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Dwarfing Gene *Rht18* from Tetraploid Wheat Responds to Exogenous GA_3 in Hexaploid Wheat

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Rht18, derived from *Triticum durum* (tetraploid) wheat, is classified as a gibberellic acid (GA)-responsive dwarfing gene. Prior to this study, the responses of *Rht18* to exogenous GA on agronomic traits in hexaploid wheat were still unknown. The response of *Rht18* to exogenous GA_3 on coleoptile length, plant height, yield components and other agronomic traits were investigated using $F_{4:5}$ and $F_{5:6}$ hexaploid dwarf lines with *Rht18* derived from two crosses between the tetraploid donor Icaro and tall Chinese winter wheat cultivars, Xifeng 20 and Jinmai 47. Applications of exogenous GA_3 significantly increased coleoptile length in both lines and their tall parents. Plant height was significantly increased by 21.3 and 10.7% in the GA_3-treated dwarf lines of Xifeng 20 and Jinmai 47, respectively. Compared to the untreated dwarf lines, the partitioning of dry matter to ears at anthesis was significantly decreased while the partitioning of dry matter to stems was significantly increased in the GA_3-treated dwarf lines. There were no obvious changes in plant height and dry matter partitioning in the GA_3-treated tall parents. Exogenous GA_3 significantly decreased grain number spike^{-1} while it increased 1000-kernel weight in both the dwarf lines and tall parents. Thus, applications of exogenous GA_3 restored plant height and other agronomic traits of *Rht18* dwarf lines to the levels of the tall parents. This study indicated that *Rht18* dwarf mutants are GA-deficient lines with impaired GA biosynthesis.

Keywords: agronomic traits, GA_3-response, plant height, *Triticum aestivum*

Introduction

Gibberellins (GAs) are important hormones that regulate many developmental processes, such as seed germination, and root and shoot elongation, flowering and fruit patterning (Yamaguchi 2008). It is important for plants to produce and maintain optimal levels of bioactive GAs to ensure normal growth and development. Mutant plants that are deficient in GA or interfere with the signal transduction pathway of GA exhibit dwarf, late flowering phenotypes whereas plants with increased GA levels or GA signaling are tall and spindly (Richards et al. 2001; Fleet and Sun 2005). GA-deficient mutants can be restored

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to normal growth by application of exogenous GAs whereas GA-insensitive mutants cannot (Magome et al. 2004).

The role of GA in control of plant stature has had major impacts on agriculture in increasing grain yield during the "Green Revolution" (Hedden 2003). The Green Revolution gene *sd1* in rice is a loss-of-function mutation in one of the GA biosynthetic genes (*GA20ox2*) (Spielmeyer et al. 2002), and GA-insensitive *Rht-B1b* and *Rht-D1b* in wheat are gain-of-function alleles caused by mutations in a transcription factor that is associated with the GA signaling pathway (Peng et al. 1999). However, in addition to reducing height, *Rht-B1b* and *Rht-D1b* also reduce coleoptile length and seedling leaf area and therefore decrease seedling vigor (Rebetzke et al. 2004; Botwright et al. 2005). Thus, replacing the GA-insensitive *Rht-B1b* and *Rht-D1b* with GA-responsive dwarfing genes might be instrumental in avoiding their negative impact on seedling vigor.

A number of GA-responsive dwarfing genes, including *Rht18*, with potential to reduce plant height without compromising seedling vigor have been identified (Konzak 1988; Ellis et al. 2004; Rebetzke et al. 1999, 2012). Even though GA-responsive dwarfing genes have been studied for many years, their molecular characteristics remain obscure and the physiological processes resulting in dwarfing are not well understood. *Rht18* is a semidominant dwarfing gene from durum wheat cultivar (cv.) Icaro, itself a mutant of durum wheat cv. Anhinga induced by fast-neutrons (Konzak 1988). *Rht18* was classified as a GA-responsive dwarfing gene (Konzak 1987, 1988). We earlier reported the effects of *Rht18* on agronomic traits in hexaploid wheat using hexaploid $F_{3:4}$ and $F_{4:5}$ semidwarf lines derived from crosses between Icaro and tall Chinese winter wheat cultivars Xifeng 20, Fengchan 3 and Jinmai 47 (Yang et al. 2015). *Rht18* reduced plant height by an average 18% in the dwarf lines compared with the tall parents. It significantly increased the partitioning of dry matter to ears at anthesis in the dwarf lines resulting in a higher harvest index compared to the tall parents, significantly increased grain number $spike^{-1}$ by 9.2% in dwarf lines of Xifeng 20 and significantly decreased 1000-kernel weight. In contrast, *Rht18* decreased grain number $spike^{-1}$ by 4.9 and 2.0% and increased 1000-kernel weight by 4.0 and 7.7% in dwarf lines of Fengchan 3 and Jinmai 47, respectively.

Although *Rht18* has been classified as a GA-responsive dwarfing gene, studies of the responses of *Rht18* lines to exogenous GAs were confined to tests on Icaro seedlings (Konzak 1988; Ellis et al. 2004). The objective of this study was to investigate the responses of *Rht18* to exogenous GA_3 on plant height and other agronomic traits in hexaploid wheat, in order to further explore its role in GA biosynthesis or signaling pathways.

Materials and Methods

Plant materials

Two populations were developed by crossing hexaploid Chinese winter wheat cultivars, Xifeng 20 and Jinmai 47 with cv. Icaro. F_1 plants were self-pollinated by covering the spikes before flowering with paper bags to generate F_2 populations. F_2 plants were se-

lected based on plant height, appearance and presence of the *Rht18* allele using the corresponding molecular marker *Xbarc3* (Yang et al. 2015).

Chromosome counts on selected F_2 individuals from both populations were made by the root-tip squash method to confirm their hexaploid status. Hexaploid F_2 individuals carrying *Rht18* were used to develop $F_{2:3}$, $F_{3:4}$, $F_{4:5}$ and $F_{5:6}$ lines, from which the homozygous $F_{4:5}$ and $F_{5:6}$ *Rht18*-dwarf lines were used for further study. In this study, 7 and 6 $F_{4:5}$ homozygous dwarf lines and 6 and 5 homozygous $F_{5:6}$ dwarf lines, respectively, from Xifeng 20/Icaro and Jinmai 47/Icaro were used in field experiments to evaluate the effects of exogenous GA_3 on *Rht18* on agronomic traits by comparing them with their respective tall parents.

Field experiments

Field experiments were carried out during two growing seasons (2013–2014 and 2014–2015) at the Institute of Water-Saving Agriculture for Arid Areas of China at Northwest A&F University (34°17′ N, 108°3′42″ E). Fungicides and insecticides were applied as needed to prevent disease and insect damage.

Complete randomized-block designs with two replications were utilized for the $F_{4:5}$ and $F_{5:6}$ lines which were sown with their parents in October, 2013 and 2014, respectively. $F_{4:5}$ and $F_{5:6}$ lines were sown in 3-row plots, with rows 2 m long and a spacing of 25 cm between rows. All seeds were planted by hand and spaced at 6.7 cm within rows.

Exogenous GA_3 treatments

Exogenous GA_3 treatments on dwarf lines and parents were conducted according to Chen et al. (2014). GA_3 solutions (100 μM) were applied with a small aerosol to the leaf and culm surfaces at seven developmental stages, viz. 5-leaf (Z15) (Zadoks et al. 1974), tillering (Z21), stem elongation (Z31), early booting (Z41), early heading (Z51), early anthesis (Z61) and at early kernel and milk development (Z71). For each plant, 1–2 mL of GA_3 solution was applied at the first 2 developmental stages and 3–5 mL at the later 5 stages. Control plants were sprayed with the same solution lacking GA_3.

Trait assessments

Plant height and internode length

Height and the lengths of stem internodes were recorded at maturity (Z90) (Zadoks et al. 1974). Plant height was determined as the distance from the soil surface to the top of the spike (awns excluded). The lengths of internodes were measured from the mid-point of their subtending nodes. The internode length below the spike is defined here as peduncle length and the lengths of subsequent internodes below the peduncle are referred to as the second, third, fourth and fifth internode length, respectively.

Dry matter partitioning at anthesis

At anthesis (Z65) five plants in the central row of each plot were harvested to determine ear dry weight (EDW), leaf dry weight (LDW), stem dry weight (SDW, including leaf sheaths) and above-ground total dry weight (TDW). The ratios of ear dry weight to total dry weight (EDW/TDW), leaf dry weight to total dry weight (LDW/TDW) and stem dry weight to total dry weight (SDW/TDW) were then calculated.

Grain yield and yield components

Ten plants from each dwarf line and tall parent were hand-harvested from each plot at maturity (Z90). The main shoot ears were assessed for spike length, spikelet number spike^{-1} and grain number spike^{-1}. Fertile tillers plant^{-1} were also recorded. The total above-ground dry biomass of the ten plants was measured before threshing and then the average biomass plant^{-1}, average yield plant^{-1}, harvest index (ratio of grain yield to total above-ground biomass) and 1000-kernel weight were determined.

Assays for response of coleoptile length to GA_3

Good quality seeds of similar size from each $F_{4:5}$ dwarf line and parent were used to investigate coleoptile length as described by Rebetzke et al. (1999); 10 seeds were planted in a mix containing perlite, vermiculite and peat and grown in a darkroom irrigated with water or GA_3 solution (100 μM) at 20 °C for 10 days after germination. Coleoptile length was then measured.

Data analysis

The mean values of all traits investigated for the four classes (dwarf, dwarf+GA, tall, tall+GA) were calculated and statistical analyses was carried out by the GLM procedure with Duncan's test for multiple comparisons at $P = 0.05$ using the SAS statistical package.

Results

Coleoptile length

Exogenous GA_3 significantly increased coleoptile lengths in both $F_{4:5}$ dwarf lines (9.2 and 27.0%) and their tall parents (11.4 and 14.7%) (Table 1). There was no significant difference in coleoptile length of *Rht18* dwarf lines of Xifeng 20 compared with their tall parent Xifeng 20, either with or without exogenous GA_3. There were significant reductions (–16.0 and –7.0%) in coleoptile length in the dwarf lines of Jinmai 47 compared to Jinmai 47, either with or without exogenous GA_3 (Table 1). There was no difference in coleoptile length between *Rht18* dwarf lines and their corresponding tall parents in responsiveness to exogenous GA_3.

Table 1. Coleoptile length, plant height and internodes lengths of $F_{4:5}$ and $F_{5:6}$ *Rht18* dwarf lines and their corresponding tall parents, with and without GA_3 treatment

Progeny	Genotype	CL (cm)	PH (cm)	PL (cm)	I2L (cm)	I3L (cm)
$F_{4:5}$	Dwarf	7.6±0.17c	81.6±2.15b	26.3±1.89b	20.5±1.18c	11.5±0.34b
	Dwarf+GA	8.3±0.33ab	99.6±3.67a	30.3±2.66a	27.7±0.94a	17.0±1.69a
	Xifeng 20	7.9±0.03bc	96.4±0.77a	28.1±0.36ab	22.2±1.40bc	17.9±0.71a
	Xifeng 20+GA	8.8±0.31a	100.1±1.38a	27.9±0.70ab	23.9±2.32b	18.7±1.23a
$F_{5:6}$	Dwarf		91.5±0.92c	32.4±1.45b	20.5±0.15c	13.9±0.54c
	Dwarf+GA		110.3±2.17b	39.0±2.21a	27.2±1.04b	18.7±1.52b
	Xifeng 20		113.4±2.30b	37.0±0.45a	26.4±0.39b	19.6±0.51ab
	Xifeng 20+GA		121.4±0.80a	37.4±0.31a	29.4±1.94a	21.6±0.02a
$F_{4:5}$	Dwarf	6.3±0.31c	70.1±3.32c	20.2±1.82b	17.7±1.81b	12.5±1.53c
	Dwarf+GA	8.0±0.15b	77.0±1.99b	24.0±1.56a	18.1±1.16b	13.9±0.69bc
	Jinmai 47	7.5±0.18b	79.6±0.65ab	17.2±0.31c	23.4±0.93a	15.2±0.98ab
	Jinmai 47+ GA	8.6±0.17a	80.9±0.81a	17.7±0.18c	22.6±0.73a	16.3±1.00a
$F_{5:6}$	Dwarf		91.8±1.27c	28.0±1.16b	23.8±0.87c	16.1±0.88b
	Dwarf+GA		102.3±3.79b	35.4±1.36a	25.7±0.82b	17.6±1.29b
	Jinmai 47		104.0±1.53ab	25.7±0.10c	26.0±0.77b	17.6±0.10b
	Jinmai 47+GA		107.8±1.41a	25.3±0.58c	28.0±0.41a	19.9±0.33a

Data are means ± SD (standard deviation) of each genotype, the same small letter within the same column group indicates no significant difference determined by Duncan's test at $\alpha = 0.05$.

CL – coleoptile length; PH – plant height; PL – peduncle length; I2L – length of the second internode from top; I3L – length of the third internode from top.

Plant height and internode length

Rht18 dwarf lines were significantly shorter than their corresponding tall parents in both populations (Table 1, Fig. 1). After exogenous GA_3 application, *Rht18* dwarf lines produced long internodes and achieved a final plant height similar to that of the tall parent. Compared with the untreated *Rht18* dwarf lines, plant height was increased by 18 cm (22.1%) and 18.8 cm (20.5%), 7 cm (10.0%) and 10.5 cm (11.4%) in the GA_3-treated $F_{4:5}$ and $F_{5:6}$ dwarf lines of Xifeng 20 and Jinmai 47, respectively (Table 1). The lengths of the top three internodes were significantly increased in the GA_3-treated dwarf lines of Xifeng 20 compared with the untreated ones. For the Jinmai 47/Icaro population, the peduncle length of the GA_3-treated dwarf lines was significantly greater than the untreated ones. The lengths of the second and third internodes of GA_3-treated dwarf lines were always greater compared to the untreated ones, however, these differences were not significant (Table 1). Plant height and internode lengths of the tall parents were generally not significantly affected by GA_3 treatment, which suggests that the tall parents were less sensitive than the *Rht18* dwarf plants to exogenous GA_3.

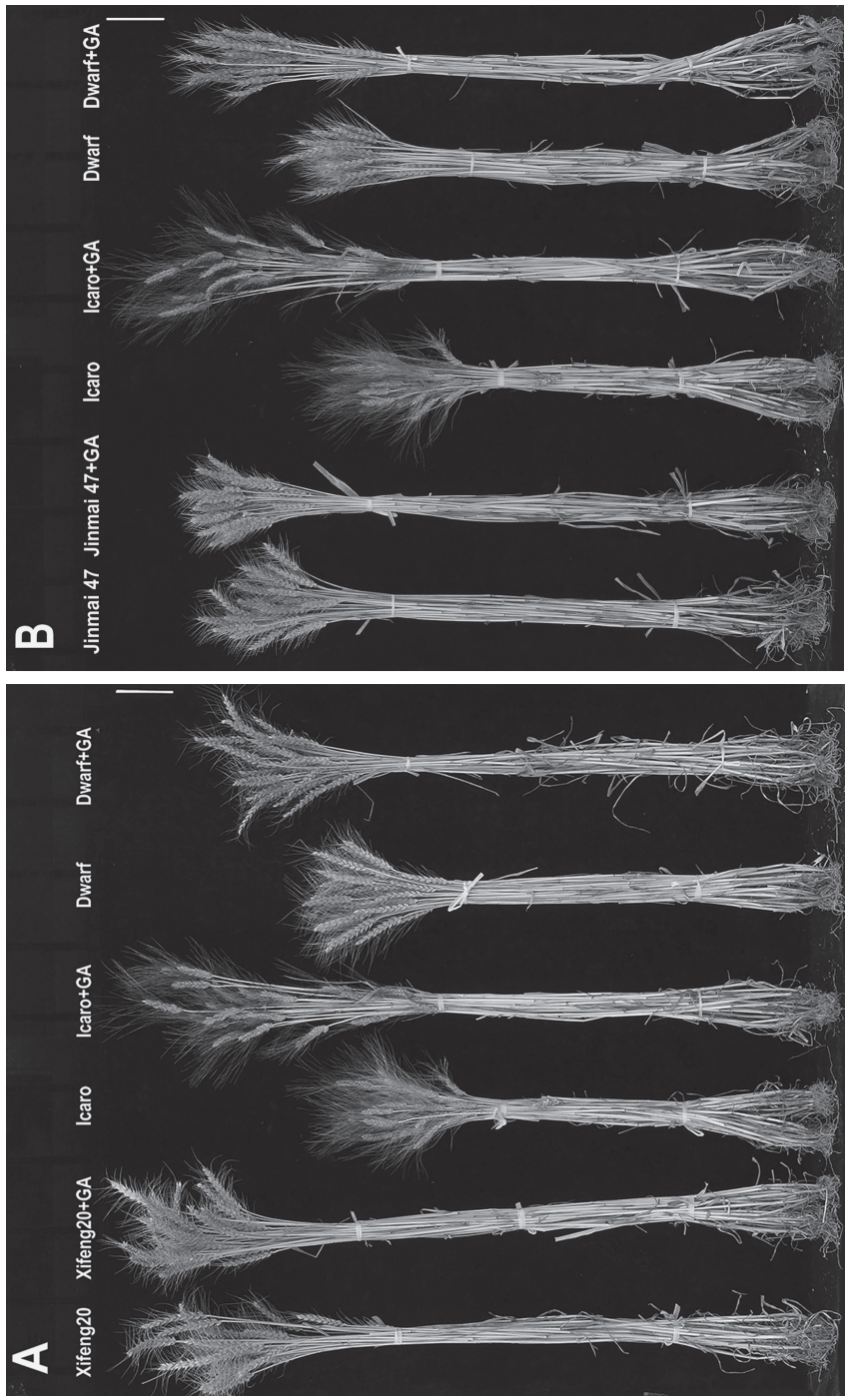

Figure 1. Plant morphologies of $F_{5:6}$ *Rht18* dwarf lines and parents when tested with GA_3 in population of Xifeng 20/Icaro (A) and Jinmai 47/Icaro (B). Scale bar, 10 cm

Table 2. Dry matter partitioning at anthesis of $F_{5:6}$ *Rht18* dwarf lines and their corresponding tall parents, with and without GA_3 treatment

Genotype	EDW (g)	LDW (g)	SDW (g)	TDW (g)	EDW/TDW	LDW/TDW	SDW/TDW
Dwarf	7.0±0.26b	3.5±0.28c	15.1±0.73d	25.5±1.10d	0.274±0.003a	0.136±0.010a	0.590±0.008c
Dwarf+GA	7.1±0.21b	4.2±0.32bc	22.0±0.46c	33.4±0.94c	0.214±0.004b	0.126±0.007ab	0.660±0.007b
Xifeng 20	7.4±0.66ab	4.9±0.48ab	26.3±2.25b	38.6±3.39b	0.191±0.001c	0.127±0.002ab	0.682±0.002a
Xifeng 20+GA	8.1±0.51a	5.1±0.31a	30.0±1.68a	43.3±2.51a	0.188±0.001c	0.118±0.001b	0.694±0.001a
Dwarf	4.1±0.16c	3.3±0.44c	12.1±0.70c	19.6±1.25c	0.212±0.006a	0.172±0.012a	0.616±0.011b
Dwarf+GA	9.1±0.37a	6.6±0.46a	31.2±0.55a	46.8±1.09a	0.194±0.005b	0.140±0.008b	0.666±0.006a
Jinmai 47	6.6±0.75b	4.7±0.39b	23.3±2.47b	34.6±3.61b	0.191±0.002b	0.137±0.003b	0.673±0.001a
Jinmai 47+GA	8.1±0.65a	5.8±0.45a	28.8±2.58a	42.8±3.68a	0.190±0.001b	0.136±0.001b	0.674±0.002a

Data are means ±SD (standard deviation) of each genotype, the same small letter within the same column group indicates no significant difference determined by Duncan's test at $\alpha = 0.05$.

EDW – ear dry weight; LDW – leaf dry weight; SDW – stem dry weight; TDW – above-ground total dry weight.

Table 3. Spike traits and yield traits of $F_{4:5}$ and $F_{5:6}$ *Rht18* dwarf lines and their corresponding tall parents, with and without GA_3 treatment

Progeny	Genotype	Spike length (cm)	Spikelet number spike^{-1}	Grain number spike^{-1}	1000-kernel weight (g)	Biomass plant^{-1} (g)	Grain yield plant^{-1} (g)	Harvest index
$F_{4:5}$	Dwarf	10.4±0.46ab	21.3±1.21a	59.0±0.89a				
	Dwarf+GA	10.8±0.70a	20.4±0.69a	54.0±1.56b				
	Xifeng 20	9.5±0.49b	20.3±0.42a	53.8±0.35b				
	Xifeng 20+GA	9.6±0.07ab	19.9±0.57a	51.2±0.57c				
$F_{5:6}$	Dwarf	11.6±0.31ab	19.6±0.54ab	53.2±2.15a	40.2±1.26b	49.2±4.46c	21.8±1.89a	0.443±0.011a
	Dwarf+GA	12.0±0.46a	19.0±0.46b	47.9±1.73b	44.4±2.34a	43.3±2.81d	18.1±1.03b	0.417±0.008ab
	Xifeng 20	10.0±0.20c	20.4±0.12a	51.3±1.41ab	43.4±1.11ab	56.1±1.41b	22.0±1.35a	0.393±0.034b
	Xifeng 20+GA	11.1±0.33b	19.3±0.49b	48.5±1.06b	46.0±0.57a	62.2±1.56a	20.1±0.78ab	0.322±0.004c
$F_{4:5}$	Dwarf	9.4±0.17b	16.7±0.31b	53.5±1.42a				
	Dwarf+GA	9.8±0.23ab	15.9±0.28b	50.1±1.22c				
	Jinmai 47	9.7±0.28ab	21.2±0.28a	53.0±0.57ab				
	Jinmai 47+GA	9.9±0.07a	20.5±0.71a	50.5±0.71c				
$F_{5:6}$	Dwarf	9.5±0.05c	17.3±0.49b	50.3±1.42a	50.1±1.61ab	37.4±3.01b	19.0±1.58a	0.470±0.014a
	Dwarf+GA	10.2±0.21b	16.1±0.66b	44.5±2.41b	52.6±2.18a	42.6±3.49a	16.8±1.08a	0.426±0.021b
	Jinmai 47	10.2±0.03b	20.8±0.35a	52.7±0.49a	46.8±0.11b	42.4±0.49a	18.8±0.18a	0.444±0.001ab
	Jinmai 47+GA	10.6±0.16a	20.4±0.94a	50.1±1.06a	49.1±0.14ab	43.8±1.06a	18.1±0.34a	0.414±0.002b

Data are means ± SD (standard deviation) of each genotype, the same small letter within the same column groups indicates no significant difference determined by Duncan's test at $\alpha = 0.05$.

Dry matter partitioning at anthesis

For the Jinmai 47/Icaro population, GA_3 treatment increased stem dry weight (SDW), ear dry weight (EDW), leaf dry weight (LDW) and above-ground total dry weight (TDW) at anthesis both in the dwarf lines and tall parent. For the Xifeng 20/Icaro population, GA_3 treatment had no effect on EDW or LDW, but significantly increased SDW and TDW (Table 2). Due to the increase in SDW, the ratios of SDW to TDW in the GA_3-treated dwarf lines were significantly increased whereas the ratios of EDW to TDW and LDW to TDW were significantly decreased. GA_3 treatment on the tall parents also increased SDW, EDW and LDW, but the ratios were not significantly changed (Table 2).

Due to the reduced height caused by *Rht18*, partitioning of dry matter to ears at anthesis was significantly increased in the dwarf lines compared to the tall parents (Table 2). Exogenous GA_3 significantly increased plant height as well as above-ground total dry weight of *Rht18* dwarf lines, resulting in a higher SDW : TDW ratio, but lower EDW : TDW ratio. Thus, after GA_3 application the partitioning of dry matter at anthesis in the dwarf lines was similar to that in the tall parents.

Yield components and yield

Exogenous GA_3 treatment of both populations significantly decreased grain number spike^{-1} in the $F_{4:5}$ and $F_{5:6}$ dwarf lines compared with the untreated dwarf lines (Table 3). However, 1000-kernel weight was increased by 10.4 and 5.0% in the GA_3-treated $F_{5:6}$ dwarf lines compared to the untreated Xifeng 20 and Jinmai 47 dwarf lines, respectively. Exogenous GA_3 increased plant biomass as a consequence of increased plant height but reduced grain yield plant^{-1} resulting in a lower harvest index in the *Rht18* dwarf lines. An exception was that the biomass plant^{-1} of the GA_3-treated dwarf lines was lower than that of the untreated dwarf lines of Xifeng 20. This might be due to the reduced number of fertile tillers plant^{-1} after application of exogenous GA_3. Additionally, GA_3 treatment slightly increased spike length in both the dwarf lines and tall parents, but did not affect spikelet number spike^{-1}. Likewise, exogenous GA_3 reduced grain number spike^{-1} as well as grain yield plant^{-1} while increasing biomass plant^{-1} and 1000-kernel weight of the tall parents (Table 3).

Discussion

The current study is part of a series of experiments carried out to achieve a comprehensive understanding of the GA-responsive dwarfing gene *Rht18* in hexaploid wheat. In previous studies, the effects of *Rht18* on agronomic traits in hexaploid wheat were evaluated using $F_{3:4}$ and $F_{4:5}$ hexaploid dwarf lines in three different populations (Yang et al. 2015). In this study, the responses of *Rht18* to exogenous GA_3 were investigated by comparing the *Rht18* dwarf lines of two different $F_{4:5}$ and $F_{5:6}$ hexaploid selections with their corresponding tall parents.

Rht18 is classified as a GA-responsive dwarfing gene based on the response of coleoptile length to exogenous GA at the seedling stage (Konzak 1988). We investigated the responses of *Rht18* to exogenous GA_3 during the complete growth cycle. GA_3 application significantly increased the internode lengths of *Rht18* dwarf lines to the extent that height was recovered to similar levels as the parents. Coleoptile length was significantly increased after GA_3 treatment in both the *Rht18* dwarf lines and tall parents and no significant difference was observed between them in response to GA_3. These results were consistent with the report of the response of *Rht12* to exogenous GA_3 by Chen et al. (2014) in that treatment of *Rht12* dwarf plants with exogenous GA_3 compensated the lost ability to produce long internodes and achieved a final plant height similar to that of the tall lines.

Height reduction by dwarfing genes is often associated with increased partitioning of assimilates to the ears and a greater number of fertile florets per spikelet (Brooking and Kirby 1981). As with other dwarfing genes, *Rht18* significantly increased grain number $spike^{-1}$ in the dwarf lines of Xifeng 20/Icaro (Yang et al. 2015). This may be due to greater dry matter partitioning to the ears at anthesis in the *Rht18* dwarf lines, which resulted in more fertile florets and more grains per spike. Although more grains per spike were produced, the grain size was smaller. In this study, GA_3 application increased grain size (higher 1000-kernel weight) and significantly decreased grain number $spike^{-1}$. This suggested that the possible deficiency of endogenous GA in *Rht18* dwarf lines might be responsible for smaller seeds. The application of exogenous GA_3 to *Rht12* dwarf lines significantly shortened the duration to double ridge formation and promoted earlier flowering, thus possibly extending the period of favorable conditions for grain development prior to harvest (Worland et al. 1994; Chen et al. 2014). GA application leads to elongation of floret lemmas and paleas, especially in the third and fourth florets in spikelets (Wang et al. 2001) and may increase cell length in the pericarp (Keyes et al. 1989). Some studies also reported that GA_3 induced male sterility (Colombo and Favret 1996; Fleet and Sun 2005) that might lead to lower seed-set. Colombo and Favret (1996) reported that GA_3 induced high levels of male sterility both in GA-sensitive and GA-insensitive genotypes, while Wang et al. (2001) found that application of GA_3 increased the fertile floret number but decreased the final grain set. Thus, the larger grain size caused by GA_3 application might be related to lower grain set.

Previous studies showed that GA-insensitive *Rht-B1* and *Rht-D1* were a consequence of mutant alleles with altered function (rather than loss-of-function) of the *Rht-1* height regulating genes (Peng et al. 1999). These mutant alleles increased endogenous GA levels and reduced response to GA by encoding DELLA proteins that acted to repress GA signaling. However, the molecular mechanisms of GA-responsive dwarfing gene effects on height are still unknown. As the reduction in leaf elongation rate and stem elongation caused by GA-responsive *Rht* genes could (at least partially) be recovered by the application of GA (Ellis et al. 2004; Chen et al. 2014), it was predicted that these *Rht* mutants were deficient in GA biosynthesis. Quantifying GAs and their precursors in these mutants would test this hypothesis and could pinpoint the biochemical block leading to reduction in GA. Assuming that *Rht18* is involved in GA biosynthesis pathways it would be instructive to use a quantitative real-time PCR strategy to investigate the expression patterns of

known GA metabolism genes (such as *Ta20ox*, *Ta13ox*, *Ta3ox* and *Ta2ox*) in a search for potential candidate genes for *Rht18*. There are many dwarf mutants resulting from changes in genes encoding GA biosynthetic enzymes in Arabidopsis and rice (Sasaki et al. 2002; Spielmeyer et al. 2002; Magome et al. 2004) and the same can be expected for wheat. Indeed, the major semi-dwarfing gene (*sd1*) used in rice production is due to a defect in a late step of GA biosynthesis (Spielmeyer et al. 2002).

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