

## ЕФЕКТ НА ГЕНИТЕ ЗА НИСКО СТЬБЛО ВЪРХУ ДЪЛЖИНАТА НА КОЛЕОПТИЛА ПРИ МЕКАТА ПШЕНИЦА В УСЛОВИЯ НА ИНДУЦИРАН ВОДЕН ДЕФИЦИТ

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### Резюме

Ланджева С., Т. Кърцева, В. Корзун, Г. Ганева, 2012. Ефект на гените за ниско стъбло върху дължината на колеоптила при меката пшеница в условия на индуциран воден дефицит FCS 8(1):15-21

Проучен е ефекта на гибберелин-нечувствителните гени за ниско стъбло *Rht-B1b* и *Rht-D1b* върху растежа на колеоптила в условия на полиетилен гликол-индуциран воден дефицит при четири серии от линии пшеница, изогенни по тези алели чрез сравняване със съответните линии, носещи дивия алел, *rht*. С доказан ефект е генотипа (*Rht* гена и сортовете особености) и третирането с осмолитик. Показано е, че редуцирането на дължината на колеоптила както в контролни условия, така и при стрес е в реда *rht* > *Rht-D1b* > *Rht-B1b* > *Rht-B1b+-D1b*. Изследвано е генетичното вариране по отношение дължината на колеоптила в нестресови условия и при индуциран воден дефицит при 68 български сорта мека пшеница, създадени в периода 1960-2000 г. С помощта на молекулни маркери е показано, че 43 сорта носят гибберелин-чувствителния ген *Rht8*, шест сорта носят гибберелин-нечувствителния ген *Rht-B1b/d* и 19 сорта носят комбинация от двата гена. Установено е, че както при нестресови условия, така и при воден дефицит най-дълъг колеоптил имат сортовете с *Rht8*, следвани от тези с *Rht-B1b/d*, а с най-къс колеоптил са сортовете, носещи двата гена. Редуциращият ефект на комбинацията от два *Rht* гена върху дължината на колеоптила е потвърден при линии пшеница сорт “Садово-1”, изогенни по гена *Rht-B1b/d*.

### Abstract

Landjeva S., T. Karceva, V. Korzun, G. Ganeva, 2012. Effect of wheat semi-dwarfing genes on coleoptile length in response to induced water deficit stress FCS 8(1):15-21

The effect of the gibberellin-insensitive wheat semi-dwarfing genes *Rht-B1b* and *Rht-D1b* on coleoptile elongation in response to polyethylene glycol-induced water deficit was studied in four series of *Rht* near-isogenic lines by comparing them with the corresponding tall isolines, *rht*. The genotype (*Rht* allele and cultivar background) and the osmotic treatment had significant effects on coleoptile growth. The coleoptile length was reduced under both non-stress and stress conditions in the order *rht* > *Rht-D1b* > *Rht-B1b* > *Rht-B1b+-D1b*. Furthermore, the genetic variation of coleoptile length in non-stress and water deficit conditions was investigated in 68 Bulgarian bread wheat cultivars, released in the

period 1960-2000. Using molecular marker technologies, the occurrence of the gibberellin-responsive gene *Rht8* was determined in 43 cultivars, *Rht-B1b/d* alleles were present in 6 cultivars, and 19 cultivars carried a combination of both genes. Under both non-stress and water deficit conditions, the longest coleoptile was produced by *Rht8* carriers, followed by those with *Rht-B1b/d*, while the double dwarfs had the shortest coleoptile. The reducing effect of the *Rht8* + *Rht-B1b/d* combination on coleoptile length was further verified in cv. “**Sadovo-1**” *Rht-B1b/d* near-isogenic lines.

**Key words:** Coleoptile length – Semi-dwarfing genes – Water deficit – Wheat

**Abbreviations:** PEG – polyethylene glycol; GA – gibberellic acid; NILs – near-isogenic lines

## INTRODUCTION

A long-term analysis of climate variability and change in Bulgaria during the 20<sup>th</sup> century showed a consistent negative trend for summer and autumn precipitation (Alexandrov et al. 2004). The early season drought is critical for emergence and successful establishment of winter wheat. The semi-dwarfing gibberellic acid (GA)- insensitive genes *Rht-B1b/d* (*Rht1*), and *Rht-D1b* (*Rht2*) reduce the final plant height to maximize yield, but at the same time reduce cell size in all organs (Miralles et al. 1998). This, in turn, is associated with shorter coleoptile that might compromise the delivering of the growing shoot to the soil surface (Schillinger et al. 1998). The GA-responsive gene *Rht8* reduces plant height and conditions the desired partitioning of assimilates to the reproductive organs, while having small effect on coleoptile length (Botwright et al. 2001; Rebetzke & Richards 2000). Longer coleoptiles are of particular importance for the fast seedling emergence in conditions with insufficient soil moisture (Rebetzke et al. 2007).

In this paper, we determined the occurrence of the three most widespread semi-dwarfing genes, *Rht-B1b/d*, *Rht-D1b* and *Rht8*, in 68 Bulgarian bread wheat cultivars, and in two *Rht* near-isogenic lines of cultivar “**Sadovo-1**”. Using four sets of *Rht* near-isogenic lines we investigated the effect of the two major GA-insensitive alleles, *Rht-B1b* and *Rht-D1b*, on coleoptile elongation under water deficit, simulated by polyethylene glycol (PEG). Furthermore, we compared the cultivars and lines carrying different *Rht* genes by their coleoptile length when growing seedlings under PEG-induced drought stress.

## MATERIALS AND METHODS

### Plant material

A collection of 68 cultivars, introduced in Bulgaria during the period 1960-2000, and two near-isogenic lines (NILs) of the standard cultivar ‘Sadovo-1’ were used in the DNA analysis and in the stress tests. Seed samples were kindly supplied by Dr. K. Kolev, IPGR, Sadovo, and maintained in the field of the former Institute of Genetics, BAS. The cv. “**Sadovo-1**” NILs were developed by one of us (G. Ganeva) using cv. “**Mercia**” lines, isogenic for *Rht-B1b* and *Rht-B1d*. Four sets of wheat lines near-isogenic for *Rht-B1b*, *Rht-D1b* and *Rht-B1b+D1b*, developed in the cultivars “**April Bearded**”, “**Bersée**”, “**Maris Huntsman**”, and “**Maris Widgeon**” were used to elucidate the effects of the *Rht* genes on coleoptile growth as compared to the tall controls, *rht*. This material was kindly provided by Dr. A. Börner, IPK, Gatersleben, Germany.

### Identification of *Rht* genes

To identify the *Rht-B1b/d* and *Rht-D1b* alleles, polymerase chain reaction based molecular markers were used according to the method described by Ellis et al. (2002). The microsatellite marker WMS261 was used to study the allelic variation at the *Xgwm261* locus according to Korzun et al. (1998) and to determine the presence of the 192 bp allele, diagnostic for *Rht8*.

### Stress tests

Water deficit was imposed by treating germinating seeds and seedlings with 12%, 15% and 18% (the four sets of NILs) or 15% solution of PEG 6000 (wheat cultivars and cv. "Sadovo-1" NILs) at 20-21°C. Distilled water was used as control. Germination was performed in covered plastic boxes (NILs) or Petri dishes (cultivars) on two layers of filter paper, moistened and replenished daily with a fixed volume of PEG solution or water. The coleoptile length was obtained on 5 (NILs) and 15 (cultivars) median size seedlings after 7-day-long treatment. Three (NILs) or two (cultivars) independent experiments were conducted under the same environmental conditions. Statistical analysis was performed using the software package STATISTICA, StatSoft, Inc., version 7.1.

## RESULTS

### Distribution of *Rht* genes

**Table 1.** Distribution of *Rht-B1b/d* and *Rht8* genes in 68 Bulgarian bread wheat cultivars and in two *Rht* near-isogenic lines of cv. "Sadovo-1"

Cultivar	Rht-B1b/d or wild allele	Xgwm261 allele (bp)	Cultivar	Rht-B1b/d or wild allele	Xgwm261 allele (bp)
Aglika	Rht-B1b/d	202	Nivyana	wild	192
Albena	Rht-B1b/d	192	Ogosta	Rht-B1b/d	192
Altimir-67	wild	192	Pliska	Rht-B1b/d	192
Asenovka	wild	192	Pobeda	wild	192
Bononia	wild	192	Prelom	Rht-B1b/d	192
Boryana	wild	192	Preslav	wild	192
Diamant	wild	192	Pryaspa	wild	192
Dobrotitsa	wild	192	Rekviem	Rht-B1b/d	192
Eliitsa	Rht-B1b/d	192	Rubin	wild	192
Enola	Rht-B1b/d	192	Russalka	Rht-B1b/d	192
Erythroperm 19-16	wild	192	Sadovo-1	wild	192
Hebros	wild	192	- NIL-1	Rht-B1b/d	192
Galateya	Rht-B1b/d	202	- NIL-2	Rht-B1b/d	192
Gladiator-113	Rht-B1b/d	174	Sadovo-552	wild	192
Ideal	wild	192	Sadovo-772	Rht-B1b/d	192
Iveta	Rht-B1b/d	192	Sadovska Beliya	Rht-B1b/d	192
Kaliakra	wild	192	Skitiya	wild	192
Kaloyan	wild	192	Slavyanka	wild	192
Kardam	wild	192	Sredets-68	wild	192
Katya	wild	192	Svilena	Rht-B1b/d	192
Kiten	wild	192	Tangra	wild	192
Krapets	wild	192	Todora	Rht-B1b/d	174
Kremena	wild	192	Toshevka	wild	192
Kristal	Rht-B1b/d	192	Trakiya	Rht-B1b/d	192
Lada	Rht-B1b/d	192	Trayana	wild	192
Laska	Rht-B1b/d	174	Vega	wild	192
Levent	wild	192	Yantar	wild	192
Ludogorie	Rht-B1b/d	192	Yassen	wild	192
Ludogorka	wild	192	Yubilej	wild	192
Medeya	wild	192	Yubilejna-3	Rht-B1b/d	174
Milena	wild	192	Yunak	Rht-B1b/d	192
Miziya	wild	192	Zagore	wild	192
Momchil	wild	192	Zdravko	Rht-B1b/d	192
Murgavets	Rht-B1b/d	192	Zlatoklas	wild	192
Mustang	wild	192	Zora	wild	192

The screening for allelic variation at the *Rht-B1*, *Rht-D1* and *Rht8* loci showed that 43 cultivars (63%) carried the *Xgwm261*<sup>192bp</sup> / *Rht8* allele, while only 9% contained *Rht-B1b/d*. The rest of cultivars carried a combination of *Rht8* and *Rht-B1b/d* (Table 1). The *Rht-D1b* was not found in any of the cultivars. The two NILs of cultivar “**Sadovo-1**” scored positive with the primers specific for *Rht-B1b* (Table 1). Although the NIL derived from the cross of cv. “**Sadovo-1**” to the *Rht-B1d* near-isogenic line of cv. “**Mercia**” was expected to carry this allele, the two NILs were undistinguishable using these markers. Since *Xgwm261*<sup>192bp</sup> / *Rht8* was present in cv. “**Sadovo-1**”, the two NILs were therefore determined as double dwarfs, carrying the combination *Rht-B1b/d* + *Rht8*.

### Coleoptile growth under water deficit stress

**Table 2.** Coleoptile length (mean±SE, standard error) in four sets of *Rht* near-isogenic lines, 68 Bulgarian bread cultivars, grouped according to the *Rht* gene/s, and *Rht* near-isogenic lines of cultivar “**Sadovo-1**” (*Rht-B1b/d+Rht8*) measured after 7-day treatment with polyethylene glycol (PEG) and compared to the corresponding controls (distilled water). Values within each column followed by different letters denote significant ( $p < 0.05$ ) differences between the carriers of various *Rht* alleles.

Cultivar	Rht allele	Treatments			
		Control	PEG 12%	PEG 15%	PEG 18%
A. <i>Rht</i> near-isogenic lines					
April Bearded	<i>rht</i>	3.4±0.10a	3.3±0.03a	3.6±0.06a	3.1±0.10a
	<i>Rht-B1b</i>	2.7±0.04c	2.8±0.02b	2.9±0.07b	2.7±0.10b
	<i>Rht-D1b</i>	2.9±0.05b	2.9±0.04b	2.9±0.03b	2.8±0.07b
	<i>Rht-B1b+-D1b</i>	2.3±0.04d	2.5±0.02c	2.4±0.03c	2.4±0.05c
Bersée	<i>rht</i>	3.3±0.07a	3.6±0.07a	3.2±0.06a	3.0±0.06a
	<i>Rht-B1b</i>	2.8±0.08b	2.8±0.04b	2.6±0.04b	2.6±0.03b
	<i>Rht-D1b</i>	2.8±0.07b	2.9±0.04b	2.7±0.04b	2.7±0.03b
	<i>Rht-B1b+-D1b</i>	2.3±0.03c	2.3±0.04c	2.4±0.04c	2.3±0.04c
Maris Huntsman	<i>rht</i>	3.2±0.05a	3.4±0.05a	3.3±0.07a	2.8±0.10a
	<i>Rht-B1b</i>	2.7±0.04b	2.6±0.05b	2.5±0.04c	2.3±0.04c
	<i>Rht-D1b</i>	2.8±0.03b	2.7±0.03b	2.6±0.03b	2.5±0.06b
	<i>Rht-B1b+-D1b</i>	2.2±0.05c	2.2±0.02c	2.1±0.03d	2.0±0.03d
Maris Widgeon	<i>rht</i>	3.3±0.09a	3.2±0.04a	3.2±0.06a	3.3±0.06a
	<i>Rht-B1b</i>	2.9±0.07b	2.7±0.04c	2.8±0.08c	2.9±0.08b
	<i>Rht-D1b</i>	2.8±0.06b	2.9±0.04b	3.0±0.05b	3.0±0.05b
	<i>Rht-B1b+-D1b</i>	2.5±0.05c	2.5±0.02d	2.5±0.05d	2.5±0.05c
Allele mean:	<i>rht</i>	3.3±0.04a	3.3±0.03a	3.3±0.04a	3.1±0.09a
	<i>Rht-B1b</i>	2.8±0.03b	2.7±0.02c	2.7±0.04c	2.6±0.05b
	<i>Rht-D1b</i>	2.8±0.03b	2.8±0.02b	2.8±0.03b	2.7±0.06b
	<i>Rht-B1b+-D1b</i>	2.3±0.02c	2.4±0.02d	2.4±0.03d	2.3±0.04c
B. Bulgarian cultivars					
Group of 43	<i>Rht8</i>	3.7±0.01a		2.8±0.03a	
Group of 6	<i>Rht-B1b/d</i>	3.5±0.04b		2.5±0.08b	
Group of 19	<i>Rht-B1b/d+Rht8</i>	3.3±0.01c		2.3±0.04c	
C. cv. “ <b>Sadovo-1</b> ” near-isogenic lines					
Sadovo-1	<i>Rht8</i>	3.3±0.04a		3.1±0.06a	
Sadovo-1 NILs	<i>Rht-B1b/d+Rht8</i>	2.9±0.02b		2.8±0.03b	

Coleoptile length varied depending on cultivar and *Rht* allele (Table 2). The presence of an *Rht* allele caused significant reduction in the coleoptile length compared to the tall isolate, carrying the wild allele *rht* (Table 2, A). The reducing effect of the *Rht* alleles was in the order: *rht* > *Rht-D1b* ≥ *Rht-B1b* > *Rht-B1b+-D1b* in both non-stress (control) and at all levels of water deficit stress. In some cultivar / treatment combinations, the *Rht-B1b* and *Rht-D1b* did

not differ in their effects. The effects of genotype (*Rht* allele and cultivar background), and the treatment, as well as their interactions were highly significant (Table 3).

Within Bulgarian bread wheat germplasm, the longest coleoptile was produced by the GA-responsive (*Rht8*) cultivars, while the GA-insensitive cultivars carrying the combination *Rht-B1b/d +Rht8* produced the shortest coleoptile (Table 2, B). This trend was consistent in control and under water deficit stress. The cv. “**Sadovo-1**” NILs (*Rht-B1b/d+Rht8*) produced significantly shorter coleoptile compared to cv. “**Sadovo-1**” (*Rht8*) in both non-stress and stress conditions (Table 2, C).

**Table 3.** Mean squares (MS) from analysis of variance for coleoptile length in wheat lines of four cultivars, carrying different *Rht* genes (*rht*, *Rht-B1b*, *Rht-D1b* and *Rht-B1b+-D1b*), following 7-day-long treatment with PEG solution in concentrations 0, 12%, 15%, and 18%.

Source of variation	df	MS	p
Cultivar (C)	3	3.256	0.000000
Rht allele (A)	3	33.954	0.000000
Treatment (T)	3	0.994	0.000000
C x A	9	0.149	0.000483
C x T	9	0.501	0.000000
A x T	9	0.192	0.000017
C x A x T	27	0.142	0.000000
Error	896	0.045	

## DISCUSSION

The vast majority of cultivars studied (91%) carried *Rht8*. In 31% of them the *Rht8* allele was combined with *Rht-B1b/d*. Only 6 cultivars carried the alleles *Rht-B1b/d* alone. The other major GA-insensitive allele, *Rht-D1b*, was not found in our sample, neither alone, nor in combination with other semi-dwarfing alleles. The dominance of the GA-responsive *Rht8* in wheat germplasm, developed and grown in regions of warm and dry climate was reported by other authors, as well (Chebotar et al. 2001, Ganeva et al. 2005, Toshovič-Marič et al. 2008). The agronomic significance of *Xgwm261<sub>192bp</sub>* / *Rht8* is mostly associated with its linkage to the *Ppd-D1* gene for photoperiod response (Worland et al. 1998). By reducing the sensitivity to day length, the dominant *Ppd-D1a* allele confers early flowering, resulting in yield benefits under the unfavourable high temperature and dry conditions (Worland et al. 1998). The non-occurrence of *Rht-D1b* could be explained by the limited utilization of the CIMMYT semi-dwarfing lines – the major carriers of this allele - by the Bulgarian wheat breeders. Another reason might be related to the findings that the genomic region on chromosome 4D, containing *Rht-D1b*, significantly contributes to the susceptibility to Fusarium head blight, a world-wide serious crop wheat threat (Voss et al. 2008).

The analysis of the temporal changes in the frequency of GA-responsive and GA-insensitive *Rht* alleles within Bulgarian wheat germplasm revealed a trend of increased introduction of *Rht-B1b/d* alleles into cultivars, registered after 1990s (Ganeva et al. 2005). Since the GA-insensitive allele *Rht-B1b* was reported to reduce size of all plant organs (Miralles et al. 1998), this observation raised the question about seedling emergence and establishment under insufficient soil moisture during or shortly after sowing. The undertaken screening of wheat lines, near-isogenic for the two major GA-insensitive alleles and their combination, in four genetic backgrounds showed that the osmotic treatments had either slight reducing effect or no effect at all on coleoptile length. The effects of water deficit were modified by the *Rht* allele and the cultivar genetic background. Both *Rht-B1b* and *Rht-D1b* reduced in a similar degree the coleoptile length in comparison with the tall (*rht*)

genotypes, while the accumulation of both alleles had more severe reducing effect. These effects were observed under all levels of stress suggesting that genotypes with longer coleoptiles under non-stress conditions as determined by the *Rht* status (*rht*) maintain their potential under stress. The GA-insensitive *Rht* alleles have been shown to reduce coleoptile and leaf elongation in comparison with either tall (*rht*) genotypes or with carriers of GA-responsive dwarfing genes such as *Rht8* (Botwright et al. 2001). The negative effects of the GA-insensitive alleles at the locus *Rht-B1* on coleoptile length was further confirmed in the present survey on the Bulgarian bread wheat cultivars and the *Rht* NILs of cv. "Sadovo-1". The effect of *Rht-B1b* allele is associated with inhibited GA signalling pathway (Peng et al. 1999) and is expressed in reduced cell elongation in all vegetative organs, including the coleoptile, whereas the *Rht8* allele has no effect on the responsiveness to GA, and hence does not reduce coleoptile length (Ellis et al. 2004). Since the seedling emergence is positively correlated with the length of coleoptile (Schillinger et al. 1998), *Rht8* is considered one of the key traits for improving drought tolerance (Rebetzke et al. 2007). The better ability to maintain coleoptile growth under limited soil water availability is beneficial where deeper sowing is practiced to utilize moisture from greater soil depth. The coleoptile length is under complex genetic control. Recently, two quantitative trait loci (QTL) were identified on chromosome 4B (Rebetzke et al. 2001), and few other QTL determining coleoptile length in either normal growing conditions or under water deficit stress were described on chromosomes 1A, 1B, 6B and 7D (Landjeva et al. 2008). The expression of these QTL may be differentially affected by the stress applied, so that a wide variation in response could be observed in addition to the variation in coleoptile growth response accounted for the *Rht* genes.

The present comparative analysis showed that under the climatic conditions of Bulgaria, the preferential introduction of *Rht8* is associated with its potential to maintain longer coleoptiles, in addition to the agronomic advantage of its linkage to the *Ppd-D1a* allele for photoperiod insensitivity. These characteristics of *Rht8* benefit to solve the problem of yield instability related to drought trends around sowing and post anthesis.

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