Interactive effects of multiple vernalization (Vrn-1)- and photoperiod (Ppd-1)-related genes on the growth habit of bread wheat and their association with heading and flowering time

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Abstract

Background: The precise identification of Winterness/Springness (growth habit) for bread wheat, which is determined by genes involved in vernalization and photoperiod, will contribute to the effective utilization of bread wheat varieties. Here, 198 varieties from the Yellow and Huai wheat production region (YHW) in China were collected to identify their vernalization (Vrn-1) and photoperiod (Ppd-1) gene composition via a series of functional markers and their association with vernalization and photoperiod requirements at three locations during two years of experiments. The growth habits were measured during the spring sowing season.

Results: The results showed that the semi-winter varieties (grades 1–4) were most prevalent in the population. The relative effects of single Vrn alleles on the growth period, such as heading date (HD) and/or flowering date (FD), were as follows: Vrn-B1b > Vrn-B1a > Vrn-D1b > Vrn-D1a > vrn-D1 = vrn-B1. The interactive effects of Vrn-B1 and Vrn-D1 on HD and FD were identical to those of Vrn-B1b. Approximately 35.3% of the cultivars carried Ppd-B1a (photoperiod-insensitive) and exhibited the earliest HD and FD. The Ppd-D1a-insensitive allele (Hapl II) was carried by just 0.5% of the varieties; however, the other two sensitive alleles were present at a higher frequency, and their effects were slightly weaker than those of Ppd-B1a. In addition, strong interactive effects between Ppd-B1 and Ppd-D1 were detected. In terms of mean values among various genotypes, the effects followed the order of Vrn-1 > Ppd-1.

Conclusions: According to the results of ANOVA and least significant range (LSR) tests, we can conclude that Vrn-1 rather than Ppd-1 played a major role in controlling vernalization and photoperiod responses in this region. This research will be helpful for precisely characterizing and evaluating the HD, FD and even growth habit of varieties in the YHW at molecular levels.

Keywords: Winter wheat, The yellow and Huai wheat production region (YHW), Growth habit, Growth period, Vernalization, Photoperiod

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Background

Because of the ease of determining its optimum time for flowering and maturation, bread wheat (*Triticum aestivum* L., AABBD, 2n = 42) is cultivated worldwide. Day length and low temperature act as environmental cues affecting the time to heading and flowering. The ability to perceive and respond to these signals is controlled by molecular pathways that regulate early growth habits in response to abiotic stress (*Vrn* alleles) and photoperiod (*Ppd* alleles) [1, 2].

In the past, the characterization of the growth habits of winter wheat in China, especially in the Yellow and Huai wheat production region, which is the largest winter wheat production region, relied on field evaluations or in-house artificial identification [3, 4]. However, these identification procedures are tedious and costly, and thus, the practicality of field evaluation and in-house artificial identification methods is limited. Furthermore, inconsistencies between breeders’ descriptions and government registrations sometimes occur due to inexact phenotypic identification methods. In contrast, molecular identification methods are relatively credible, but more novel types of vernalization alleles must be surveyed, and their interactive effects among each other remain unclear.

Indeed, the molecular basis for flowering time regulation has been extensively studied in wheat and other crops [5]. In hexaploid wheat, vernalization requirements are controlled by three major orthologous *Vrn* alleles—*Vrn-A1*, *Vrn-B1*, and *Vrn-D1*—which have been mapped onto the long arm of chromosomes 5A, 5B, and 5D, respectively [6–8]. Each of these loci encodes a MADS-box transcription factor orthologous to *API* in *Arabidopsis*, which is reported to be involved in floral meristem development during the transition from the vegetative phase to the reproductive phase [8]. The *VRN-1* gene is dominant for the spring growth habit, and it is upregulated by vernalization in winter lines [9, 10]. A homologue of the *Arabidopsis* FT gene, the *Vrn-3* gene, has been mapped to the short arm of chromosome 7 in wheat; this gene upregulated the *Vrn-1* genes and thus accelerated heading and flowering indirectly [11].

The emergence of dominant alleles at the *Vrn-A1* locus is a result of insertions and deletions within the promoter or a deletion within intron 1, which have been designated *Vrn-A1a*, *Vrn-A1b*, and *Vrn-A1c*, respectively [9, 10, 12, 13]. Spring-growth habits can also be attributed to deletions at the *Vrn-B1* and *Vrn-D1* loci, which have been classified as insensitive vernalization types, and have been designated *Vrn-B1a* [10, 14, 15], *Vrn-D1a*, and *Vrn-D1b* [16]. The *Vrn-B1c* (novel) allele, which is due to the deletion of 0.8 kb and the duplication of 0.4 kb within intron 1, differs from *Vrn-B1a* [17, 18]. Another spring allele, *Vrn-B1b*, has also been described; this allele contains two deletions in the promoter region and is present in spring variety ‘Alpowa’ [14]. The various vernalization requirements of the *Vrn-1* alleles or combinations can result in variations in flowering time and spring growth habit [19]. In wheat and other temperate grasses, *VRN1* is also expressed in the leaves, where it acts as a repressor of *VRN2* [20, 21]. The detailed pathway of the vernalization genes involved in controlling wheat flowering was reviewed by Chen and Dubcovsky (2012) [20].

Photoperiod response is another vital factor affecting flowering time under long-day conditions. For wheat, photoperiod insensitivity (*Ppd-1a*) is widespread and especially prevalent in regions where crops grow during short days or when the crops mature before the onset of high summer temperatures [22]. Three semi-dominant orthologous *Ppd-1* loci—*Ppd-A1*, *Ppd-B1*, and *Ppd-D1*—have been mapped onto the short arm of chromosomes 2A, 2B, and 2D, respectively [23, 24]; these loci are all members of the *Pseudo-Response Regulator* (PRR) gene family, which is orthologous to the *Ppd-H1* gene family in barley [25]. A series of diagnostic markers have been used to efficiently screen for several variants [10, 13, 16, 24, 26].

The Yellow and Huai valley wheat production region (YHW) covers 45% of China’s total cultivation area but contributes 60–70% of the country’s wheat production. Varieties that flower and mature early are helpful in sustaining China’s double-harvest cropping system. In this study, we collected and identified a total of 198 popular varieties, elite lines, and landraces from China to (i) accurately identify the growth habits of the varieties via the field spring sowing method and evaluate their association with heading date (HD; growth period) and flowering date (FD; growth period) at three locations within the YHW during a two-year period (Zhengzhou, Zhumadian and Shangqiu in 2014 and 2015); (ii) use diagnostic molecular markers to determine the main allelic frequencies of *Vrn-1* and *Ppd-1*; and (iii) specifically determine the interactive effects between *Vrn-1* and *Ppd-1* allelic combinations on heading and flowering times. This study contributes knowledge concerning the effective selection of various types of growth habits of varieties and will be of service to the selection of early-maturation cultivars at the molecular level.

Results

Semi-winter varieties were predominant in the YHW according to the field spring sowing method.

The results of the two-year growth habits were very similar, and the order ranks recorded in 2015 strongly correlated with those recorded in 2016 (Pearson coefficient = 0.96). In general, the ranks of two accessions (Xinong979 and Yumai47) were inconsistent between
years, but the discrepancies were only 1–2 grades. Our method separated 10 accessions (Yannong19, Beijing841, etc.) into winterness (grade 0) in 2015 and 2016, which accounted for 5.05%. One hundred and forty-seven accessions (74.24%) were identified as semi-winter types in 2015, and 145 (73.23%) were identified in 2016. In contrast, 41 (20.71%) and 43 (21.72%) grade 5 accessions belonged to the spring type in 2015 and 2016. Overall, the semi-winter varieties (grades 1–4) were predominant in the YHW (Table 1; Fig. 1).

In comparison with the data on regional trials (registered, Table 1), our data showed that the consistency was 89.39% when the accessions were divided into winter and spring groups, although 21 varieties need to be re-examined. In detail, of the varieties identified in the winter group, 10 varieties, including Huangming116, Lankaoai20a, Yumai4, Taikong6, Zhengmai101, Zhongchuang805, Zhoulai23, Zhongyanmai 0708, Huaimai 19 and Xiaoyan 22, qualified as week spring (spring) type according to the registered results. The 11 spring growth habit accessions, which included Jihan2, Hengguan55, Zhengyou6, Luohan6, Luomai23, Pu2056, Shanyou225, Xinong9871, Yunong416, Zhoumai26 and Huairui 00712, were misclassified as accessions having winter (or semi-winter) growth habits. The reason is that these varieties were registered ten years ago, and winter-spring identification was not evaluated during registration tests at that time.

**Growth habits were highly associated with growth periods in six environments**

The results of the joint ANOVA analysis revealed that significant differences in the mean values of the HD or FD grouped by grades 0–5 in all six environments (Table 2). Briefly, grade 5 exhibited the shortest length of the growth period, while grade 0 exhibited the greatest length. In detail, significant differences in the average values of growth period data were also found between different levels of each trait. The trend was similar to that revealed by the joint ANOVA results. Generally, the smaller the value of the HD or FD is, the greater the value of the grade (Fig. 2).

Significant negative correlations were detected between growth habit and growth period in six environments (Table 2, p < 0.01). According to the results of joint variance analysis, the mean values of HD and FD were also correlated with growth habit; the Pearson correlation coefficients were −0.915 and −0.886, respectively. Generally, these results also indicated that HD were more tightly, though negatively, related with growth habits. Furthermore, the range of correlation coefficients in six environments were from −0.813 (FD_15_ZMD) to −0.938 (HD_15_ZZ). Thus, we could conclude that the duration of the heading and flowering time of cultivars was tightly associated with growth habits (Additional file 1: Table S2).

**Distribution frequency of Vrn-1 alleles in varieties**

Because no polymorphisms were found in the Vrn-A1 and Vrn-B3 alleles, we focused on Vrn-B1 and Vrn-D1. The distribution frequency of the dominant alleles was Vrn-D1a (23.70%) > Vrn-D1b (8.10%) > Vrn-B1a (2.50%) > Vrn-B1b (2.00%) (Table 3). Only one accession was found to carry Vrn-B1b + Vrn-D1a, and 125 accessions presented no dominant alleles. We also used the “consistency index” to evaluate the reliability between the allelic detection and speculated results as described by Stelmakh [19]. According to Stelmakh’s report, the accessions that contained at least one dominant allele were classified as spring types, whereas they were classified as winter types if they had three recessive alleles. Then, we found that nine accessions harbouring dominant Vrn-B1a or Vrn-B1b alleles as well as one accession harbouring Vrn-B1b + Vrn-D1a exhibited the highest rate of consistency (100%). Therefore, these accessions were classified as spring types, which were identical to the results of identification of growth habit in this research. The genotype rate of vrn-B1 + vrn-D1 (63.1%) dominated in all tested panels, and its consistency (96.8% or 95.2%) was also higher than that of Vrn-D1a (48.9%) and Vrn-D1b (25.0%). Therefore, Vrn-D1, especially Vrn-D1b, could not accurately estimate the growth habit.

**Effects of Vrn-1 combinations on HD and FD**

The effects of Vrn-B1 + Vrn-D1 combinations concerning HD and FD were examined. In total, there were 6 different types of genotypes grouped by combinations. Among them, 125 accessions had double-recessive vrn-B1 + vrn-D1 alleles. However, Vrn-B1a + vrn-D1, Vrn-B1b + vrn-D1, vrn-B1 + Vrn-D1a, vrn-B1 + Vrn-D1b alleles were carried by 5, 4, 47 and 16 varieties, respectively. Only 1 accession harboured double-dominant Vrn-B1b + Vrn-D1a alleles. Least significant range (LSR, a method of multiple comparison) tests revealed significant differences among the six groups with respect to the mean values of HD and FD in almost all environments (P < 0.05), with the exception that LSR tests for FD_15_ZMD were not significant (highlighted with yellow). However, no significant differences in the mean values of each group across environments were revealed by the joint ANOVA results (highlighted with green) (Table 4).

Because the low frequency of the Vrn-B1b + Vrn-D1a type (0.5%) made it difficult to exactly compare this allelic combination with other genotypes, we focused on the other five combinations. With respect to their effects, we found that accessions with the vrn-B1 + vrn-D1 genotype presented the latest HD and FD (178.9 d), while varieties that harboured the Vrn-B1b + vrn-D1
| ID  | Taxa          | Registered   | Origination | W/S_2016 | W/S_2015 | Vrn-A1 | Vrn-B1 | Vrn-D1 | Vrn-B3 |
|-----|---------------|--------------|-------------|-----------|-----------|--------|--------|--------|--------|
| Q201| Shanyou 225   | winter       | Shaanxi     | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| Q202| Aikang 58     | semi-winter  | Henan       | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q203| Zhoumai 24    | semi-winter  | Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q204| Xiaoxiang 158 | semi-winter  | Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q205| Zhengmai 366  | semi-winter  | Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q206| Bainong 418   | semi-winter  | Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q207| Taihema 1     | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q208| Anmai 8       | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q209| Huaimai 05159 | semi-winter  | Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q210| Zhoumai 16    | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q211| Xinmai 18     | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q212| Xianmai 13    | weak spring  | Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| Q213| Yandian 9433  | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q214| Zhonghuang 805| weak spring  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q215| Zhengjumai 9987| semi-winter | Henan       | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| Q216| Luoma 31      | semi-winter  | Henan       | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q217| Yimai 6       | semi-winter  | Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1b| vrn-B3 |
| Q218| Zhongzhong 17 | semi-winter  | Henan       | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| Q219| Fanmai 803    | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q220| Zhoumai 27    | semi-winter  | Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q221| Luoma 28      | semi-winter  | Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q222| Yunong 982    | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q223| Zhengmai 1023 | semi-winter  | Henan       | 0         | 0         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q224| BN 160        | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q225| 04 zhong 36   | weak spring  | Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| Q226| Lankao198     | weak spring  | Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1b| vrn-B3 |
| Q227| Fengdecunmai 5| semi-winter  | Henan       | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q228| Guoyu 101     | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q229| Wen 0418      | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q230| Yunong 202    | semi-winter  | Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q231| Zhongyu 9307  | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q232| Ruzhou 0319   | semi-winter  | Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q233| Yumai 41      | semi-winter  | Henan       | 0         | 0         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q234| Yumai 55      | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q235| Yunong 186    | semi-winter  | Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q236| Junda 106     | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q237| Yumai 49      | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q238| Zhengmai 7698 | semi-winter  | Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q239| Lunxuan 1298  | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q240| Mengmai 023   | weak spring  | Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| Q241| Luoma 23      | semi-winter  | Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| Q242| Jiyanmai 7    | semi-winter  | Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q243| Fengdecunmai 8| semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q244| Zhoumai 9     | semi-winter  | Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
Table 1 The phenotypic variants in winter/spring growth habits and allelic variants on Vrn-1 alleles (Continued)

| ID  | Taxa                  | Registered | Origination | W/S_2016 | W/S_2015 | Vrn-A1 | Vrn-B1 | Vrn-D1 | Vrn-B3 |
|-----|-----------------------|------------|-------------|-----------|-----------|--------|--------|--------|--------|
| QZ45| Zhengyumai 0519       | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| QZ46| Xuke 316              | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ47| Luomai 18             | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ48| Xinong 979            | semi-winter| Henan       | 5         | 4         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ49| Cunmai 11             | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ50| Fengdecunmai 12       | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ51| Huachuan 919          | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ52| Zou 84258             | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ53| Yunong 211            | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ54| LS 6109               | semi-winter| Shandong    | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ55| Lankao 182            | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ56| Xumai 1242            | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ57| Zhoumai 26            | semi-winter| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ58| Yamai 1               | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ59| Yujiao 5              | semi-winter| Henan       | 0         | 0         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ60| Fanmai 11             | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ61| Zhengmai 103          | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ62| Purnai 053            | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ63| Tunfeng 802           | semi-winter| Henan       | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ64| Fengdecunmai 1        | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ65| Pu2056                | semi-winter| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1b| vrn-B3 |
| QZ66| Xinxia 19             | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ67| Wenliang 1            | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ68| Zhongyu 9302          | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ69| Zhengmai 583          | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ70| Hengguan 35           | semi-winter| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1b| vrn-B3 |
| QZ71| Luomai 24             | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ72| Yunong 416            | semi-winter| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ73| Zhoumai 22            | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ74| Yumai 52              | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ75| FS 059                | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ76| Pinging 11            | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ77| Bonong 6              | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ78| Zhoumai 18            | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ79| Xuke 168              | semi-winter| Henan       | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ80| Purnai 10             | semi-winter| Henan       | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ81| Xumai 0054            | semi-winter| Jiangsu     | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ82| Zhengyou 6            | semi-winter| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ83| Yunong 9901           | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ84| Zhengmai 0856         | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ85| Luomai 8              | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ86| Zhoumai 13            | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ87| Luo 10 T07            | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| QZ88| Yuanyu 3              | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
Table 1 The phenotypic variants in winter/spring growth habits and allelic variants on Vrn-1 alleles (Continued)

| ID  | Taxa              | Registered | Origination | W/S_2016 | W/S_2015 | Vrn-A1 | Vrn-B1 | Vrn-D1 | Vrn-B3 |
|-----|------------------|------------|-------------|-----------|-----------|--------|--------|--------|--------|
| Q289| Fengdecunmai 10  | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q290| Hongmai 118      | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q291| Yumai 14 You     | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| Q292| Zhoumai 19       | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | Vrn-D1b| vrn-B3 |
| Q293| Guomai 301       | semi-winter| Henan       | 0         | 0         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q294| Zhengmai 113     | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| Q295| Pumai 9          | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| Q296| Pingan 8         | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q297| Xinmai 20        | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| Q298| Zhongmai 875     | semi-winter| Beijing     | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q299| Zhongyanmai 0708 | weak spring| Jiangsu     | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q300| Zhongmai 1       | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q301| Nongda 1108      | semi-winter| Beijing     | 1         | 1         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| Q302| Yumai 47         | weak spring| Henan       | 5         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q303| Zhengmai 98      | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| Q304| Xinong 889       | semi-winter| Shaanxi     | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q305| Neixiang 188     | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q306| Jirmai 22        | semi-winter| Shandong    | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q307| Fanmai 5         | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| Q308| Huayu 198        | semi-winter| Henan       | 0         | 0         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q309| Huangming 116    | weak spring| Henan       | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q310| Zhengmai 9694    | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q311| Yunong 949       | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| Q312| Shan 160         | semi-winter| Shaanxi     | 3         | 3         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q313| Luomai 4         | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q314| Luoxin 998       | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| Q315| Yunong 201       | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q316| Kaimai 21        | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q317| Yubao 1          | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | Vrn-D1b| vrn-B3 |
| Q318| Bainong 207      | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q319| Luomai 21        | semi-winter| Henan       | 3         | 3         | vrn-A1 | vrn-B1 | Vrn-D1b| vrn-B3 |
| Q320| Zhengmai 101     | weak spring| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q321| Xinmai 9         | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q322| Xinmai 208       | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | Vrn-D1b| vrn-B3 |
| Q323| Yumai 34         | weak spring| Henan       | 5         | 5         | vrn-A1 | vrn-B1 | Vrn-D1b| vrn-B3 |
| Q324| Kaimai 18        | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q325| Xuke 793         | semi-winter| Henan       | 1         | 1         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q326| Fanmai 7030      | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q327| Shi 4185         | semi-winter| Hebei       | 3         | 3         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| Q328| Xin 0208         | semi-winter| Henan       | 2         | 2         | vrn-A1 | vrn-B1 | vrn-D1 | vrn-B3 |
| Q329| Zhengmai 379     | semi-winter| Henan       | 3         | 3         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| Q330| Jirmai 20        | semi-winter| Shandong    | 4         | 4         | vrn-A1 | vrn-B1 | vrn-D1a| vrn-B3 |
| Q331| Qiule 2122       | semi-winter| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| Q332| Zhoumai 23       | weak spring| Henan       | 4         | 4         | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
Table 1 The phenotypic variants in winter/spring growth habits and allelic variants on Vrn-1 alleles (Continued)

| ID   | Taxa                | Registered Origination | W/S_2016 | W/S_2015 | Vrn-A1 | Vrn-B1 | Vrn-D1 | Vrn-B3 |
|------|---------------------|------------------------|----------|----------|--------|--------|--------|--------|
| QZ133| Zhengyumai 043      | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ134| Zhengmai 004        | semi-winter Henan       | 3        | 3        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ135| Wennong 14          | semi-winter Shandong    | 2        | 2        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ136| Xinhan 1            | semi-winter Henan       | 4        | 4        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ137| Yumai 18            | weak spring Henan       | 5        | 5        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ138| Xue 415             | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ139| Pingan 9            | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ140| Shangmai 156        | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ141| Xioning 9871        | semi-winter Shaanxi     | 5        | 5        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ142| Pingan 3            | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ143| Zhongyu 12          | semi-winter Henan       | 2        | 2        | vrn-A1 | vrn-B1 | Vrn-D1b| vrn-B3 |
| QZ144| Yumai 58            | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ145| Xun 9917            | semi-winter Henan       | 4        | 4        | vrn-A1 | vrn-B1 | Vrn-D1b| vrn-B3 |
| QZ146| Ping’an 6           | weak spring Henan       | 5        | 5        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ147| Xinmai 26           | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ148| Lankaoaizao 8       | weak spring Henan       | 2        | 2        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ149| Fengyou 6           | weak spring Henan       | 5        | 5        | vrn-A1 | Vrn-B1a| Vrn-D1 | vrn-B3 |
| QZ150| Huaimai 0882        | semi-winter Jiangsu     | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ151| Taikong 6           | weak spring Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ152| Xinong 529          | weak spring Shaanxi     | 5        | 5        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ153| Yumai 51            | weak spring Henan       | 5        | 5        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ154| Luo 6073            | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ155| Bainong 64          | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ156| Jinan 17            | winter                  | 2        | 2        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ157| Yanzhan 4110        | weak spring Henan       | 5        | 5        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ158| Weilai 0818         | semi-winter Anhui       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ159| Yanke 028           | weak spring Henan       | 5        | 5        | vrn-A1 | Vrn-B1a| Vrn-D1 | vrn-B3 |
| QZ160| 86(79)-128          | semi-winter Henan       | 4        | 4        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ161| Yammiai864          | weak spring Henan       | 5        | 5        | vrn-A1 | Vrn-B1b| Vrn-D1 | vrn-B3 |
| QZ162| Xiaoyan 81          | semi-winter Beijing     | 2        | 2        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ163| Liangxing 99        | semi-winter Shandong    | 2        | 2        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ164| Tainong 8968        | semi-winter Shandong    | 0        | 0        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ165| Huayumai 118        | semi-winter Henan       | 2        | 2        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ166| Yanshi 16           | weak spring Henan       | 5        | 5        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ167| Xue 1               | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1a| vrn-B3 |
| QZ168| Zhoumai 30          | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1b| vrn-B3 |
| QZ169| Yunong 4023         | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ170| Zhengpinmai 8       | semi-winter Henan       | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ171| Zhengmai 9023       | weak spring Henan       | 5        | 5        | vrn-A1 | Vrn-B1b| Vrn-D1 | vrn-B3 |
| QZ172| Shiluan 02–1        | semi-winter Hebei       | 0        | 0        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ173| Aifeng 3            | winter                  | 1        | 1        | vrn-A1 | vrn-B1 | Vrn-D1b| vrn-B3 |
| QZ174| Han 6172            | winter                  | 2        | 2        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ175| Luohan 3            | semi-winter Henan       | 4        | 4        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
| QZ176| Yannong 19          | winter                  | 0        | 0        | vrn-A1 | vrn-B1 | Vrn-D1  | vrn-B3 |
allelic combination presented the earliest HD and FD (174.3 d) as well as the shortest growth habit. This method could be useful for precisely identifying the differences in growth habits in each group individually. Then, analyses of Vrn-1 combinations revealed that the effects of the dominant Vrn-B1 genotype on HD and FD were stronger than those of the dominant Vrn-D1 genotype. Finally, we concluded that the rank order of the effects on the growth period was as follows: Vrn-B1b > Vrn-B1a > Vrn-D1b > Vrn-D1a > vrn-D1 = vrn-B1 (Table 4).

### Allelic variations of Ppd-1 alleles

No polymorphisms were found in the promoter of Ppd-A1 or Ppd-B1; thus, we focused on their internal variants. Here, variations in the junction sequences of Ppd-B1 were investigated to analyse their allelic variations and effects, which were considered copy number variations (CNVs) [28]. For Ppd-B1, the Ppd-B1a gene has three types in terms of CNV, accounting for 33.8% (Truncated CS type), 8.6% (Intact CS type) and 35.3% (Sonora 64 type) (Table 5). According to a previous report, the first two genotypes were named Ppd-B1c and the Sonora 64 type was named Ppd-B1a [30]. For variations within Ppd-B1, 6 types of genotypic combinations were all detected because of their different types of combination. As to percentage, the “S: N: N” type constituted the largest proportion (34.34%, “Sonora 64 type” only), while the percentages of “N: I: N” (1.01%, “Intact CS type” only) and “S: N: T” (1.01%, “Sonora 64 type + Truncated CS type” for short) were the lowest (Additional file 1: Table S2; Table 5).
For Ppd-D1, the haplotypes identified among the materials were divided into three types in accordance with the reports of Guo et al. (2010) [31] and Chen et al. (2013) [13]: Hapl I (34.85%, sensitive), Hapl II (0.5%, Chinese Spring, insensitive), and Hapl VII (64.6%, sensitive). Only one variety (Chinese Spring) had a 2.0-kb deletion in the promoter region and thus should be designated Ppd-D1a (insensitive, theoretical relatively short HD and FD). Thus, Hapl I and Hapl VII could be considered recessive alleles in this study (Table 6).

**Effects of single Ppd-1 alleles on HD and FD**

One hundred and twenty-five materials that had double-recessive Vrn alleles (vrn-B1 + vrn-D1) were selected to evaluate the influence of Ppd-B1 or Ppd-D1 on plant traits. There were four genotypes, but no significant differences were found among groups according to ANOVA results. Because inconsistencies between the ANOVA and LSR test results were sometimes detected, multiple comparisons were subsequently performed. The average values for phenotypes among the four groups differed significantly for only five traits (three traits in Zhumadian). Furthermore, varieties that harboured “Sonora 64 type” (“N: N: S”) showed the shortest HD and FD. This result was consistent with those reported by Díaz et al. (2012) [28] (Table 7).

**Table 2** The joint ANOVA analysis in HD and FD grouped by growth habit in six environments

| Traits   | 0          | 1          | 2          | 3          | 4          | 5          | r          |
|----------|------------|------------|------------|------------|------------|------------|------------|
| HD_14_SQ | 183.3 ± 2.0(a) | 182.3 ± 2.2(ab) | 181.9 ± 2.2(ab) | 181.3 ± 1.9(b) | 181.7 ± 2.9(ab) | 178.5 ± 2.9(c) | −0.871*    |
| HD_15_SQ | 189.3 ± 2.4(a) | 187.9 ± 2.0(b)  | 186.9 ± 2.0(b)  | 187.1 ± 2.0(b)  | 186.5 ± 2.1(b)  | 183.7 ± 2.2(c) | −0.922**   |
| HD_14_ZMD| 172.0 ± 1.8(a) | 171.3 ± 1.4(a)  | 171.3 ± 1.2(a)  | 171.3 ± 1.7(a)  | 171.0 ± 1.7(a)  | 169.9 ± 1.1(b) | −0.884**   |
| HD_15_ZMD| 159.1 ± 1.5(a) | 158.3 ± 1.1(b)  | 157.9 ± 1.5(bc) | 157.9 ± 1.1(bc) | 158.1 ± 1.2(b)  | 157.2 ± 1.5(c) | −0.869*    |
| HD_14_ZZ | 181.7 ± 1.9(a) | 180.9 ± 1.1(ab) | 180.8 ± 1.3(ab) | 180.1 ± 1.3(b)  | 180.1 ± 1.5(b)  | 177.6 ± 1.6(c) | −0.897**   |
| HD_15_ZZ | 190.2 ± 3.5(a) | 187.9 ± 2.3(b)  | 187.6 ± 1.8(bc) | 186.8 ± 1.8(bc) | 186.4 ± 1.9(c)  | 183.1 ± 2.2(d) | −0.938*    |
| HD_average| 180.3 ± 11.4(a) | 179.1 ± 10.7(a) | 178.8 ± 10.7(a) | 178.4 ± 10.4(ab) | 178.3 ± 10.3(bc) | 176.0 ± 9.5(b) | −0.915**   |
| FD_14_SQ | 193.7 ± 1.8(a) | 192.2 ± 1.8(b)  | 191.4 ± 2.0(b)  | 191.5 ± 1.5(b)  | 191.9 ± 2.6(b)  | 188.3 ± 2.5(c) | −0.838*    |
| FD_15_SQ | 198.4 ± 1.0(a) | 197.5 ± 1.0(b)  | 197.1 ± 0.8(b)  | 197.4 ± 0.7(b)  | 196.9 ± 1.0(b)  | 194.8 ± 1.4(c) | −0.867*    |
| FD_14_ZMD| 181.3 ± 1.0(a) | 179.5 ± 1.8(b)  | 179.2 ± 1.8(bc) | 179.5 ± 1.8(b)  | 178.7 ± 1.9(b)  | 177.0 ± 1.8(c) | −0.907**   |
| FD_15_ZMD| 167.4 ± 2.1(a) | 166.5 ± 1.5(ab) | 166.1 ± 1.4(b)  | 165.9 ± 1.4(b)  | 166.4 ± 1.6(ab) | 166.2 ± 1.6(b) | −0.813*    |
| FD_14_ZZ | 190.6 ± 1.6(a) | 189.5 ± 1.4(ab) | 189.0 ± 1.5(b)  | 188.8 ± 1.3(b)  | 188.6 ± 1.3(b)  | 185.4 ± 1.9(c) | −0.885**   |
| FD_15_ZZ | 199.3 ± 1.7(a) | 198.2 ± 1.6(ab) | 197.9 ± 1.6(bc) | 197.3 ± 1.6(bc) | 196.8 ± 1.6(c)  | 192.9 ± 2.6(d) | −0.889**   |
| FD_average| 189.1 ± 11.4(a) | 188.1 ± 11.2(a) | 187.6 ± 11.3(ab) | 187.6 ± 11.1(ab) | 187.4 ± 11.0(ab) | 184.9 ± 10.2(b) | −0.886**   |

Lowercase letters indicate significant differences at the 0.05 level; * and ** indicates significant differences at the 0.05 and 0.01 level, respectively. The correlation coefficients (r) were calculated between the mean values of each grade and corresponding rank (r_{0.05} = 0.754 and r_{0.01} = 0.875); the ranks of the growth habit were calculated via the arithmetic means obtained during a two-year period, and all the data of all discrepant individuals were omitted.
The rank order of their effects on growth was as follows: Ppd-1 genotypes, the rank order of their effects on growth was stronger than those of single Ppd alleles but were far weaker than those of Hapl VII or Hapl VIII.

We also examined and assessed the interactive effects of Ppd-B1, Ppd-D1, and Vrn combinations. Similarly, no significant differences were found according to the ANOVA results. A total of eight genotypes that contained two Ppd alleles were surveyed, in which the type “N: N: S + Hapl VII” constituted the largest proportion (29, 23.6%), while the percentages of “T: I: N + Hapl I or Hapl VII” were the lowest (Table 8). With respect to their effects, the LSR method revealed significant differences in the mean values among groups for four traits (three in Zhumadian and one in Zhengzhou). We suspected that mean values of HD and FD were the lowest (Table 8).

The values in parentheses represent the percentage in each group; “only” in parentheses indicates that the value is specific to the single genotype.

### Interactive effects of Ppd-1 combinations on HD and FD

We examined and assessed the interactive effects of Ppd-B1 and Ppd-D1 combinations. Similarly, no significant differences were found according to the ANOVA results. A total of eight genotypes that contained two Ppd alleles were surveyed, in which the type “N: N: S + Hapl VII” constituted the largest proportion (29, 23.6%), while the percentages of “T: I: N + Hapl I or Hapl VII” were the lowest (Table 8).

The values in parentheses represent the percentage in each group; “only” in parentheses indicates that the value is specific to the single genotype.

| Genotype | Material number (only) | Frequency (%) | Speculation of winter/spring habit | Winter | Spring | Consistency (%) |
|----------|------------------------|---------------|-----------------------------------|--------|--------|-----------------|
| Vrn-B1a (only) | 5 | 2.5 | Spring | 0 | 5 | 100.0 |
| Vrn-B1b (only) | 4 | 2.0 | Spring | 0 | 4 | 100.0 |
| Vrn-D1a (only) | 47 | 23.7 | Spring | 24 | 23 | 48.9 |
| Vrn-D1b (only) | 16 | 8.1 | Spring | 12 | 4 | 25.0 |
| Vrn-B1 + Vrn-D1a | 1 | 0.5 | Spring | 0 | 1 | 100.0 |
| Vrn-B1 + vrn-D1 | 125 | 63.1 | Winter | 119/117 | 4/6 | 96.8/95.2 |
| Total | 198 | 100.0 | | 155/153 | 41/43 | 79.8/78.2 |

### Discussion

#### Consistency of marker analysis and growth habit identification

Wheat is the major crop in the YHW in terms of yield and area in China. This region is located in the transition zone of winter and spring wheat cultivation, where semi-winter varieties and weak spring cultivars are also planted. However, inconsistencies sometimes occur between registered and empirical results. Hence, the precise identification of winter/spring growth habits for newly registered varieties is necessary and helpful not only for the rational use of varieties but also for the provision of vital information for breeders in the YHW. Here, growth habits were examined during a two-year period via a novel field spring sowing identification method and materials along with marker-assisted selection (MAS).

We believe that our identification method in the field is more practical than that conducted in greenhouses, where the materials are grown under conditions closely related to those in the field. Furthermore, correlation analysis revealed that the phenotypic data during two years was as follows: Ppd-1 > Ppd-B1 > Ppd-D1.

| Types | Frequency (%) | Speculation of winter/spring habit | Winter | Spring | Consistency (%) |
|-------|---------------|-----------------------------------|--------|--------|-----------------|
| Vrn-B1 + vrn-D1 | 125 | 63.1 | Winter | 119/117 | 4/6 | 96.8/95.2 |
| Vrn-B1a + vrn-D1 | 5 | 2.5 | Spring | 0 | 5 | 100.0 |
| Vrn-B1b + vrn-D1 | 4 | 2.0 | Spring | 0 | 4 | 100.0 |
| Vrn-D1a (only) | 47 | 23.7 | Spring | 24 | 23 | 48.9 |
| Vrn-D1b (only) | 16 | 8.1 | Spring | 12 | 4 | 25.0 |
| Vrn-B1 + Vrn-D1a | 1 | 0.5 | Spring | 0 | 1 | 100.0 |
| Vrn-B1 + vrn-D1 | 125 | 63.1 | Winter | 119/117 | 4/6 | 96.8/95.2 |
| Total | 198 | 100.0 | | 155/153 | 41/43 | 79.8/78.2 |

Letters in parentheses indicate a significant difference at the 0.05 level; decimal values preceded by “±” indicate standard deviation.
years were also consistent between years. In comparison with registered information, 10 of 155 (6.45%, 2016) winter wheat varieties were inconsistent (Zhongchuang805, Taikong6, Xiaoyan22, etc.), and similar situations were observed in other groups—in particular, 43 spring wheat varieties containing 13 (25.6%, 2016) inconsistent samples (Bainong3217, Luohan6, Xinong979, etc.). We doubted that the reason for this situation was due to their early registration before the materials were rigorously identified. In total, the consistency was approximately 90%, although some varieties presented discrepancies, and our method was more convenient than the report of Gardener and Barnett [32].

More vital clues that we wanted to examine included the consistency between vernalization alleles and growth habit. The results indicated that all ten Vrn-B1 genotypes of spring wheat varieties presented a value of 100%, whereas Vrn-D1 exhibited lower results. Among the 43 cultivars ranked as grade 5 (data from 2016, Xinzheng), 37 (86.04%) carried at least one of the tested dominant vernalization alleles and were classified as spring varieties; the other 6 varieties carried the recessive alleles at the three vernalization loci. For winter types, 131 of 155 (84.51%) accessions presented similar consistency. We predicted that there are two main factors that could be responsible for this phenomenon. First, a single individual is genotyped, whereas the phenotype is assessed on a plot scale of multiple individuals, and there may be some variation among individual seeds. Second, two other major pathways also control heading and flowering dates in plants, i.e., the phytohormone gibberellic acid (GA) and the autonomous pathways, in addition to the vernalization and photoperiod pathways [33, 34].

Allelic distributions of Vrn-1 revealed trends and orientations in the YHW.

As no dominant allele of Vrn-A1 or Vrn-B3 was detected, which are probably the two genes that have the strongest effects of those examined, the allelic distributions of the dominant Vrn-B1 and Vrn-D1 alleles are likely responsible for the spring genotypes of wheat varieties. Indeed, the scarcity and decreasing frequency of the Vrn-A1 and Vrn-B3 loci in the YHW have been previously discussed by Zhang et al. (2008) and Chen et al. (2013) [4, 13]. We suspected that the frequencies of recessive vrn-B1 and vrn-D1 likely increased via direct selection due to their contributions to yield traits because of the long maturation period. This inference was in agreement with that reported in the literature [14, 35]. Furthermore, the frequency of dominant Vrn-D1 was higher than that of Vrn-B1 in our tested materials. These results were also consistent with the previous report of Zhang et al. (2015) [36].

Although only 10 dominant Vrn-B1 alleles (5.0%) were discovered in the spring genotypes, the consistency of the marker-growth habit (100%) was better than that of Vrn-D1. Additionally, we found that accessions with single Vrn-B1b alleles exhibited the earliest HD and FD in the six environments; these effects were stronger than those for Vrn-B1a, Vrn-D1b and Vrn-D1a. These results were consistent with those of previous studies in which the rank order was Vrn-A1 > Vrn-B1 > Vrn-D1 [37]. Santra et al. (2009) reported that a novel Vrn-B1b allele that resulted from a 36-bp deletion within intron 1, which is

### Table 5 Allelic variants of Ppd-B1

| Genotype | Sonora 64 type | Intact CS type | Truncated CS type | N | Proportion (%) |
|----------|----------------|----------------|-------------------|---|----------------|
| Ppd-B1   | No             | No             | No                | 61 | 30.81          |
|          | Yes            | No             | No                | 68 | 34.34          |
|          | No             | Yes            | No                | 2  | 1.01           |
|          | No             | No             | Yes               | 50 | 25.25          |
|          | Yes            | No             | Yes               | 2  | 1.01           |
|          | No             | Yes            | Yes               | 15 | 7.58           |
| Total    |                |                |                   | 198| 100            |

### Table 6 Allelic variants of Ppd-D1

| Genotype | 2-kb deletion | Transposable element (TE)insertion | 5-bp deletion | 16-bp insertion | Number | Proportion (%) |
|----------|---------------|-----------------------------------|---------------|-----------------|--------|----------------|
| Ppd-D1   | Hapl I        |                                   |               |                 | 69     | 34.85          |
|          | Hapl II       |                                   |               |                 | 1      | 0.51           |
|          | Hapl VII      |                                   |               |                 | 128    | 64.65          |
| Total    |               |                                   |               |                 | 198    | 100            |

The method for dissecting the haplotypes of Ppd-D1 was described by Guo et al. (2010)
referred to as ‘Alpowa’ and carries the winter growth habit alleles *vrn-A1* and *vrn-B1*, was likely to cause a spring growth habit; however, the authors did not provide sufficient evidence [14].

The dominant *Vrn-D1* locus occurs in the most popular types and is distributed throughout nearly the entire wheat production region [4, 13]. The previous data also established that carriers of *Vrn-A1* or *Vrn-D1* tend to produce longer spikes than do carriers of *Vrn-B1*. As a result, the *Vrn-D1* genotypes were prevalent in China [38, 39]. In the present study, in terms of *Vrn-D1*, the allelic frequency reached 81.8%, which represented the most dominant allele distribution in the population. Thus, the proportion was similar to previous reports; however, poor consistency in the growth habit of Zhengzhou was observed. Thus, it would be interesting to test whether *Vrn-D1* and other alleles interact to influence growth habits and period (HD and FD).

The results of the *Vrn-1* combination analysis revealed that only one accession carried *Vrn-B1B + Vrn-D1a* alleles; thus, the samples were so limited that phenotypic data were not statistically representative. For genotypic analysis, in combination with growth habit identification, 63.1% of the materials that had three recessive alleles all belonged to winter or semi-winter wheat (grade 0–4). This tendency was in accordance with that reported by Sun et al. (2009, 61.1%). Moreover, the ANOVA and LSR tests revealed that the mean values of the HD, FD and growth habit among six genotypes differed significantly, which also indicated that the *Vrn-1* combinations were tightly associated with phenotypes. Although the effects of these combinations on HD and FD were not definitively stronger than those of single *Vrn-B1* alleles, the growth habit level was divided in greater detail. This division will enable a more precise identification of vernalization requirements for accessions at molecular levels.

### Table 7 The effects of single *Ppd-B1* and *Ppd-D1* alleles on HD and FD

| Type     | N   | HD$_{14}$SQ | HD$_{15}$SQ | HD$_{14}$ZMD | HD$_{15}$ZMD | HD$_{14}$ZZ | HD$_{15}$ZZ | HD_average |
|----------|-----|-------------|-------------|--------------|--------------|-------------|-------------|------------|
| NNN      | 39  | 185.2 ± 2.7(ab) | 188.7 ± 2.6(a) | 172.1 ± 1.5(b) | 159.2 ± 1.4(b) | 181.8 ± 1.3(ab) | 189.2 ± 2.6(a) | 179.1 ± 10.9(a) |
| TNN      | 30  | 183.3 ± 2.1(ab) | 186.6 ± 1.9(a) | 172.7 ± 1.7(ab) | 159.2 ± 1.2(b) | 181.9 ± 1.4(ab) | 188.4 ± 2.6(a) | 179.0 ± 10.6(a) |
| TIN      | 10  | 184.1 ± 2.1(a) | 189.3 ± 1.9(a) | 173.6 ± 1.3(a) | 160.2 ± 0.8(a) | 182.7 ± 0.7(a) | 189.7 ± 1.3(a) | 179.9 ± 10.5(a) |
| NNS      | 44  | 182.6 ± 2.1(b) | 188.2 ± 2.1(a) | 171.8 ± 1.4(b) | 158.8 ± 1.3(b) | 181.7 ± 1.3(b) | 188.2 ± 2.5(a) | 178.6 ± 10.6(a) |
| Type     | N   | FD$_{14}$SQ | FD$_{15}$SQ | FD$_{14}$ZMD | FD$_{15}$ZMD | FD$_{14}$ZZ | FD$_{15}$ZZ | FD_average |
| NNN      | 39  | 193.3 ± 2.5(a) | 197.3 ± 1.3(a) | 180.4 ± 2.1(a) | 167.5 ± 1.9(ab) | 190.5 ± 1.8(a) | 199.2 ± 2.1(a) | 188.1 ± 11.3(a) |
| TNN      | 30  | 192.7 ± 1.6(a) | 197.5 ± 0.9(a) | 180.5 ± 1.9(a) | 167.5 ± 1.4(b) | 190.2 ± 1.4(a) | 198.7 ± 2.2(a) | 187.9 ± 11.1(a) |
| TIN      | 10  | 193.1 ± 0.9(a) | 197.7 ± 0.8(a) | 181.0 ± 1.4(a) | 168.5 ± 1.4(a) | 190.9 ± 0.8(a) | 199.4 ± 0.8(a) | 188.5 ± 10.8(a) |
| NNS      | 44  | 192.7 ± 1.5(a) | 197.3 ± 0.8(a) | 180.3 ± 1.8(a) | 166.9 ± 1.6(b) | 190.3 ± 1.4(a) | 187.6 ± 1.5(a) | 187.7 ± 11.2(a) |

The dominant *Vrn-D1* locus occurs in the most popular types and is distributed throughout nearly the entire wheat production region [4, 13]. The previous data also established that carriers of *Vrn-A1* or *Vrn-D1* tend to produce longer spikes than do carriers of *Vrn-B1*. As a result, the *Vrn-D1* genotypes were prevalent in China [38, 39]. In the present study, in terms of *Vrn-D1*, the allelic frequency reached 31.8%, which represented the most dominant allele distribution in the population. Thus, the proportion was similar to previous reports; however, poor consistency in the growth habit of Zhengzhou was observed. Thus, it would be interesting to test whether *Vrn-D1* and other alleles interact to influence growth habits and period (HD and FD).

The results of the *Vrn-1* combination analysis revealed that only one accession carried *Vrn-B1B + Vrn-D1a* alleles; thus, the samples were so limited that phenotypic data were not statistically representative. For genotypic analysis, in combination with growth habit identification, 63.1% of the materials that had three recessive alleles all belonged to winter or semi-winter wheat (grade 0–4). This tendency was in accordance with that reported by Sun et al. (2009, 61.1%). Moreover, the ANOVA and LSR tests revealed that the mean values of the HD, FD and growth habit among six genotypes differed significantly, which also indicated that the *Vrn-1* combinations were tightly associated with phenotypes. Although the effects of these combinations on HD and FD were not definitively stronger than those of single *Vrn-B1* alleles, the growth habit level was divided in greater detail. This division will enable a more precise identification of vernalization requirements for accessions at molecular levels.

**Interactive effects detected between the *Ppd-B1* and *Ppd-D1* alleles**

Photoperiod responses are controlled by members of the pseudo-response regulator (PRR) gene family in plants. In general, the potential of *Ppd-1* alleles to affect insensitivity has been ranked as *Ppd-D1* > *Ppd-B1* > *Ppd-A1* [17, 38]. However, in the present study, only one accession (Chinese spring) was found to carry a 415-bp band that indicated a genotype of *Ppd-D1a*. Thus, it was difficult to precisely evaluate its effect on phenotype statistically. According to the criterion of previous research, two sensitive haplotypes (Hapl I and Hapl II) of *Ppd-D1* were used for the evaluation for their distribution and effects [13, 31].

For *Ppd-B1*, we only examined the polymorphisms of CNVs of the *Ppd-B1* locus because of its tight association with heading and flowering time. Indeed, Zhang et al. (2015) designated eight haplotypes according to the combinations of CNVs of *Ppd-B1* and found that the cultivar with *Ppd-B1_hapl-VI* demonstrated the earliest heading and flowering times [36]. However, the results were not consistent with those of Diaz et al. (2012) [28]. In the present study, 125 accessions carrying recessive *vrn-B1* and *vrn-D1* alleles were selected. With respect to *Ppd-B1*, we found that wheat cultivars with “Sonora 64” *Ppd-B1a* alleles flower earlier than those with “Chinese
Table 8: Interactive effects of Ppd-1 combinations on growth period and growth habit

| Truncated CS type | Intact CS type | Sonara64 type | Ppd-D1 | N     | HD_14_SQ | HD_15_SQ | HD_14_ZMD | HD_15_ZMD | HD_14_ZZ | HD_15_ZZ | HD_average |
|------------------|----------------|---------------|--------|-------|-----------|-----------|-----------|-----------|-----------|-----------|------------|
| No               | No             | No            | Hap-I  | 13    | 1829 ± 2.4(a) | 188.3 ± 2.6(a) | 172.1 ± 1.3(bc) | 159.0 ± 1.1(b) | 181.5 ± 1.2(b) | 188.4 ± 1.9(a) | 178.7 ± 10.6(a) |
| Yes              | No             | No            | Hap-I  | 11    | 1832 ± 2.2(a) | 188.2 ± 1.6(a) | 172.8 ± 1.9(abc) | 159.1 ± 1.2(b) | 181.8 ± 1.4(b) | 188.8 ± 2.3(a) | 179.0 ± 10.6(a) |
| Yes              | Yes            | No            | Hap-I  | 5     | 1841 ± 1.7(a) | 189.2 ± 2.2(a) | 173.6 ± 1.3(a) | 159.9 ± 0.4(ab) | 183.1 ± 0.2(a) | 190.1 ± 0.6(a) | 180.0 ± 10.7(a) |
| No               | No             | Yes           | Hap-I  | 15    | 1827 ± 2.2(a) | 188.8 ± 1.8(a) | 171.9 ± 1.1(c) | 159.0 ± 1.2(b) | 182.1 ± 1.3(ab) | 188.7 ± 2.8(a) | 178.9 ± 10.8(a) |
| No               | No             | No            | Hap-VII| 26    | 1835 ± 2.9(a) | 188.9 ± 2.6(a) | 172.1 ± 1.6(abc) | 159.3 ± 1.6(ab) | 182.1 ± 1.6(ab) | 189.6 ± 2.8(a) | 179.3 ± 11.0(a) |
| Yes              | No             | No            | Hap-VII| 5     | 1834 ± 2.0(a) | 188.8 ± 2.1(a) | 172.6 ± 1.6(abc) | 159.2 ± 1.1(b) | 181.9 ± 1.3(ab) | 188.2 ± 2.8(a) | 179.1 ± 10.7(a) |
| Yes              | Yes            | No            | Hap-VII| 19    | 1840 ± 2.7(a) | 189.5 ± 1.8(a) | 173.6 ± 1.5(ab) | 160.5 ± 1.1(a) | 182.3 ± 0.9(ab) | 189.4 ± 1.8(a) | 179.9 ± 10.5(a) |
| No               | No             | Yes           | Hap-VII| 29    | 1825 ± 2.1(a) | 187.8 ± 2.2(a) | 171.8 ± 1.6(c) | 158.7 ± 1.4(b) | 181.5 ± 1.3(b) | 187.9 ± 2.3(a) | 178.4 ± 10.5(a) |
| Truncated CS type | Intact CS type | Sonara64 type | Ppd-D1 | N     | FD_14_SQ | FD_15_SQ | FD_14_ZMD | FD_15_ZMD | FD_14_ZZ | FD_15_ZZ | FD_average |
| No               | No             | No            | Hap-I  | 13    | 1928 ± 1.8(a) | 197.1 ± 1.3(a) | 180.6 ± 1.7(a) | 167.3 ± 1.2(b) | 190.41 ± 1.3(a) | 198.9 ± 2.0(a) | 187.9 ± 11.1(a) |
| Yes              | No             | No            | Hap-I  | 11    | 1924 ± 1.1(a) | 197.3 ± 0.8(a) | 181.1 ± 2.1(a) | 167.3 ± 2.4(a) | 190.0 ± 1.2(a) | 199.0 ± 1.8(a) | 187.9 ± 11.0(a) |
| Yes              | Yes            | No            | Hap-I  | 5     | 1934 ± 0.5(a) | 197.9 ± 1.1(a) | 181.2 ± 1.4(a) | 167.8 ± 1.3(ab) | 191.1 ± 0.7(a) | 199.8 ± 0.4(a) | 188.5 ± 11.2(a) |
| No               | No             | Yes           | Hap-I  | 15    | 1926 ± 1.8(a) | 197.5 ± 0.8(a) | 180.4 ± 1.9(a) | 167.0 ± 1.7(b) | 190.6 ± 1.7(a) | 198.8 ± 1.7(a) | 187.8 ± 11.3(a) |
| No               | No             | No            | Hap-VII| 26    | 1936 ± 2.9(a) | 197.5 ± 1.4(a) | 180.3 ± 2.4(a) | 167.7 ± 2.2(b) | 190.5 ± 2.0(a) | 199.3 ± 2.1(a) | 188.2 ± 11.4(a) |
| Yes              | No             | No            | Hap-VII| 19    | 1929 ± 1.9(a) | 197.6 ± 0.9(a) | 180.1 ± 1.8(a) | 167.6 ± 1.5(b) | 190.4 ± 1.5(a) | 198.6 ± 2.4(a) | 187.9 ± 11.2(a) |
| Yes              | Yes            | No            | Hap-VII| 5     | 1928 ± 1.3(a) | 197.5 ± 0.5(a) | 180.8 ± 1.4(a) | 169.3 ± 1.1(a) | 190.8 ± 1.1(a) | 199.1 ± 1.1(a) | 188.4 ± 10.6(a) |
| No               | No             | Yes           | Hap-VII| 29    | 1928 ± 1.4(a) | 197.2 ± 0.8(a) | 180.2 ± 1.8(a) | 166.8 ± 1.6(b) | 190.2 ± 1.2(a) | 198.6 ± 1.4(a) | 187.7 ± 11.2(a) |

Letters in parentheses indicate a significant difference at the 0.05 level; decimal values preceded by "±" indicate standard deviation; 'N' indicates no target bands were amplified in 'Truncated CS type,' 'Intact CS type' and 'Sonara64' of CNVs at Ppd-B1 locus.
Spring” alleles, which was in accordance with Díaz et al. (2010). Furthermore, we found that six types of combinations emerged, and the “Truncated CS type” and “Intact CS type” did not simultaneously emerge for Ppd-B1. These results were also not the same as those reported by Chen et al. (2013). We suspected that the complex genetic background (genotypes mixed with Vrn-1 genes) would hinder us from providing definitive results. Thus, we believed that our method was possibly more reliable than previous methods because of the uniform background [30]. With respect to Ppd-D1a, the rare diversity of the Ppd-D1 allele could not be used to exactly evaluate the effects of the variants, and no significant differences were observed between the two haplotypes (Hapl I and Hapl II).

Comparison of effects on growth period (HD and FD) between the Vrn-1 and Ppd-1 alleles
Moreover, from a comprehensive perspective, we concluded that, compared with Ppd-1, Vrn-1 played a major role in regulating heading and flowering traits as well as growth habit. At the Vrn-1 locus, cultivars with the Vrn-B1b + vrn-D1 (174.3 d for HD, 183.2 d for FD) allele both headed and flowered earlier by approximately 4 days than did cultivars with the vrn-B1 + vrn-D1 (178.9 d for HD, 187.9 d for FD) allele (Table 4). Whereas at the Ppd-1 locus, cultivars with the “N: N: S” allele combination (178.6 d for HD, 187.7 d for FD) both headed and flowered approximately 1 day earlier than did cultivars with the “T: I: N” allele combination (179.9 d for HD, 188.5 d for FD) (Table 7). Indeed, the interactive effects of Vrn-1 and Ppd-1 gene combinations were also detected in our research. However, the results of ANOVA and LSR tests revealed weak interactions between Vrn-B1 and Ppd-B1, Vrn-D1 and Ppd-D1, Vrn-B1and Ppd-D1, Vrn-D1and Ppd-B1 (data not shown). We suspected that the complex genetic background in natural populations would make it difficult to reveal this interaction. In a previous study, Shcherban et al. (2014) also found that the haplotypes Ppd-D1a/Vrn-B1a or Ppd-D1a/Vrn-B1a did not differ significantly in heading time from the respective Vrn-I haplotypes harbouring the sensitive allele Ppd-D1b [40]. This finding suggests that it is better for us to examine the interaction between Ppd-1 and Vrn-1 in biparental populations.

Conclusion
In the present study, we dissected the Vrn-1 and Ppd-1 gene composition and found that Vrn-1, rather than Ppd-1, played a major role in controlling vernalization and photoperiod responses in this region. The work will be helpful for guiding the breeding of wheat in the Yellow and Huai wheat production region.

Methods
We tested 198 cultivars (lines) including historic varieties, commercial varieties, and newly bred varieties originating from the YHW. Among them, 159 accessions were from Henan, 10 accessions were from Shandong, 10 accessions were from Shaanxi, 8 accessions were from Jiangsu, 4 accessions were from Hebei, 4 accessions were from Beijing, 1 accession was from Shanxi, 1 accession was from Anhui, and 1 accession (Chinese Spring) was from Sichuan (Table 1). The entire original source of the plant materials used in our study was kindly provided by other labs. We complied with the Convention on the Trade in Endangered Species of Wild Fauna and Flora: https://www.cites.org.

Characterization of winter/spring growth habits
Although the growth habit for assessing vernalization is already well established, identification of the exact materials involved is necessary because of differences in environmental conditions. The tested materials were planted at the Zhengzhou Scientific Research and Education Center of Henan Agriculture University (113.7°E, 34.7°N) on 12 March 2015 and at another test site [ZhengHan Seed Technology Co. Ltd., XinZheng (113.7°E, 34.4°N)] on 12 March 2016. Seeds were sown in 1.0-m rows, and individual seeds were spaced 6.67 cm apart; 15 seedlings were reserved per row after wheat seedling emergence. Two replications were planted for reliable data collection. The stage of maturity and percentage of headed spikes were recorded on 25 June in the same year; we repeated these measurements one week later. The growth habit of the materials was divided into grades numbered 0 to 5. The criteria were as follows: 0, no jointing and booting; 1, partial main stem headed; 2, main stem and a few tillers headed; 3, normal heading but abnormal grain filling and immaturity; 4, normal heading and grain filling but premature; 5, normal maturity.

Identification of HD and FD
The varieties used to assess agronomic traits were planted on 9 October 2013 and 2014 in Zhengzhou (113.7°E, 34.7°N), on 15 October 2013 and 17 October 2014 in Shangqiu (115.7°E, 34.5°N), and on 19 October 2013 and 5 November 2014 in Zhumadian (114.0°E, 32.9°N). All of these locations differed significantly in day length and climatic factors in Henan Province. Each material was planted in two 1.5-m rows; there were 110 seeds per row, and the rows were spaced 23 cm apart. Two replications were planted at each location. Field management practices during our experiments were in accordance with agronomic practices commonly used in the area. The HD and FD were assessed on a plot scale of multiple individuals when more than half of the
individual seedlings exhibited classic morphological traits for these events.

**DNA extraction and diagnostic markers for Vrn-1 and Ppd-1**

DNA was extracted from the seedlings in accordance with a modified SDS-phenol-chloroform method [27]. The primers used were based on those described in many previous reports and were synthesized by Sangon Biotech Co., Ltd. (Shanghai) (Additional file 2: Table S1). To recognize the segments amplified from Ppd-B1, accessions harbouring 994 bp, 425 bp, and 223 bp were designated intact Chinese Spring type (I), truncated Chinese Spring type (T), and Sonora 64 type (S), respectively. If no bands were amplified in the materials, the genotypes were referred to as null (N) [28].

**PCR amplification and electrophoresis**

PCR amplification reactions were conducted in a 12-μL reaction flask containing 40 ng of genomic DNA, each primer at 2.5 μM, each dNTP at 200 μM, 1× buffer containing 1.5 μM MgCl₂, and 0.5 units of Taq polymerase. We used a Bio-Rad thermocycler with the following PCR conditions: 94 °C for 3 min; 34 cycles of 94 °C for 30 s, 50 °C to 65 °C for 30 s (annealing temperatures for each primer pair are listed in Additional file 2: Table S1), and 72 °C for 1 min; and a final 10-min extension at 72 °C for preservation. The PCR products were separated by electrophoresis either on a 0.8–1.2% agarose gel stained with ethidium bromide (EB) or an 8% nondenaturing polyacrylamide gel and visualized with silver staining [29].

**Statistical analysis**

A consistency index (%) was used; this index indicated the number of accessions that originated from the consistent results of both genotype and field identifications divided by the total number of materials. The phenotypic data were imported into R software (R 3.4.1) for analysis via ANOVA, Student’s t-tests and correlation; we used the “reshape” and “agricolae” packages to perform these analyses and the “ggplot2” package for graphical construction.

**Additional file**

- **Additional file 1**: Table S2. The genotypes of Ppd-1 and their effects on heading and flowering dates. (XLS 25 kb)
- **Additional file 2**: Table S1. Primers used in this study. (XLS 71 kb)

**Abbreviations**

YHW: Yellow and Huai wheat production region; HD: Heading date; FD: Flowering date; ZZ: Zhengzhou; ZMD: Zhumadian; SQ: Shangqiu; ANOVA: Analysis of variance; LSR: Least significant range method of multiple comparison; CNVs: Copy number variations; MAS: Marker-assisted selection; PRR: Pseudo-response regulator; WS: Winter/Spring growth habit.

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**Availability of data and materials**

All data are available in the additional files.

**Authors’ contributions**

KZ conceived the project and prepared the trials. JW, GD and LC performed the experiments. SC analysed the data and was a major contributor in writing the manuscript. XC and HX performed statistical analysis and edited the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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