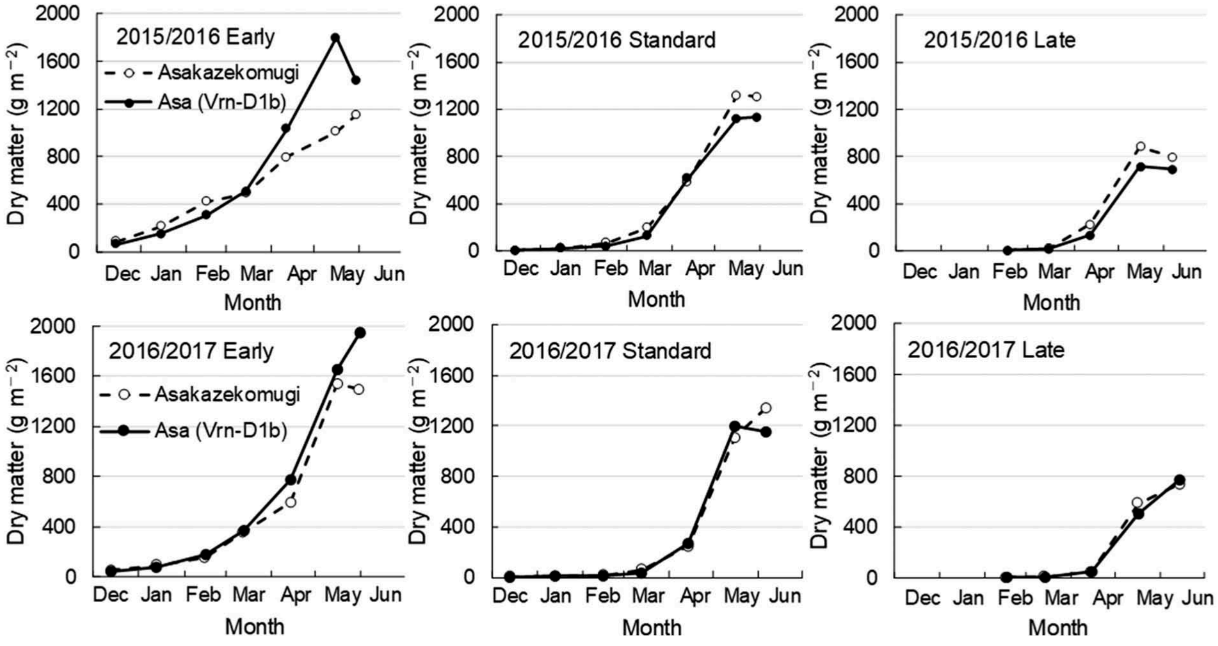

Figure 2. Time course of dry matter production in 'Asakazekomugi' and Asa (Vrn-D1b) from different sowing dates.

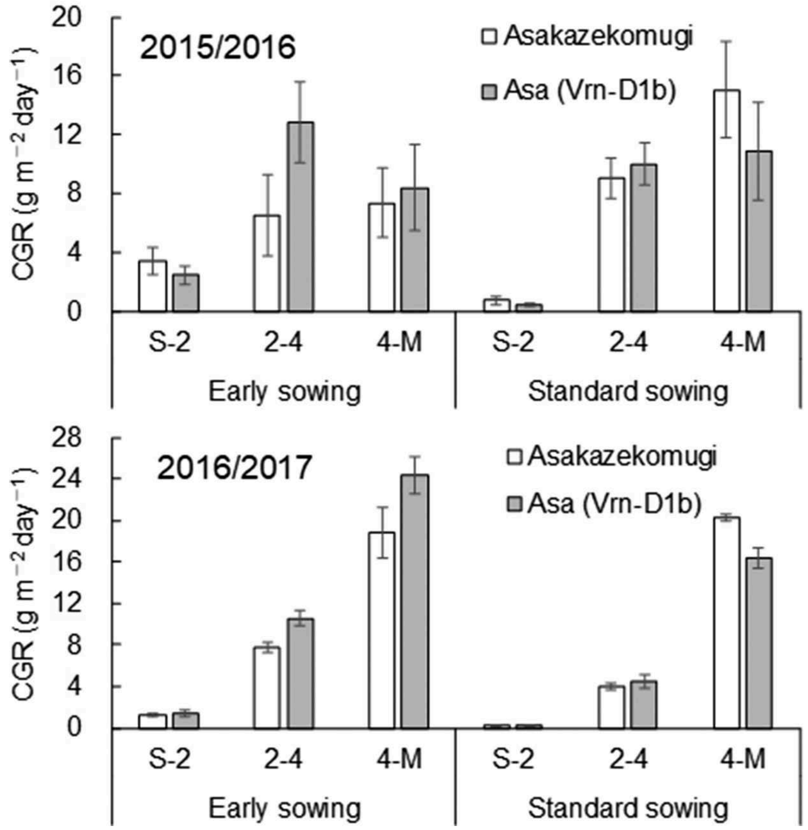

Figure 3. Crop growth rate (CGR) of 'Asakazekomugi' and Asa (Vrn-D1b) at each growth period from early- and standard-sowing dates. S-2, from sowing to mid-February; 2-4, from mid-February to mid-April; 4-M, from mid-April to maturity stage.

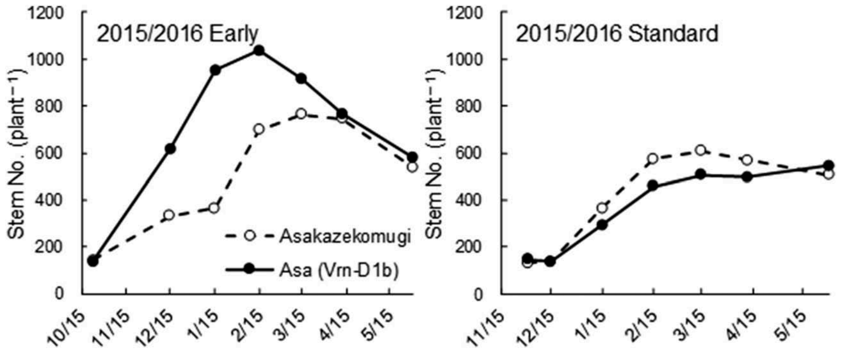

Figure 4. Time course of tiller production in 'Asakazekomugi' and Asa (Vrn-D1b) from early- and standard-sowing dates.

Table 3. Leaf area index of 'Asakazekomugi' and Asa (Vrn-D1b) from early and standard sowings.

			Mid-March	Late-March	Mid-April	Mid-May
2015/2016	Early	Asakazekomugi	—	—	4.77 *	3.5 **
		Asa (Vrn-D1b)	—	—	6.77	4.79
	Standard	Asakazekomugi	—	—	4.13 ns	2.89 ns
		Asa (Vrn-D1b)	—	—	3.30	2.62
2016/2017	Early	Asakazekomugi	1.85 ns	2.60 ns	4.46 *	4.73 *
		Asa (Vrn-D1b)	2.04	2.85	5.32	5.26
	Standard	Asakazekomugi	—	1.22 ns	2.01 ns	3.48 ns
		Asa (Vrn-D1b)	—	1.22	2.09	3.40

—: Not recorded. * and ** indicate significance at $P < 0.05$ and $P < 0.10$ level, respectively (Student's *t*-test). ns: not significant.

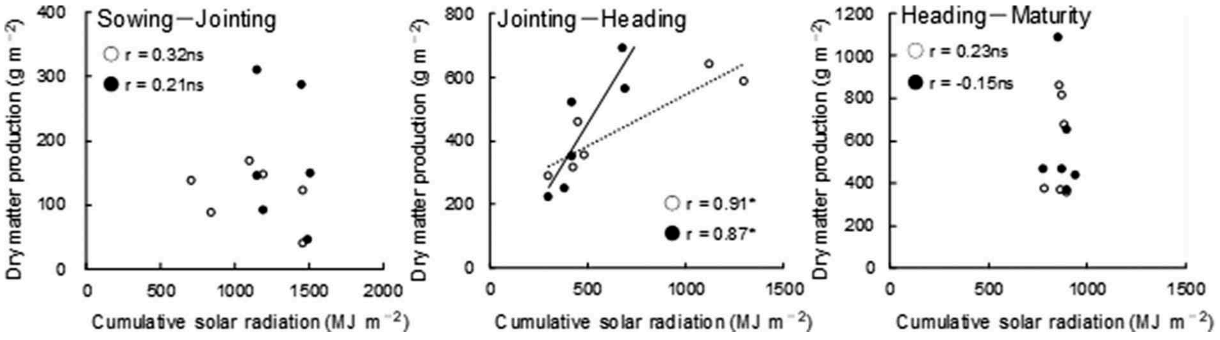

Figure 5. Relationship between cumulative solar radiation and dry matter production in 'Asakazekomugi' and Asa (Vrn-D1b) at each growth stage. ○ and ● show 'Asakazekomugi' and Asa (Vrn-D1b), respectively. * indicates significance at $P < 0.05$. ns: not significant.

stage and from heading to maturity stage. From the jointing to the heading stage, there were significant positive correlations between cumulative solar radiation and dry matter production in both lines ($P < 0.05$). The slope of the regression line between cumulative solar radiation and dry matter production for Asa (Vrn-D1b) significantly differed from that for 'Asakazekomugi' ($P < 0.05$), suggesting that dry matter production per unit cumulative solar radiation in Asa (Vrn-D1b) was significantly higher between stem elongation and heading than in 'Asakazekomugi.'

4. Discussion

We evaluated the effect of *Vrn-D1* on the response of dry matter production and grain yield to increasing temperature using spring-habit wheat 'Asakazekomugi' carrying vernalization response gene *Vrn-D1a* and its winter-type NIL carrying the winter allele of *Vrn-D1* (*Vrn-D1b*), providing the higher temperatures after sowing by sowing the crops earlier. The period from sowing to the jointing stage in 'Asakazekomugi' was more than 30 days shorter under early-sowing conditions than under standard-sowing conditions, whereas the corresponding period in the early-sown Asa (Vrn-D1b) extended very little compared with standard-sowing conditions (Table 1). This demonstrated that the vegetative growth period in Asa (Vrn-D1b) differed little with early sowing compared with 'Asakazekomugi,' findings consistent with our previous results (Matsuyama et al., 2017). The early-sown 'Asakazekomugi' began stem elongation at the coldest time of the year (Figure 1, Table 1).

Dry matter production in 'Asakazekomugi,' which was accelerated by the early-sowing date, was higher than that in the early-sown Asa (Vrn-D1b) until February especially in 2015/2016, which experienced a warm winter (Figures 2 and 3). This suggests that dry matter production in 'Asakazekomugi,' which had reached the jointing stage in the cold season, increased transiently under warm winter conditions, since dry matter production in wheat usually increases rapidly after the jointing stage. However, as shown in Figure 5, the dry matter production per unit cumulative solar radiation between the jointing and heading stages in 'Asakazekomugi' was significantly lower than that in Asa (Vrn-D1b). Dry matter productions in wheat and barley are closely related to photosynthetically active radiation absorbed during growth, with results from different sites, seasons, and cultivars all at about the same level (Gallagher & Biscoe, 1978). Therefore, we suggest that the early-sown 'Asakazekomugi' could not effectively absorb or intercept the solar radiation. One of the reasons for the low interception of radiation might be the lower tiller number exhibited in the early-sown 'Asakazekomugi.' In general, the onset of tiller mortality coincides with the beginning of stem elongation (Miralles & Slafer, 1999). The shortened duration of the tillering phase caused by the acceleration effect of stem elongation by *Vrn-D1* in the early-sown 'Asakazekomugi' led to the lower tiller frequency in 'Asakazekomugi' compared with Asa (Vrn-D1b) (Figure 4). Moreover, White et al. (1990) reported that low temperatures inhibit leaf appearance and elongation in wheat. Another reason for the low light interception might be reduced leaf appearance or leaf elongation caused by low temperatures after the jointing stage in 'Asakazekomugi' when the crop was sown early. This proposal is supported by the observation that the LAI after jointing stage in early-sown Asa (Vrn-D1b) was higher than in early-sown 'Asakazekomugi' (Table 3). Therefore, dry matter production of Asa (Vrn-D1b), which was slow to reach the jointing stage and produced many tillers and leaves, exceeded that of 'Asakazekomugi' after mid-March, when the temperature rose. In the 2016/2017 crop season, in which temperatures were lower in autumn and winter than in 2015/2016, dry matter production in 'Asakazekomugi' was at the same level as it was in Asa (Vrn-D1b) during the winter season, whereas the dry matter production in Asa (Vrn-D1b) after March exceeded that in 'Asakazekomugi.' The greater dry matter production in the early-sown Asa (Vrn-D1b) in 2016/2017 was also thought to be due to the higher numbers of tillers as a result of the extended vegetative phase (indicated by the delayed jointing stage) than in 'Asakazekomugi,' and the more rapid growth of those after the jointing stage. As indicated in the previous study (Matsuyama et al., 2017), *Vrn-D1* hardly affected duration from sowing to heading (Table 1), which means that the duration from jointing to heading in Asa (Vrn-D1b) was shorter than that in 'Asakazekomugi' under early-sowing conditions. These results suggest that it is important to ensure sufficient duration for tiller emergence before jointing stage rather than after it due to increase in dry matter production in the early-sown wheat.

The grain yields of Asa (Vrn-D1b) under the early-sowing conditions, with higher temperatures after sowing than under the standard-sowing conditions, were higher than the corresponding yields of 'Asakazekomugi' in both years (Table 2). The high yield of the early-sown Asa (Vrn-D1b) was due to the grain number per spike in the early sowing being maintained at the same level as in the standard sowing, whereas the grain number per spike in the early-sown 'Asakazekomugi' was less than that in the standard sowing. Fukushima et al. (2001)and Fukushima, Kusuda, and Furuhata (2003) compared tiller development, grain yield, and yield components among very early (late October), early (early November), and standard (late October) sowings of winter-type wheat 'Iwainodaichi' and spring-type wheat 'Chikugoizumi' in a paddy field in southwestern Japan. They reported that the very-early- and early-sown 'Iwainodaichi' showed delayed stem elongation, higher tiller number, higher LAI, and slightly greater aboveground dry weight than in the very-early- and early-sown 'Chikugoizumi.' These findings were nearly consistent with our results. However, there was no significant difference in the grain yield among very early, early, and standard sowings of both 'Iwainodaichi' and 'Chikugoizumi,' and the grain number per spike in the very-early- and early-sown 'Iwainodaichi' was less than that in the standard sowing. In contrast, Seki et al. (2007) showed that the grain yield of a winter-type NIL with *Vrn-D1b* (previously recessive *vrn*) was significantly higher than that of spring-type NILs carrying either of the *Vrn-A1, Vrn-D1a*, or *Vrn-D5* gene in a warm winter in Ibaraki, central Japan. Under the higher temperature conditions in central Japan, wheat cultivars carrying *Vrn-D1b* might be superior to those carrying *Vrn-D1a* with respect to stability of grain yield. In the present study, carried out in an upland field in central Japan, where the temperatures are lower than in southwestern Japan, it was shown that Asa (Vrn-D1b) was superior to 'Asakazekomugi' not only with respect to avoidance of frost injury to young spikes but also with regard to dry matter production and grain yield.

As a factor responsible for the lower grain number per spike in the early-sown 'Asakazekomugi,' we speculate that the increase in spikelet number per spike or floret number per spikelet was suppressed by nitrogen deficiency during the spike development period due to early dry matter growth during the cold season. Nitrogen deficiency during the spike growth period reduces grain number per m^2 and grain number per spike in wheat (Fischer, 1993; Demotes-Mainard et al., 1999). Takahashi et al. (2010) have shown that early sowing decreases grain yield by reducing spike number or grain number per spike in 'Iwainodaichi' and 'Airakomugi,' but the reduction in grain number per spike and grain number per spikelet was mitigated by nitrogen topdressing during the spike development period. Further research will be needed to identify the factor responsible for the low grain number per spike in the early-sown 'Asakazekomugi.'

HI and percentage productive stem values in crops grown from early sowings were lower in both wheat lines than those under standard-sowing conditions (Table 2), suggesting that the increase in dry matter production in the early-sown Asa (Vrn-D1b) did not contribute directly to grain yield under early-sowing conditions. The lower HI and percentage productive stem values in the early-sown Asa (Vrn-D1b) might be caused by a decrease in tiller survival due to an excessive increase in tiller number, followed by competition among tillers for assimilates. Miyama et al. (1989) showed that the amount of nitrogen absorbed by wheat at maximum tiller number stage was highly positively correlated to the percentage of productive stems. This suggests that the percentage of productive stems would be increased by nitrogen topdressing before maximum tiller number stage. Watanabe et al. (2016) reported that the percentage of productive stems and HI were higher in winter-type wheat 'Satonosora' treated with reduced basal fertilizer accompanied by increased topdressing at tillering stage than with a conventional application. It will be important to develop fertilizer treatments, which maintain an adequate number of tillers while inhibiting the formation of nonproductive tillers in order to improve yield performance in winter-type wheat.

5. Conclusion

Dry matter production in the spring-type wheat 'Asakazekomugi' carrying *Vrn-D1a* was surpassed by its NIL carrying *Vrn-D1b* (Asa (Vrn-D1b)) after the jointing stage of Asa (Vrn-D1b) under early-sowing conditions. It is suggested that the lower dry matter production of 'Asakazekomugi' is caused by the acceleration of stem elongation by *Vrn-D1a* under warm conditions after sowing, resulting in lower tiller number and LAI. Under the early-sowing conditions, grain yield of Asa (Vrn-D1b) was higher than that of 'Asakazekomugi.' It is suggested that the lower grain yield of 'Asakazekomugi' was caused by its lower dry matter production and grain number per spike. Further research is required to clarify the effect of *Vrn-D1* on the low number of grains per spike in early-sown wheat.

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References

Asseng, S., Ewert, F., Rotter, R. P., Lobell, D. B., Cammarano, D., Kimball, B. A., … Zhu, Y. (2015). Rising temperatures reduce global wheat production. *Nature Climate Change*, *5*, 143–147.

Demotes-Mainard, S., Jeuffroy, M. H., & Robin, S. (1999). Spike dry matter and nitrogen accumulation before anthesis in wheat as affected by nitrogen fertilizer: Relationship to kernels per spike. *Field Crops Research*, *64*, 249–255.

Fischer, R. A. (1993). Irrigated spring wheat and timing and amount of nitrogen fertilizer. II. Physiology of grain yield response. *Field Crops Research*, *33*, 57–80.

Fujita, M. (1997). Studies on breeding of early maturing wheat cultivars with tiller frost avoidance. *Bulletin of the Kyushu Agricultural Experiment Station*, *32*, 1–50.**In Japanese with English abstract.

Fujita, M., Taniguchi, Y., Ujihara, K., & Sasaki, A. (1995). Ear primordia development and stem elongation of near-isogenic lines for vernalization requirement in extremely-early maturing wheat cultivars (*Triticum aestivum* L.). *Breeding Science*, *45*, 97–104.

Fukushima, A., Kusuda, O., & Furuhata, M. (2001). Tiller development of winter type wheat Iwainodaichi sown early in the southwestern part of Japan. *Japanese Journal of Crop Science*, *70*, 173–178.**In Japanese with English abstract.

Fukushima, A., Kusuda, O., & Furuhata, M. (2003). Analysis of the factors determining the grain yield of winter type wheat, Iwainodaichi, sown early in the southwestern part of Japan. *Japanese Journal of Crop Science*, *72*, 149–157.**In Japanese with English abstract.

Gallagher, J. N., & Biscoe, P. V. (1978). Radiation absorption, growth and yield of cereals. *Journal of Agricultural Science*, *91*, 47–60.

Gotoh, T. (1979). Genetic studies on growth habit of some important spring wheat cultivars in Japan, with special reference to the identification of the spring genes involved. *Japanese Journal of Breeding*, *29*, 133–145.

Iwaki, K., Nakagawa, K., Kuno, H., & Kato, K. (2000). Ecogeographical differentiation in east Asian wheat, revealed from the geographical variation of growth habit and *Vrn* genotype. *Euphytica*, *111*, 137–143.

Kakizaki, Y., & Suzuki, S. (1937). Studies on the physiology of earing in wheat. *Journal of the Imperial Agricultural Experiment Station*, *3*, 41–92.**In Japanese with English abstract.

Law, C. N., & Wolfe, M. S. (1966). Location of genetic factors for mildew resistance and ear emergence time on chromosome 7B of wheat. *Canadian Journal of Genetics Cytology*, *8*, 462–470.

Matsuyama, H., Seki, M., Shimazaki, Y., Kojima, H., Kiribuchi-Otobe, C., Takayama, T., … Kato, K. (2017). Differences in growth properties between near-isogenic wheat lines carrying different types of *vrn-D1* in the genetic background of two early cultivars. *Japanese Journal of Crop Science*, *86*, 311–318.**In Japanese with English abstract.

Meng, L. Z., Liu, H. W., Yang, L., Mai, C. Y., Yu, L. Q., Li, H. J., … Zhou, Y. (2016). Effects of the *Vrn-D1b* allele associated with facultative growth habit on agronomic traits in common wheat. *Euphytica*, *211*, 113–122.

Minoda, T. (2010). Effects of climatic conditions on the yield of wheat variety 'Norin 61' in the experimental upland field in Saitama Prefecture. *Japanese Journal of Crop Science*, *79*, 62–68.**In Japanese with English abstract.

Miralles, D. J., & Slafer, G. A. (1999). Wheat development. In E. H. Satorre & G. A. Slafer (Eds.), *Wheat: Ecology and physiology of yield determination* (pp. 13–43). New York: Food Products Press.

Miyama, M., Katsukita, H., & Saito, K. (1989). A suitable nitrogen fertilization method for several crop plants based on the optimum nitrogen contents 3. The optimum amount of nitrogen for wheat crop to be absorbed at each growth stage. *Japanese Journal of Soil Science and Plant Nutrition*, *60*, 106–115.**In Japanese with English abstract.

Nakazono, K. (2010). Impact of global warming on wheat production in Japan. *Research Journal of Food and Agriculture*, *33*(8), 10–14.*In Japanese.

Pugsley, A. T. (1971). A genetic analysis of spring-winter habit of growth in what. *Australian Journal of Agricultural Research*, *22*, 21–31.

Pugsley, A. T. (1972). Additional genes inhibiting winter habit in wheat. *Euphytica*, *21*, 547–552.

Seki, M., Oda, S., Matsunaka, H., Hatta, K., Fujita, M., Hatano, T., … Kato, K. (2007). Growth and yield of near-isogenic wheat lines carrying different vernalization response genes. *Breeding Research*, *9*, 125–133.**In Japanese with English abstract.

Takahashi, H., Nishida, H., & Kato, K. (2013). Analysis on sequence variation in 5′ upstream region and first intron of *Vrn-D1* in winter wheat. *Breeding Research*, *15* (Supplement 1), 95.*In Japanese.

Takahashi, T., Zhang, L., Matsuzawa, T., Fujimoto, K., Yamaguchi, S., Hossain, M. D. A., & Araki, H. (2010). Effects of nitrogen topdressing during spike development on yield of winter wheat cultivars early sown in Yamaguchi. *Japanese Journal of Crop Science*, *79*, 468–475.**In Japanese with English abstract.

Watanabe, K., Nakazono, K., Nakamura, D., Nishitani, T., Nishimura, N., Matsushima, H., ... Ehara, H. (2016). Effects of the higher top to basal nitrogen dressing ratio on growth and yield of wheat. *Japanese Journal of Crop Science*, *85*, 373–384. **In Japanese with English abstract.

White, P. J., Cooper, H. D., Earnshaw, M. J., & Clarkson, D. T. (1990). Effects of low temperature on the development and morphology of rye (*Secale cereale*) and wheat (*Triticum aestivum*). *Annals of Botany*, *66*, 559–566.

Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., … Dubcovsky, J. (2006). The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. *Proceedings of the National academy of Sciences of the United States of America*, 103, 19581–19586

Zhang, J., Dell, B., Biddulph, B., Khan, N., Xu, Y., Luo, H., & Appels, R. (2014). Vernalization gene combination to maximize grain yield in bread wheat (*Triticum aestivum* L.) in diverse environments. *Euphytica*, *198*, 439–454.

Zhang, J., Wang, Y., Wu, S., Yang, J., Liu, H., & Zhou, Y. (2012). A single nucleotide polymorphism at the *Vrn-D1* promoter region in common wheat is associated with vernalization response. *Theoretical and Applied Genetics*, *125*, 1697–1704.

Zhang, X. K., Xiao, Y. G., Zhang, Y., Xia, X. C., Dubcovsky, J. & He, Z. H. (2008) Allelic variation at the vernalization genes *Vrn-A1, Vrn-B1, Vrn-D1*, and *Vrn-B3* in Chinese wheat cultivars and their association with growth habit. *Crop Science*, *48*, 458–470.