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Dwarfing gene *Rht4* and its effects on key agronomic and coleoptile traits in bread wheat

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Abstract

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The gibberellin sensitive gene *Rht4* is a single recessive factor and reduces plant height in wheat. To reveal its potential use in wheat breeding, the effects of *Rht4* gene on plant height and other key agronomic and coleoptile traits were investigated and compared using a bi-parental population from a cross between "BSJ-14" (semi-dwarf, *Rht4*) and Scholar (tall, no known dwarfing alleles) under the rainfed conditions. The presence of *Rht4* gene was controlled at the molecular level using SSR marker of *Rht4*. It was found that 25 out of 72 F_4 lines carried *Rht4* gene while 36 lines did not possess the gene and 11 lines were heterozygous. *Rht4* gene reduced plant height and peduncle length by 5.2% and 5.3%, respectively. Spike length, spikelet number and kernel number per spike were decreased but thousand kernels weight increased in lines with *Rht4* gene as compared to tall lines. Reduction in coleoptile length, coleoptile diameter and first leaf length was observed in lines with *Rht4* gene by 5.5%, 2.1% and 6.6%, respectively. The increase in thousand kernels weight in plants carrying *Rht4*, especially in heterozygotes, shows that this gene should be put emphasis on in wheat breeding programs, particularly in the development of hybrid varieties.

Keywords: wheat; dwarfing gene; *Rht4*; agronomic traits; coleoptile traits

Introduction

The use of major dwarfing or reduced height (*Rht*) genes has led to extreme shortening and increasing the yield potential of cereals since the 1960s. Especially, obtaining high-yielding varieties in wheat and rice breeding programs caused the Green Revolution in the World (Evans, 1993). The "Norin 10" dwarfing genes, *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) were used in a breeding program of CIMMYT germplasm and present in most grown wheat varieties worldwide (Evans, 1993; Ellis et al., 2005).

Rht1 and *Rht2* are the gibberellin insensitive genes and encode proteins involved in gibberellin (GA) signal transduction. Due to this, the pleiotropic effects of these genes on plant growth led to causing reductions in coleoptile length and seedling leaf area (Peng et al., 1999; Ellis et al., 2005). However, some other dwarfing genes such as *Rht4, Rht8, Rht9, Rht12* and *Rht13* are the gibberellin-sensitive dwarfing genes and decrease plant height by 12-50% while having small or negligible effects on coleoptile length (Rebetzke et al., 1999; Rebetzke et al., 2004; Ellis et al., 2004). In total, 20 *Rht* genes have been reported as other height-reducing genes by Konzak (1988).

Rht4 allele was induced by X-ray and fast neutron irradiation in the variety Burt and preserved in hexaploid stock Burt ert 937 (Konzak, 1976; Gale & Youssefian, 1985). Hu (1980) reported that *Rht4* segregated as a single recessive factor and reduced plant height by about 45%. Otherwise, the reducing effect of *Rht4* on coleoptile length is not consistent (Konzak, 1982; Gale & Youssefian, 1985). Liu et al. (2017) stated that *Rht4* gene alone has a 10% effect on reducing plant length while the study of Du et al. (2018) revealed

that the dwarfing gene *Rht4* had moderate effects on plant height.

Ellis et al. (2005) used the double haploid population of Vigour 18 x Burt ert (*Rht4*) cross to identify chromosomal region and molecular markers linked to *Rht4*. The researchers reported that *Rht4* was associated with molecular markers on chromosome 2BL and the highest proportion of phenotypic variation in plant height was explained by marker Wmc317 which amplified a 170 bp band in Burt ert (*Rht4*) and a 150-bp band in the Vigour 18 parent. It has been reported that this SSR marker (Wmc317) can help breeders select *Rht4* dwarf genes in wheat (Ellis et al., 2005).

This study aims to determine the effects of *Rht4* gene on plant height, peduncle length, some spike traits, thousand kernels weight and coleoptile length & diameter with the first leaf length using the F_4 lines from the cross between BSJ-14 (*Rht4*) and Scholar under the rainfed conditions to explore the potential availability of *Rht4* gene in bread wheat breeding programs.

Materials and Methods

The plant material consisted of the 72 F_4 lines from the cross between BSJ-14 (*Rht4*) and Scholar which contains no known semi-dwarf genes and is classified as a normal height cultivar (Lanning et al., 2000). The cross and the F_1, F_2, F_3 generations were produced from the wheat breeding program of the Soil and Crop Sciences Department of Colorado State University. The F_4 lines were grown in augmented design with 5 blocks in Ege University, Faculty of Agriculture, Department of Field Crops experimental fields under rainfed conditions in 2014-2015 wheat growing season. The precipitation and temperature data during the growing seasons of the wheat in the experimental site are shown in Table 1. The soil of the experimental site has a heavy soil structure with clay-silt soil at 0-20 cm depth and clay-loamy structure at 20-40 cm depth. The F_4 lines and the parents were sown in two rows of 1 m length with a spacing of 20 cm between rows and 5 cm between plants. The four regional varieties of bread wheat were used as control genotypes in each block. Recommended cultural practices were followed to raise a good crop. The fertilizer was applied as 160 kg N ha^{-1} and 60 kg P ha^{-1} equally at sowing time and during the stem elongation period.

The plant height, peduncle length, spike length, spikelet number, kernel number per spike and TKW (thousand kernels weight) were measured on ten plants representing F_4 lines, the parents and the control genotypes when the plants reached harvesting maturity. The coleoptile length, coleoptile diameter and the first leaf length of the same line were measured following Jamali & Arain (2008).

The presence/absence of the *Rht4* gene was controlled at the molecular level using SSR marker Wmc317 (Ellis et al., 2005). Genomic DNA from young leaves of the 72 F_4 lines was isolated by DNA mini-extraction from fresh leaf tissue (Doyle & Doyle, 1987). Purity of the DNA template was checked by the 260:280 nm absorbance ratio. Dilutions that gave 25 ng/µl were calculated from the absorbance measured at 260 nm. The polymerase chain reaction (PCR) consisted of 50 ng/µl genomic DNA, 2 mM $MgCl_2$, 0.5 mM deoxyribonucleotide triphosphates (dNTPs) (Sigma-Aldrich, St. Louis, MO, USA), 1x PCR buffer, 0.5 U Taq DNA polymerase (Sigma-Aldrich) and 0.5 µM each of forward and reverse primers in a total volume of 10 µl (Tonk et al., 2016). Amplification was carried out in an Eppendorf thermocycler (Eppendorf, Hamburg, Germany) using the protocol described by Tonk et al. (2016). The PCR products were separated on 8% non-denatured polyacrylamide gel using a Bio-Rad Protean II xi Cell electrophoresis system (Bio-Rad, Hercules, CA, USA). Gels were electrophoresed at 90 V for 16 h with 0.5X TBE buffer, stained with ethidium bromide and visualized using a UV transilluminator (Vilber Lourmat, Marne-la-Vallee, France) (Tonk et al., 2016).

Table 1. Average monthly rainfall and temperature during growing seasons of wheat in the experimental site

Months		Average temperature, °C	Long-term average temperature, °C	Rain distribution, kg/m²	Long-term rain distribution, kg/m²
2014	November	13.2	13.6	15.2	73.0
	December	11.1	10.0	206.8	161.9
2015	January	8.9	9.0	125.1	123.7
	February	9.5	9.1	101.9	82.4
	March	11.7	11.6	75.6	88.9
	April	15.9	15.6	46.4	56.0
	May	20.8	20.3	30.9	19.5
	June	25.6	25.1	9.8	30.0
Average /Total		14.5	14.2	611.7	635.4

All data recorded were subjected to analysis of variance (ANOVA) according to Steel & Torrie (1980), to determine significant differences among the genotypes using JMP 7.0 statistical software from SAS. The relative effect of *Rht4* gene was compared as a percent change for the tall group.

$$\%\ change\ of\ Rht4\ gene = \frac{Mean_{dwarf} - Mean_{tall}}{Mean_{tall}} \times 100$$

The SSR marker bands of the lines were scored as maternal band presence (A), paternal band presence (B), or both maternal and paternal band presence i.e., heterozygous (H) (Tonk et al., 2016; Zuki et al., 2020). The marker results were analyzed for conformity with an expected 7A: 2H: 7B genotypic segregation ratio for a codominant marker in an F_4 population using chi-square (χ^2) goodness-of-fit tests.

$$\chi^2 = \Sigma \frac{(Observed - Expected)^2}{Expected},$$

where χ^2 is chi-square.

The chi-square values were compared with the values in chi-square Table and the hypothesis was accepted or rejected based on those values.

Results and Discussion

The most important difference between the gibberellin sensitive and insensitive dwarfing genes is that the gibberellin sensitive genes reduce the plant height without affecting coleoptile length (Ellis et al., 2004; Liu et al., 2017). The gibberellin sensitive gene *Rht4* has not been used widely in commercial wheat varieties, however, it has a potential for short plant height with long coleoptile. In this study, we utilized 72 F_4 lines from the cross between BSJ-14 (*Rht4*) and Scholar to evaluate the effects of the dwarfing gene *Rht4* on a range of agronomic traits and coleoptile features under rainfed conditions.

The presence of *Rht4* gene was controlled at the molecular level using SSR marker Wmc317 and the mother (BSJ-14 - *Rht4*) and the father (Scholar) of the lines were used as control. Wmc317 marker amplified a 150-bp band in BSJ-14 (*Rht4*) and a 140-bp band in Scholar parent. Ellis et al. (2005) mapped *Rht4* on chromosome 2BL and found a 170-bp band in Burt ert (*Rht4*) and a 150-bp band in the Vigour 18 parent of mapping population using Wmc317 marker. Results showed that 25 out of 72 lines carried *Rht4* gene while 36 lines did not possess the gene and 11 lines were heterozygous (Table 2). The chi-square test showed a non-significant difference between expected and observed ratios of monohybrid segregation (Table 2) which proved that *Rht4* is a single gene. These results are in agreement with Hu (1980) who reported that *Rht4* segregated as a single recessive factor in wheat.

The estimation of significant mean squares of the F_4 lines from BSJ-14 (*Rht4*) × Scholar cross for investigated traits is shown in Table 3. Results indicated that differences for plant height, peduncle length, spike length, spikelet number and kernel number per spike were non-significant among the lines while differences for thousand kernels weight, coleoptile length, coleoptile diameter and the first leaf length were significant. The presence of *Rht4* gene in some F_4 lines did not make a statistical difference among all the lines in the population for some investigated traits. *Rht4* gene is one of the rarely studied genes among the dwarfing genes in wheat. However, Liu et al. (2017) investigated and compared the effects of *Rht4*, *Rht-B1b*, and *Rht4* + *Rht-B1b* on plant height and key agronomic traits using the wheat RILs and they found significant differences among the three types of dwarfing lines, however, spikelet number spike did not differ statistically.

The mean values for key agronomic and coleoptile traits for the F_4 lines carrying the *Rht4* gene, tall (without *Rht4*) and heterozygous are displayed in Table 4 and Figure 1. Plant height for maternal parent BSJ-14 (*Rht4*) was significantly smaller (69.2 cm) than that of paternal parent Scholar (104.20 cm). Although the parental lines are quite different, plant height was reduced by 5.2% in *Rht4* lines and 0.9% in heterozygous lines compared with the tall lines. This effect of *Rht4* on plant height reduction was weaker than that re-

Table 2. The expected and observed values of segregation and chi-square values for the F_4 lines of BSJ-14 (*Rht4*) × Scholar cross

	Number of lines		Chi-square value (χ^2)
	Observed value	Expected value	
Lines with mother band (*Rht4*)	25	31.5	1.341
Lines with father band (Tall)	36	31.5	0.643
Heterozygous	11	9	0.444
Total	72	72	2.428*

* Significance limit of χ^2 (P = 0.05, df = 2 for F_4) = 5.99. Total χ^2 value lies between P value 0.3 > 0.05.

Table 3. Analysis of variance (Mean Squares) of investigated traits in F_4 lines of BSJ-14 (*Rht4*) × Scholar cross

Source of Variation	PH	PL	SL	SN	KN	TKW	CL	CD	FL
Blocks	57.43^{ns}	57.58^{ns}	1.58^{ns}	0.63^{ns}	5.24^{ns}	8.65^{ns}	1.42^{ns}	0.04**	2.07^{ns}
Genotypes	134.89^{ns}	37.87^{ns}	1.23^{ns}	1.92^{ns}	47.20^{ns}	346.63**	1.89**	0.17**	13.76**
Error	146.05	24.80	1.35	1.42	30.62	9.77	0.70	0.007	2.76

ns: non-significant, **: significant at α = 0.01, PH: plant height (cm), PL: peduncle length (cm), SL: spike length (cm), SN: spikelet number, KN: kernel number per spike, TKW: thousand kernels weight (g), CL: coleoptile length (cm), CD: coleoptile diameter (mm), FL: first leaf length (cm).

Table 4. Effects of *Rht4* gene on the investigated traits in the F_4 lines of BSJ-14 (*Rht4*) × Scholar cross

Traits	BSJ14 (*Rht4*)	Scholar	Tall	*Rht4*	Heterozygous
PH	69.2±6.99	104.2±10.89	101.1±11.83	95.85±14.48 (-5.2%)	100.17±9.84 (-0.9%)
PL	13.03±3.71	13.38±3.15	39.17±6.76	37.09±6.88 (-5.3%)	40.72±6.37 (3.9%)
SL	7.27±1.66	9.90±1.68	9.26±1.22	8.91±0.96 (-3.8%)	10.05±1.28 (8.5%)
SN	16.2±2.35	19.4±2.72	18.30±1.72	17.80±1.17 (-2.7%)	17.91±1.23 (-2.1%)
KN	29.6±7.66	31.5±6.11	43.83±8.59	42.65±6.80 (-2.7%)	43.95±4.61 (0.3%)
TKW	40.0±0.61	28.0±0.62	65.66±14.45	66.60±9.66 (1.4%)	73.95±16.67 (12.6%)
CL	6.38±1.03	6.16±0.91	5.41±1.36	5.11±1.13 (-5.5%)	5.05±1.85 (-6.6%)
CD	1.39±0.18	1.52±0.16	1.46±0.52	1.43±0.38 (-2.1%)	1.44±0.24 (-1.4%)
FL	13.04±1.65	15.77±2.13	9.99±3.85	9.33±4.39 (-6.6%)	9.09±3.37 (-9.0%)

The data represented as the mean ± standard deviation (SD), where the percentage in parentheses shows the reduction compared with the tall genotype. PH: plant height (cm), PL: peduncle length (cm), SL: spike length (cm), SN: spikelet number, KN: kernel number per spike, TKW: thousand kernels weight (g), CL: coleoptile length (cm), CD: coleoptile diameter (mm), FL: first leaf length (cm).

ported in previous studies i.e., 17% (Rebetzke et al., 2012b) and 11.5% (Liu et al., 2017). The reason for this difference may be that *Rht4* gene has a different effect in varied genetic backgrounds and growth environments. Plant height reductions by *Rht* genes can be attributed to the length of the internodes, particularly the peduncle length and the length of the second internode (Rebetzke et al., 2012a; Liu et al., 2017). However, the peduncle length of the parental lines was estimated as 13.03 cm in BSJ-14 (*Rht4*) and 13.38 in Scholar. Compared with the tall lines, peduncle length was decreased by 5.3% in *Rht4* lines whereas it was increased by 3.9% in heterozygous lines.

The effect of *Rht4* gene on spike length, spikelet and kernel number per spike and thousand kernels weight were shown in Table 4 and Figure 1. The highest spike length was found in heterozygous lines (8.5%) while the lowest value was determined in the lines with *Rht4* (3.8%) compared with the tall lines. The spike lengths of BSJ-14 (*Rht4*) and Scholar parents were 7.27 and 9.90 cm, respectively. Spikelet number for maternal parent BSJ-14 (*Rht4*) was significantly less (16.2 cm) than that of paternal parent Scholar (19.4 cm). Spikelet number reduced by 2.7% in *Rht4* lines and 2.1% in heterozygous lines compared with the tall lines. The lines that had higher spikelet number, possessed a concomitant increase in their kernel number per spike. Compared with the tall lines, kernel number per spike was decreased by 2.7% and 0.3% for *Rht4* and heterozygous, respectively. However, Liu et al. (2017) found that dwarfing lines with *Rht4* exhibited the greatest increasing spike length, spikelet and kernel number per spike compared with tall lines. The highest thousand kernel weight was found in heterozygous lines (12.6%) while the increasing value was determined in the lines with *Rht4* (1.4%) compared with the tall lines. Besides, the thousand kernel weights of BSJ-14 (*Rht4*) and Scholar parents were 40.0 g and 28.0 g, respectively (Table 4). Our results are not in agreement with several reports about the effect of *Rht4* on thousand kernel weights (Rebetzke et al., 2012b; Liu et al., 2017) who reported the significant reduction by *Rht4*.

The length of the coleoptile determines the depth of seed sowing and is under the control of many genes (Rebetzke et al., 2007; Rebetzke et al., 2014). Coleoptile length, coleoptile diameter and the first leaf length of the lines were compared (Table 4 and Figure 1). Although the coleoptile length of BSJ-14 (*Rht4*) was higher (6.38 cm) than that of Scholar (6.16 cm), coleoptile length was reduced by 5.5% in *Rht4* lines and by 6.6% in heterozygous lines compared with the tall lines. *Rht4* reduced coleoptile length in this study, which was consistent with the results obtained by (Rebetzke et al., 2012b). Compared with the tall lines, coleoptile diameter was decreased by 2.1% and 1.4% for *Rht4* and heterozygous, respectively. Despite this small decrease in the lines, coleoptile diameter was measured as 1.39 mm in BSJ-14 (*Rht4*) and 1.52 mm in Scholar. The first leaf length of *Rht4* and heterozygous lines was shorter than that of the tall lines, and

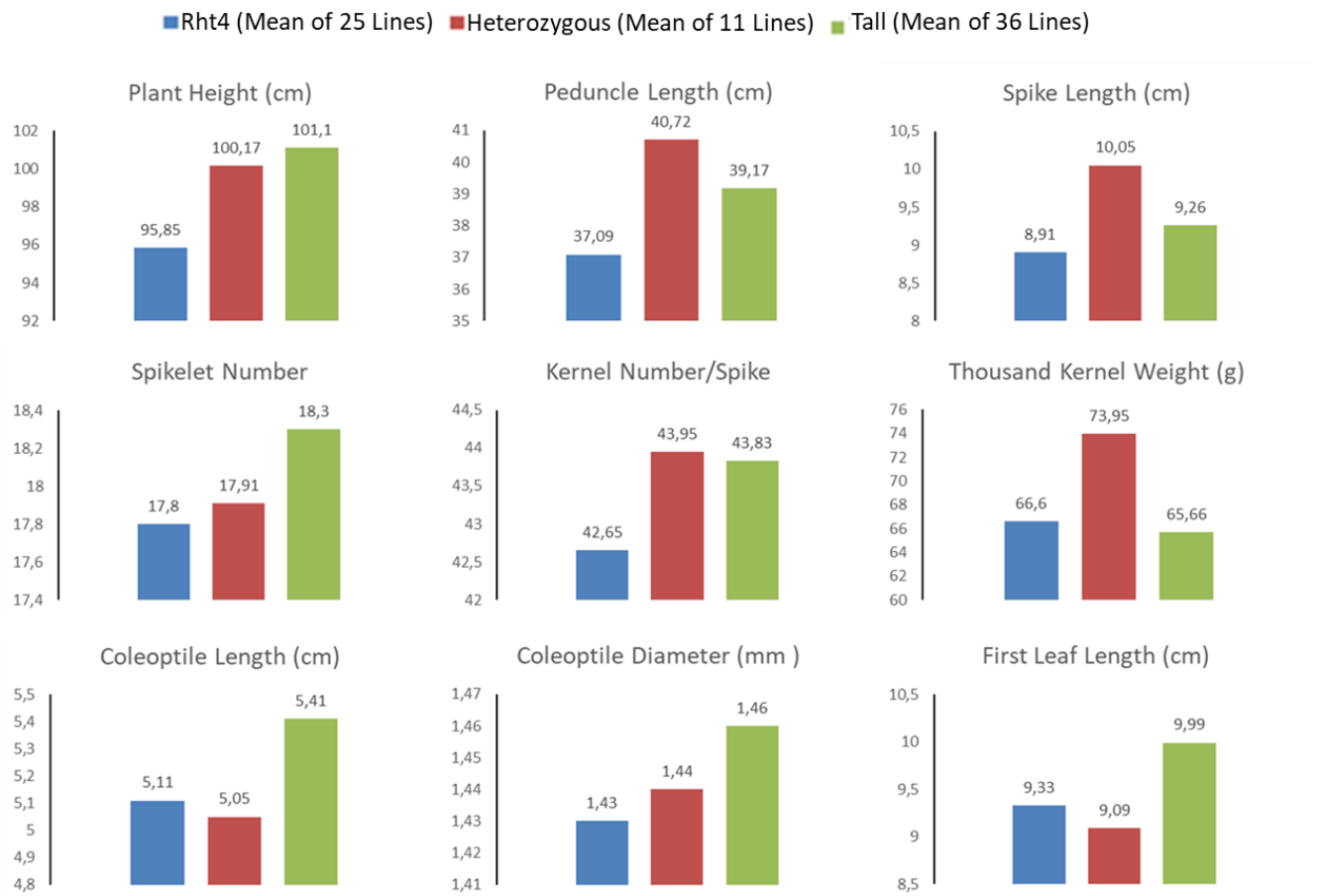

Fig. 1. Effects of *Rht4* gene on the investigated traits in the F_4 lines of BSJ-14 (*Rht4*) × Scholar cross

the greatest reduction (9.9%) in the first leaf length was observed in *Rht4* lines. The first leaf length for maternal parent BSJ-14 (*Rht4*) was significantly shorter (13.04 cm) than that of paternal parent Scholar (15.77 cm).

Conclusion

Rht4 gene reduced plant height, peduncle length, coleoptile length and first leaf length in the lines while slightly decreased spike length, spikelet number, kernel number per spike and coleoptile diameter. However, significantly higher thousand kernels weight was observed in the lines with *Rht4*. The heterozygous lines showed higher peduncle length, spike length, kernel number per spike and thousand kernels weight compared to the tall and *Rht4* lines. These results indicate that heterozygotes are superior in terms of these traits. The increase in thousand kernels weight in plants carrying *Rht4*, especially in heterozygotes, shows that this gene should be put emphasis on in wheat breeding programs, particularly in the development of hybrid varieties.

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