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EFFECTS OF DWARFING GENE *RHT5* ON COLEOPTILE LENGTH, SOME AGRONOMIC AND SEED TRAITS IN BREAD WHEAT

Abstract. *Rht5* is a gibberellin-sensitive dwarfing gene and reduces plant height without affecting early growth. In this study, the effects of dwarfing gene *Rht5* on coleoptile length, some agronomic and seed traits were revealed in an F_4 population obtained from a cross between Marj12 (semi-dwarf, *Rht5*) and Scholar (tall). The genotypes of F_4 individuals were screened using SSR marker BARC102 linked with the *Rht5* gene and the dwarf, tall and heterozygous individuals were identified. Analysis of variance in the F_4 population indicated highly significant differences among the lines for all traits studied, suggesting genetic differences existed for all traits among these lines. In general, the *Rht5* dwarfing gene was associated with plant height and peduncle length reduction of 25.44 and 22.28 %, respectively, as well as decreases in spike length, number of spikelets, and kernels per spike. Higher values of kernel weight, seed width and seed length could be observed in the tall lines when compared to the dwarf lines. The dwarfing gene *Rht5* increased coleoptile length by 6.03 % in dwarf lines and also increased primary root number and first leaf length. The results of the study could contribute to the proper use of dwarfing gene *Rht5* in breeding programs aimed at modifying plant height and coleoptile length in wheat.

Keywords: wheat, dwarfing gene, *Rht5*, agronomic traits, coleoptile length

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ВЛИЯНИЕ ГЕНА КАРЛИКОВОСТИ *RHT5* НА ДЛИНУ КОЛЕОПТИЛЯ, НЕКОТОРЫЕ АГРОНОМИЧЕСКИЕ И СЕМЕННЫЕ ПРИЗНАКИ ХЛЕБНОЙ ПШЕНИЦЫ

Аннотация. Ген карликовости *Rht5*, чувствительный к гиббереллину, снижает высоту растений, не влияя на ранний рост. В данном исследовании изучалось влияние гена карликовости *Rht5* на некоторые агрономические признаки, длину колеоптиля и характеристики семян в популяции F_4, полученной в результате скрещивания сортов Marj12 (полукарликовый, *Rht5*) и Scholar (высокорослый). Генотипы гибридов F_4 были проверены с использованием SSR-маркера BARC102, сцепленного с геном *Rht5*, и идентифицированы карликовые, высокорослые и гетерозиготные особи. Дисперсионный анализ в популяции F_4 показал высокозначимые различия между линиями по всем изученным признакам, что свидетельствует о наличии генетических различий по всем признакам между ними. В целом ген карликовости *Rht5* был связан с уменьшением высоты растения и длины подколосового междоузлия на 25,44 и 22,28 % соответственно, а также с уменьшением длины колоса, количества колосков и зерен в колосе. У высоких линий наблюдались более высокие значения массы зерна, ширины и длины семян по сравнению с карликовыми линиями. Ген карликовости *Rht5* увеличил длину колеоптиля на 6,03 % у карликовых линий, а также количество первичных корней и длину первого листа. Результаты исследования могут помочь в правильном использовании гена карликовости *Rht5* в селекционных программах для изменения высоты растения и длины колеоптиля у пшеницы.

Ключевые слова: пшеница, ген карликовости, *Rht5*, агрономические признаки, длина колеоптиля

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Introduction. Various plant genetic resources were discovered in the 1900s and have been used, developed, and preserved to the present day. In the 20th century, scientists developed the Norin 10 variety by crossing Turkish Red Winter wheat with Daruma wheat [1]. The dwarf Norin 10 variety was crossed with Brevor, a local American variety, and high-yielding varieties were obtained from these lines,

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which were investigated by Dr. Borlaug and his colleagues [2]. Transferring dwarfing genes to existing high-yielding varieties has resulted in a net increase in harvest index of over 50 % [3]. Cultivation of these high-yielding varieties began to spread rapidly worldwide in the 1950s, and they have also found widespread cultivation in Türkiye.

After the Green Revolution, semi-dwarf wheat varieties played an important role in wheat breeding [4]. Genes that eliminated lodging problems and shortened plant height in high-yielding varieties known as Mexican wheat were designated as *Rht* (Reduced height) genes. The development of semi-dwarf wheat genotypes resulted in increased grain yield [5]. Coleoptile length and plant height were independent traits in varieties lacking the *Rht* gene, while in varieties carrying the *Rht* gene, a positive correlation was observed between plant height and coleoptile length, and a positive correlation was also observed between grain yield and plant height [6].

To date, 21 *Rht* genes have been identified [7], the most widely known and used genes worldwide being *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) [8, 9]. The *Rht2* gene has been shown to have a more dominant effect on plant height than the *Rht1* gene [10, 11]. Other dwarfing genes, *Rht3*, *Rht4*, *Rht5*, *Rht6*, *Rht7*, *Rht8*, *Rht12* and *Rht13*, have also been reported to reduce plant height (7–55 %) and grain yield [8, 12–15].

Rht genes are divided into two groups, depending on their response to gibberellic acid, as gibberellic acid sensitive or insensitive genes [16]. It is known that *Rht1*, *Rht2*, *Rht3* and *Rht10* genes are insensitive to gibberellic acid, while other dwarfing genes are sensitive. The gibberellin-sensitive dwarfing genes decrease plant height while having small or negligible effects on coleoptile length [17–19]. Similarly, *Rht5* is a gibberellin-sensitive dwarfing gene that reduces plant height without affecting early growth [19]. Significant relationships have been identified between the *Rht5* gene and plant height [13, 15]. One study reported that the *Rht5* gene causes a 55 % reduction in plant height [13]. Although some researchers have observed dwarfing genes in the F_1 generation, dwarfism is generally observed in the F_2 generation and subsequent generations [20–22].

In order to further increase yield in wheat and to create a broader genetic base for dwarfing genes, it is essential to search for additional dwarfism sources. In this study, the SSR marker for the *Rht5* gene was used in the F_4 generation of a cross between the Marj12 genotype carrying the *Rht5* gene and the Scholar bread wheat variety to identify plants carrying the gene. The effects of the *Rht5* dwarfing gene on seed characteristics, coleoptile lengths, plant heights and some other agronomic traits of the plants in the F_4 generation lines were investigated.

Materials and Methods. The material for this study consisted of the F_4 generation of a cross between Marj12 and Scholar bread wheat varieties. The Marj12 genotype possesses the *Rht5* gene, while Scholar lacks this gene and carries no dwarfing genes [23]. The crossing and F_1, F_2, F_3 generations were acquired in a wheat breeding program of the Soil and Crop Sciences Department of Colorado State University. The 65 F_4 lines were grown under rainfed conditions at Ege University, Faculty of Agriculture, Department of Field Crops experimental fields in the 2014–2015 wheat growing season. The trial was conducted using an augmented design with 5 blocks. Four regional standard varieties (Cumhuriyet, Ziyabey, Basribey and Sagittario) were repeated in each block as controls. The 65 F_4 lines, their parents and control genotypes were planted in two rows of 1 m length with a spacing of 20 cm between rows and 5 cm between plants. The soil of the experimental area has a heavy soil structure; silty clay soil at 0–20 cm depth and clay-loam soil at 20–40 cm depth. Fertilizer was applied in equal amounts at planting time and stem elongation period, totaling 160 kg N ha^{-1} and 60 kg P ha^{-1}.

The precipitation and temperature averages of the growing seasons and long-term average in the experimental area are presented in Table 1. The average temperatures and rainfall of the growing season did not differ significantly from the long-term average values.

The plant height, peduncle length, spike length, spikelet number and kernel number per spike were measured on ten plants of each F_4 line, the control genotypes and the parents at maturity. Also, kernel weight, seed width, seed length, coleoptile length, primary root number and first leaf length were evaluated in ten seeds each from each genotype. Coleoptile length, primary root number, and the first leaf length were measured following Jamali and Arain [24]. The seeds were soaked in a 2.5 % sodium hypochlorite (NaClO) solution for 2–3 minutes for sterilization, then rinsed in double-distilled pure water for 2–3 minutes. The sterilized seeds were placed in a PVC and paper towel-lined container and rolled

up. The rolls were then placed in a germination chamber and kept in a container filled with sufficient water in complete darkness for 13 days under observation. Coleoptile length, primary root number, and the first leaf length were measured at the end of the observation period.

T a b l e 1. **Climate data for Izmir province in the 2014–2015 wheat growing season**

Month, year	Average temperature (°C)	Long years avg. (°C)	Rain distribution (mm)	Long years avg. (mm)
November 2014	13.2	13.6	15.2	73.0
December 2014	11.1	10.0	206.8	161.9
January 2015	8.9	9.0	125.1	123.7
February 2015	9.5	9.1	101.9	82.4
March 2015	11.7	11.6	75.6	88.9
April 2015	15.9	15.6	46.4	56.0
May 2015	20.8	20.3	30.9	19.5
June 2015	25.6	25.1	9.8	30.0
Average / Total	14.5	14.2	611.7	635.4

DNA isolation from young leaves of the 65 F_4 lines was performed using DNA mini-extraction from fresh leaf tissue [25]. The purity of the DNA template was investigated using A260 : A280 absorbance ratio. DNA of all samples was diluted to 25 ng/µl using the absorbance value measured at 260 nm. The presence of the *Rht5* gene was confirmed at the molecular level using SSR marker Barc102 published by Ellis et al. [26] (Table 2). The polymerase chain reaction (PCR) was performed in a total volume of 10 µl according to Tonk et al. [27]. Amplification was performed in an Eppendorf thermocycler (Eppendorf, Germany) using the protocol described by Tonk et al. [27]. After PCR, the samples were separated on 8 % non-denatured polyacrylamide gel at 90 V for 16 h with 0.5× TBE buffer using a Bio-Rad Protean II xi Cell electrophoresis system (Bio-Rad, Hercules, CA, USA). The gels were stained with ethidium bromide and visualized using a UV transilluminator (Vilber Lourmat, Marne-la-Vallee, France).

T a b l e 2. **Base sequence and chromosome location of Barc102 SSR primer**

Marker	Base sequence	Primer type	Chromosome Location
Xbarc102	GGAGAGGACCTGCTAAAATCGAAGACA	Forward Primer	3B
	GCGTTTACGGATCAGTGTTGGAGA	Reverse Primer	

To determine significant differences among the genotypes, all data were subjected to analysis of variance (ANOVA) according to Steel and Torrie [28], using JMP 7.0 statistical software from SAS. SSR marker bands of F_4 lines were scored as the presence of maternal band (A), paternal band (B) or both maternal and paternal bands i.e., heterozygous (H) [27, 29]. The results were analyzed for conformity with the expected 7A : 2H : 7B genotypic segregation ratio for a codominant marker in an F_4 population using chi-square (χ^2) goodness-of-fit test:

$$\chi^2 = \sum \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}.$$

The chi-square values found were compared with the chi-square table values and the hypothesis was accepted or rejected according to these values. The relative effect of *Rht5* gene was compared as a percent change between the tall and the dwarf groups [22]:

$$\%\text{change}_{Rth5\text{gene}} = \frac{\text{Mean}_{\text{tall}} - \text{Mean}_{\text{dwarf}}}{\text{Mean}_{\text{tall}}} 100.$$

Results and Discussion. The estimation of the mean squares for the investigated traits in the F_4 lines from Marj12 (*Rht5*) × Scholar cross are shown in Table 3. As seen in the Table, the differences among the lines for all traits were statistically significant ($p \leq 0.01$), suggesting genetic differences existed for

all traits among the F_4 lines. Similar to our results, Daoura et al. [14] reported highly significant differences among all the $F_{2:3}$ lines of Ningchun10 (*rht5*) × Marfed M (*Rht5*) for all the traits investigated except for biomass, grain yield and harvest index using ANOVA.

T a b l e 3. **Analysis of variance (Mean Squares) of investigated traits in F_4 lines of Marj12 (*Rht5*) × Scholar cross**

Source of variation (SV)	Plant height (PH), cm	Peduncle length (PL), cm	Spike length (SpL), cm	Spikelet number (SN)	Kernel number per spike (KN)	Kernel weight (KW), g	Seed width (SW), mm	Seed length (SeL), mm	Coleoptile length (CL), cm	Primary root number (PRN)	First leaf length (FL), cm
Block	1.82	7.10	2.20	8.75	1.83	1.04	0.00	0.00	5.56	5.70	0.02
Genotype	31207.80*	4298.01*	234.54*	365.78*	9646.79*	0.01*	36.31*	28.39*	114.06*	141.92*	425.43*

* Significant at $\alpha = 0.01$.

The presence of *Rht5* gene was confirmed at the DNA level using SSR marker Barc102 in the F_4 lines; the mother parent Marj12 was used as a positive control and the father parent Scholar as a negative control. Barc102 marker produced a band approximately 150 bp long in Scholar. Marj12 parent, which possesses the *Rht5* gene, had a smaller band of 140 bp. Ellis et al. [26] mapped *Rht5* on chromosome 3BS and they amplified a 200 bp band in Marfed M (*Rht5*) and a 165 bp band in the Vigour 18 parent of mapping population using Barc102 marker. Most of the lines in the Marj12 × Scholar population could be readily classified as tall (*rht5*), short (*Rht5*) or heterozygous. According to the Barc102 marker results, 19 individuals from the 65 F_4 lines carried the *Rht5* gene, while 34 lines carried the *rht5* gene and 12 individuals were observed to be heterozygous (Table 4). The chi-square test showed the significant differences between the expected and the observed ratios of *Rht5* gene segregation ($\chi^2 = 6.067 > \chi^2 = 5.99$, $p = 0.05$, df = 2). This means that the observed values do not match the expected values of the segregation of a single gene in the F_4 generation.

T a b l e 4. **Distribution of F_4 population individuals in terms of *Rht5* gene according to Xbarc102 marker**

Genotype class	Number of individuals		Chi-square values
	Observed	Expected	
Maternal band pattern	19	28.438	3.132
Paternal band pattern	34	28.438	1.087
Heterozygous band pattern	12	8.125	1.848
Total	65	65.000	6.067^{ns}

N o t e. ^{ns} – Non-significant of χ^2 ($p = 0.05$, df = 2 for F_4) = 5.99.

The mean values for the tall, dwarf and heterozygous F_4 plants and the mean differences between the tall and dwarf group, and the effects of *Rht5* on the investigated traits were calculated and shown in Table 5. The mean values of all traits except coleoptile length, primary root number and first leaf length were higher in tall lines than in dwarf lines. Notably, the heterozygous lines had higher values for peduncle length and the seed characteristics such as kernel weight, seed width, seed length. The plant height and peduncle length of the tall lines were higher than those of the dwarf F_4 lines by 25.44 % and 22.28 %, respectively. This means that the dwarfing gene *Rht5* has a high effect on plant height and peduncle length of the lines. In a study investigating the genetic effects of the *Rht5* gene, plant height and peduncle length of the $F_{2:3}$ lines from the Marfed M (*Rht5*) cross were found to be 22.4 % and 20.4 % lower, respectively [14]. In another study by the same researchers, an average of 25.84 % reduction in plant height could be associated with *Rht5* [15]. However, Rebetzke et al. [13] reported the largest reduction (–55 %) in plant height associated with *Rht5*. Cui et al. [30] emphasized that the plant height of *Rht5* dwarf lines was reduced by 32.67 % and 27.84 % in the Jinmai47 and Ningchun45 population, respectively. As can be seen from these comparisons, the results obtained from this study were more consistent with those of both Daoura et al. studies [14, 15].

T a b l e 5. **The effects of *Rht5* gene on the investigated traits in the F_4 lines of Marj12 (*Rht5*) × Scholar cross**

Traits	Tall (*rht5*)	Range	Dwarf (*Rht5*)	Range	Heterozygous (*Rht5rht5*)	Range	Difference (tall-dwarf)	Effect (%)
Plant height (PH), cm	100.45 ± 14.11	59.00–127.75	74.89 ± 21.96	44.50–117.16	95.46 ± 8.52	81.80–108.12	25.56	25.44
Peduncle length (PL), cm	37.88 ± 5.21	22.12–48.18	29.44 ± 8.99	20.37–51.05	39.77 ± 5.62	33.10–47.75	8.44	22.28
Spike length (SpL), cm	8.45 ± 1.46	5.41–12.25	7.00 ± 1.57	4.35–9.20	8.08 ± 1.72	5.60–10.50	1.45	17.16
Spikelet number (SN)	18.64 ± 1.93	15.20–23.40	16.70 ± 2.89	11.40–22.80	17.90 ± 1.66	14.80–19.40	1.94	10.41
Kernel number per spike (KN)	40.07 ± 9.11	23.20–71.00	30.23 ± 15.16	12.40–64.80	36.30 ± 10.37	23.20–54.00	9.84	24.56
Kernel weight (KW), g	0.0423 ± 0.0084	0.0201–0.0579	0.0363 ± 0.0100	0.0306–0.0390	0.0429 ± 0.0070	0.0464–0.0520	0.006	14.18
Seed width (SW), mm	3.22 ± 0.31	2.54–3.81	2.93 ± 0.41	2.60–3.34	3.28 ± 0.31	3.52–3.57	0.29	9.00
Seed length (SeL), mm	6.02 ± 0.38	6.75–5.14	5.18 ± 0.51	4.85–6.81	6.12 ± 0.29	5.75–6.50	0.84	13.95
Coleoptile length (CL), cm	7.46 ± 0.98	5.50–10.25	7.91 ± 0.98	5.83–9.50	7.17 ± 1.37	5.25–9.50	–0.45	–6.03
Primary root number (PRN)	4.66 ± 0.73	3.00–6.00	4.73 ± 0.73	3.50–6.00	4.69 ± 0.75	3.30–6.00	–0.07	–1.50
First leaf length (FL), cm	13.00 ± 2.39	7.70–18.75	13.87 ± 2.10	9.58–16.83	13.44 ± 2.64	10.00–17.00	–0.87	–6.69

N o t e. The data represented as the mean ± standard deviation (SD).

The spike length of the tall F_4 lines was 1.45 cm (17.16 %) greater than that of the dwarf F4 lines. The effect of the *Rht5* gene on the number of spikelets per spike was observed to be lower compared to spike length, and 10 % more spikelets were observed in tall lines than in dwarf F_4 lines. Higher spike length caused a significant increase in the number of kernels per spike. The kernel number per spike of the tall lines was 9.84 kernels (24.56 %) greater than that of the dwarf F_4 lines. The effect of the *Rht5* gene on spike traits has been previously reported, with similar trends: spike length, spikelet number, and grain number were reported as higher values in tall groups than in the dwarf groups by 16.35 %, 7.53 %, and 13.84 % [14] and 21.16 %, 11.51 %, and 38.1 % [15], respectively.

Although the seed traits such as kernel weight, seed width and seed length had the highest values in heterozygotes, the tall lines had higher values than the dwarf lines by 14.18 %, 9 % and 13.95 %, respectively. Similar findings were reported by Cui et al. [30] that the grains of dwarf lines had significantly lower grain length, grain width and thickness in all progenies of the two populations.

In the study, one of the features for which higher values were measured in dwarf lines was the coleoptile length and it was obtained as 6.03 % higher in dwarf lines. Coleoptile length is an important factor determining sowing depth in wheat. A long coleoptile is a desirable trait in wheat, but as coleoptile length increases, there is a parallel increase in plant height [31–33] and this is undesirable. From this perspective, in our study dwarf lines carrying the *Rht5* gene had longer coleoptile lengths. The dwarfing genes *Rht1* and *Rht2*, which are commonly found in wheat varieties, are the gibberellin-insensitive genes and cause reductions in coleoptile length and seedling vigor [22, 26, 34]. Although the GA-responsive *Rht5* gene caused a decrease in plant height, it caused an increase in coleoptile length in this study. Similar results were observed for primary root number and first leaf length. The primary root number and first leaf length of the dwarf lines had higher values than those of the tall lines by 1.5 % and 6.69 %. This shows that the lines carrying the *Rht5* gene may show improved seedling vigor.

Conclusion. The present study revealed the association of the GA-responsive *Rht5* gene with reductions in plant height, peduncle length, spike length, number of spikelets, kernels per spike, kernel weight, seed width, and seed length. However, in contrast to these reductions, the *Rht5* gene had an enhancing effect on coleoptile length, primary root number, and first leaf length. Coleoptile length is an important factor determining sowing depth in wheat. The high-yielding modern wheat varieties widely

used today have short coleoptile lengths. Therefore, they will not be able to meet expectations in a world that is becoming increasingly arid and where deep seeding is becoming increasingly important. For this reason, it is recommended that the dwarfing gene *Rht5* be taken into consideration in the development of new wheat varieties suitable for changing climatic and environmental conditions.

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