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CHANGES IN THE SEEDLING GROWTH PARAMETERS IN THREE COMMON WHEAT (*Triticum aestivum* L.) CULTIVARS SUBJECTED TO DROUGHT STRESS AND SUBSEQUENT RE-HYDRATION

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Abstract

Landjeva, S., G. Ganeva, 2006. Changes in the seedling growth parameters in three common wheat (Triticum aestivum L.) cultivars subjected to drought stress and subsequent re-hydration.

Three common wheat cultivars, **"Lozen 6"**, carrying a gibberellin-responsive height reducing (*Rht*) gene, and **"Todora"** and **"Gladiator113"**, both carrying gibberellin-insensitive *Rht* genes, were compared for drought tolerance based on growth parameters at early seedling stage. The drought was induced by polyethylene glycol (PEG 10 % and 15 %). The seedling potential to resume the growth processes after subsequent re-hydration was assessed. Under stress shoot growth was mostly inhibited at both concentrations, followed by root growth inhibition, while the coleoptile growth was the least affected. Among the cultivars, **"Todora"** had the longest coleoptile and shoots, and the highest tolerance index (TI) for shoots and coleoptile under stress conditions. The TI for roots was highest in **"Lozen 6".** Significant genotypic differences were found regarding root and shoot elongation at both PEG concentrations. Activation of growth processes after the subsequent re-hydration was observed for coleoptile and shoots, while the growth of roots was less recovered, or even ceased. Significant genotypic differences were revealed regarding coleoptile elongation during the recovery after the PEG 15 %-stress and further shoot elongation during the recovery after the PEG 10 %-stress. The obtained results suggest that cultivars **"Todora"** and **"Lozen 6"** are drought tolerant at early seedling stage. The associations between *Rht* alleles and coleoptile length under control conditions are also discussed.

Резюме

Ланджева, С., Г. Ганева, 2006. Промени в растежните параметри при три сорта обикновена пшеница (Triticum aestivum L.), подложени на последователно засушаване и ре-хидратиране

Направен е сравнителен анализ по отношение на толерантност към засушаване между три български сорта обикновена пшеница, **"Лозен 6"** - носител на гиберелин-чувствителен ген за ниско стъбло (*Rht*), **"Тодора"** и **"Гладиатор113"** - носители на гиберелин-нечувствителен *Rht* ген. Сравнението е направено на база изменение в растежните параметри в стадий поникване. Засушаването е симулирано чрез добавяне на полиетилен гликол в две концентрации (ПЕГ10 % и 15 %). Проучена е и възможността за възобновяване на растежните процеси след ре-хидратация. В условия на стрес в най-силна степен е инхибиран растежа на надземната част, следван от този на корените, докато растежът на колеоптила е потиснат в най-малка степен. От изследваните сортове в условия на стрес **“Тодора”** се характеризира с най-дълъг колеоптил и надземна част и най-висок индекс на толерантност (ИТ) по отношение на колеоптила и надземната част. При **“Лозен 6”** е установен най-висок ИТ по отношение на корените. Установени са статистически доказани генотипни различия по отношение изменение дължината на корените и надземната част и при двете ПЕГ концентрации. Активиране на растежните процеси след ре-хидратиране е наблюдавано относно растежа на колеоптила и надземната част, докато растежа на корените се възобновява в по-слаба степен или дори окончателно се преустановява. Статистически доказани различия между сортовете са установени по отношение растежа на колеоптила при възстановяване след третиране с ПЕГ 15 %, а по отношение растежа на надземната част - при възстановяване след третиране с ПЕГ 10 %. Получените резултати показват, че сортовете **“Тодора”** и **“Лозен 6”** са толерантни на засушаване в ранни онтогенетични фази. В представената работа се обсъжда и взаимовръзката между *Rht* гените и дължината на колеоптила в нормални условия.

Key words: Wheat – Drought tolerance – Seedling stage - *Rht*-genes

INTRODUCTION

Under continental climate, drought is a frequent abiotic stress that can negatively affect plant growth at different critical stages, which can finally result in a considerable reduction of crop productivity. Seedling growth is one of the most sensitive stages in wheat establishment. The genetic potential of plants to produce longer coleoptiles and to guarantee rapid growth and early ground cover is of priority value for the efficient use of limited soil water reserves (Reynolds et al. 2000; Richards 1996). Studies on the effects of height-reducing genes (*Rht*) on the early stages of plant development showed that gibberellic acid (GA) insensitive alleles *Rht-B1b* and *Rht-D1b* tend to restrict coleoptile and leaf elongation, while GA-responsive alleles can reduce plant height to an extent similar to that of GA-insensitive ones, and yet can produce longer coleoptiles and greater leaf biomass (Botwright et al. 2001; Ellis et al. 2004). This is considered a beneficial trait under unfavorable soil conditions at sowing (Richards et al. 2001).

Recent studies on the distribution of *Rht* alleles among 89 Bulgarian common wheat cultivars showed that 55 % of the modern semi-dwarf cultivars carry GA-responsive alleles, and the rest carry either the GA-insensitive alleles *Rht-B1b, Rht-B1d* or *Rht-D1b*, or a combination of both *Rht* classes (Ganeva et al. 2005).

The aim of this work was to study the seedling growth response in terms of root, coleoptile and shoot elongation of three Bulgarian common wheat cultivars carrying different *Rht* alleles to drought stress simulated by addition of polyethylene glycol (PEG 6000), and further, to evaluate the seedling potential for recovery of growth processes after rehydration.

MATERIAL AND METHODS

Plant material

Three Bulgarian common wheat (*Triticum aestivum* L.) cultivars were examined: **“Todora”**, **“Lozen 6”** and **“Gladiator 113”**. Genotypes were chosen on the basis of their reaction to exogenous gibberellic acid (GA): “Todora” and “Gladiator 113” are GA-insensitive, and “Lozen 6” is GA-responsive (Ganeva et al. 2005).

Experimental design

For each cultivar, 100 seeds were surface sterilized and germinated at 23±1 °C in dark. On the 4-th day measurements (length of roots and coleoptile) were taken for each genotype on 45 seedlings of similar size (I measurement). They were divided into three groups, each of 15 seedlings, which were placed in Petri dishes on two treatment variants (10 % and 15 % PEG) and on a control variant (distilled water). The effect of PEG-treatment was evaluated after 5 days incubation at 23±1 °C by measuring of root, coleoptile and shoot length (II measurement). To assess the recovery potential of the three genotypes, the stressed seedlings were transferred to distilled water for another 3 days, followed by measuring of root, coleoptile and shoot length (III measurement).

Data analysis

For each trait, mean and standard deviation were calculated. Student's *t*-test was applied to compare the means within each treatment and each measurement. Tolerance index (TI) was determined for the root, coleoptile and shoot length as a ratio between the values obtained under stress and the corresponding values obtained from the control group of seedlings (Macnair 1993). Analysis of covariance (Dowdy & Wearden 1983) was applied to study the existence of genotypic differences regarding root, coleoptile, or shoot elongation in the control and under drought stress. We tested the null hypothesis H_0, which states that the three genotypes show the same growth response against Ha: At least one inequality.

RESULTS

The analysis of variance revealed that under non-stressed conditions considerable genotypic differences exist regarding coleoptile length; the longest coleoptile in 10-day-old seedlings was obtained for **"Gladiator 113"** (Table 1). Analysis of covariance applied to reduce the within-cultivar variability revealed significant genotypic differences in the control regarding coleoptile and shoot elongation, **"Lozen 6"** showing the highest growth rate (Table 2).

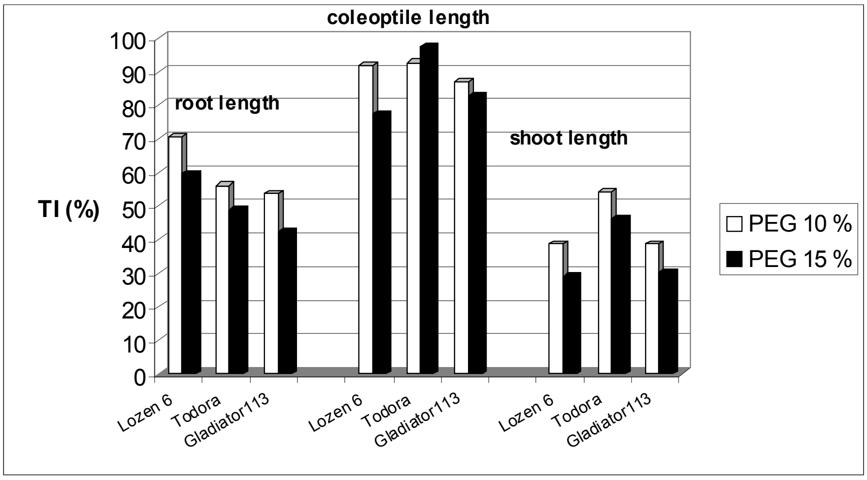

Fig.1. Tolerance index for seedling root, coleoptile and shoot length in three common wheat cultivars subjected to PEG-induced drought

Under PEG-simulated drought, shoot growth was mostly inhibited at both concentrations, followed by root growth inhibition, while the coleoptile growth was the least affected (Fig. 1, Table 1). Under stress the longest coleoptile and longest shoots were recorded for **"Todora"**. The highest TI for shoots and coleoptile was also calculated for **"Todora"**, while the TI for roots was highest in **"Lozen 6"**. By the analysis of covariance significant genotypic differences were found regarding root and shoot elongation at both concentrations (Table 2). The highest root elongation was recorded for **“Lozen 6”** and the highest shoot elongation for **“Todora”**.

Table 1. Mean values and standard deviation of the seedling growth parameters of three wheat cultivars before starting stress (I), after exposure to PEG –induced drought (10 % and 15 %) (II), and after the recovery period (III)

Cultivar	*Treatment*	*Root length (cm)*		
		I	II	III
„Lozen 6“	Control	2.66±0.70b*	12.45±2.77a	15.74±3.77a
	PEG 10%	3.21±0.94ef	8.76±1.06d	8.74±1.03d
	PEG 15%	2.86±0.60hi	7.44±1.31g	8.01±1.46g
„Todora“	Control	3.16±0.60a	13.99±1.86ab	16.45±2.30a
	PEG 10%	3.11±0.62e	7.85±0.87e	7.59±1.08e
	PEG 15%	2.85±0.67h	6.83±1.27gh	8.00±1.60g
„Gladiator 113“	Control	3.05±1.28ab	14.64±3.01b	17.70±3.58a
	PEG 10%	2.47±0.92d	7.83±1.24e	7.88±1.21e
	PEG 15%	2.25±0.96g	6.19±1.06h	6.76±1.22h
		Coleoptile length (cm)		
„Lozen 6“	Control	0.85±0.10b	3.37±0.31c	3.46±0.35c
	PEG 10%	0.82±0.11e	3.09±0.56d	3.15±0.53d
	PEG 15%	0.73±0.11h	2.60±0.62h	2.90±0.73g
„Todora“	Control	1.09±0.10a	3.15±0.14b	3.17±0.15b
	PEG 10%	1.16±0.16d	2.91±0.40d	3.02±0.22d
	PEG 15%	1.15±0.13g	3.06±0.48g	3.08±0.33g
„Gladiator 113“	Control	0.73±0.19c	3.75±0.44a	3.76±0.44a
	PEG 10%	0.70±0.15e	3.26±0.55d	3.34±0.46d
	PEG 15%	0.71±0.27h	2.70±0.81gh	3.34±0.72g
		Shoot length (cm)		
„Lozen 6“	Control	0.85±0.10b	9.00±0.95a	12.78±1.62a
	PEG 10%	0.82±0.11e	3.49±0.53e	4.39±1.23e
	PEG 15%	0.73±0.11h	2.60±0.62h	5.28±1.61h
„Todora“	Control	1.09±0.10a	9.19±0.44a	12.10±0.60a
	PEG 10%	1.16±0.16d	4.98±0.72d	7.13±0.99d
	PEG 15%	1.15±0.13g	4.25±0.94g	8.28±1.77g
„Gladiator 113“	Control	0.73±0.19c	9.01±1.95a	12.30±2.18a
	PEG 10%	0.70±0.15e	3.47±0.84e	4.27±1.50e
	PEG 15%	0.71±0.27h	2.70±0.81h	4.58±1.90h

Means followed by a various letter differ significantly at P>0.05 by *t*-test, performed within each measurement and each treatment (letters sets “abc”, “def” and “ghi” were used to depict significant differences within treatments: control, PEG 10% and PEG 15%, respectively).

After the subsequent re-hydration of the stressed seedlings, the results indicated activation of growth processes for coleoptile and shoots, while the growth of roots was less recovered, or even ceased (Table 1). We applied analysis of covariance using the corresponding values of the growth parameters from the II measurement as a covariate variable to test the null hypothesis. Significant genotypic differences were revealed regarding coleoptile elongation during the recovery after treatment with PEG 15 %, and further shoot elongation after PEG 10 %-recovery. The highest coleoptile elongation was recorded in **“Gladiator 113”**, while the highest shoot elongation was observed in **“Todora”** (Table 2).

Table 2. Analysis of covariance of the changes in seedling growth parameters In three wheat cultivars after PEG-induced drought stress and subsequent recover

Treatment	*Root length*		*Coleoptile length*		*Shoot length*	
	F value	*H_0 against H_a*	*F value*	*H_0 against H_a*	*F value*	*H_0 against H_a*
Control	3.079		20.149***	$F>F_{0.001,2,40}$	21.556***	$F>F_{0.001,2,40}$
PEG 10 %	4.258*	$F>F_{0.05,2,40}$	0.140		12.415***	$F>F_{0.001,2,40}$
PEG 15 %	4.038*	$F>F_{0.05,2,40}$	2.045		9.676***	$F>F_{0.001,2,40}$
Control - dH_20	0.311		5.436**	$F>F_{0.01,2,40}$	0.728	
PEG 10% -dH_20	0.769		1.187		4.462*	$F>F_{0.05,2,40}$
PEG 15% -dH_20	2.557		5.271**	$F>F_{0.01,2,40}$	2.982	

DISCUSSION

The present investigation indicates that the three cultivars display distinct responses to drought stress at early seedling stage and to the subsequent re-hydration. This implies that different mechanisms might be activated to overcome the stress. The GA-insensitive cultivar **"Todora"** produces the longest coleoptile and shoots under drought stress. It is also characterized with the highest TI for coleoptile and shoots under both PEG treatments and the highest potential to resume the growth of shoots after re-hydration. The more rapid shoot elongation in **"Todora"** would enable faster ground cover, thus conserving soil moisture. In a study on physiological responses of Bulgarian cultivars to drought at later developmental stages, Yordanov et al. (2001) showed that **"Todora"** had minimal inhibition of photosynthesis under mild stress, while under severe stress it was among the most sensitive ones.

Under drought stress the GA-responsive cultivar **"Lozen 6"**showed the highest TI for roots and the most intensive root growth. We suppose that the higher TI in this cultivar is a result of the slower growth rate of roots observed under control conditions. This is in compliance with Blum et al. (1997), who suggested that greater stress tolerance of small plants is derived from their relatively slower growth rate. The more extensive root growth of **"Lozen 6"** under stress combined with moderate reduction of shoot elongation would be of importance to increase the soil moisture use efficiency and to diminish its loss through evaporation.

Our previous studies showed that the GA-insensitive cultivars **"Todora"** and **"Gladiator 113"** both carry the *Rht-D1b* allele, while the GA-responsive *Rht* allele in **"Lozen 6"** has not been identified yet (Ganeva et al. 2005). According to the same study, the three cultivars lack the 192-bp allele at locus *Xgwm261* on chromosome 2D, diagnostic for *Rht8* (Korzun et al. 1998), and carry the 174-bp allele. The results of the present investigation do not show clear association between response to GA and the coleoptile length under control conditions. The GA-insensitive cultivar **"Gladiator 113"** exhibited the longest coleoptile, while the GA-responsive **"Lozen 6"** has shorter coleoptile, though showing the highest coleoptile elongation rate in control. These results do not fully confirm the association of GA-insensitive *Rht* alleles with reduction of coleoptile length, reported by Ellis et al. (2004), but agree with the findings of higher potential of GA-responsive genotypes to produce longer coleoptiles (Botwright et al. 2001). Recently, Clayshulte (2005) studied the associations between coleoptile length, GA-insensitive *Rht* genes and the major allelic classes at *Xgwm261.* They found no relationships between *Rht-B1b* and *Rht-D1b* and coleoptile length and were unable to verify the diagnostic potential of WMS261 microsatellite marker to identify genotypes with long coleoptiles. **"Lozen 6"** has a complex genealogy involving *Aegilops crassa, T. dicoccoides* and the common wheat cv. **"Avrora"** as ancestors (Ganeva et al. 2005). It might be suggested that the GA-responsive *Rht* allele in **"Lozen 6"**, associated with shorter coleoptile, has its origin either from the wild parent, or from the tetraploid wheat. This is in accordance with Ellis et al. (2004), who reported about a class of GA-responsive genes originated from durum wheats, which had reduced coleoptile length.

CONCLUSIONS

The observed genetic variability among the three studied wheat genotypes offers a valuable tool for investigating the mechanisms of drought tolerance. Based on the data, cultivars **"Todora"** and **"Lozen 6"** can be considered drought tolerant at early seedling stage, the tolerance being achieved through different mechanisms. The results of this study suggest that breeding could target creating of genotypes with potential for less reduced root system and less reduced shoots under unfavorable conditions.

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