
Effects of Gibberellic Acid Responsive Dwarfing Gene *Rht9* on Plant Height and Agronomic Traits in Common Wheat

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To cite this article:

Tay Zar Linn, Daoura Goudia Bachir, Liang Chen, Yin-Gang Hu. Effects of Gibberellic Acid Responsive Dwarfing Gene *Rht9* on Plant Height and Agronomic Traits in Common Wheat *American Journal of Agriculture and Forestry*. Vol. 5, No. 4, 2017, pp. 102-111.

doi: 10.11648/j.ajaf.20170504.14

Received: May 11, 2017; Accepted: May 20, 2017; Published: July 7, 2017

Abstract: To explore the potential use of GA-responsive dwarfing gene *Rht9* in common wheat breeding program, its effects on plant height, seedling vigor, photosynthesis and yield traits were investigated and compared in field experiments using hexaploid *Rht9* dwarf lines derived from two crosses of Chinese winter wheat cultivars Xifeng 20 and Jinmai 47 with the *Rht9* tetraploid donor Granaoto. Xifeng20-*Rht9* dwarf lines reduced plant height on average by 25.38%, while on average by 9.39% in Jinmai47-*Rht9/Rht8* dwarf lines. Compared with taller parents, coleoptile length was reduced by 19.80% in Xifeng20-*Rht9* dwarf lines, while it was increased by 14.22% in Jinmai 47-*Rht9/Rht8* dwarf lines. There were no adverse effects of *Rht9* on root characters and flag leaf characters, though slightly increased relative leaf chlorophyll content (SPAD) observed. Grain numbers per spike was increased on average by 19.63%, and biomass per plant was slightly decreased on average by 3.37% in Xifeng 20-*Rht9* dwarf lines, while, grain number per spike was decreased on average by 11.49%, and biomass per plant was increased on average by 8.57% in Jinmai47-*Rht9/Rht8* dwarf lines. Compared with taller parents, *Rht9* increased fertile tillers on average by 11.25% and 11.19%, grain yield on average by 10.11% and 14.10%, harvest index on average by 12.67% and 6.85%, while decreased spike length on average by 4.80% and 16.23%, slightly decreased 1000 kernels weight by 4.43% and 4.61%, in the *Rht9* dwarf lines of Xifeng 20 and Jinmai 47, respectively. The results of current study could be useful for proper use of dwarfing gene *Rht9* to improve lodging resistance, grain yield potential in wheat breeding programs for water limited area.

Keywords: Dwarfing Genes, *Rht8*, *Rht9*, Plant Height, Seedling Vigor, Yield, Wheat

1. Introduction

Wheat is grown mostly in arid and semiarid regions, taking about one fifth of all crop acreage worldwide, and is the staple food for 40% of the world's population [1]. Due to the increasing global population and limited arable land, wheat production and yield improvement become more and more important. The introduction of semi-dwarfing genes into rice (*Oryza sativa*) and wheat (*Triticum aestivum*) contributes prominent yield increases during the Green Revolution [2]. High yields were associated with improved lodging resistance and the ability to tolerate higher rates of inorganic

nitrogen-based fertilizers [3]. The use of dwarfing genes to reduce plant height, increase harvest index, improve lodging resistance and increase grain yield has been one of the major strategies in developing modern bread wheat cultivars.

Most of the dwarf and semi-dwarf wheat lines in Europe have inherited dwarfing genes from the Japanese wheat landrace Akakomugi, which is the donor of the dwarf gene *Rht8* and the photoperiod-insensitive gene *Ppd-D1*, both genes are closely linked and located on chromosome 2D [3]. Donor of dwarfing genes *Rht-B1* and *Rht-D1* from Japanese origin variety Norin 10 was first introduced to United State, then to CIMMYT, where new semi-dwarf wheat varieties were developed by Norman Bourlax (1968) and then

followed by other wheat breeding programs worldwide [3, 4]. Dwarfing genes are classified according to their sensitivity to externally applied gibberellins (GAs) [5]. *Rht-B1b* and *Rht-D1b* are GA-insensitive (GAI) genes, conferring the dwarf phenotype, and the wild type alleles are *Rht-B1a* and *Rht-D1a*, while *Rht8* is a GA-responsive gene. *Rht-B1b* and *Rht-D1b* are probably present in around 90% of the world's semi-dwarf wheat crops and responsible for the worldwide green revolution in wheat [6]. *Rht-B1b* and *Rht-D1b*, located on homoeologous chromosome arms of 4BS and 4DS, had pleiotropic effects on plant growth, causing reductions in coleoptile length and seedling leaf area [7]. The effect of the *Rht-B1* and *Rht-D1* on height reduction is commonly reported at around 20-25% of the wild-type allele but it varies with different genetic background and environment [8-10]. Apart from reducing the plant height of wheat, dwarfing genes *Rht-B1b*, *Rht-D1b* also decrease the coleoptiles length, the seedling rate and population density [11, 12]. Therefore the use of GAI dwarfing genes is not recommended in dry land environments. The development of wheat cultivars with greater seedling vigor and the capacity to emerge from deep sowing becomes very interesting for wheat breeders [13, 14].

Replacement of the *Rht-B1b* and *Rht-D1b* GAI-dwarfing alleles with alternate gibberellic acid-responsive (GAR) dwarfing genes shows its potential in reducing plant height without compromising seedling vigor [14-17]. Several studies have been conducted to demonstrate the potential of *Rht8* in the development of semi-dwarf, long coleoptiles wheat targeted at sowing depths exceeding 100 mm [11, 14]. *Rht8* has a smaller effect on height reduction (8-12%) than the GAI ones *Rht-B1b*, *Rht-B1c* and *Rht-D1b* [13, 17, 18]. *Rht8* allele has been shown to reduce plant height and increase carbon-partitioning in grains to increase the grain number and yield [13]. In addition to *Rht8*, there are some other major GAR dwarfing genes (e.g. *Rht4*, *Rht5*, *Rht9*, *Rht12*, *Rht13*, *Rht14* and *Rht18*) that reduce plant height by as much as 50% when compared with tall-parental or near-isogenic controls [17-20]. However, they are seemingly neutral in their effects on coleoptile length and seedling vigor [17, 21, 22]. Coleoptile length for progenies derived from *Rht8* and *Rht9* donors is generally reduced (7 to 13%) but is still 47% longer than that of *Rht-B1b* and *Rht-D1b* controls on average [16]. However, genetic and agronomic studies have demonstrated the potential for *Rht8* and *Rht9* in the development of high-yielding, reduced height wheat with long coleoptiles [13, 14].

Marker assisted selection (MAS) is efficient in selecting the traits contributed by the specific genes in their segregating populations. PCR-based markers have been developed for discriminating between mutant (dwarf) *RhtB1b* and *RhtD1b* and their wild type alleles [23], while *Rht8* was linked to markers WMC503 and WMS 261 on chromosome 2DS and *Rht9* was linked to marker BARC 151 on chromosome 5AL in the Chuan Mai 18 (*Rht8*) × Mara

(*Rht8/Rht9*) population, and *Rht9* semi-dwarf lines were on average 5 to 7 cm shorter than *Rht8* lines, with a small but significant height reduction effect [18].

In this study, two Chinese winter wheat cultivars Xifeng 20 (without known dwarfing genes) and Jinmai47 (with *Rht8*) were crossed with *Rht9* donor Granato (AABB), and the SSR markers BARC 151 for *Rht9* and WMC503 for *Rht8* were used to identify the genotypes of each hexiploid dwarfing lines to evaluate the effects of dwarfing gene *Rht9* and its combination with *Rht8* on plant height, coleoptile length, root length, photosynthesis-related traits and yield traits of common wheat in two genetic backgrounds, to better understand the potential use of *Rht9* in common wheat improvement.

2. Materials and Methods

2.1. Plant Materials

The *Rht9* dwarfing lines used in this study were initially derived from two crosses between two Chinese winter wheat cultivars, Xifeng 20 and Jinmai 47 with Granato (AABB), the *Rht9* donor with tetraploid wheat background. Jinmai47, carrying *Rht8*, and Xifeng 20, with no known dwarfing genes, are winter wheat cultivars widely grown in the dryland areas of northern China. F₁ plants from the two crosses were self-pollinated to produce F₂ progeny by covering the spikes with paper bags before flowering. Hexaploid individuals of each F₂ populations were selected based on the presence of targeted gene with the aid of the linked markers and plant height. The hexaploid F₂ individuals carrying *Rht9* allele were used to develop the *Rht9* dwarf lines of Xifeng 20 and Jinmai 47 for successive generations of self-pollinating. BC₁F₁ population was developed by crossing F₁ plants of Jinmai 47/Granato with Jinmai 47, and then the hexaploid BC₁F₂ individuals were selected to develop BC₁ dwarfing lines. The numbers of lines from the two crosses of Xifeng 20/Granato, Jinmai47/Granato used in this study are shown in Table 1. As *Rht8* located in chromosome 2DS of Jinmai47 [18], so all the *Rht9* dwarfing lines of Jinmai47 carried *Rht8*.

2.2. Field Experiments

Field experiments were conducted at the experimental farm of Northwest A&F University (Shaanxi, P. R. China). Eleven *Rht9* dwarfing lines of Xifeng 20 and 13 *Rht9* dwarfing lines of Jinmai 47, which were identified with *Rht9*, were sown next to their corresponding parental varieties during the growing seasons of 2014-2015 and 2015-2016. Complete randomized block designs were adopted with two replications. Each line was sown by hand in 3 rows of 2 m long with an interval of 25 cm between rows and 6.7 cm within plants. No irrigation was provided, so wheat growth relied on the moisture in the soil at sowing and the rainfall in the season.

Table 1. Number of entries of the hexaploid *Rht9* dwarf lines from two crosses between Chinese winter-wheat cultivars, Xifeng20 and Jinmai47 with Granato.

Crosses	Generations (2014-15)	Generations (2015-16)	Number of Lines	Genotypes
Xifeng20 × Granato	F ₆	F ₇	11	
<i>Rht9</i> dwarf lines of Xifeng20	F ₆	F ₇	11	<i>Rht9</i>
(Jinmai47 × Granato)	F ₅	F ₆	4	
(Jinmai47 × Granato) × Jinmai47	BC ₁ F ₄	BC ₁ F ₅	9	
<i>Rht9</i> dwarf lines of Jinmai47	F ₅ &BC ₁ F ₄	F ₆ &BC ₁ F ₅	13	<i>Rht9</i> + <i>Rht8</i>

2.3. Genotyping *Rht9* with Linked SSR Marker

Genomic DNA was extracted from the mixed fresh young leaves of 5 individuals for each line using CTAB method [24]. Polymerase chain reaction (PCR) were performed with the SSR marker BARC 151 to identify the presence of *Rht9*, while WMC 503 for *Rht-8*, as previously reported [18, 25]. The PCR products were separated on 8% denatured polyacrylamide gels and were visualized by silver staining following the procedure described [26].

2.4. Traits Evaluation

2.4.1. Plant Height and Internode Characters

Ten plants were randomly selected at maturity (Z80) [27] from each line and their tall parents. Plant height and internode length were measured individually to calculate the means of plant height and internode length. The length of the internode below the spike was defined as peduncle length (PL), length of the second internode from top was defined as (I2L), followed by the length of the third (I3L), fourth (I4L), and fifth (I5L) internode, respectively. Each internode length was measured from the mid-point of their subtending nodes.

2.4.2. Coleoptile Length and Seedling Root Characters

Good quality seeds of similar seeds and free of any visible damage from F₇ lines of Xifeng20, F₆&BC₁F₅ lines of Jinmai47 and parents were used to investigate coleoptile length (CL) and seedling root characteristics with two replications. Briefly, ten uniform-sized seeds without physical damage were placed on the filter paper in the Petri-dishes. The Petri-dishes were then placed in the dark room at 20°C for 10 days. Coleoptile length (CL) was recorded to the nearest millimeter measuring from the base of the seed to the coleoptile tip.

An additional ten uniform-sized seeds were pre-sterilized (soaked in 75% ethanol for 30 seconds and rinsed with sterile de-ionized water 5-6 times) and placed in a row 2 cm from the edge of a 20 cm × 20 cm germination paper. The germination paper were rolled and placed vertically, with seeds at the top, into a container with 1 cm of water at the bottom. The container was then placed into an incubator at 20°C until the accumulated temperature reached 200°C d. Root number per plant (RN) were determined, and root length (RL) were measured with a ruler. Total root length (TRL), average root diameter (ARD), root surface area (RSA) and root volume (RV) were recorded using a flatbed scanner (Epson Expression 4990) and analyzed with WinRHIZO Pro (Regent Instruments, QC, Canada) [28].

2.4.3. Assessment of Photosynthesis-Related Traits

Flag leaf length (FLL), flag leaf width (FLW), the relative content of leaf chlorophyll (SPAD), the photosynthesis rate (A), stomatal conductance (gs), internal CO₂ concentration (C_i), transpiration rate (E) of the flag leaf were recorded from plots of F₇ lines of Xifeng20, F₆&BC₁F₅ lines of Jinmai47 and parents in 2015-2016 growth season.

Flag leaf length (FLL), flag leaf width (FLW) and the relative content of leaf chlorophyll (SPAD) were recorded at early grain-filling stage (Z70) [27]. Flag leaf width (FLW) was recorded by measuring at the middle of the leaf. SPAD value was determined as the average at the base, middle and top of the flag leaf with a SPAD-502 chlorophyll meter (Minolta Co., Ltd, Japan) [29].

At anthesis (Z65) and early grain-filling (Z73) stages, the photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E) of the flag leaf were measured with a portable photosynthesis system (Li-6400, USA). Conditions in the leaf chamber were as reference CO₂ concentration at 400 μmol mol⁻¹, Photosynthetic Photon Flux Density (PPFD) at 1800 μmol m⁻² s⁻¹, relative humidity of 50-70% and block temperature of 20°C. Photosynthetic traits were measured for five plants of each line at 9:00-11:00 am in sunny and windless weather.

2.4.4. Assessment of Agronomic Traits

From plots of F₆ and F₇ lines of Xifeng20, F₅&BC₁F₄ and F₆&BC₁F₅ lines of Jinmai47 and their corresponding parents, ten plants were randomly selected and measured individually to estimate the means of the spike length (SL), spikelet number per spike (SNPS), grain number per spike (GNPS) and the number of fertile tillers per plant (FT), biomass per plant (BM), grain yield per plant (GY), harvest index (HI) in the two growing seasons. Distance from spike to flag leaf ligule (DSL), internodes length and 1000-kernel weight (TKW) were recorded from plots of F₇ lines of Xifeng20, F₆&BC₁F₅ lines of Jinmai47 and parents in 2015-2016 growth season.

Ten plants were randomly selected from each line and hand harvested at maturity. The main shoot from each plant was used to measure the spike length (SL), spikelet number per spike (SNPS), grain number per spike (GNPS) and the number of fertile tillers per plant (FT). Biomass and grain yield were recorded for the 10 plants and then calculated as the biomass per plant (BM) and grain yield per plant (GY). Harvest index (HI) was calculated as a ratio of grain weight to aboveground biomass. 1000-kernel weight (TKW) was

recorded.

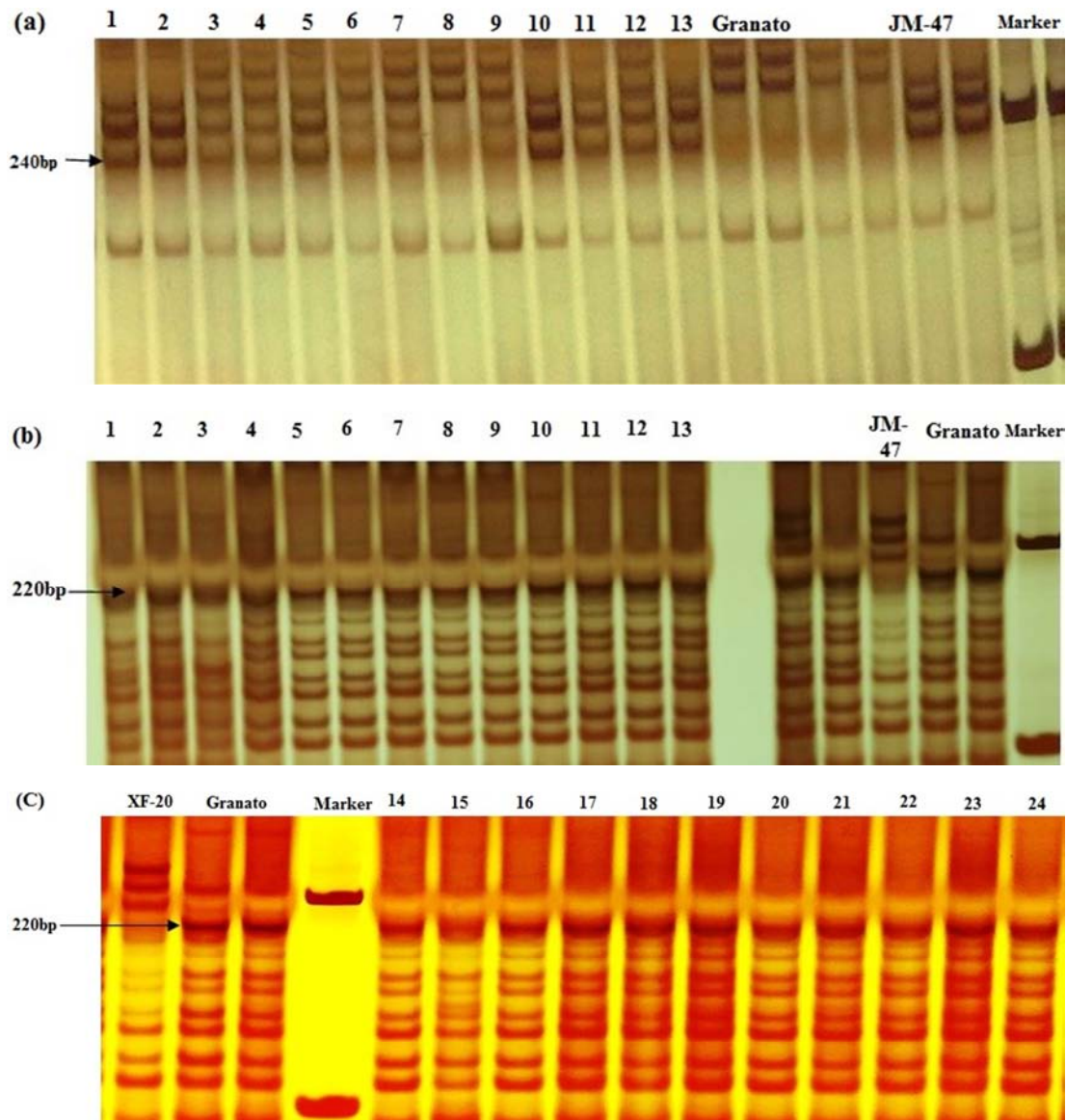
2.5. Statistical Analysis

The mean values of all traits for each line were determined and the effects of dwarfing gene *Rht9* were estimated following the formula, $\text{effect} = (X_{\text{Dwarf}} - X_{\text{Tall}})/X_{\text{Tall}} \times 100\%$, where X_{Dwarf} is the mean value of the *Rht9* lines and X_{Tall} is the mean value of the taller parent. Statistical analysis was carried out by t- test using SAS procedure to compare the significance of difference between the mean of dwarf lines and their corresponding tall parents (SAS Institute Inc, Cary, NC, USA). Phenotypic correlation coefficients between plant heights with other agronomic traits were worked out with the help of SPSS 16.0 for Windows. Significance of difference was designated by $p < 0.05$ (*) and $p < 0.01$ (**), respectively.

3. Results

3.1. Genotyping of *Rht9* with SSR Marker

Rht9 dwarf lines of Xifeng20 and Jinmai47 were identified by the PCR products using the linked SSR marker BARC151 for *Rht9*, while the presence of *Rht8* in the dwarfing lines of Jinmai47 was also confirmed using WMC503, which suggested that the *Rht9* dwarfing lines of Jinmai47 was under the *Rht8* background (Figure 1). Genotyping with SSR marker BARC151 showed a 220bp product in Granato and 230bp product in Xifeng20 and Jinmai 47, and confirmed the presence of *Rht9* the dwarf lines of Xifeng20 and Jinmai 47. Genotyping with SSR marker WMC503 showed a 240bp product in Jinmai47 (*Rht8*) and a 270bp product in Granato (*rht8*), and confirmed the presence of *Rht8* in the dwarf lines of Jinmai47 as well. Thus, the dwarfing lines of Jinmai47/Granato were identified as double dwarf (*Rht9/Rht8*).



JM-47; Jinmai47, XF-20; Xifeng20, 1-13; dwarf individuals of Jinmai47 (*Rht9/Rht8*), 14-24; dwarf individuals of Xifeng20 (*Rht9*).

Figure 1. Detection of dwarfing genes *Rht8* and *Rht9* with their linked SSR marker WMC503 and BARC151 (a) Detection of *Rht8* with WMC503 in the dwarf lines of Jinmai47; (b) Detection of *Rht9* with BARC151 in the dwarf lines of Jinmai47; (c) Detection of *Rht9* with BARC151 in the dwarf lines of Xifeng20.

Table 2. Plant height and main agronomic traits of *F₆* *Rht9* dwarfing lines of Xifeng20 and *F₅&BC₁F₄* *Rht9* dwarf lines of Jinmai47 with their corresponding tall parents.

Traits	Xifeng20/Granato			Jinmai47/Granato		
	Dwarf	Xifeng20	Difference	Dwarf	Jinmai47	Difference
PH (cm)	91.31±2.83	120.89±5.54	-29.58(-24.47%)**	96.02±4.81	102.58±0.38	-6.56(-6.40%)*
SL (cm)	10.27±0.23	11.17±0.07	-0.90(-8.06%)**	9.22±0.67	11.20±0.13	-1.98(-17.68%)**
SNPS	21.21±0.61	20.16±0.70	1.05(5.21%)ns	18.26±0.98	18.33±0.76	-0.07(-0.38%)ns
FT	13.35±1.41	12.25±0.25	1.10(8.98%)*	14.67±1.31	13.39±1.08	1.28(9.56%)*
GNPS	67.02±2.92	54.16±2.02	12.86(23.74%)**	53.24±6.15	58.83±8.51	-5.59(-9.50%)*
BM (gm)	47.36±7.51	47.61±0.00	-0.25(-0.53%)ns	52.32±6.00	46.70±0.00	5.62(12.04%)*
GY (gm)	21.99±3.05	19.67±0.00	2.32(11.79%)*	23.61±2.09	19.80±0.00	3.81(19.19%)*
HI %	0.46±0.25	0.41±0.00	0.05(12.00%)**	0.46±0.03	0.42±0.00	0.04(9.52%)ns

PH, plant height (cm), SL, The spike length (cm); SNPS, Spikelet number per spike; FT, The number of fertile tillers per plant; GNPS, Grain number per spike; BM, The biomass per plant (gram); GY, Grain yield per plant (gram); HI, Harvest Index %. Data are means ±SD (standard deviation) of each genotype. Difference was calculated as the value of

dwarf lines minus that of the tall parent; the percentage difference was estimated as the difference to tall parent and is shown in parentheses. ns indicates that differences are not significant. * and ** indicates differences between the dwarfing lines and tall parent significant at $p < 0.05$, and $p < 0.01$, respectively.

Table 3. Plant height and main agronomic traits of *F₇* *Rht9* dwarfing lines of Xifeng20 and *F₆&BC₁F₅* *Rht9* dwarf lines of Jinmai47 with their corresponding tall parents.

Traits	Xifeng20/Granato			Jinmai47/Granato		
	Dwarf	Xifeng20	Difference	Dwarf	Xifeng20	Difference
PH (cm)	76.35±3.02	103.58±3.57	-27.23(-26.29%)**	82.50±6.33	94.16±1.33	-11.66(-12.38%)**
SL (cm)	8.95±0.34	9.11±0.58	-0.16(-1.54%)ns	7.50±0.85	8.80±0.33	-1.30(-14.77%)*
SNPS	18.72±0.41	17.97±0.91	0.75(4.17%)ns	16.50±1.25	17.78±0.98	-1.28(-7.20%)ns
FT	8.98±0.60	7.91±0.36	1.07(13.53%)*	7.83±0.59	6.94±0.47	0.89(12.82%)*
GNPS	54.80±2.77	47.44±5.64	7.36(15.51%)*	45.95±5.16	53.11±3.42	-7.16(-13.48%)*
BM (gm)	31.11±1.62	33.18±7.31	-2.07(-6.21%)ns	31.73±3.10	30.19±1.67	1.54(5.10%)ns
GY (gm)	16.09±1.44	14.84±2.40	1.25(8.42%)ns	15.97±1.79	14.65±0.97	1.32(9.01%)ns
HI %	0.51±0.02	0.45±0.01	0.06(13.33%)**	0.50±0.02	0.48±0.02	0.02(4.17%)ns
TKW (gm)	41.55±1.02	43.47±0.98	-1.92(-4.43%)ns	47.81±4.88	50.12±0.45	-2.31(-4.61%)ns

PH, plant height (cm), SL, The spike length (cm); SNPS, Spikelet number per spike; FT, The number of fertile tillers per plant; GNPS, Grain number per spike; BM, The biomass per plant (gram); GY, Grain yield per plant (gram); HI, Harvest Index %; TKW, 1000 kernel weight (gram). Data are means±SD (standard deviation) of each genotype. Difference was calculated as the value of dwarf lines minus that of the tall parent; the percentage difference was estimated as the difference to tall parent and is shown in parentheses. ns indicates that differences are not significant.

* and ** indicates differences between the dwarfing lines and tall parent significant at $p < 0.05$, and $p < 0.01$, respectively.

3.2. The Effects of *Rht9* on Plant Height and Internode Lengths

Compared with their tall parental varieties, plant height (PH) was significantly reduced in the *Rht9* dwarfing lines of the two populations (Table 2, Table 3). PH was significantly reduced by 24.47% and 26.29% in the *F₆* and *F₇* *Rht9* dwarf lines of Xifeng 20, respectively (Table 2) and by 6.40% and 12.38% in the *F₅&BC₁F₄* and *F₆&BC₁F₅* *Rht9* dwarf lines of Jinmai47, respectively compared with their taller parents (Table 3). The peduncle length (PL), the lengths of the second (I2L), third (I3L), fourth (I4L) and fifth (I5L) internode from the top were decreased significantly by 23.40%, 30.60%, 31.20%, 36.50% and 25.10%, respectively in the *F₇* *Rht9* dwarf lines of Xifeng 20 than Xifeng 20 (Table 4). The lengths of the second (I2L), and third (I3L) internode from the top were significantly decreased by 15.80% and 13.13% in *F₆&BC₁F₅* *Rht9* dwarf lines of Jinmai 47 than Jinmai 47, and there were no significant differences on the peduncle length (PL), and other internodes (Table 4). The distance from spike to flag-leaf ligule (DSL) was significantly reduced by 34.10% in *F₇* *Rht9* dwarf lines of

Xifeng 20, while it was significantly increased by 50.60% in *F₆&BC₁F₅* *Rht9* dwarf lines of Jinmai47 (Table 4). The effects of *Rht9* on the lengths of different internodes varied in the two genetic backgrounds, which were stronger in the background of Xifeng 20, a variety with no known dwarfing genes, than that in the background of Jinmai47, which carried the GAR dwarfing gene *Rht8*. This indicated that there may have some interactions between *Rht9* and *Rht8*.

3.3. The Effects of *Rht9* on Agronomic Traits

Compared with Xifeng 20, the number of fertile tillers per plant (FT) was significantly increased by 8.98% and 13.53% in *F₆* and *F₇* *Rht9* dwarf lines of Xifeng20, respectively and grain number per spike (GNPS) was also significantly increased by 23.74% and 15.51% in *F₆* and *F₇* *Rht9* dwarf lines of Xifeng 20, respectively (Table 2 and Table 3). The biomass per plant (BM) was slightly reduced, grain yield per plant (GY) was slightly increased in the *F₆* and *F₇* lines of Xifeng 20, while harvest index (HI) were significantly increased by 12.00% and 13.33% in *F₆* and *F₇* *Rht9* dwarf lines of Xifeng 20, respectively (Table 2 and Table 3). The *F₇* *Rht9* dwarf lines of Xifeng 20 showed 4.43% decrease in

1000 kernels weight (TKW) (Table 3).

Compared with Jinmai47, spike length (SL) was significantly decreased by 17.68% and 14.77% and grain number per spike (GNPS) was significantly decreased by 9.50% and 13.48%, while the number of fertile tillers per plant (FT) was significantly increased by 9.56% and 12.82% in F_5 & BC_1F_4 and F_6 & BC_1F_5 *Rht9* dwarf lines of Jinmai47, respectively (Table 2 and Table 3). Biomasses per plant (BM), grain yield per plant (GY) were significantly increased by 12.04% and 19.19% and harvest index was slightly

increased in the F_5 & BC_1F_4 *Rht9* dwarf lines of Jinmai 47 (Table 2). F_6 & BC_1F_5 *Rht9* dwarf lines of Jinmai 47 showed 5.10% increase in biomass per plant (BM), 9.01% increase in grain yield per plant (GY) and 4.17% increase of harvest index (HI) but their differences between the dwarf lines and tall parent were not significant (Table 3). 1000 kernels weight (TKW) was slightly decreased by 4.61% in *Rht9* dwarf lines of Jinmai 47 (Table 3). These results indicated that the effects of *Rht9* on grain number per spike (GNPS) and biomass per plant (BM) varied with different genetic backgrounds.

Table 4. Plant height related traits of F_7 *Rht9* dwarfing lines of Xifeng20 and F_6 & BC_1F_5 *Rht9* dwarf lines of Jinmai47 with their corresponding tall parents.

Traits	Xifeng20/Granato			Jinmai47/Granato		
	Dwarf	Xifeng20	Difference	Dwarf	Xifeng20	Difference
PH (cm)	76.35±3.02	103.58±3.57	-27.23(-26.29%)**	82.50±6.33	94.16±1.33	-11.66(-12.38%)**
PL (cm)	24.46±1.43	31.92±4.57	-7.46(-23.40%)**	22.44±1.57	23.88±1.31	-1.44(-6.00%)ns
I2L (cm)	17.53±0.60	25.26±1.92	-7.73(-30.60%)**	20.94±1.99	24.88±0.29	-3.94(-15.80%)**
I3L (cm)	12.27±0.67	17.84±1.18	-5.57(-31.20%)**	15.63±2.29	18±1.15	-2.37(-13.13%)*
I4L (cm)	7.92±0.62	12.48±1.54	-4.56(-36.50%)**	10.28±2.18	11.55±0.75	-1.27(-10.90%)ns
I5L (cm)	5.50±0.68	7.34±1.96	-1.84(-25.10%)*	4.81±0.96	4.75±2.06	0.06(1.30%)ns
DSL (cm)	8.34±1.70	12.67±2.25	-4.33(-34.10%)**	5.27±1.63	3.5±0.9	1.77(50.60%)**

PH, plant height (cm); PL, peduncle length (cm); I2L, I3L, I4L, I5L, length of the second, third, fourth and fifth internode from top (cm), respectively; DSL, distance from base of the spike to the flag ligule (cm);

Data are means±SD (standard deviation) of each genotype. Difference was calculated as the value of dwarf lines minus that of the tall parent; the percentage difference was estimated as the difference to tall parent and is shown in parentheses. ns indicates that differences are not significant. * and ** indicates differences between the dwarfing lines and tall parent significant at $p<0.05$, and $p<0.01$, respectively.

3.4. The Effects of *Rht9* on Seedling Root Characters and Coleoptile Length

Coleoptile length (CL) was significantly reduced, by 19.80% (about 10.09mm), for the single dwarfing gene *Rht9*, in F_7 lines of Xifeng 20, while it was even significantly increased by 14.22% in F_6 & BC_1F_5 *Rht9* dwarf lines of Jinmai47 which also carried *Rht8* compared with taller parents (Table 5).

Rht9 dwarf lines of Jinmai 47 also showed 16.20%, 18.18% and 33.33% significant decrease in the root surface area (RSA), average root diameter (ARD) and root volume (RV), respectively (Table 5). As to the *Rht9* dwarf lines of Xifeng 20, no significant difference was observed on those root characters. These results indicated that the combination of *Rht9* and *Rht8* improved coleoptiles length.

Table 5. Root characters and coleoptile length of the F_7 *Rht9* dwarfing lines of Xifeng20 and F_6 & BC_1F_5 *Rht9* dwarf lines of Jinmai47 with their corresponding tall parents.

Traits	Xifeng20/Granato			Jinmai47/Granato		
	Dwarf	Xifeng20	Difference	Dwarf	Xifeng20	Difference
RN	3.33±0.25	3.66±0.28	-0.33(-9.00%)ns	4.00±0.58	3.75±0.35	0.25(6.70%)ns
RL (cm)	34.25±2.73	32.5±1.38	1.75(5.40%)ns	27.14±1.87	25±3.37	2.14(8.60%)ns
TRL (cm)	87.39±3.99	82.62±6.41	4.77(5.80%)ns	78.03±11.19	77.99±9.75	0.04(0.10%)ns
RSA (cm ²)	12.40±0.94	11.78±1.05	0.62(5.30%)ns	8.96±1.30	10.69±2.12	-1.73(-16.20%)*
ARD (mm)	0.20±0.03	0.22±0.01	-0.02(-9.09%)ns	0.18±0.01	0.22±0.01	-0.04(-18.18%)**
RV (cm ³)	0.14±0.01	0.13±0.02	0.01(7.69%)ns	0.08±0.01	0.12±0.03	-0.04(-33.33%)**
CL (mm)	44.68±1.37	55.67±2.51	-10.99(-19.80%)**	57.11±7.78	50.00±3.46	7.11(14.22%)*

RN, Root number per plant; RL, Root length (cm); TRL, total root length (cm); RSA, Root surface area (cm²); ARD, Average root diameter (mm); RV, Root volume (cm³); CL, coleoptile length (mm).

Data are means±SD (standard deviation) of each genotype. Difference was calculated as the value of dwarf lines minus that of the tall parent; the percentage difference was estimated as the difference to tall parent and is shown in parentheses. ns indicates that differences are not significant. * and ** indicates differences between the dwarfing lines and tall parent significant at $p<0.05$, and $p<0.01$, respectively.

3.5. The Effects of *Rht9* on Photosynthesis-Related Traits

Rht9 dwarf lines of Xifeng 20 showed 10.30% significant increase in flag leaf width (FLW), but 9.70% significant decrease in flag leaf length (FLL) than Xifeng 20. As to the *Rht9* dwarf lines of Jinmai47, no significant differences were observed on the flag leaf characters (Table 6). Relative leaf chlorophyll content (SPAD) was slightly increased by 2.20%

and 2.90% in *Rht9* dwarf lines of Xifeng20 and Jinmai47 dwarf lines, respectively (Table 6). Photosynthetic rate (*A*), stomatal conductance (*g_s*), and transpiration rate (*E*) at grain filling stage were significantly reduced by 19.30%, 36.20% and 25.80%, respectively in *Rht9* dwarf lines of Xifeng20, while photosynthetic rate (*A*), and stomatal conductance (*g_s*), and transpiration rate (*E*) at grain filling stage were significantly increased by 26.40%, 45.80% and 19.50%

respectively, and transpiration rate (*E*) at anthesis stage was significantly reduced by 13.30% in *Rht9* dwarf lines of

Jinmai47, compared with Xifeng 20 and Jinmai 47, respectively (Table 6).

Table 6. Flag leaf characters, SPAD and gas exchange parameters of *F₇* *Rht9* dwarfing lines of Xifeng20 and *F₆*&*BC₁F₅* *Rht9* dwarf lines of Jinmai47 with their corresponding tall parents.

Traits	Xifeng20/Granato			Jinmai47/Granato		
	Dwarf	Xifeng20	Difference	Dwarf	Xifeng20	Difference
FLL (cm)	15.33±1.13	16.98±1.19	-1.65(-9.70%)*	15.71±1.27	15.52±1.46	0.19(1.20%)ns
FLW (cm)	1.61±0.06	1.46±0.05	0.15(10.30%)**	1.55±0.19	1.44±0.05	0.11(7.60%)ns
SPAD	54.86±0.98	53.68±1.16	1.18(2.20%)ns	51.24±3.10	49.8±1.67	1.44(2.90%)ns
At anthesis				At anthesis		
<i>A</i>	18.07±1.67	19.62±0.52	-1.55(-7.80%)ns	18.47±2.36	16.67±1.92	1.80(10.80%)ns
<i>g_s</i>	0.26±0.02	0.26±0.02	0.00(0.80%)ns	0.24±0.04	0.27±0.02	-0.03(-7.40%)ns
<i>E</i>	2.18±0.12	2.07±0.13	0.11(5.30%)ns	1.95±0.22	2.25±0.11	-0.30(-13.30%)*
At grain filling				At grain filling		
<i>A</i>	8.56±0.99	10.62±0.34	-2.06(-19.30%)**	10.53±1.17	8.33±3.27	2.20(26.40%)*
<i>g_s</i>	0.29±0.03	0.47±0.05	-0.18(-36.20%)**	0.35±0.05	0.24±0.10	0.11(45.80%)**
<i>E</i>	5.59±0.55	7.53±0.32	-1.94(-25.80%)**	7.23±1.12	6.05±1.77	1.18(19.50%)*

FLL, flag leaf length (cm); FLW, Flag leaf width (cm); SPAD, relative leaf chlorophyll content; *A*, Photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); *g_s*, stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$); *E*, transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$).

Data are means±SD (standard deviation) of each genotype. Difference was calculated as the value of dwarf lines minus that of the tall parent; the percentage difference was estimated as the difference to tall parent and is shown in parentheses. ns indicates that differences are not significant. * and ** indicates differences between the dwarfing lines and tall parent significant at $p < 0.05$, and $p < 0.01$, respectively.

3.6. Correlations Between Plant Height and Other Agronomic Traits

Correlation analysis on plant height (PH) and internode lengths showed that PH was positively and significantly correlated with the length of the third (I3L) and fourth (I4L) internode with $r = 0.571$, 0.478 in the *F₇* *Rht9* dwarf lines of Xifeng20; while plant height (PH) was positively and significantly correlated with the length of the second (I2L), third (I3L), fourth (I4L) and fifth (I5L) internode with $r = 0.681$, 0.585 , 0.715 , 0.422 in the *F₆* & *BC₁F₅* *Rht9* dwarf lines of Jinmai47, respectively, but no significant correlation between PH and peduncle length (PL) (Table 7).

Table 7. Simple correlation coefficients (*r*) between plant height and internode length and agronomic traits in the *F₇* *Rht9* dwarfing lines of Xifeng20 and *F₆* & *BC₁F₅* *Rht9* dwarf lines of Jinmai47.

Traits	Xifeng20/Granato	Jinmai47/Granato
I5L	0.230ns	0.422**
I4L	0.478*	0.715**
I3L	0.571**	0.585**
I2L	0.268ns	0.681**
PL	0.248ns	-0.068ns
Spike length	0.522*	-0.148ns
Grain numbers spike ⁻¹	-0.188ns	-0.215ns
Fertile tillers plant ⁻¹	-0.463*	-0.322*
Biomass plant ⁻¹	-0.128ns	0.227ns
Grain yield plant ⁻¹	-0.345ns	0.099ns
Harvest index	-0.373*	-0.381*

PL, peduncle length (cm); I2L, I3L, I4L, I5L, length of the second, third, fourth and fifth internode from top, respectively;

ns indicates correlations that are not significant, * indicates Significant at $p < 0.05$, ** indicates Significant at $p < 0.01$

Correlation on plant height (PH) with yield related traits indicated that in the *F₇* *Rht9* dwarf lines of Xifeng 20, PH was negatively and significantly correlated with harvest index and the number of fertile tillers per plant ($r = 0.373$, 0.463), and was positively and significantly correlated with

spike length ($r = 0.522$); while in the *F₆* & *BC₁F₅* *Rht9* dwarf lines of Jinmai 47, PH was negatively and significantly correlated with harvest index and the number of fertile tillers per plant ($r = 0.381$, 0.322) (Table 7).

4. Discussion

Dwarfing in wheat breeding has been emphasized under situations of improved or more intensive husbandry methods, particularly increased use of fertilizers. Dwarfing genes *RhtB1b*, *RhtD1b* and *Rht8* were widely used throughout the world to reduce the plant height, increase the lodging resistance, harvest index and yield potential. Dwarfing effects on plant height vary with genetic backgrounds and environments. For instance, GA-insensitive *Rht-B1b* and *Rht-D1b* alleles reduced around 20-25% of plant height to their wild-type allele [8-10]. The GAR dwarfing genes *Rht4*, *Rht8*, *Rht9*, *Rht12*, and *Rht13* reduced plant height by 12-50% while having small or negligible effects on coleoptile length [17, 30, 31]. Extreme dwarfism was associated with reductions in interception of photosynthetic active radiation, above ground biomass and harvest index and increased weed prevalence [32, 33]. To gain a more comprehensive understanding of dwarfing genes, *Rht9* dwarf lines were developed from two populations resulting from crosses of tall Chinese winter wheat cultivars with Granaoto, the tetraploid donor of *Rht9*. In this study, *Rht9* dwarf lines of Xifeng20 reduced plant height by 25.38%, averaged across two seasons and generations than taller parent, while *Rht9* dwarf lines of Jinmai47 reduced plant height by 9.39%, averaged across two seasons and generations than taller parent, with final plant height at around 80cm, which were stronger than previous reported [18], as the experiments were conducted in field conditions without irrigation, but the rainfall in season affected greatly on plant height. The result of this study indicated that *Rht9* alone could approximately have the

similar height reducing effect with *Rht-B1b*, but much weaker effect when combined with *Rht8* allele in the double *Rht9/Rht8* dwarfing lines. It should be noted that the reduction of plant height on average by 9.39% in the dwarf lines of Jinmai 47 was the effect of *Rht9* in a cultivar that also carries *Rht8*. Peduncle length was suggested as a useful indicator of yield capacity in dry environment [17]. Various effects of *Rht9* on different internodes may contribute to varied levels of height reduction by *Rht9*. Investigation revealed that lengths of peduncle and all the internodes were significantly reduced in *Rht9* dwarf lines of Xifeng 20 to significantly reduce overall plant height, while, only the second and third internode length from the spike were significantly reduced in *Rht9* dwarf lines of Jinmai 47. These results showed that *Rht9* had a moderately strong height reducing effect in common wheat which could be used to create germplasm with improved stature in wheat breeding. The effects by *Rht9* alone and by *Rht9/Rht8* on plant height reduction varied greatly with the genetic backgrounds, which suggested that selection of parents was important for its effective utilization.

The dwarf cultivars of wheat were mainly planted in irrigated environments or regions with abundant rainfall during the growing season. However, with the occurrence of severe water shortage and global warming, water resource for wheat planting become more and more limited. Thus the main challenges for the breeding of wheat cultivars for water limited areas is to increase the drought resistance, reduce plant height and increase the grain yields. The effect of different dwarfing genes on coleoptile length is an important indicator for breeding wheat with characteristics of drought resistance and water savings. Seedling vigor and coleoptile length have been important in successful crop establishment, especially in water limited areas. Short coleoptiles can reduce seedling emergence when sowing deep as shown by semi-dwarf wheats with GAI dwarfing genes *Rht-B1b* and *Rht-D1b* [11]. GAR dwarfing genes *Rht4*, *Rht12*, *Rht13* and *Rht18* do not affect coleoptile length [11, 22, 32, 34]. In this study, coleoptile length was significantly reduced by 19.80% in the *Rht9* dwarf lines of Xifeng20 than Xifeng20, while it was significantly increased by 14.22% in the *Rht9/Rht8* double dwarf lines of Jinmai47 than Jinmai47. These results suggested that *Rht8* may compensate the negative effect of *Rht9* on coleoptile length, offering opportunity to breed lines with double dwarfing genes *Rht9/Rht8* for better coleoptile development. Root characters affect plant establishment, water and nutrient uptake in the field, especially in water limited regions. The effects of GAI *Rht* genes on wheat root system varied in previous studies, as little or only a slightly negative effect [35, 36] and strong negative effects observed in semi dwarf wheat cultivars with GAI dwarfing genes *Rht-B1b* and *Rht-D1b* [37, 38]. Most works on GAR dwarfing genes indicated that less negative effects were observed on wheat root system [22]. In this study, most root characters in the seedlings were slightly improved in *Rht9* dwarf lines of Xifeng 20; while root surface area, average root diameter and root volume were significantly reduced in *Rht9/Rht8* dwarf

lines of Jinmai 47. The lack of negative effects from *Rht9* on root traits indicated that *Rht9* may have potential for improving root traits in wheat breeding for water limited regions.

Flag leaf assimilates are the most important contributor to the dry weight accumulation in grain [39]. Higher photosynthetic rate is associated with higher crop yield [40]. The flag leaf contributes greatly to the grain filling and grain yield as it has the highest potential for photosynthesis at the later growth stage of wheat. Though GAR dwarfing gene *Rht13* was associated with narrower flag leaf width, it was improved in the *RhtD1b* and *Rht13* double dwarfing lines [21]. In this study, *Rht9* was associated with shorter and wider flag leaf in Xifeng20 background, while it was associated with longer and wider flag leaf in Jinmai47 background. Relative leaf chlorophyll content (SPAD) was also slightly higher in both *Rht9* dwarf lines of Xifeng20 and Jinmai47 while photosynthetic rate (*A*), stomatal conductance (*g_s*), and transpiration rate (*E*) at grain filling stage were significantly reduced in *Rht9* dwarf lines of Xifeng20, but photosynthesis rate (*A*), stomatal conductance (*g_s*) were significantly increased in *Rht9/Rht8* double dwarf lines of Jinmai47. This result may suggest that the combination of *Rht9* with *Rht8* may improve photosynthetic capacity of flag leaf.

Primary determinants of grain yield are the number of fertile tillers per plant, grain number per spike, and grain weight. Fertile tillers per plant was increased by *Rht5* [41], but it was decreased by either *Rht8* (11.2%) or *Rht13* (5.5%) alone [42]. The presence of GAI dwarfing genes *Rht-B1b* and *Rht-D1b* is commonly associated with greater grain number per spike to increase grain number per unit area [43, 44], and GAR dwarfing genes *Rht13*, *Rht12*, *Rht4* were also associated with increased grain number, while *Rht8* had little effect on grain number [32]. *Rht13* did not affect 1000-kernel weight whereas *Rht8* significantly increased 1000-kernel weight [42]. In this study, the number of fertile tillers per plant was significantly increased, while 1000 kernels weight was slightly reduced in both *Rht9* dwarf lines of Xifeng20 and Jinmai47, and grain number per spike was significantly increased in *Rht9* dwarf lines of Xifeng20 but was decreased in *Rht9/Rht8* dwarf lines of Jinmai47. *Rht8* slightly decreased biomass and grain yield, finally the harvest index (5.7%) [32]. In this study, biomass was slightly decreased in *Rht9* dwarf lines of Xifeng20 but it was increased in *Rht9/Rht8* dwarf lines of Jinmai47; and grain yield per plant was increased in both *Rht9* dwarf lines of Xifeng20 and Jinmai47. Harvest index was significantly increased in *Rht9* dwarf lines of Xifeng20, while it was slightly increased in *Rht9/Rht8* dwarf lines of Jinmai47. These results may reflect effects of genetic backgrounds. This study indicated both single *Rht9* and its combination with *Rht8* increased the number of fertile tillers per plant, grain yield and harvest index.

5. Conclusions

As investigated in two populations derived from Xifeng 20

and Jinmai47, Xifeng 20-*Rht9* dwarf lines reduced plant height on average by 25.38%, while on average by 9.39% in Jinmai47-*Rht9/Rht8* dwarf lines. Height reduction of *Rht9* with the background of Xifeng 20 was greater than that of *Rht9* with the background of Jinmai47 that also carried *Rht8*. Coleoptile length was reduced in *Rht9* dwarf lines of Xifeng20 while coleoptile length was even increased in *Rht9* dwarf lines of Jinmai47 that also carries *Rht8*. Combination of *Rht9* with *Rht8* had slightly longer coleoptile than Jinmai47. *Rht8* may partially compensate for the negative effect of *Rht9* on coleoptile length. Peduncle length and several internodes length were associated with reduction of plant height in both of two populations, which may have potential for freeing-up assimilates for partitioning to the growing ear. There were no adverse effects of *Rht9* on root characters and flag leaf characters, though slightly increased relative leaf chlorophyll content (SPAD) was observed.

Rht9 was associated with increased number of fertile tillers on average by 11.25% in dwarf lines of Xifeng20 and 11.19% in dwarf lines of Jinmai47. *Rht9* was associated with increased grain yield and harvest index in both two populations while *Rht9* was associated with decreased spike length in both two populations. The influence of *Rht9* on spike number per spike, grain numbers per spike and harvest index was varied with different genetic backgrounds of Xifeng 20 and Jinmai 47. 1000- Kernel weight was slightly decreased by 4.43% and 4.61% in both *Rht9* dwarf lines of Xifeng 20 and Jinmai 47 while *Rht9* was associated with increased relative leaf chlorophyll content (SPAD) in both of two populations. This study indicated that the use of *Rht9* should be combined with proper parental lines selection for better coleoptile length, spike characters, especially improved spike length, grain numbers per spike and gas exchange parameters. As the works were carried out in single plant level grown in small plots of one location, further experiments for evaluating the effects of *Rht9* on grain yield should be tested in big plots under more conditions.

Acknowledgements

This study was supported by the National High Technology Research and Development Program of China (2013AA102902)", the Program of Introducing Talents of Discipline to Universities (B12007); Science and Technology Innovation Team Plan of Shaanxi Province (2014KCT-25); as well as ACIAR Project (CIM/2005/111) of Australia.

Abbreviations: PH, plant height (cm); SL, The spike length (cm); SNPS, Spikelet number per spike; FT, The number of fertile tillers per plant; GNPS, Grain number per spike; BM, The biomass per plant (gram); GY, Grain yield per plant (gram); HI, Harvest Index %; TKW, 1000 kernel weight (gram); DSL, distance from base of the spike to the flag ligule (cm); PL, peduncle length (cm); I2L, length of the second internode from top (cm); I3L, length of the third internode from the top (cm); I4L, length of the fourth internode from top (cm); I5L, length of the fifth internode from top (cm); RN, Root numbers; RL, Root length (cm); TRL, total root length

(cm); RSA, Root surface area (cm²); ARD, Average root diameter (mm); RV, Root volume (cm³); CL, coleoptile length (cm); FLL, flag leaf length (cm); FLW, Flag leaf width (cm); SPAD, relative leaf chlorophyll content; *A*, Photosynthetic rate (μmol m⁻² s⁻¹); *gs*, stomatal conductance (mol m⁻² s⁻¹); *E*, transpiration rate (mmol m⁻² s⁻¹).

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