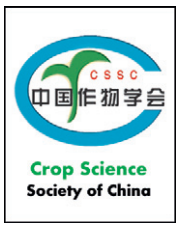
THE CROP JOURNAL 7 (2019) 845–856



Available online at www.sciencedirect.com

ScienceDirect

Assessment of the individual and combined effects of *Rht8* and *Ppd-D1a* on plant height, time to heading and yield traits in common wheat

Kunpu Zhang[a,b,*], *Junjun Wang*[a], *Huanju Qin*[a], *Zhiying Wei*[c], *Libo Hang*[c], *Pengwei Zhang*[c], *Matthew Reynolds*[d], *Daowen Wang*[a,b,*]

[a]*State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*

[b]*College of Agronomy and State Key Laboratory of Wheat and Maize Crop Science, Henan Agricultural University, Zhengzhou 450002, Henan, China*

[c]*Zhaoxian Institute of Agricultural Sciences, Zhaoxian 051530, Hebei, China*

[d]*CIMMYT, Int. Apdo. Postal 6-641, 06600 México, DF, Mexico*

ARTICLE INFO

Article history:
Received 19 December 2018
Received in revised form
11 March 2019
Accepted 2 August 2019
Available online 10 August 2019

Keywords:
Association analysis
QTL mapping
Grain number
Grain yield
Triticum aestivum

ABSTRACT

Grain yield in cereal crops is a complex trait controlled by multiple genes and influenced by developmental processes and environment. Here we report the effects of alleles *Rht8* and *Ppd-D1a* on plant height, time to heading, and grain yield and its component traits. Association analysis and quantitative trait locus mapping using phenotypic data from 15 environments led to the following conclusions. First, both *Rht8* and *Ppd-D1a* reduce plant height. However, *Ppd-D1a* but not *Rht8* causes earlier heading. Second, both *Rht8* and *Ppd-D1a* promote grain yield and affect component traits. Their combined effects are substantially larger than those conferred by either allele alone. Third, promotion of grain yield by *Rht8* and *Ppd-D1a* is through increasing fertile spikelet number. We speculate that *Rht8* and *Ppd-D1a* act independently and additively in control of plant height, grain yield and yield component. Combination of the two alleles is desirable for adjusting plant height and enhancing grain yield and abiotic stress tolerance.

© 2019 Crop Science Society of China and Institute of Crop Science, CAAS. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Abbreviations: DH, double haploid; FSNS, fertile spikelet number per spike; GNS, grain number per spike; GY, grain yield; HS, Hengshui; TH, time to heading; IF, irrigated and fertilized; JY, Jiyuan; MLM, mixed linear model; PH, plant height; QTL, quantitative trait loci; RF, rainfed; RN, reduced nitrogen; RP, reduced phosphorus; SL, spike length; SNS, spikelet number per spike; SZ, Suzhou; TA, Tai'an; YHRV, Yellow and Huai river valley

* *Corresponding authors at: State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.*

E-mail addresses: kpzhang@genetics.ac.cn, (K. Zhang), dwwang@genetics.ac.cn. (D. Wang).

Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

https://doi.org/10.1016/j.cj.2019.06.008

2214-5141 © 2019 Crop Science Society of China and Institute of Crop Science, CAAS. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Increased cereal yield is a major goal of agricultural research. However, efforts to reach such a goal have met considerable difficulties in recent years, because the rate of genetic gain in grain yield (GY) is slowing down [1]. Furthermore, crop productivity is increasingly limited by reduced availability of natural resources (such as water for irrigation) and ecological concerns (e.g., water pollution by excessive use of chemical fertilizers) [2]. It is well known that GY and its related component traits are generally controlled by polygenes whose expression is affected by plant development and environmental factors [3,4]. It is essential, therefore, to study the genes controlling plant development, GY and related traits and their interactions with environmental factors in order to increase the efficiency of enhancing GY by breeding.

Common wheat (*Triticum aestivum* L, AABBDD, 2*n* = 6× = 42) is the most widely cultivated staple crop. Considerable effort has been devoted to investigating the genes and quantitative trait loci (QTL) involved in controlling wheat development and GY [5–8]. The most frequently studied wheat development genes include those controlling vernalization response (*Vrn1*, *Vrn2* and *Vrn3*), photoperiod sensitivity (*Ppd1*), and GA-insensitive plant height (*Rht-B1b* and *Rht-D1b*) [9–11]. The function of these genes tends to be allele-specific and environmentally sensitive. Moreover, they often interact in affecting plant development and GY.

The GA-sensitive *Rht8* located on wheat chromosome arm 2DS has long been known for its involvement in regulating plant height (PH) in common wheat [12,13]. The *Rht8* allele that originated from the Japanese variety Akakomugi confers a semi-dwarf phenotype [14]; the contrasting allele *rht8* is associated with a taller phenotype [14]. Compared to *Rht-B1b* and *Rht-D1b* (located on chromosomes 4B and 4D, respectively) that decrease PH by 15%–30% [15–18], the effect of *Rht8* on height is less (8%–15%) [13,14,17,19,20]. Although *Rht8* has not yet been characterized at the molecular level, it has been tagged by specific genetic markers. The 192 bp amplicon of the microsatellite marker *Xgwm261* has been widely used for identifying *Rht8* in global wheat germplasm [13,20–23]. A recent study revealed that the semi-dwarf phenotype conferred by *Rht8* is associated with reduced sensitivity to the brassinosteroid hormone [14]. That study also developed new gene-based markers more closely linked to *Rht8* than *Xgwm261*. *Rht8* does not significantly affect GY in European environments although it slightly improves spike fertility under some conditions [19,24–26]. Rebetzke et al. [17] found that *Rht8* improves GY and grain number per spike (GNS) in Australian environments. Several quantitative genetic studies in China have reported QTL controlling GY and related traits around *Xgwm261*, indicating that *Rht8* might promote GY and its component traits [27–29].

The molecularly characterized *Ppd-D1* on chromosome arm 2DS plays an important role in regulation of photoperiod sensitivity [30,31]. Two main alleles, *Ppd-D1a* and *Ppd-D1b*, are distinguished in most wheat varieties [30,32]. *Ppd-D1a* confers photoperiod insensitivity and promotes early heading and flowering [30,31,33]. It decreases PH possibly due to a shortened vegetative growth period and accelerated ear emergence [14,25,33–35] and tends to increase grain number and final grain yield, particularly in the environments with post-anthesis high temperature and drought stress [19,33,36].

The combination of *Rht8* and *Ppd-D1a* likely leads to plants with reduced height and early flowering [14]. These traits are important for adaptation to the environments with risk of summer heat and drought. This is consistent with the observation that both alleles are present in a high proportion of the wheat lines from Southern European countries, where summer heat and drought stress commonly occur during the late stages of growth and grain development [19]. The close association between *Rht8* and *Ppd-D1a* (i.e., both located on 2DS and frequently found together) has caused researchers to question whether it is possible to separate the effects of the two genes. Rebetzke et al. [17] suggested that the positive effects of *Rht8* on GY and GNS might be associated with its linkage with *Ppd-D1a*. Wilhelm et al. [18] speculated that *Rht8* might be the main cause of height reduction associated with *Ppd-D1a* in their analysis of 372 worldwide common wheat accessions. Hence, there was a need for a systematic investigation of the effects of *Rht8* and *Ppd-D1a* on key developmental and yield traits under diverse cultivation conditions, as the outcomes could aid the development of the wheat varieties with improved water and nutrient use efficiencies.

The main objective of this study was to assess the individual and combined effects of *Rht8* and *Ppd-D1a* on PH, TH, GY, and GY-related traits using data from two types of genetic analysis. The first was an association analysis of 94 elite common wheat varieties grown in 12 environments differing in location and water and fertilizer applications [37]. The second was a QTL analysis involving 168 double haploid lines (DHLs) grown in three fertilized and irrigated environments [38]. The genotypes included in both analyses came mainly from the Yellow and Huai river valley (YHRV), and encompassed the major wheat growing regions in Henan and Shandong provinces and some parts of Hebei, Shaanxi, Shanxi, Jiangsu and Anhui, with a total annual wheat cultivation area of about 12 Mha [39]. Wheat productivity in the YHRV is adversely affected by drought and heat stress particularly during flowering and grain filling stages [39]. *Rht8* and *Ppd-D1a* have each been detected in >50% of the varieties planted in YHRV [21,22,33], suggesting that both alleles were selected by breeders in coping with drought and heat stresses. Considering that both *Rht8* and *Ppd-D1a* have been reported to exert beneficial effects on spike fertility and grain number [17,19,24,33], the effects of the individual alleles and their combination on GNS, spikelet number per spike (SNS), fertile spikelet number per spike (FSNS) and spike length (SL) were investigated.

2. Materials and methods

2.1. Genetic populations and field trials

The population for association analysis contained 94 winter wheat varieties, 89 of which came from the YHRV region. The other five lines were from the neighboring Northern winter wheat zone. They were grown at Hengshui (HS) in Hebei

province in 2008/2009 and 2009/2010, and at Jiyuan (JY) in Henan province during 2009/2010 where they were subjected to four treatments, namely irrigated and fertilized (IF), rainfed (RF), reduced nitrogen (RN), and reduced phosphorus (RP) as detailed previously [37].

The QTL mapping population consisted of 168 DHLs derived from a cross between Huapei 3 and Yumai 57 [38]. Huapei 3 has higher grain weight and relatively high yield potential [40], whereas Yumai 57 has broad environmental adaptation and comparatively high yield potential at multiple locations in the YHRV [41]. This DH population had been grown in three IF environments, two at Tai'an (TA) in Shandong province (during 2005/2006 and 2006/2007) and one at Suzhou (SZ) in Anhui province (during 2006/2007) [38].

2.2. Trait evaluation

The PH of each genotype was measured as the average height (cm) of 10 stems from the soil surface to the tip of the spike (excluding awns). TH was recorded as the number of days from sowing to 50% spike emergence. Ten main-stem spikes from the middle of each replication were used for investigating GNS, SNS, FSNS and SL (excluding awns). GY was measured as the weight of all grains harvested from the entire plot.

2.3. Molecular markers and chromosomal linkage map

Alleles *Rht8* and *rht8* were detected using microsatellite marker *Xgwm261* [42] and the gene-based marker *DG273* [14]. Allelic variants at *Ppd-D1* were amplified using a multiplex PCR assay [30]. The primer set *Ppd-D1*_F (5′-ACGCCTCCCACTACACTG-3′) and *Ppd-D1*_R1 (5′-TGTTGGTTCAAACAGAGAGC-3′) yielded a 414 bp fragment diagnostic for *Ppd-D1b*, whereas primer pair *Ppd-D1*_F and *Ppd-D1*_R2 (5′-CACTGGTGGTAG CTGAGATT-3′) gave a 288 bp fragment specific for *Ppd-D1a*. The results of genotyping of the 94 cultivars with the three markers are provided in Table S1. Polymorphisms of the three markers among the 168 DHLs enabled estimation of genetic distances among *Xgwm261*, *DG273*, and *Ppd-D1*. The genotypic data were analyzed by MAPMAKER/Exp version 3.0b [43]. Map positions of the three markers were integrated with those of previously published markers on 2D [44] using Mapchart version 2.1 [45].

2.4. Association analysis and QTL mapping

The mixed linear model (MLM) model with population structure (Q) and kinship (K) in the TASSEL 2.1 software package (http://www2.maize genetics.net/) was used for genome wide association analysis of PH, TH, GY, and GY component traits (GNS, SNS, FSNS, and SL) for each of the 12 environments as described by Zhang et al. [37]. Q and K parameters were estimated previously [37]. The R^2 (percentage of phenotypic variance explained) values and their significance levels were computed for the examined markers (loci).

QTL mapping of PH, TH, GY, and GY component traits was performed for each of the three IF environments in which the DH population was cultivated using IciMapping software (http://www.isbreeding.net/). The walk speed was 1.0 cM for all QTL. QTL declaration was based on a LOD score threshold of ≥2.5. Marker intervals and peak positions were computed for the *Rht8* and *Ppd-D1* QTL.

2.5. Statistical analysis

All statistical analyses were carried out using IBM SPSS for Windows 17.0 (SPSS, www.spss.com). Environmental and genetic variations of the traits were examined by analysis of variance (ANOVA) and the least significant difference rank test ($\alpha = 0.05$) followed by a Behrens-Fish multiple comparison test. Genotyping and comparison of genotypic means were performed to examine the allelic effects of detected loci as described previously [37].

3. Results

3.1. Assessment of phenotypic data

Phenotypic data for seven traits (PH, TH, GY, GNS, SNS, FSNS, and SL) were obtained from all 12 environments in the association analysis experiment (Table S2). All seven traits showed variation. In general, trait means were highest under IF conditions, followed by those under RP or RN conditions, with that in RF environments being the lowest. There was substantial genotypic variance (δ^2_G) for all seven traits and broad sense heritabilities (H^2) were relatively high (Table S2).

To validate the findings from the association analysis, we used the phenotypic data for PH, TH, GY, GNS, SNS, FSNS and SL from the 168 DHLs and their parents grown in three IF environments [38]. The ranges in trait variation and broad sense heritabilities (Table S3) were generally comparable to those determined for the association mapping population (Table S2).

3.2. Association of *Rht8* and *Ppd-D1* with PH, TH and GY and related traits

We previously conducted an association analysis of chromosome loci controlling grain weight in the 94 common wheat varieties using 1171 microsatellite and DArT markers [37]. Here, we incorporated the new *Rht8*-co-segregating marker and the *Ppd-D1* locus into the linkage map of chromosome 2D. Among the five markers *DG273* was clearly amplified in all 94 varieties with alleles of 391 and 421 bp. The positions of *DG273* and *Ppd-D1* on chromosome 2D were then verified by genotyping the 168 Huapei 3 × Yumai 57 DHLs. Huapei 3 carried the $DG273_{391bp}$ and *Ppd-D1b* alleles, whereas Yumai 57 had $DG273_{421bp}$ and *Ppd-D1a*. The genetic distance between *Xgwm261* and *DG273* in the 2D genetic map (Fig. S1) was 0.5 cM, whereas that between *DG273* and *Ppd-D1* was 11 cM. With the incorporation of *DG273* and *Ppd-D1*, the total number of markers used for association analysis changed from 1171 in the previous study [37] to 1173 in the present work.

Using the MLM program and considering both Q and K, a larger number of loci were associated with each of the seven traits (i.e., PH, TH, GY, GNS, SNS, FSNS, and SL). *Xgwm261*, *DG273* and *Ppd-D1*, all located on 2DS, were selected for further analysis because they were stably associated with PH, GY,

Table 1 – Phenotypic effects (R^2, %) of *Xgwm261*, *DG273*, and *Ppd-D1* loci on PH, TH, GY, GNS, SNS, FSNS, and SL as detected by association analysis.

Locus	Environment	PH	TH	GY	GNS	SNS	FSNS	SL
Xgwm261	09HS-IF	8.70**	0.76	4.41*	8.93**	8.13**	9.10**	0.49
(192 bp)	10HS-IF	8.21**	0.92	5.48*	10.73***	9.76***	10.94***	0.61
	10JY-IF	8.12**	1.18	5.94*	13.82***	12.58***	14.09***	0.67
	09HS-RF	6.83**	0.98	14.45***	11.16***	10.43***	12.28***	1.62
	10HS-RF	6.21*	0.47	6.53**	5.37*	5.02*	5.91*	0.73
	10JY-RF	6.80**	0.45	7.65**	5.14*	4.81*	5.65*	0.86
	09HS-RN	8.36**	0.54	9.75***	6.17*	5.77*	6.79**	1.09
	10HS-RN	10.73***	0.49	14.60***	5.56*	5.20*	6.12*	1.64
	10JY-RN	8.18**	1.51	7.30**	17.22***	16.10***	18.94***	0.82
	09HS-RP	8.90**	1.11	7.70**	12.68***	11.86***	13.95***	0.86
	10HS-RP	8.08**	0.79	4.60*	8.95**	8.37***	9.85***	0.52
	10JY-RP	8.31**	1.14	6.28*	12.95***	12.11***	14.25***	0.70
DG273	09HS-IF	10.53***	0.90	7.52**	10.57***	9.62***	10.77***	0.84
(421 bp)	10HS-IF	9.94***	1.25	7.94**	14.63***	13.31***	14.91***	0.89
	10JY-IF	9.83***	0.82	16.94***	9.54***	8.68**	9.72***	1.90
	09HS-RF	8.26**	0.82	8.35**	9.58***	8.72**	9.76***	0.94
	10HS-RF	7.51**	0.54	14.53***	6.26*	5.70*	7.38**	1.63
	10JY-RF	8.23**	0.53	9.82***	6.18*	5.62*	7.30**	1.10
	09HS-RN	10.12***	1.31	10.25***	15.36***	13.98***	15.65***	1.15
	10HS-RN	12.98***	1.41	13.76***	16.48***	15.00***	16.80***	1.54
	10JY-RN	9.90***	1.00	8.39**	11.72***	10.67***	11.95***	0.94
	09HS-RP	10.77***	0.87	12.77***	10.19***	9.27***	10.39***	1.43
	10HS-RP	9.78***	0.66	13.78***	7.72**	7.03**	7.87**	1.54
	10JY-RP	10.05***	0.85	10.68***	9.99***	9.09**	10.18***	1.20
Ppd-D1	09HS-IF	11.35***	6.82**	6.09*	12.35***	11.24***	12.59***	0.68
(288 bp)	10HS-IF	9.61***	13.32***	11.89***	10.61***	9.66***	10.81***	1.33
	10JY-IF	11.56***	9.80***	8.75**	7.37**	6.71**	8.51**	0.98
	09HS-RF	9.61***	14.66***	13.09***	9.72***	8.85**	9.91***	1.47
	10HS-RF	6.01**	9.00**	8.04**	5.01*	4.56*	6.11*	0.90
	10JY-RF	7.48**	10.71***	9.56**	6.26*	5.70*	6.38*	1.07
	09HS-RN	9.36**	10.65***	9.51**	9.66***	8.79**	9.85***	1.07
	10HS-RN	7.99**	11.31***	10.10***	7.96**	7.24**	8.11**	1.13
	10JY-RN	10.88***	17.35***	15.49***	7.98**	7.26**	8.13**	1.73
	09HS-RP	6.89**	10.66***	9.52***	6.19*	5.63*	6.31*	1.07
	10HS-RP	7.87**	15.86***	14.16***	7.13**	6.49*	7.27**	1.59
	10JY-RP	8.34**	8.76**	7.82**	8.04**	7.32**	8.19**	0.88

The 12 environments were created by growing 94 common wheat varieties in three crop seasons (09HS, 10HS and 10JY) under four conditions (IF, RF, RN and RP) at the experimental sites HS and JY. The elite alleles of the three loci were each indicated in the brackets. FSNS, fertile spikelet number per spike; GNS, grain number per spike; GY, grain yield; TH, time to heading; IF, irrigated and fertilized; HS, Hengshui; JY, Jiyuan; PH, plant height; RF, rainfed; RN, reduced nitrogen; RP, reduced phosphorus; SL, spike length; SNS, spikelet number per spike.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

GNS, SNS and FSNS across all 12 environments ($P \leq 0.01$) (Table 1). The phenotypic variance explained (R^2) by *DG273* for all five traits tended to be larger than that explained by *Xgwm261*, consistent with the fact that *DG273* was more closely linked to *Rht8* than *Xgwm261* [14] (Fig. S1). The average effects of *DG273* on the five traits were generally larger than those conferred by *Ppd-D1* (Table 1). In contrast to the results described above, *Ppd-D1*, but not *Xgwm261* and *DG273*, was consistently associated with TH (Table 1). Neither *Rht8* nor *Ppd-D1* was significantly associated with SL.

$DG273_{421bp}$ (*Rht8*), the superior allele, was found in 37 of the 94 cultivars (39.4%, Table S4). *Ppd-D1a*, the elite allele of

Fig. 1 – Assessing the allelic effects of *Rht8* on plant height (PH), grain yield (GY), grain number per spike (GNS), spikelet number per spike (SNS), and fertile spikelet number per spike (FSNS) using the phenotypic data from 94 common wheat varieties cultivated in 12 environments. These environments were created by growing the varieties in different cropping cycles (08/09 and 09/10) and locations (HS and JY) under four conditions (IF, RF, RN, and RP). The 94 varieties were divided into *Rht8* and *rht8* groups. Means (±SD) of PH, GY, GNS, SNS, and FSNS were calculated for each group and each environment, and statistically compared (*$P \leq 0.05$, **$P \leq 0.01$). Percentage differences between means are given at the top of each histogram. The percentages at the right indicate averaged effects of *Rht8* relative to *rht8* across all 12 environments. IF, irrigated and fertilized; HS, Hengshui; JY, Jiyuan; RF, rainfed; RN, reduced nitrogen; RP, reduced phosphorus.

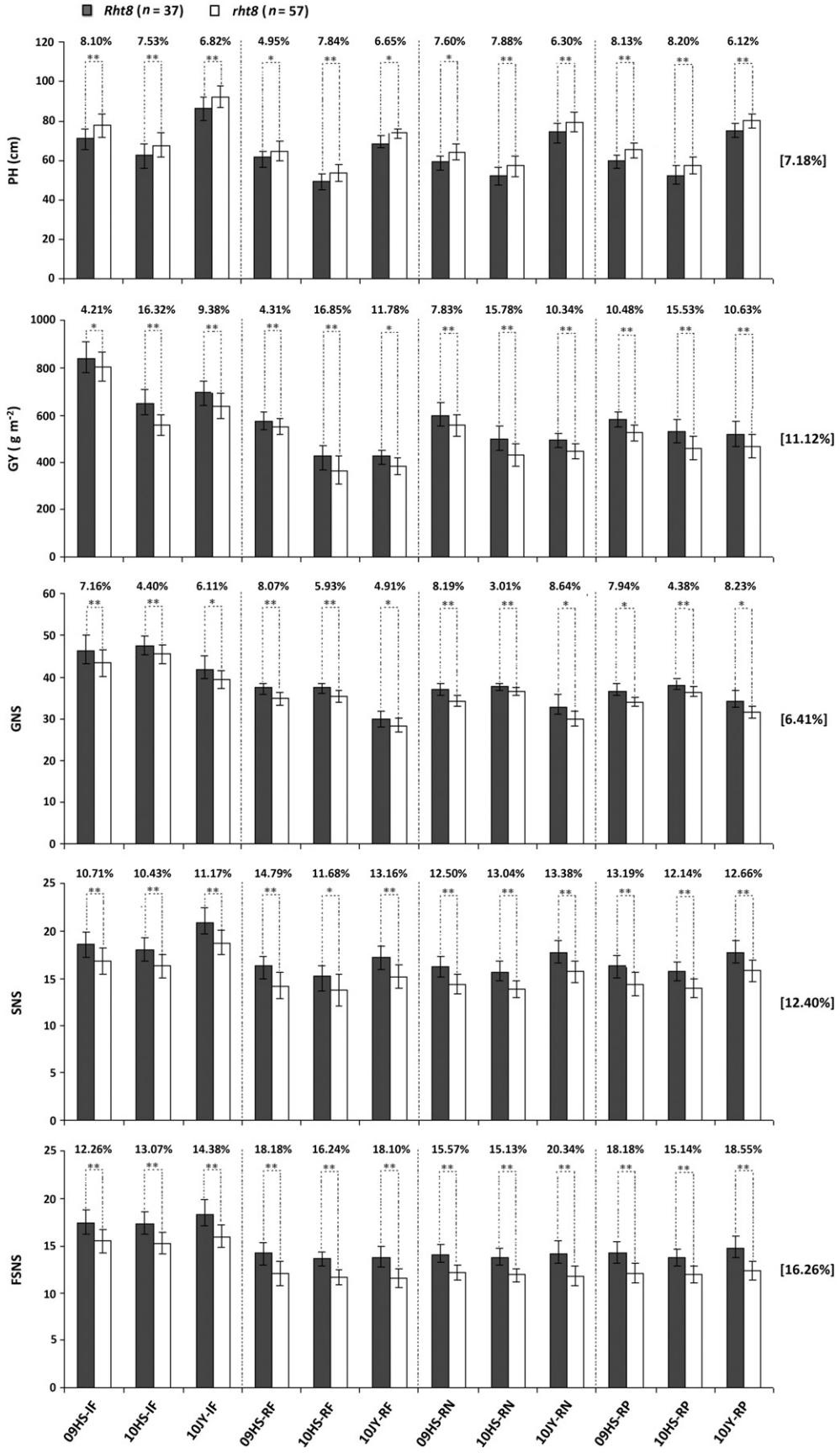

Rht8 (n = 37)
rht8 (n = 57)
PH (cm)
8.10% 7.53% 6.82% 4.95% 7.84% 6.65% 7.60% 7.88% 6.30% 8.13% 8.20% 6.12%
[7.18%]
GY (g m-2)
4.21% 16.32% 9.38% 4.31% 16.85% 11.78% 7.83% 15.78% 10.34% 10.48% 15.53% 10.63%
[11.12%]
GNS
7.16% 4.40% 6.11% 8.07% 5.93% 4.91% 8.19% 3.01% 8.64% 7.94% 4.38% 8.23%
[6.41%]
SNS
10.71% 10.43% 11.17% 14.79% 11.68% 13.16% 12.50% 13.04% 13.38% 13.19% 12.14% 12.66%
[12.40%]
FSNS
12.26% 13.07% 14.38% 18.18% 16.24% 18.10% 15.57% 15.13% 20.34% 18.18% 15.14% 18.55%
[16.26%]
09HS-IF
10HS-IF
10JY-IF
09HS-RF
10HS-RF
10JY-RF
09HS-RN
10HS-RN
10JY-RN
09HS-RP
10HS-RP
10JY-RP

Table 2 – Effects of *Rht8* and *Ppd-D1* loci on PH, TH, GY, GNS, SNS, FSNS. and SL revealed by QTL mapping.

Trait	Environment	*Rht8*			*Ppd-D1*		
		LOD	Additive effect	PVE (%)	LOD	Additive effect	PVE (%)
PH	06TA	5.59	+6.78**	9.97	4.88	+5.92**	8.59
	07TA	3.74	+4.54**	7.34	2.87	+3.36*	5.42
	07SZ	3.62	+4.39**	7.11	3.16	+3.84*	6.21
TH	06TA	1.02NS	0.18NS	0.91NS	4.44	+1.56**	7.82
	07TA	0.68NS	0.12NS	0.68NS	2.61	+0.95*	4.93
	07SZ	0.66NS	0.12NS	0.65NS	2.88	+1.01*	5.65
GY	06TA	9.80	−44.59***	19.24	6.87	−34.59***	13.48
	07TA	5.67	−30.81***	11.13	3.97	−26.81**	7.80
	07SZ	3.98	−26.95**	7.81	2.79	−20.95*	5.48
GNS	06TA	9.42	−4.05***	18.49	7.82	−3.36***	15.35
	07TA	6.64	−2.85***	13.04	5.51	−2.36***	10.82
	07SZ	4.85	−2.23**	9.52	4.03	−1.85**	7.90
SNS	06TA	7.72	−1.49***	15.16	6.41	−1.24***	12.59
	07TA	5.44	−1.05***	10.69	4.52	−0.87**	8.87
	07SZ	3.98	−0.82**	7.81	3.30	−0.68*	6.48
FSNS	06TA	6.87	−1.33***	13.49	5.71	−1.10***	11.20
	07TA	4.85	−0.94**	9.52	4.06	−0.77**	7.95
	07SZ	3.59	−0.73**	6.99	2.94	−0.61*	5.77
SL	06TA	0.96NS	0.41NS	0.85NS	0.68NS	0.34NS	0.57NS
	07TA	0.59NS	0.29NS	0.63NS	0.51NS	0.24NS	0.48NS
	07SZ	0.40NS	0.26NS	0.55NS	0.34NS	0.19NS	0.35NS

Three environments (06TA, 07TA and 07SZ) were created by growing the double haploid population in three seasons at TA and SZ. In the "Additive effect" column, "+" and "-" indicate increasing alleles from Huapei 3 and Yumai 57, respectively. QTL marker intervals and peak positions are given in Fig. S2. FSNS, fertile spikelet number per spike; GNS, grain number per spike; GY, grain yield; TH, time to heading; PH, plant height; PVE, phenotypic variance explained; SL, spike length; SNS, spikelet number per spike; SZ, Suzhou; TA, Tai'an; NS, not significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Ppd-D1, was present in 86 of the 94 cultivars (91.5%, Table S4). Among the 94 varieties examined, 34 (36.2%) possessed both *Rht8* and *Ppd-D1a* (Table S4). The relatively high numbers of varieties carrying *Rht8* (37) or *rht8* (57) facilitated a reliable statistical comparison of their effects on PH, GY, GNS, SNS, and FSNS (Fig. 1). *Rht8* was significantly more effective than *rht8* in decreasing PH, and in promoting GY, GNS, SNS, and FSNS. *Rht8* decreased PH by an average 7.18%, and promoted GY by 11.12%, GNS by 6.41%, SNS by 12.40% and FSNS by 16.26% (Fig. 1).

The number of varieties with *Ppd-D1b* was small ($n = 8$), making it difficult to statistically compare the allelic effects of *Ppd-D1a* and *Ppd-D1b*. Nevertheless, a provisional assessment revealed that *Ppd-D1a* tended to be more effective than *Ppd-D1b* in reducing PH, shortening TH, and promoting GY, GNS, SNS, and FSNS in all 12 environments (Table S5).

Stringent statistical comparisons among the means of the four groups of genotypes carrying different allelic combinations of *Rht8* and *Ppd-D1* (i.e., *Rht8* + *Ppd-D1a*, *rht8* + *Ppd-D1a*, *Rht8* + *Ppd-D1b* and *rht8* + *Ppd-D1b*) were also difficult because the numbers of varieties with *Rht8* + *Ppd-D1b* ($n = 3$) or *rht8* + *Ppd-D1b* ($n = 5$) were low. A tentative examination showed that, on average, genotypes with *Rht8* and *Ppd-D1a* appeared to have the lowest PH and the highest values for GY, GNS, SNS, and FSNS, whereas those carrying *rht8* and *Ppd-D1a* or *Rht8* and *Ppd-D1b* were comparatively less effective in reducing PH and promoting GY, GNS, SNS, and FSNS (Table S6). Genotypes with *rht8* and *Ppd-D1b* had the highest PH and the lowest GY, GNS, SNS, and FSNS values (Table S6).

3.3. *Validation of association analysis findings by QTL mapping*

Quantitative genetic mapping with 168 DHLs showed that both *Rht8* and *Ppd-D1* represented stably expressed QTL for PH, GY, GNS, SNS and FSNS in all three IF environments, and that *Ppd-D1* but not *Rht8* was a significant QTL for TH (Table 2). Neither the marker interval nor the peak position of the *Rht8* QTL overlapped with those of the *Ppd-D1* QTL (Table 2, Fig. S2). According to the percentage of phenotypic variance explained, *Rht8* was generally more effective than *Ppd-D1* in affecting PH, GY, GNS, SNS and FSNS (Table 2). This analysis also indicated highly significant ($P < 0.01$) additive interactions between *Rht8* and *Ppd-D1* on PH, GY, GNS, SNS and FSNS (Table 2).

DHL genotypes carrying *Rht8* ($n = 93$), *rht8* ($n = 75$), *Ppd-D1a* ($n = 90$), or *Ppd-D1b* ($n = 78$) were sufficiently large for rigorous statistical comparisons of the allelic effects of the two genes. It is clear that *Rht8* was superior to *rht8* in lowering PH and increasing GY, GNS, SNS and FSNS (Fig. 2). Across the three environments, the mean effects of *Rht8* on the five traits were 6.94% (PH), 9.38% (GY), 9.39% (GNS), 10.94% (SNS), and 11.79% (FSNS). *Ppd-D1a* showed much stronger effects than *Ppd-D1b* on advancing TH, reducing PH, and increasing GY, GNS, SNS, and FSNS (Fig. 3). When averaged across the three environments, the effects of *Ppd-D1a* on the six traits were 1.68% (TH), 4.32% (PH), 8.83% (GY), 8.86% (GNS), 10.32% (SNS), and 11.16% (FSNS). The combination of *Rht8* and *Ppd-D1a* was significantly more effective than that of *rht8* and *Ppd-D1b* in reducing PH,

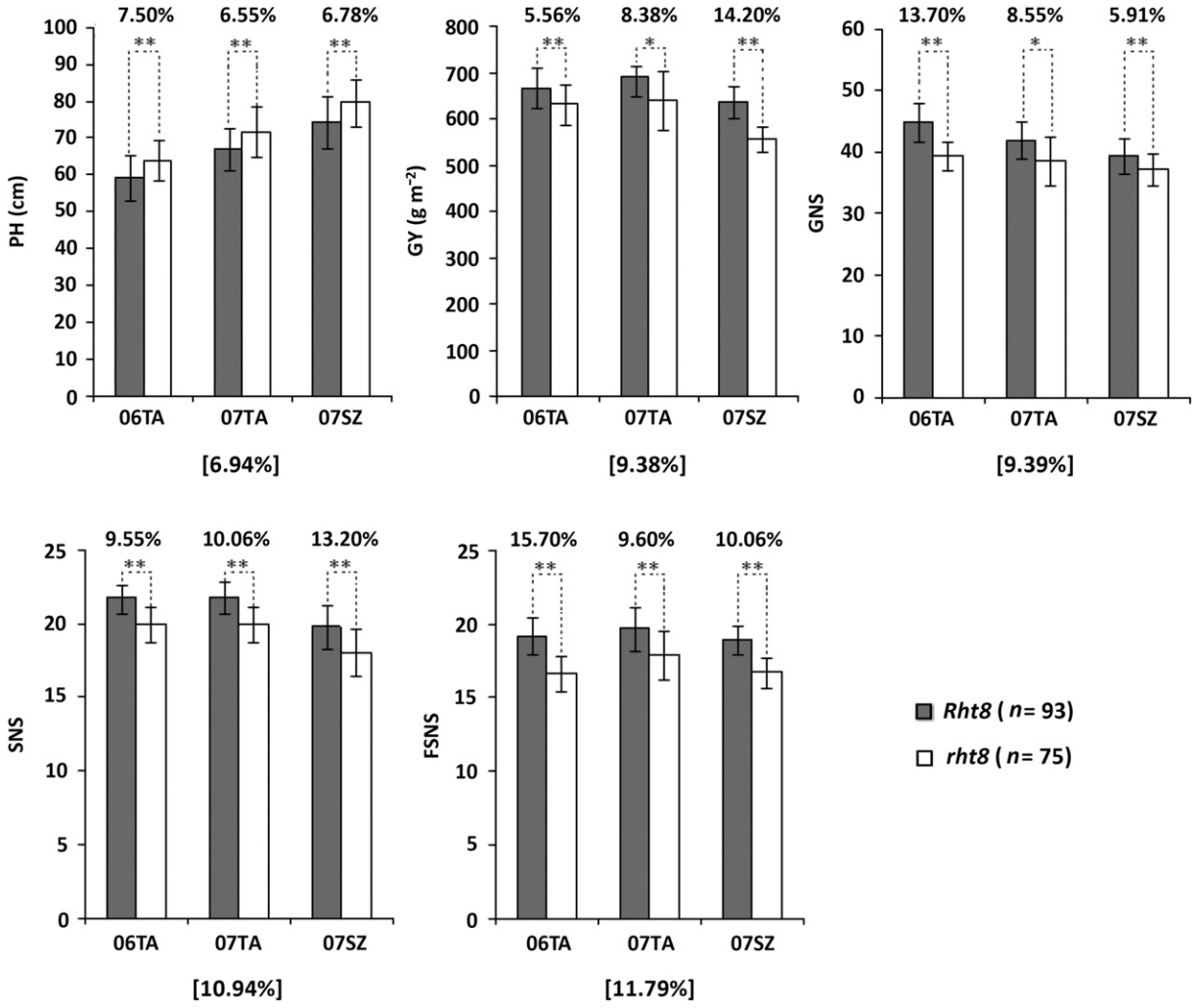


Fig. 2 – Verification of the allelic effects of *Rht8* on plant height (PH), grain yield (GY), grain number per spike (GNS), spikelet number per spike (SNS), and fertile spikelet number per spike (FSNS) with the phenotypic data from 168 DHLs grown in three irrigated and fertilized environments (06TA, 07TA and 07SZ). The 168 lines were divided into two genotype groups based on *Rht8* alleles (*Rht8* or *rht8*). The means (±SD) of PH, GY, GNS, SNS and FSNS were calculated for each group and for each environment and were statistically compared (* $P \leq 0.05$, ** $P \leq 0.01$). The number of lines (*n*) for each group is given in the brackets. For each environment, the percentage of difference between the means of the two groups is given at the top of the figure. Values in square brackets show the averaged effect of *Rht8* (relative to *rht8*) across all three environments. SZ, Suzhou; TA, Tai'an.

and increasing GY, GNS, SNS, and FSNS (Fig. 4). The *Rht8* + *Ppd-D1a* combination was significantly more effective than *Rht8* or *Ppd-D1a* alone in promoting PH, GY, GNS, SNS, and FSNS (Table 3). The allelic effects of *Rht8* on the five traits tended to be higher than those of *Ppd-D1a*, *Rht8*, *Ppd-D1a*, and *Rht8* + *Ppd-D1a* exhibited the highest effect on FSNS (Table 3).

The effect of an allele is the percentage increase (+) or decrease (–) over the inferior allele. The mean values (±SD) being compared are derived from Fig. 2 (*Rht8*), Fig. 3 (*Ppd-D1a*) and Fig. 4 (*Rht8* + *Ppd-D1a*). FSNS, fertile spikelet number per spike; GNS, grain number per spike; GY, grain yield; PH, plant height; SNS, spikelet number per spike. Different letters following SD indicate statistical significance ($P < 0.05$) among the three genotypes.

Consistent with the lack of association of *Rht8* and *Ppd-D1* with SL among the 94 elite varieties grown in 12 environments (Table 1), allelic variation of *Rht8* (*Rht8* vs. *rht8*) had no significant effect on SL, and genotypes carrying *Rht8* + *Ppd-D1a* or *rht8* + *Ppd-D1b* did not differ substantially from each other in mean SL (Table S7). For the 168 DHLs grown in three IF environments, SL was not markedly influenced by allelic variation at the *Rht8* or *Ppd-D1* loci, nor did it vary significantly between genotypes carrying *Rht8* + *Ppd-D1a* or *rht8* + *Ppd-D1b* (Table S8).

4. Discussion

In this work we assessed the effects of *Rht8* and *Ppd-D1a* on common wheat traits PH, TH and GY by association analysis

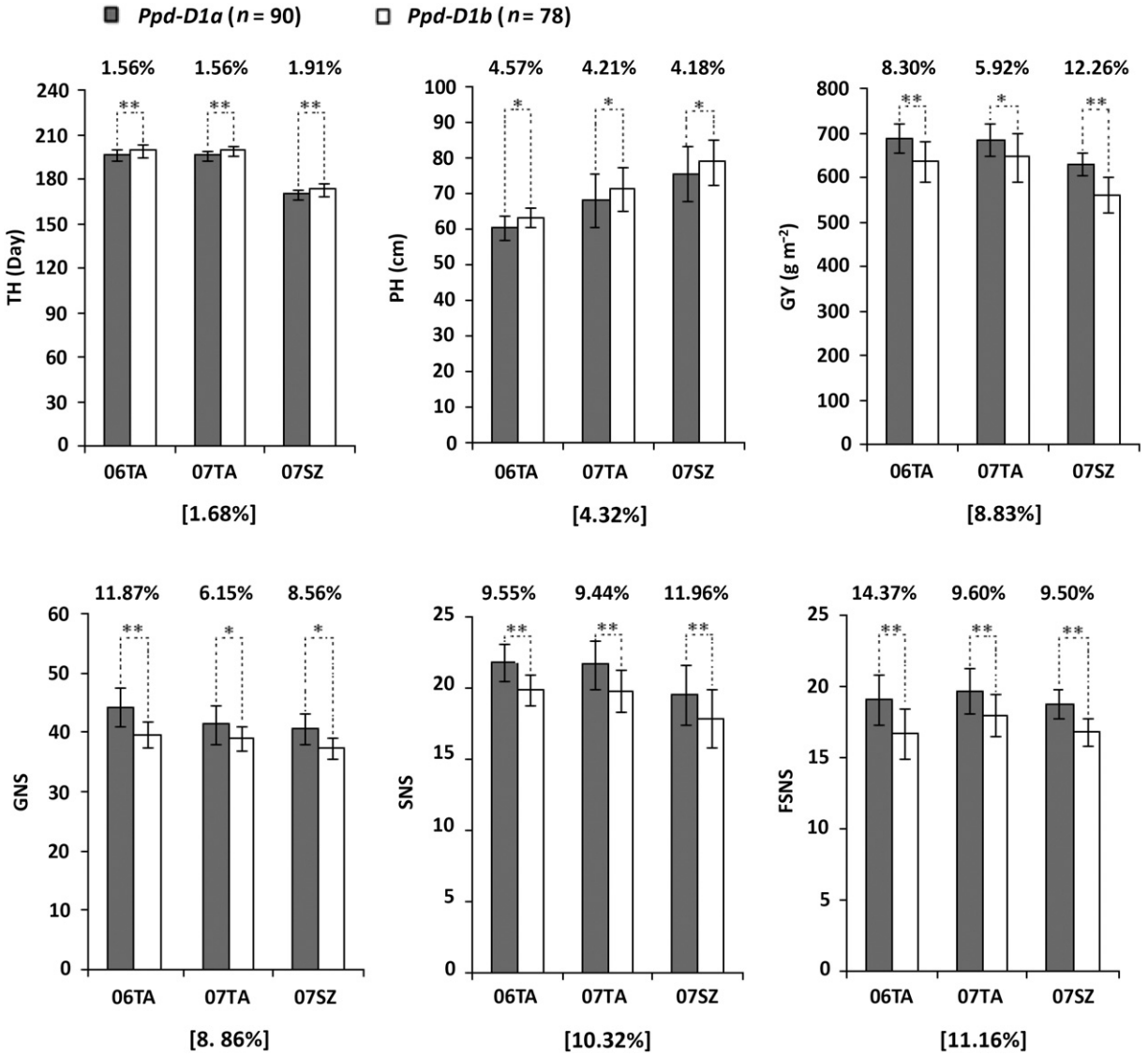


Fig. 3 – Allelic effects of *Ppd-D1* on time to heading (TH), plant height (PH), grain yield (GY), grain number per spike (GNS), spikelet number per spike (SNS), and fertile spikelet number per spike (FSNS) using phenotypic data from 168 DHLs grown in three irrigated and fertilized environments (06TA, 07TA, and 07SZ). The lines were divided into two genotype groups based on *Ppd-D1* alleles (*Ppd-D1a* or *Ppd-D1b*). Means (±SD) of PH, GY, GNS, SNS, FSNS and TH calculated for each group and for each environment were statistically compared (* $P \leq 0.05$, ** $P \leq 0.01$). The number of lines (*n*) for each group is given at the top. The percentage difference between means for the two groups is given above each histogram. Values in square brackets indicate averaged effects of *Ppd-D1a* (compared to *Ppd-D1b*) across all three environments. SZ, Suzhou; TA, Tai'an.

and QTL mapping with phenotypic data from 15 environments. The information obtained and implications for wheat improvement are discussed below.

4.1. Distribution of *Rht8*, *Ppd-D1a* in common wheat

The frequency of *Rht8* in the 94 varieties (39.4%) was lower than reported previously (42.3%–64.0%) [22,46]. This work employed marker *DG273* to identify the presence of *Rht8*, whereas the 192 bp amplicon of *Xgwm261* was used in previous studies as an indicator of *Rht8*. Since *DG273* is more closely linked to *Rht8* [14] (Fig. S1), its use should provide more accurate identification of *Rht8*. The frequency of *Ppd-D1a* in the 94 cultivars (91.5%) was comparable to previous reports (77.9%–90.6%) for the improved winter wheat varieties in China [33,47]. *Rht8* and *Ppd-D1a* were simultaneously detected in 36.2% of the 94 varieties (Table S3), which is quite low considering the beneficial effects of the combination on GY and related traits revealed in this work.

4.2. *Rht8* and *Ppd-D1a* appear to act independently and additively

Considering the data obtained in this work, we speculate that *Rht8* and *Ppd-D1a* act independently and additively in control of PH, GY, GNS, SNS, and FSNS. The marker interval and peak

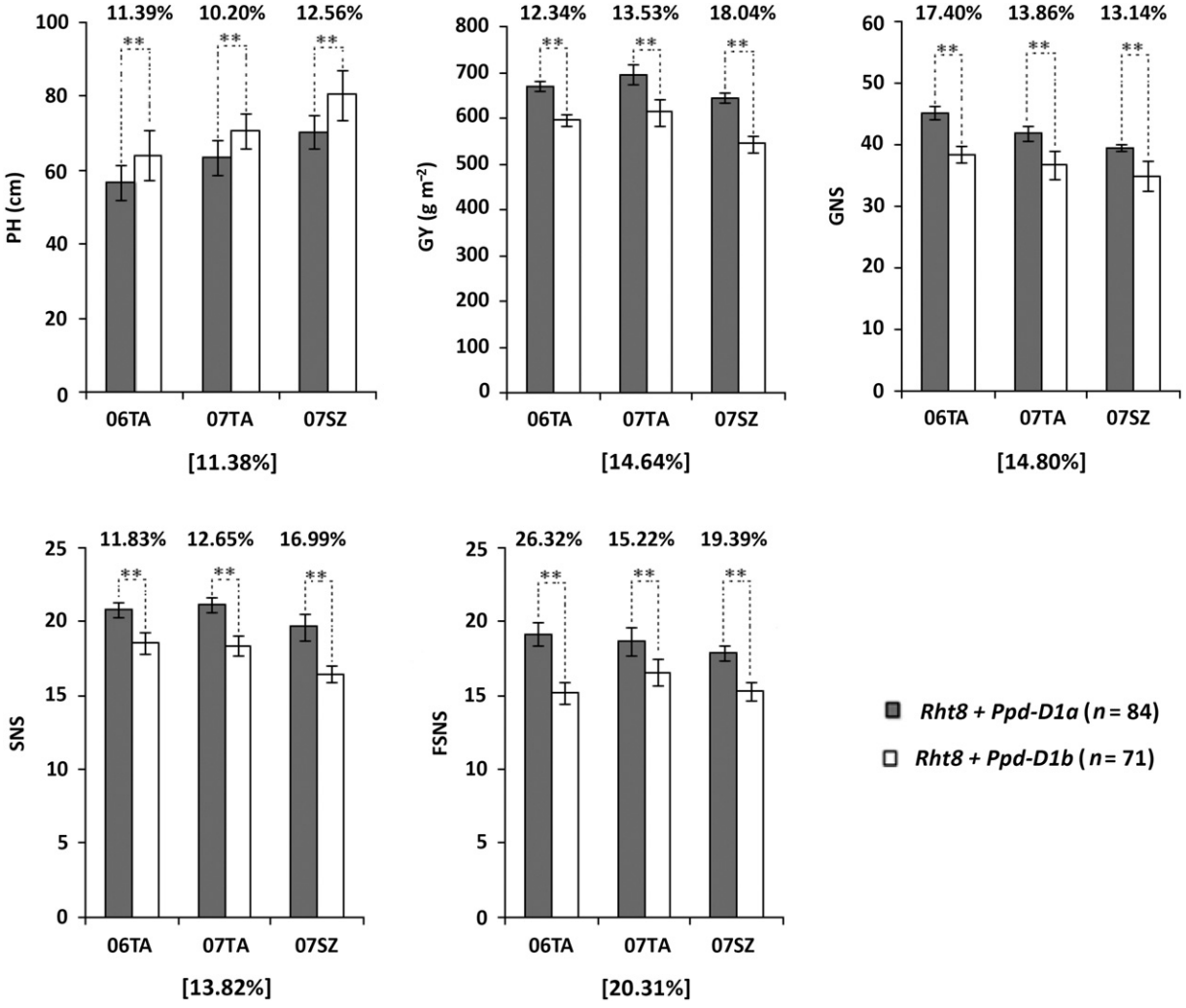


Fig. 4 – Comparisons of plant height (PH), grain yield (GY), grain number per spike (GNS), spikelet number per spike (SNS) and fertile spikelet number per spike (FSNS) between lines carrying *Rht8* + *Ppd-D1a* and those with *rht8* + *Ppd-D1b*. The phenotypic data used were from 168 DHLs grown in three IF environments (06TA, 07TA and 07SZ). These lines were divided into genotype groups carrying *Rht8* + *Ppd-D1a* or *rht8* + *Ppd-D1b*. The means (±SD) of PH, GY, GNS, SNS, and FSNS calculated for each group and environment were statistically compared (* $P \leq 0.05$, ** $P \leq 0.01$). The number of lines (*n*) for each group is shown at the top. The percentage differences between the means for the two groups are given above the histograms. Values in square brackets illustrate the averaged effects of *Rht8* + *Ppd-D1a* (compared to *rht8* + *Ppd-D1b*) across the three environments. SZ, Suzhou; TA, Tai'an.

position of the *Rht8* QTL did not overlap with those of the *Ppd-D1* QTL (Table 2, Fig. S2). Moreover, *Ppd-D1a*, but not *Rht8*, significantly affected TH in all 15 environments (Table 1, Table 2). The fact that the genetic effects of *Rht8* on PH, GY, GNS, SNS, and FSNS were generally not identical to those of *Ppd-D1a* (Table 3) provides additional support for functional difference of the two loci.

The possibility that *Rht8* and *Ppd-D1a* act additively on PH, GY, GNS, SNS, and FSNS is mainly supported by two findings. First, highly significant ($P < 0.01$ or $P < 0.001$) additive interactions on PH, GY, GNS, SNS, and FSNS were detected for *Rht8* and *Ppd-D1* by QTL mapping (Table 2). Second, the additive effect of *Rht8* + *Ppd-D1a* on each of the five traits was generally close to the sum of their individual effects (Table 3).

Table 3 – Effects of *Rht8*, *Ppd-D1a* and their combination (*Rht8* + *Ppd-D1a*) on PH, GY, GNS, SNS, and FSNS.

Genotype	Compared with	PH (–)	GY (+)	GNS (+)	SNS (+)	FSNS (+)
Rht8	*rht8*	6.94 ± 0.50 a	9.38 ± 4.41 a	9.39 ± 3.96 a	10.94 ± 1.98 a	11.77 ± 3.39 a
Ppd-D1a	*Ppd-D1b*	4.32 ± 0. 22 b	8.83 ± 3.20 a	8.86 ± 2.87 a	10.32 ± 1.42 a	11.16 ± 2.78 a
Rht8 + *Ppd-D1a*	*rht8* + *Ppd-D1b*	11.38 ± 0.16 c	14.64 ± 3.00 b	14.80 ± 2.28 b	13.82 ± 2.77 b	20.31 ± 5.60 b

4.3. Comparison with previous studies

Both *Rht8* and *Ppd-D1a* conferred substantial improvement on GY and related traits relative to their respective inferior counterparts (*rht8* and *Ppd-D1b*). This contrasts with previous findings of limited positive effects of *Rht8* on spike fertility [19,24–26] and of *Ppd-D1a* on GY and grain number in some European environments [9,19]. Because post anthesis drought and high temperature stresses are generally severe and common in YHRV environments [39], the beneficial effects of *Rht8* and *Ppd-D1a* on GY and related traits might have been higher under the conditions of our study. Additionally, our analysis used a comprehensive phenotypic data set collected from 94 varieties and 168 DHLs cultivated in 15 different environments, which may be more conducive to uncovering larger allelic effects of *Rht8* and *Ppd-D1a* on GY and related traits.

In agreement with our observations, two genetic studies involving five Chinese wheat populations consistently mapped significant QTL for GY-related traits around *Xgwm261* (indicative of *Rht8*) [28,29]. In another study by Wu et al. [27], wheat yield-related QTL were also detected in the chromosome 2D region of *Rht8* and *Ppd-D1a* in multiple rainfed environments. Furthermore, genetic variation associated with *Ppd-D1* has been found to affect GY in a wide range of European conditions [48,49], and a recent study suggested that *Ppd-D1a* was associated with significant positive effects on GY in both Australian and Mexican environments [8].

4.4. Implications for wheat improvement

From the discussion above, it seems that *Rht8* and *Ppd-D1a* can be used to adjust PH and to enhance GY and tolerance to post-anthesis heat and drought stress of common wheat in the YHRV region and in other areas (e.g., southern European countries) with similar wheat production environments (Fig. 5). Because the combined effects of *Rht8* and *Ppd-D1a* are generally larger than the effect of either allele alone, it would be preferable to pyramid them. This can be achieved using DNA markers developed previously [14,30,33]. Since the proportion of cultivars carrying both *Rht8* and *Ppd-D1a* is currently low (36.2%, Table S4), pyramiding the two alleles may enable rapid development of more superior common wheat lines with higher yield potential and better environmental adaptability. In agreement with this view, *Rht8* was recently recommended as a useful height reducing gene for breeding common wheat varieties adapted to the environments with recurrent drought problems [50]. The number of common wheat varieties carrying both *Rht8* and *Ppd-D1a* is rising rapidly in southern European regions where post-anthesis drought and high temperature stress are frequently encountered [35].

The wide-ranging beneficial effects conferred by *Rht8* + *Ppd-D1a* on wheat developmental and yield traits deserve further study using larger segregating populations. We have now developed a multiparent advanced generation intercross population with >4000 recombinant lines, which should be useful for a more comprehensive elucidation of the individual

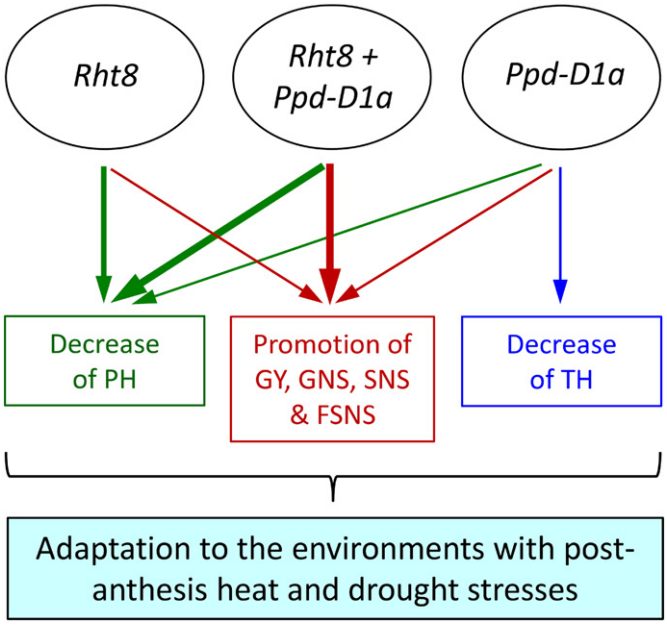


Fig. 5 – A diagram depicting the individual and combined effects of *Rht8* and *Ppd-D1a* on plant height (PH), grain yield (GY), grain number per spike (GNS), spikelet number per spike (SNS), fertile spikelet number per spike (FSNS), and time to heading (TH) revealed in this work. *Rht8*, *Ppd-D1a* and their combination (*Rht8* + *Ppd-D1a*) cause decrease of PH and promote GY mainly through increasing FSNS. In addition, *Ppd-D1a* decreases TH. The thicker arrows illustrate the larger effects conferred by *Rht8* + *Ppd-D1a* relative to those by *Rht8* or *Ppd-D1a* alone. *Rht8*, *Ppd-D1a* and especially their combination (*Rht8* + *Ppd-D1a*) may be useful for developing the common wheat cultivars adaptable to the environments with post-anthesis heat and drought stresses.

and combined effects of *Rht8* and *Ppd-D1a* in the growth, development, environmental adaptation and yield performance of wheat in future research.

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2019.06.008.

Declaration of competing interest

There is no conflict of interest.

Acknowledgments

This study was supported by the Ministry of Science and Technology of China (2017YFD0101000), Science and Technology Service Network Program (STS Program) of Chinese Academy of Sciences (KFJ-STS-ZDTP-024) and National Natural Science Foundation of China (31371611). The authors thank Professor Jiankang Wang (Institute of Crop Science, Chinese Academy of Agricultural Sciences) for advice on QTL mapping.

REFERENCES

[1] P. Grassini, K.M. Eskridge, K.G. Cassman, Distinguishing between yield advances and yield plateaus in historical crop production trends, Nat. Commun. 4 (2013) 2918.
[2] J.A. Foley, N. Ramankutty, K.A. Brauman, E.S. Cassidy, J.S. Gerber, M. Johnston, N.D. Mueller, C. O'Connell, D.K. Ray, P.C. West, C. Balzer, E.M. Bennett, S.R. Carpenter, J. Hill, C. Monfreda, S. Polasky, J. Rockström, J. Sheehan, S. Siebert, D. Tilman, D.P. Zaks, Solutions for a cultivated planet, Nature 478 (2011) 337–342.
[3] J.L. Araus, G.A. Slafer, C. Royo, M.D. Serret, Breeding for yield potential and stress adaptation in cereals, Crit. Rev. Plant Sci. 27 (2008) 377–412.
[4] N. Sreenivasulu, T. Schnurbusch, A genetic playground for enhancing grain number in cereals, Trends Plant Sci. 17 (2012) 91–101.
[5] K. Neumann, B. Kobiljski, S. Denčić, R.K. Varshney, A. Börner, Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L.), Mol. Breed. 27 (2011) 37–58.
[6] D. Bennett, A. Izanloo, M. Reynolds, H. Kuchel, P. Langridge, T. Schnurbusch, Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments, Theor. Appl. Genet. 125 (2012) 255–271.
[7] D. Bennett, M.P. Reynolds, D.A. Mullan, A. Izanloo, H. Kuchel, P. Langridge, T. Schnurbusch, Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments, Theor. Appl. Genet. 125 (2012) 1473–1485.
[8] L. Maphosa, P. Langridge, H. Taylor, B. Parent, L.C. Emebiri, H. Kuchel, M.P. Reynolds, K.J. Chalmers, A. Okada, J. Edwards, D. E. Mather, Genetic control of grain yield and grain physical characteristics in a bread wheat population grown under a range of environmental conditions, Theor. Appl. Genet. 127 (2014) 1607–1624.
[9] M.J. Foulkes, R. Sylvester-Bradley, A.J. Worland, J.W. Snape, Effects of a photoperiod-response gene *Ppd-D1* on yield potential and drought resistance in UK winter wheat, Euphytica 135 (2004) 63–73.
[10] U.M. Quraishi, M. Abrouk, F. Murat, C. Pont, S. Foucrier, G. Desmaizieres, C. Confolent, N. Rivière, G. Charmet, E. Paux, A. Murigneux, L. Guerreiro, S. Lafarge, J. le Gouis, C. Feuillet, J. Salse, Cross-genome map based dissection of a nitrogen use efficiency ortho-metaQTL in bread wheat unravels concerted cereal genome evolution, Plant J. 65 (2011) 745–756.
[11] M. Reynolds, J. Foulkes, R. Furbank, S. Griffiths, J. King, E. Murchie, M. Parry, G. Slafer, Achieving yield gains in wheat, Plant Cell Environ. 35 (2012) 1799–1823.
[12] A.J. Worland, C.N. Law, Genetic analysis of chromosome 2D of wheat. 1. The location of genes effecting height, day length insensitivity, hybrid dwarfism and yellow rust resistance, Plant Breed. 96 (1986) 331–345.
[13] V. Korzun, M.S. Roder, M.W. Ganal, A.J. Worland, C.N. Law, Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.), Theor. Appl. Genet. 96 (1998) 1104–1109.
[14] D. Gasperini, A. Greenland, P. Hedden, R. Dreos, W. Harwood, S. Griffiths, Genetic and physiological analysis of *Rht8* in bread wheat: an alternative source of semi-dwarfism with a reduced sensitivity to brassinosteroids, J. Exp. Bot. 63 (2012) 4419–4436.
[15] J.E. Flintham, A. Börner, A.J. Worland, M.D. Gale, Optimizing wheat grain yield: effects of *Rht* (gibberellin-insensitive) dwarfing genes, J. Agric. Sci. 128 (1997) 11–15.
[16] G.J. Rebetzke, R. Appels, A. Morrison, R.A. Richards, G. McDonald, M.H. Ellis, W. Spielmeyer, D.G. Bonnett, Quantitative trait loci on chromosome 4B for coleoptile length and early vigor in wheat (*Triticum aestivum* L.), Aus. J. Agric. Res. 52 (2001) 1221–1234.
[17] G.J. Rebetzke, M.H. Ellis, D.G. Bonnett, A.G. Condon, D. Falk, R. A. Richards, The *Rht13* dwarfing gene reduces peduncle length and plant height to increase grain number and yield of wheat, Field Crops Res. 124 (2011) 323–331.
[18] E.P. Wilhelm, M.I. Boulton, N. Al-Kaff, F. Balfourier, J. Bordes, A.J. Greenland, W. Powell, I.J. Mackay, *Rht-1* and *Ppd-D1* associations with height, GA sensitivity, and days to heading in a worldwide bread wheat collection, Theor. Appl. Genet. 126 (2013) 2233–2243.
[19] A.J. Worland, V. Korzun, M.S. Roder, M.W. Ganal, C.N. Law, Genetic analysis of the dwarfing gene *Rht8* in wheat. Part II. The distribution and adaptive significance of allelic variants at the *Rht8* locus of wheat as revealed by microsatellite screening, Theor. Appl. Genet. 96 (1998) 1110–1120.
[20] M.H. Ellis, D.G. Bonnett, G.J. Rebetzke, A 192 bp allele at the *Xgwm261* locus is not always associated with the *Rht8* dwarfing gene in wheat (*Triticum aestivum* L.), Euphytica 157 (2007) 209–214.
[21] Y. Liu, D.C. Liu, H.Y. Zhang, J. Wang, J.Z. Sun, X.L. Guo, A.M. Zhang, Allelic variation, sequence determination and microsatellite screening at the *XGWM261* locus in Chinese hexaploid wheat (*Triticum aestivum*) varieties, Euphytica 145 (2005) 103–112.
[22] X.K. Zhang, S.J. Yang, Y. Zhou, Z.H. He, X.C. Xia, Distribution of the *Rht-B1b*, *Rht-D1b* and *Rht8* reduced height genes in autumn-sown Chinese wheats detected by molecular markers, Euphytica 152 (2006) 109–116.
[23] L. Asplund, M.W. Leino, J. Hagenblad, Allelic variation at the *Rht8* locus in a 19th century wheat collection, Sci. World J. 2012 (2012), 385610.
[24] A. Börner, A.J. Worland, J. Plaschke, E. Schumann, C.N. Law, Pleiotropic effects of genes for reduced height (*Rht*) and day length insensitivity (*Ppd1*) on yield and its components for wheat grown in middle Europe, Plant Breed. 111 (1993) 204–216.
[25] A.J. Worland, E.J. Sayers, V. Korzun, Allelic variation at the dwarf gene *Rht8* locus and its significance in international wheat breeding programmes, Euphytica 119 (2001) 155–159.

[26] M. Addisu, J.W. Snape, J.R. Simmonds, M.J. Gooding, Effects of reduced height (*Rht*) and photoperiod insensitivity (*Ppd*) alleles on yield of wheat in contrasting production systems, Euphytica 172 (2010) 169–181.
[27] X. Wu, X. Chang, R. Jing, Genetic insight into yield-associated traits of wheat grown in multiple rain-fed environments, PLoS One 7 (2012), e31249.
[28] C. Zhao, F. Cui, Z. Fan, J. Li, A. Ding, H. Wang, Genetic analysis of important loci in the winter wheat backbone parent Aimengniu-V, Aus. J. Crop Sci. 7 (2013) 182–188.
[29] Y. Xu, R. Wang, Y. Tong, H. Zhao, Q. Xie, D. Liu, A. Zhang, B. Li, H. Xu, D. An, Mapping QTLs for yield and nitrogen-related traits in wheat: influence of nitrogen and phosphorus fertilization on QTL expression, Theor. Appl. Genet. 127 (2014) 59–72.
[30] J. Beales, A. Turner, S. Griffiths, J.W. Snape, D.A. Laurie, A pseudo-response regulator is mis-expressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.), Theor. Appl. Genet. 115 (2007) 721–733.
[31] L.M. Shaw, A.S. Turner, D.A. Laurie, The impact of photoperiod insensitive *Ppd-D1a* mutations on the photoperiod pathway across the three genomes of hexaploid wheat (*Triticum aestivum*), Plant J. 71 (2012) 71–84.
[32] K. Cane, H.A. Eagles, D.A. Laurie, B. Trevaskis, N. Vallance, R. F. Eastwood, N.N. Gororo, H. Kuchel, P.J. Martin, *Ppd-B1* and *Ppd-D1* and their effects in southern Australian wheat, Crop Pasture Sci. 64 (2013) 100–114.
[33] Z.A. Guo, Y.X. Song, R.H. Zhou, Z.L. Ren, J.Z. Jia, Discovery, evaluation and distribution of haplotypes of the wheat *Ppd-D1* gene, New Phytol. 185 (2010) 841–851.
[34] A.J. Worland, The influence of flowering time genes on environmental adaptability in European wheats, Euphytica 89 (1996) 49–57.
[35] G.O. Chebotar, S.V. Chebotar, I.I. Motsnyy, Y.M. Sivolap, Clarification of the *Rht8-Ppd-D1* gene linkage on the 2D chromosome of winter bread wheat, Cytol. Genet. 47 (2013) 70–74.
[36] M.J. Foulkes, G.A. Slafer, W.J. Davies, P.M. Berry, R. Sylvester-Bradley, P. Martre, D.F. Calderini, S. Griffiths, M.P. Reynolds, Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance, J. Exp. Bot. 62 (2011) 469–486.
[37] K.P. Zhang, J.J. Wang, L.Y. Zhang, C.W. Rong, F.W. Zhao, T. Peng, H.M. Li, D.M. Cheng, X. Liu, H.J. Qin, A.M. Zhang, Y.P. Tong, D.W. Wang, Association analysis of genomic loci important for grain weight control in elite common wheat varieties cultivated with variable water and fertiliser supply, PLoS One 8 (2013), e57853.
[38] K.P. Zhang, G.F. Chen, L. Zhao, B. Liu, X.B. Xu, J.C. Tian, Molecular genetic analysis of flour color using a doubled haploid population in bread wheat (*Triticum aestivum* L.), Euphytica 165 (2009) 471–484.
[39] Z.H. He, A.P.A. Bonjean, Cereals in China, CIMMYT, Mexico D. F., Mexico, 2010.
[40] Y. Hai, M.H. Kang, Breeding of a new wheat variety Huapei 3 with high yield and early maturing, Henan Agric. Sci. 5 (2007) 36–37 (in Chinese with English abstract).
[41] C.Q. Guo, Z.A. Bai, P.A. Liao, W.K. Jin, New high quality and yield wheat variety Yumai 57, China Seed Ind. (4) (2004) 54 (in Chinese).
[42] G.J. Bryan, A.J. Collins, P. Stephenson, A. Orry, J.B. Smith, M.D. Gale, Isolation and characterization of microsatellites from hexaploid bread wheat, Theor. Appl. Genet. 94 (1997) 557–563.
[43] S.E. Lincoln, M.J. Daly, E.S. Lander, Constructing Genetic Maps with MAPMAKER/EXP Version 3.0: A Tutorial and Reference Manual, 3rd ed Whitehead Institute, Cambridge, MA, USA, 1993.
[44] K.P. Zhang, L. Zhao, J.C. Tian, G.F. Chen, X.L. Jiang, B. Liu, A genetic map constructed using a doubled haploid population derived from two elite Chinese common wheat varieties, J. Integr. Plant Biol. 50 (2008) 941–950.
[45] R.E. Voorrips, MapChart, software for the graphical presentation of linkage maps and QTLs, J. Hered. 93 (2002) 77–78.
[46] G.H. Bai, M.K. Das, B.F. Carver, X.Y. Xu, E.G. Krenzer, Co-variation foe microsatellite alleles associated with *Rht8* and coleoptile length in winter wheat, Crop Sci. 44 (2004) 1187–1194.
[47] F.P. Yang, X.K. Zhang, X.C. Xia, D.A. Laurie, W.X. Yang, Z.H. He, Distribution of the photoperiod insensitive *Ppd-D1a* allele in Chinese wheat cultivars, Euphytica 165 (2008) 445–452.
[48] S.A. Quarrie, A. Steed, C. Calestani, A. Semikhodskii, C. Lebreton, C. Chinoy, N. Steele, D. Pljevljakusic, E. Waterman, J. Weyen, J. Schondelmaier, D.Z. Habash, P. Farmer, L. Saker, D.T. Clarkson, A. Abugalieva, M. Yessimbekova, Y. Turuspekov, S. Abugalieva, R. Tuberosa, M.C. Sanguineti, P.A. Hollington, R. Aragués, A. Royo, D. Dodig, A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese spring × SQ1 and its use to compare QTLs for grain yield across a range of environments, Theor. Appl. Genet. 110 (2005) 865–880.
[49] A. Laperche, M. Brancourt-Hulmel, E. Heumez, O. Gardet, E. Hanocq, F. Devienne-Barret, J. le Gouis, Using genotype × nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints, Theor. Appl. Genet. 115 (2007) 399–415.
[50] S. Liu, J.C. Rudd, G.H. Bai, S.D. Haley, A.M.H. Ibrahim, Q. Xue, D.B. Hays, R.A. Graybosch, R.N. Devkota, P.S. Amand, Molecular markers linked to important genes in hard winter wheat, Crop Sci. 54 (2014) 1304–1321.