Multi-environmental population phenotyping suggest the higher risks of wheat *Rht-B1b* and *Rht-D1b* cultivars in global warming scenarios

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Short Title: The filed phenotyping of *Rht-B1b* and *Rht-D1b*

One-sentence Summary: The *Rht-B1b* and *Rht-D1b* controls broadly wheat agronomic traits, which are vulnerable to temperature increase.

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ABSTRACT

After six decades practice, the semi-dwarf alleles *Rht-B1b* and *Rht-D1b*, (also called *Rht-1* and *Rht-2*) had been applied into more than 70% current wheat cultivars, laid the foundation for the worldwide wheat production supply, then the phenotypes of the two alleles still keeps unclear. Here 400 wheat accessions, including 106 *Rht-B1b* accessions, 99 *Rht-D1b* accessions, were systematic investigated on 16 agronomic traits in seven environments, uncover the effects of two well-known alleles on wheat structure and yield traits in different genetic backgrounds and ecosystems, providing important reference for further application in different genetic and environmental conditions. The regression analysis based on the phenotypic and their meteorological data showed the temperature increase at seedling stage severely reduce the tiller number and result in huge yield penalty per plant. The accession containing *Rht-D1b* may lost about 40% yield if the temperature increases 2□ in average. This population phenotyping provides the global phenotypes to dissect the molecular mechanism of *Rht-B1b*, *Rht-D1b* and GA signaling in wheat development and environmental adaptation. This study recommends a new approach to get the global phenotypes conferred by a specific allele with natural environmental conditions, which benefits the data from field to lab.

INTRODUCTION

The introduction of the semi-dwarfing trait into wheat cultivars during the 1960s and 1970s, confers great lodging resistance, allowing high fertilizer input, thereby significantly enhance yield of wheat, was a defining as the “Green Revolution” (Peng et al., 1999; Hedden, 2003). Approximately 70% of cultivars in a worldwide winter wheat panel that were released in the 21st century carry one of these two “Green Revolution” alleles (Wurschum et al., 2017).

There are two Rht (Reduced Height) alleles, *Rht-B1b* and *Rht-D1b* (also named as *Rht1* and *Rht2*), which introduce premature stop codons in the N-terminal coding region of DELLA proteins to alter the GA signaling (Wu et al., 2011; Liu et al., 2021). Accumulating data showed the Gibberellins interacts with multiple phytohormones, such as auxin (Löfke et al., 2013), strigolactones (Marzec, 2017; Liao et al., 2019), brassinosteroids (Ross and Quittenden, 2016) and ethylene (Qin et al., 2022), to plays broadly role in regulating plant development and environmental adaptation (Claeys et al., 2014). However, the GA synthesis, transportation and regulatory role is spatiotemporally dependent (Topham et al., 2017; Binenbaum et al., 2018; Van De Velde et al., 2021; Qin et al., 2022), challenges the functional study of *Rht-B1b* and *Rht-D1b*. Recent study suggests the *Rht-B1b* and *Rht-D1b* produce N-terminal truncated proteins through translational re-initiation, which may confer new function beyond of GA signaling (Van De Velde et al., 2021). All these prevent our understanding on the regulatory mechanism of the green alleles in wheat.

On the other side, after sixty years application the green alleles were found implication in a broad agronomic traits in addition to reducing the plant height (Lanning et al., 2012; Sherman et al., 2014). However, limited by few genetic backgrounds and environmental conditions in previous studies, the agronomic traits controlled by green alleles always showed inconsistent conclusion (Jobson et al., 2019). In addition, two green alleles, the *Rht-B1b* and *Rht-D1b*, showed minor difference in controlling wheat traits (Bush and Evans, 1988; Velu et al., 2017; Jobson et al., 2019), which may related their adoption to the distinct wheat region in USA

(Guedira et al., 2010), Europe (Wurschum et al., 2015; Wurschum et al., 2018) and China (Zhang et al., 2006; Gao et al., 2015), then the underlying mechanism still keeps unknown. These observations suggested the agronomic phenotypes of *Rht-B1b* and *Rht-D1b* are far from fully dissected, which prevent the study on underlying regulatory mechanism and its precisely application in wheat breeding and production.

Current practices had demonstrated that the wheat cultivars containing *Rht-B1b* and *Rht-D1b* are more vulnerable to drought stress (Monneveux et al., 2012; Jatayev et al., 2020), indicating a potential weakness of *Rht-B1b* and *Rht-D1b* in adapt to environmental stress, which may influence the green alleles application in futural global warming scenario. As the most adopted wheat alleles, their adaptation to temperature increase has to be evaluated to ensure wheat production security for the future.

Here, 16 agronomic traits of *Rht-B1b* and *Rht-D1b* were investigated in seven environments across three years on 400 wheat accessions, demonstrating the global effects of *Rht-B1b* and *Rht-D1b* on wheat traits and its environmental stability, providing the global phenotypes for studying the molecular mechanism of *Rht-B1b* and *Rht-D1b* in wheat development and environmental adaptation, and the potential risk for futural wheat breeding and production.

RESULTS

The bread wheat population for agronomic phenotyping

A total 400 wheat accessions were collected from all over the world, and were genotyped by KASP assay, including 105 *Rht-B1b* (*B1b)* accessions, 98 *Rht-D1b* (*D1b*) accessions, and 197 accessions without the two alleles (refer to *NO* thereafter) (Figure 1A). Sixteen agronomic traits on plant structure and yield components were investigated in seven environments across three years (Table S1). The correlations analysis suggests the plant height in this population is significantly correlated with peduncle length, total tiller number, flag leaf length, flag leaf width, kernel number per spike and thousand kernel weight (Figure 1B), indicating that these traits may be

affected by the dwarf gene introduction.

The effects of *Rht-B1b* and *Rht-D1b* introduction on wheat plant structure and yield

To acquire the phenotypes of *Rht-B1b* and *Rht-D1b*, the BLUE (best linear unbiased estimator) value of the agronomic traits in seven environments were compared to that of *NO* accessions. Meanwhile, 1000 permutation tests were performed on the 50% random samples combination in each group to evaluate genetic background dependence. We found that all the plant structure related traits are significantly altered by the green alleles (Figure 2A) with low genetic background dependences (> 800 times significantly difference out of 1000 times test). Meanwhile, all the traits also showed significantly difference and low genetic background dependence between the *Rht-B1b* and *Rht-D1b* accessions, except the trait flag leaf area, which showed significantly difference between *Rht-B1b* and *Rht-D1b* but this difference is medium dependent on the genetic background (540 significant difference in 1000 permutation test). Moreover, the *Rht-D1b* generally displayed a stronger effect in altering structure traits, consistent with the previous observation that the dwarf effect from *Rht-D1b* is significantly and stably higher than that in *Rht-B1b* (Wurschum et al., 2017).

However, the general significant difference can't be detected on the yield related traits between the dwarf and *NO* group (Figure 2B). The *Rht-D1b* has a role in reducing the spike length and the spikelet spacing but still maintain the similar spikelet number, suggesting a compact spike structure in the wheat cultivars containing *Rht-D1b*. Meanwhile, the *Rht-D1b* confers higher kernel length, kernel width and final kernel weight, result in 2.1 microgram increase for each kernel (Figure. 2B), equal to 6% yield increase. In contrast, the *Rht-B1b* showed higher fertile spikelet rate and kernel number per spike, which result in 4.2 kernels increase per spike, contribute 8% yield increase compared to that of *NO*. The significantly difference of kernel number and kernel weight are also observed between the group of *Rht-B1b* and *Rht-D1b* with medium genetic background dependence, highlighting that

the distinct positive effect of *Rht-B1b* and *Rht-D1b* on the wheat yield, providing new insights for higher yield conferred by green alleles (Hedden, 2003; Jatayev et al., 2020).

Environmental variations of Green Revolution genes

To evaluate the environmental stability of the green alleles, the difference of traits was further compared in different environment (Figure 2, Table. S1). We found the effect of green alleles were stable on plant structure traits in all environments, especially *Rht-D1b*, which showed the significant difference in five environments at least out of seven environments compared to *NO* group, indicating that the effects of green alleles on wheat structure traits is stable in different environments.

For yield related trait, only the kernel number per spike controlled by *Rht-B1b* showed high environmental stability (>70%), while the effect of *Rht-D1b* on spike length, thousand kernel weight and kernel width showed medium environmental stability (≥43.9%) (Figure 2). These results suggested the effects of green alleles on yield traits tend to be affected by the environments.

Spatiotemporal application of the Green Revolution genes in China

In our wheat population, including 87 landraces (LA) and 259 historical/modern cultivars (MC) (Table. S1), which contains 30 Chinese landraces and 163 Chinese modern cultivars (after 1970s) (Figure 3A). In China, which have no accession in LA contains the Green Revolution genes, while more than 67% accessions in MC contains one of Green Revolution alleles. The higher frequency of *Rht-D1b* (44.8%) was observed in Chinese cultivars, and enriched in the Yellow and Huai wheat zone (81%) out of 10 Chinese agroecological wheat zones (Table S2). Which further supporting that a distinct environmental application of two Green Revolution genes (Wurschum et al., 2015; Grogan et al., 2016; Zhao et al., 2019), and highlighting that the importance of *Rht-D1b* in wheat production and breeding in the biggest wheat region of China.

The application frequency of *Rht-B1b* or *Rht-D1b* increased from 57.3% to 77.8%

in MC which is released before and after 2000 (Figure 3B), highlighting the green alleles are the major dwarf gene applied in current wheat varieties.

The heat adaptation potential of *Rht-B1b* and *Rht-D1b*

To evaluate the thermotolerance of green alleles for facing the Global warming challenges, Late-sowing field assays (Chauhan et al., 2009; Tiwari et al., 2012) were performed in three years, which conferred higher temperatures for each growth stage of wheat (Figure 4A). Almost investigated traits significantly changed with the late-sowing (Figure 4B, Table S5), indicating that wheat traits are susceptible to late-sowing.

For plant structure, the plant height and flag leaf area were decreased about 30%, and the tiller number were decreased more than 50% in late-sowing condition, however, for yield traits, only the thousand kernel weight were decreased more than 15%, indicating that the vegetative growth stage of wheat was more sensitive to the environmental alterations (Figure 4B, Table S5).

Notably, the agronomic traits of green alleles accessions showed more severe influences by the late-sowing, indicating the green alleles are more sensitive to the temperature increase than *NO* (Figure 4B), especially the accessions with *Rht-D1b*.

The effect of green alleles in response to increasing temperature

To further confirm the effects of high temperature on green alleles, we collected all traits data of 400 accessions in seven environments and the available meteorological data in seven environments for multivariate regression analysis (Chmielewski and Potts, 1995; Tao et al., 2018). We found relative humidity and solar radiation is not significantly contribute influence to wheat traits variation, while the temperature in different developmental stage contributes the substantial effect to all traits of wheat population (Table S8).

The temperature showed the necessary variations on the different accessions at different developmental stage in seven environments (Figure 5A). The regression analysis shows that the average temperature at different stage significantly affected

wheat traits (Table S9). Based on the regression equations, we predict the traits alterations when the average temperature increase 1 and 2 □ (Figure 5B and C). We found the tilling number, flag leaf angle, grain weight and plant height are the most temperature sensitive traits, generally consistent to the estimation by the late sowing (Figure 4B). Both *Rht-B1b* and *Rht-D1b* were more severe influenced by the increasing temperature, compared to *NO* accessions.

This regression analysis showed the temperature increase in seedling stage is the most severe disruption for the wheat growth, except the flag leaf angle is mostly influenced by the temperature in filling stage (Table S8). The yield per plant reduces around 20% and 38% if the average temperature increases 1 and 2 □ (Figure 5D). The yield penalty of cultivars containing green alleles is about 25% higher compared to that in *NO* cultivars. Further calculation demonstrates that the temperature increases at seedling stage cause the most penalty for the yield per plant, which directly inhibits the tiller number (Figure 5E). This regression analysis strongly suggest that green alleles is more sensitive to the temperature increase, will cause a severe yield penalty in the global warming scenarios.

DISCUSSION

Decades application had proved the capability of green alleles in controlling plant structure and increasing wheat yield (Kertesz et al., 1991; Jin et al., 2015; Jobson et al., 2019). Our results further showed the *Rht-B1b* and *Rht-D1b* reduced tillers, flag leaf length, and flag leaf angle, resulted in more compact wheat plant structure, which allow a higher planting density and contribute to the significant yield increase per unit area, as demonstrated in rice and maize (Sinclair and Sheehy, 1999; Evenson and Gollin, 2003). Meanwhile, these two alleles caused the increase of flag leaf width, which made the leaf shape more reasonable and increased the transverse light receiving area for enhancing photosynthetic efficiency (Gale et al., 1974; Morgan et al., 1990), these findings may provide references for wheat structure improvement. Notably the more erect flag leaf was observed in accessions containing green alleles,

which is unknown traits related to the GA signaling, need further clarified. It would play valuable role in improving modern wheat cultivars.

In rice, the GA negatively regulated tiller number through SLR1-MOC1 mechanism (Xu et al., 2021), showing the interaction mechanism between GA and strigolactones (Marzec, 2017). However, we found the green alleles reduce the tiller number in wheat, indicating a different mechanism in wheat to control the tiller number through the DELLA. The reduced tiller may confer a benefit for the dry-land wheat yield, but limited the yield potential under well water conditions (Houshmandfar et al., 2020). We speculate that the less tiller number would be another reason to require a higher plant density for the wheat cultivars containing green alleles.

Although many studies have reported that the semi-dwarfing genes affect wheat yield related traits such in number, grain number per spike and thousand kernel weight (Pinthus and Levy, 1983; Gale and Youssefian, 1985; Richards, 1992; Zhang et al., 2013), but no convinced conclusions have been reached so far. In this study, we found the *Rht-B1b* contribute more the kernel number per spike while *Rht-D1b* confers higher kernel weight in different genetic background and environments (Figure 2), highlighting the distinct way to increase wheat yield, this finding is great significance for wheat production and breeding.

Our data suggested that the phenotypes controlled by green alleles is easily influenced by the temperature increase, consistent to GA signaling participate in environmental perception (Mo et al., 2018; Bao et al., 2020; Tang et al., 2021; Xu et al., 2021). The increased temperature confers broad negative effect on the wheat structure and yield traits (Figure 5). We found the tiller number is the most heat sensitive traits for wheat yield. The reduced tiller number would require higher planting density for maintaining wheat production. However, the flag leaf angle is also significantly increased by the temperature increase (Figure 5), which would prevent the higher planting density practice. Thus the dilemma between tiller number and flag leaf angle has to resolved in futural wheat breeding. For the cultivars containing green alleles, especially containing *Rht-D1b*, temperature increase would cause more yield loss to

risk futural food supply.

MATERIALS AND METHODS

Plant materials and growing conditions

400 accessions of bread wheat were collected in this study, including modern cultivars and landraces from worldwide regions (Table S1). All materials were planted in seven environments (E1--E7) at Yangling (34°28′N, 108°07′E, altitude 517 m) and Chongzhou (30°63′N, 103°67′E, altitude 1300 m) of China in 2018-2021 (Table S10). The experiments were arranged in a randomized complete block design with each variety planted in three replicates. Each accession was sown with 1 m in length and a row spacing of 20 cm, with 10 seeds per row. Field management was consistent with local practices for wheat production.

Agronomic traits measurements

The traits of plant structure and yield were measured at physiological maturity. Three healthy individuals selected from each row were used to investigate plant height (PH), peduncle length (PL), total tiller number (TTN), productive tiller number (PTN), flag leaf length (FLL), flag leaf width (FLW), flag leaf angle (FLAN), spike length (SL), total spikelet number (TSN), fertile spikelet number (FSN) and kernel number per spike (KNS) according to established protocols (Fan et al., 2015; Sun et al., 2017; Guan et al., 2019; Jobson et al., 2019). Days to heading was assessed as the interval between the date of seeding emergence and the date at which 50% of spikes per row emerged from the flag leaf, days to flowering was assessed as the interval between the date of seeding emergence and the date at which 50% of spikes per row have extruded at least one anther (Kiseleva et al., 2016; Li et al., 2019). Further, we calculated flag leaf area (FLA, FLL × FLW × 0.83), spikelet spacing (SS, SL / TSN) and yield per plant (YPP, PTN×KNS×TKW). In addition, thousand kernel weight (TKW), kernel length (KL) and kernel width (KW) were recorded using the rapid SC-G grain appearance quality image analysis system developed by Hangzhou WSeen Detection

Technology Co, Ltd, China (Guan et al., 2019).

DNA extraction and genotyping

Genomic DNA was extracted from wheat flag leaves of heading stage plants following the modified Cetyl Trimethylammonium Bromide (CTAB) method for genotyping (Wehner et al., 2015). Kompetitive allele-specific PCR (KASP) markers were developed by the mutation site of *Rht-B1b* and *Rht-D1b* to identify all the accessions (Ma et al., 2019). All primers were listed in Supplementary Table S11. The KASP assay was performed as following: assays were set up as 5 μL reactions containing 2.2 μL template DNA dried to assay plate, 2.5 μL of V4.0 2x Kaspar mix (LGC Group, Teddington, UK), 2.444 μL ddH_2O and 0.056 μL primer mix; and the PCR cycling included hotstart at 94 °C for 15 min, followed by 10 touchdown cycles (94 °C for 20 s; touchdown 61°C, -0.6 °C per cycle, 1 min), then followed by 35 cycles of amplification (94 °C for 20 s; 55 °C for 1 min).

Statistical analysis

The best linear unbiased estimate (BLUE) values for phenotypic under seven environments were obtained using a mixed linear model by genotype as a fixed effect and replicates as random effects. Analysis of variance (ANOVA) was performed to test the homogeneity of genotype and phenotypic based on linear mixed model using the Python SciPy module. Differences between phenotypic of different genotypes were tested by *t-test* (Virtanen et al., 2020). If there is homogeneity of variance between the two sets of data, the default argument to the function *stats.ttest_ind* is used; otherwise, the parameter *equal_var* is set to *False*. All analyses were conducted using *lme4* (Bates et al., 2015) and *lsmeains* packages (Lenth, 2016) in R3.6.2 (Bates et al., 2014).

Permutation test

To avoid the co-selected SNPs and/or genes interferes, the permutation test was performed on each genotype pairs to evaluate the effect of different genomic background. The wheat population was divided into three groups by genotypes (*NO*,

B1b, *D1b*), 50% of the accessions were randomly selected to test the significance between different groups. 1000 permutations were performed to examine how often significant differences between groups occurred in by chance alone. Significant differences between groups were considered to be reliable if they occurred in a frequency more than 800; not vice versa (Wang et al., 2018; Xu et al., 2020).

Multivariate regression analysis

Multiple linear regression analysis of climate factors

Weather data were taken from the China Meteorological Data Service Center (https://data.cma.cn). We collected 6 climate variables, that is, the maximum and minimum temperatures, precipitation, sunlight hours and relative humidity and the solar radiation in seven environments. According to the heading and flowering time of each accession and 6 climate variables, the average maximum temperature, the average minimum temperature, the mean precipitation, the sunlight hours, the average relative humidity and the solar radiation at seeding stage, flowering stage and filling stage for every accessions were collected (Table S6). Multiple regression analysis was used to examine the effect of meteorological factors on phenotypes (Chmielewski and Potts, 1995; Tao et al., 2018). The equation was:

$$Y=a+bX_1+cX_2+dX_3+eX_4+fX_5+gX_6+hX_7+iX_8+jX_9+kX_{10}+lX_{11}+mX_{12}+nX_{13}+oX_{14}+pX_{15}+qX_{16}+rX_{17}+sX_{18}$$

where Y represents the phenotypic data. X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16}, X_{17}, X_{18} indicate the average maximum temperature, the average minimum temperature, the mean precipitation, the sunlight hours, the average relative humidity and the solar radiation at seeding stage, flowering stage and filling stage, respectively. b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s are the regression coefficients, and a is a constant.

Multiple linear regression analysis of average temperature at different stage

Multiple regression analysis was used to examine the effect of average temperature at different stage on phenotypes. The equation was:

$$Y=m_1+m_2Xa+m_3Xb+m_4Xc$$

where Y represents the phenotypic data. Xa, Xb, Xc represents the average temperature at seeding stage, flowering stage and filling stage, respectively. m_2, m_3, m_4 are the regression coefficients, and m_1 is a constant.

We used *fit()* function to construct the general equation for regression analysis, and then *drop1()* function was used to conduct stepwise regression for the constructed equation. Meteorological factors with the smallest values from Akaike Information Criterion (AIC) were always removed until the equation was constructed successfully. The criteria of the constructed equation were as follows: the significance of T-test for the regression coefficient of independent variables was $Pt < 0.05$; the significance of F-test for the whole equation was $Pf < 0.05$, and the R-squared > 0.8.

Supplemental Data

Supplemental Table 1. The information and phenotypes of 400 wheat accessions in seven environments.

Supplemental Table 2. The distributions of wheat accessions in different agroecological regions.

Supplemental Table 3. The durations days for all wheat accessions at different stage.

Supplemental Table 4. The average temperature of wheat different sowing date at different growth stages.

Supplemental Table 5. The alterations of traits with late-sowing.

Supplemental Table 6. The meteorological data in different periods and years for all wheat accessions.

Supplemental Table 7. The heading and flowering time for all wheat accessions.

Supplemental Table 8. The multiple linear regression equations with multiple meteorological factors.

Supplemental Table 9. The multiple linear regression equations with temperatures.

Supplemental Table 10. The sowing information of seven environments.

Supplemental Table 11. The KASP primer sequence.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China [31571756 and 31501380]. We thank Guoyue Chen and Zhien Pu for wheat field investigation in Sichuan.

AUTHOR CONTRIBUTIONS

S.X. designed the concept and experiments. Z.L., X.L., J.Z. and X.M. performed field investigation. J.C and X.Z performed the phenotypic investigation of transgenic lines in field. Z.L., Z.H., W.J., X.L. and S.X. analyzed the data and wrote the manuscript. All the authors were involved in the revision of the manuscript and approved the final manuscript.

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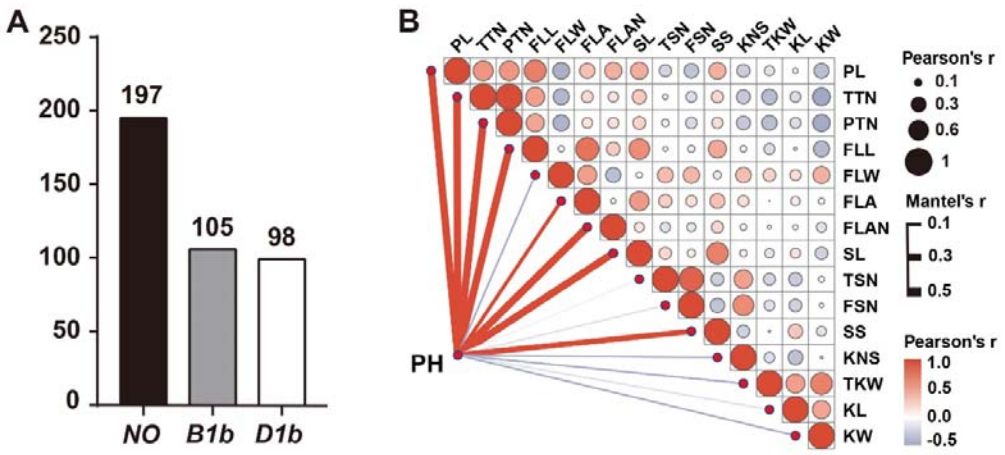

Figure. 1 The number of accessions with different genotypes in population and the correlation analysis between different agronomic traits.

(A) The number of wheat accessions with different genotypes in the population by KASP assay. *NO*, wheat accessions without *Rht-B1b* and *Rht-D1b*; *B1b*, wheat accessions containing *Rht-B1b*; *D1b*, wheat accessions containing *Rht-D1b*. The number indicates wheat accessions number in each genotype.

(B) The correlations analysis between different agronomic traits by the best linear unbiased estimate (BLUE) values. PH, plant height; PL, peduncle length; TTN, total tiller number; PTN, productive tiller number; FLL, flag leaf length; FLW, flag leaf width; FLA, flag leaf area; FLAN, flag leaf angle; SL, spike length; TSN, total spikelet number; FSN, fertile spikelet number; SS, spikelet spacing; KL, kernel length; KW, kernel width; KNS, kernel number per spike; TKW, thousand kernel weight. Red lines mean positive correlation and gray lines mean negative correlation. The correlations were analyzed by the “corrplot” in the R package using the BLUE values in Table S1.

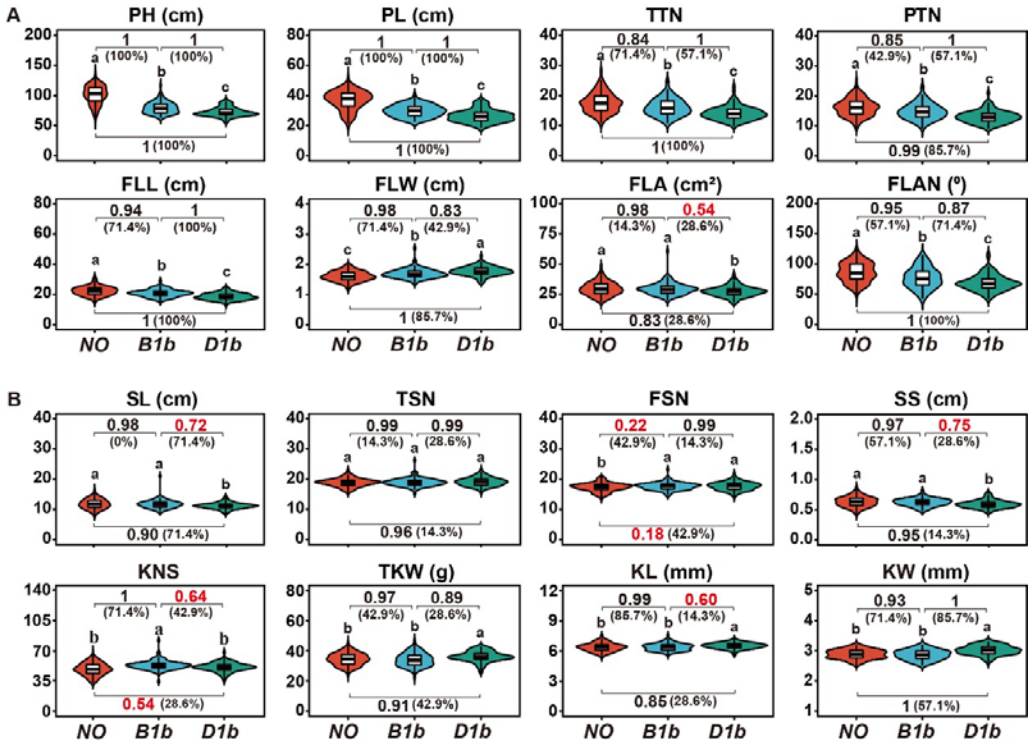

Figure. 2 The function of the *Rht-B1b* and *Rht-D1b* on wheat traits.

(A) The function of the *Rht-B1b* and *Rht-D1b* on wheat structure. The letters at the top of violin plots indicate the significant difference between each group (two-sided Dunn's Kruskal–Wallis test, $p < 0.01$). The numbers represent the ratio of comparison showing significantly difference / all comparison, the 1000 permutation tests. Red number marks the ratio less 800. The percentages in parentheses indicate environmental stability, the number of environments that have the same effect divided by the total number of environments. The data come from the BLUE values (n=400) of seven conditions, as shown in Table S1.

(B) The function of the *Rht-B1b* and *Rht-D1b* on wheat yield.

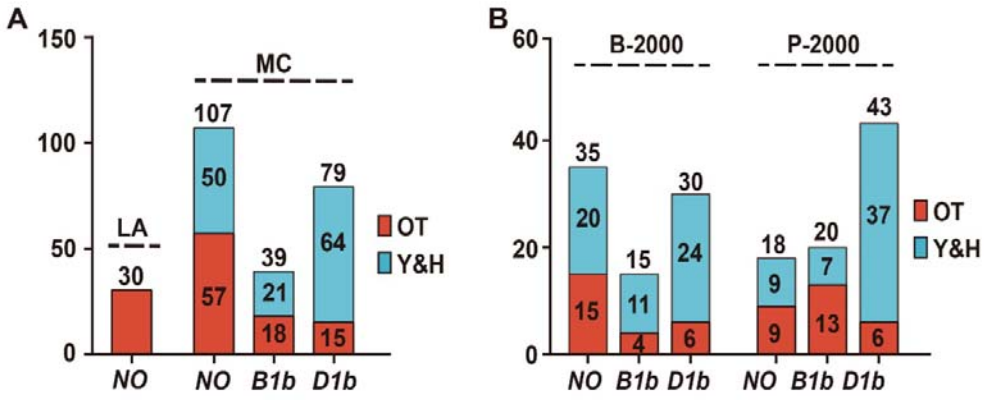

Figure. 3 The spatiotemporal distribution of the *Rht-B1b* and *Rht-D1b* in China.

(A) The introduction and distribution of the *Rht-B1b* and *Rht-D1b* in Chinese landraces and Chinese modern cultivars. Y&H, Yellow and Huai wheat zone (blue); OT, Other agroecological wheat zone (red). The numbers at the top of the bars means the total number of wheat accessions. The numbers in the blue and red bar indicate the number of wheat accessions in different agroecological wheat zone, respectively.

(B) The application time of the *Rht-B1b* and *Rht-D1b* in Chinese modern cultivars (MC). B-2000, the cultivars was released from 1960's to 2000; P-2000, post 2000.

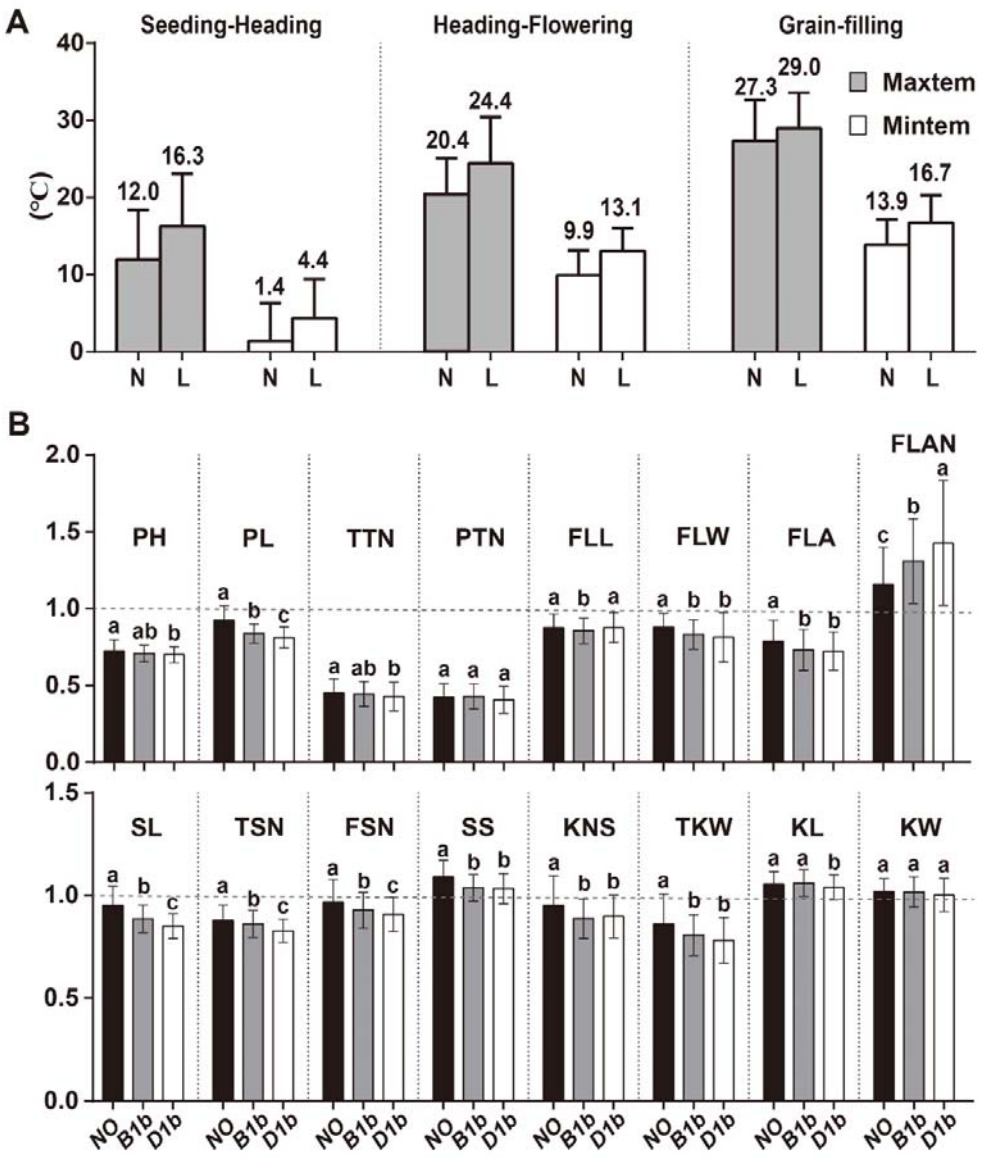

Figure. 4 The phenotypic variations with late-sowing.

(A) The temperature variations with late-sowing. N, normal sowing time conditions; L, late sowing time conditions. The sowing date and temperature was shown in Table S3 and S4.

(B) The phenotypic alteration with late sowing. The data is the phenotypic data in late-sowing/ phenotypic data in normal-sowing. Different letters on the bars indicate the significant differences ($p < 0.05$, two-sided Dunn's Kruskal–Wallis test), as shown in Table S5. The gray dash lines indicate the value 1.

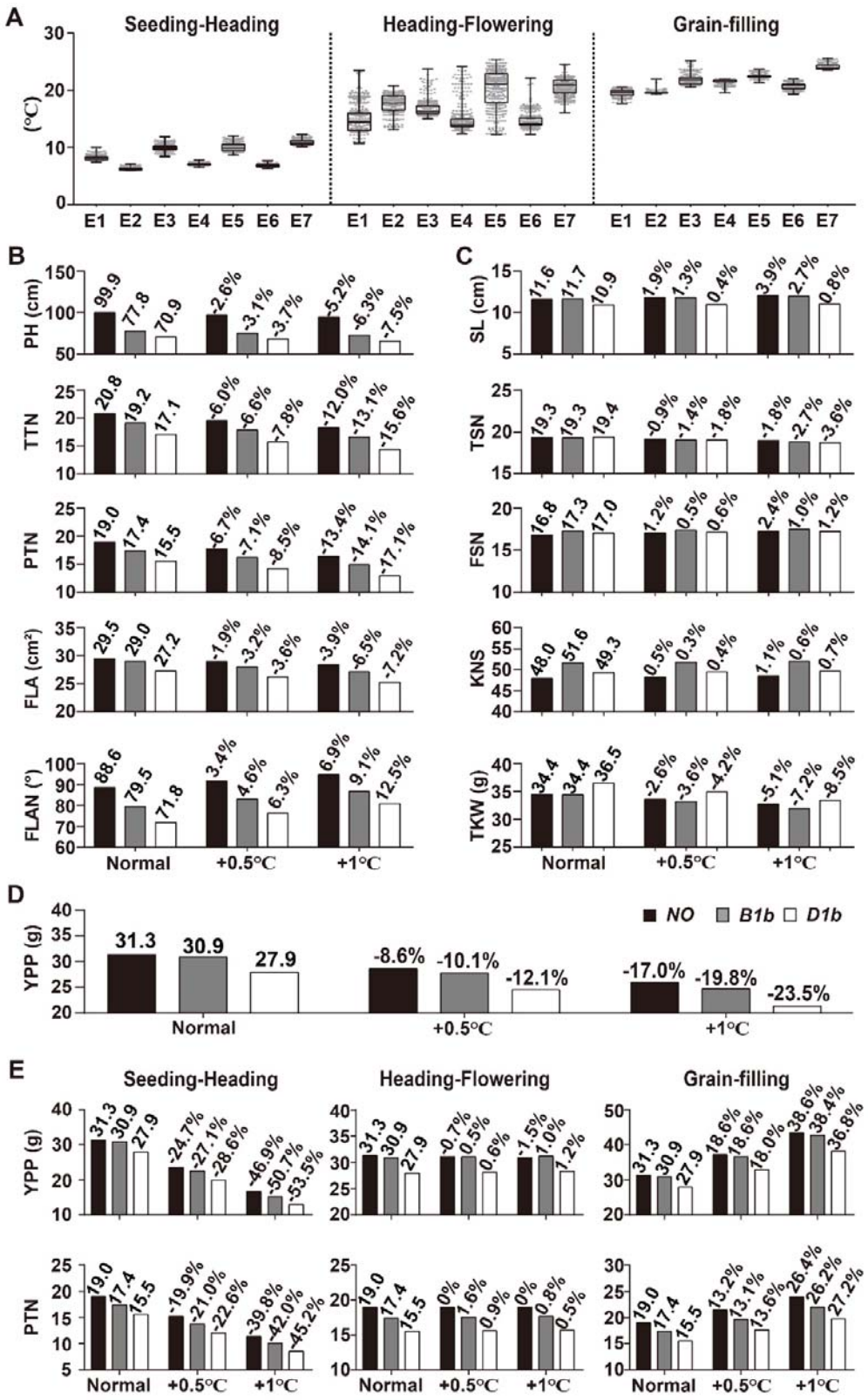

Figure. 5 The phenotypes of the *Rht-B1b* and *Rht-D1b* in response to temperature increase.

(A) The temperature variations in seven different environments. The environments information, temperature and the developmental stage of each accession as shown in Table S6, S7 and S11.

(B) The structure traits alterations with average temperature increased 0.5 and 1℃. The phenotypic value of Normal, +0.5℃ and +1℃, was calculated by regression equation with average temperatures in observed temperature, the average temperature increased +0.5℃, +1℃ respectively. The percentages the bars indicate the phenotypic changed ratio of corresponding genotype wheat accessions when the average temperature increased 0.5 and 1℃.

(C) The alterations of wheat yield traits when the average temperature increased 0.5 and 1℃. The phenotypic value of Normal, +0.5℃ and +1℃, was calculated by regression equation with average temperatures in observed temperature, the average temperature increased +0.5℃, +1℃ respectively.

(D) The alterations of wheat yield per plant (YPP) with temperature increase. The YPP was estimated by productive tiller number * kernel number per spike * kernel weight in (C).

(E) The alterations of wheat yield per plant with temperature increased 0.5 and 1℃ at different developmental stage. The YPP was estimated by productive tiller number * kernel number per spike * kernel weight, which was calculated by the regression equation with the average temperature at sowing-heading, heading-flowering and grain-filling stages of wheat, respectively.