

The contribution of the gibberellin-insensitive semi-dwarfing (*Rht*) genes to genetic variation in wheat seedling growth in response to osmotic stress

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SUMMARY

The effects of various gibberellin-insensitive semi-dwarfing (*Rht*) alleles and background genotypes on the growth response of wheat seedlings to simulated low water potential were investigated. Four sets of near-isogenic lines, each consisting of six members (*Rht-B1a* + *Rht-D1a* (*rht*), *Rht-B1b*, *Rht-B1c*, *Rht-D1b*, *Rht-B1b* + *Rht-D1b* and *Rht-B1c* + *Rht-D1b*), and one set of five members (*rht*, *Rht-B1b*, *Rht-B1c*, *Rht-B1d* and *Rht-D1b*) were germinated in the presence of polyethylene glycol (PEG). The growth responses were assessed by measuring the lengths of the longest root, coleoptile and longest leaf (shoot) and calculating the root length:shoot length ratio and a tolerance index (TI). Seedling growth was significantly affected by the allelic status at the *Rht* loci, background genes and the water potential. The PEG treatment had major effects on root and shoot growth. Coleoptile growth was mainly affected by the *Rht* alleles. There were significant interactions of the *Rht* allele and variety on the growth response to low water potential. Genotypes with longer roots, coleoptiles and shoots when grown in water, as determined by the *Rht* allelic status (*rht*, *Rht-B1b* and *Rht-D1b*) and varietal background, had the highest TI and maintained this advantage under stress, while genotypes with smaller seedlings (*Rht-B1c* and *Rht-B1c* + *Rht-D1b*) when grown in water were more strongly affected.

INTRODUCTION

In continental climates, autumn-sown bread wheat frequently experiences low water potential early in the season prior to and/or shortly after seedling emergence. Over the course of the 20th century the annual and, in particular, the summer and autumn precipitation has significantly declined in south-eastern Europe (Quarrie *et al.* 1999; Alexandrov *et al.* 2004). Early season drought inhibits plant growth, affects subsequent development and can lead to a substantial loss in yield. Although progress is being made in sensing the soil physical environment (Clark *et al.*

2005), nevertheless the identification early in breeding programmes of genotypes with useful traits is crucial. Thus, the inclusion in selection programmes of drought tolerance-related traits, acting at various stages of plant development, would serve to achieve the important breeding goal of yield stability in the face of the probable occurrence of water deficit. Specific drought tolerance traits include plant size, root elongation, long coleoptiles and early seedling vigour (Quarrie *et al.* 1999; Reynolds *et al.* 2000, 2006; Richards *et al.* 2000). The ability to extend the root system under drought conditions helps the plant to exploit stored water deeper in the soil profile and avoids the inevitable water deficiency closer to the soil surface. Longer coleoptiles ensure successful seedling emergence where deep sowing is employed as an

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Temporal trends in the microsatellite-based genetic diversity of 91 bread wheat varieties released in Bulgaria since 1925¹

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SUMMARY – Patterns of the molecular genetic diversity changes within groups of old and modern Bulgarian bread wheat varieties were analyzed using 19 wheat microsatellite markers and one secalin-specific sequence tagged site marker. A total of 91 genotypes representing almost the complete spectrum of varieties released in Bulgaria during the period 1925-2003 were included in the study. Analysis of microsatellite allele polymorphisms revealed quantitative (different allele frequency) and qualitative (loss of alleles and/or appearance of novel alleles) changes in genetic diversity over decades of variety release. Genetic diversity was high among both old and modern varieties with an average of 0.65. An average heterogeneity of 10.1% was established, this being highest in the old varieties. The genetic diversity was affected by the interaction between the breeding centre and the applied breeding strategy directed towards increasing the productivity or grain quality improvement.

Public concern over potential decline in crop diversity and the necessity of developing appropriate science-based breeding strategy motivated the assessment of current levels of and temporal trends in genetic diversity of a number of crops, including bread wheat (Donini *et al.*, 2000; Chebotar *et al.*, 2003; Khlestkina *et al.*, 2004). Quantitative estimation of the genetic diversity and characterization of diversity trends over time is fundamental for the efficient conservation, maintenance and rational utilization of genetic resources. The present study is an extended analysis of dimensions of genetic diversity and temporal trends in the diversity changes over periods of variety release among Bulgarian bread wheat germplasm as revealed by microsatellites.

The experimental material included 91 winter bread wheat (*Triticum aestivum* L.) varieties released in Bulgaria during the period 1925-2003 at the main breeding centres: Dobrudzha Agricultural Institute (DAI), General Toshevo; Institute of Plant Genetic Resources (IPGR), Sadovo, and Institute of Genetics (IG), Sofia. A total of 19 wheat microsatellite markers covering 17 chromosomes and one secalin-specific STS-marker for rye chromosome arm 1RS were used. A modification of the PCR protocol by Röder *et al.* (1998) was followed. The patterns of genetic variation were analyzed using Nei's measure of genetic diversity (Nei, 1987).

Microsatellite alleles polymorphisms

Analysis of microsatellite allele polymorphisms revealed quantitative and qualitative changes in the number of alleles per locus and the favoured alleles within the groups of old and modern varieties registered before 1960 and afterwards, respectively (Table 1). For six markers the dominating alleles have been maintained during the whole period under investigation, suggesting their importance and selective advantage. For six other, the prevailing allele among the sub-groups of modern releases is different in comparison to the early years of breeding. For the rest, the alleles which dominate within the group of old varieties prevail in the modern varieties at random. The total number of alleles detected in varieties from each period shows changes over time with the highest number of alleles found in varieties registered after 1990, and the least number of alleles present in 1960s-varieties. Four different patterns of changes in the number of alleles per locus over decades of variety release were recorded. Alleles detected with markers WMS46, WMS155, WMS389, WMS577 and WMS631 displayed a random pattern of change over time, meaning that alleles found in the early years of wheat breeding were randomly present in those registered later on. For markers WMS165, WMS357 and WMS458, a shifting pattern of predominance in two or three of their alleles was displayed. Seven

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Genetic variability of seed longevity in wheat and its implications for biodiversity preservation

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ABSTRACT: Knowledge on the extent of genetic variation in seed longevity is beneficial for the development of strategies for long-term preservation of plant genetic resources. We studied the genetic variability in seed germinability, vigour and deterioration, and seedling growth traits after long-term natural ageing in 75 wheat (*Triticum* sp.) accessions stored for up to 15 years at 0°C and ambient room temperature (RT) in the seed genebank at IPK, Gatersleben, Germany. Germination, vigour (electrical conductivity, EC, and accelerated ageing, AA) and seedling growth tests were performed on all or selected accessions. The study revealed a considerable genetic variation in seed longevity, determined by the genotype, duration and temperature of storage. Correlation between EC and germinability observed for accessions after 5 and 7 years in storage was not preserved with ageing. The subjection of 0°C-seed lots to AA resulted in deterioration effects, which were comparable to those caused by 10 years in storage at RT. The AA tests provided additional tools to differentiate between high and lower vigour accessions among high-germinating ones.

Keywords: accelerated ageing, electrical conductivity, seed longevity, seed vigour, variability, wheat.

Introduction

Plant genetic resources (PGR) comprise diversity of landraces, traditional and modern cultivars, and related wild species. Currently, the majority of plant germplasm is maintained as seed in genebanks under specified conditions. The imperative demands to preserve the natural and introduced genetic variation prompted worldwide actions of the national seed genebanks. A substantial part of their programmes alongside collection and conservation is the comprehensive characterization and evaluation of the PGR (Börner, 2006). The identification of genetic variation within germplasm is indispensable to the effective management and rational utilization of genetic resources. Seed longevity is a function of both storage environment and the genetically imposed seed vigour. The existence of genetic variability for seed longevity and its identification is related to the regeneration frequency, integrity maintenance, avoidance of genetic shifts and contamination, and, therefore, has an impact on both resources preservation strategies and genebank management. Here we present results of a study on genetic

MINERAL TOXICITY STRESS

Physiological Responses of Wheat (*Triticum Aestivum* L.) – *Aegilops Sharonensis* Introgression Lines to Excess CopperV. Nenova¹, M. Merakchiyska¹, G. Ganeva², E. Zozikova¹ & S. Landjeva²¹ Institute of Plant Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria² Institute of Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria**Keywords***Aegilops sharonensis*; excess copper; peroxidase; phenolics; photosystem II efficiency; wheat**Correspondence**

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Abstract

Two wheat lines (TL3 and TL5) derived from selection for Cu tolerance among wheat – *Aegilops sharonensis* hybrids were compared when grown as hydroponics at zero (control), 10^{-6} and 10^{-5} M CuSO_4 . The morphometric measurements showed that the line TL5 with a more inhibited root system under 10^{-6} M CuSO_4 had a less inhibited shoot growth under 10^{-5} M CuSO_4 as compared to TL3. The decreased maximum efficiency of photosystem II and the more pronounced trend towards increased catalase activity suggested that despite the better shoot growth, the leaves of TL5 were more functionally injured. The increased content of carotenoids at excess Cu and the higher peroxidase and catalase activities of TL3 in control plants might contribute to its better stress tolerance. While no significant changes in enzyme activities were found at 10^{-6} M Cu, at 10^{-5} M the activities tended to increase. Although the close values of free phenolics concentrations in control plants, at Cu excess their content was higher in TL5 compared to TL3. The free phenolics content in roots at 10^{-6} M Cu decreased, and although higher at 10^{-5} M Cu, it remained below the control in TL3, and above the control in TL5. From the obtained results TL3 emerged to be more tolerant to excess Cu than TL5.

Introduction

Copper (Cu) is a microelement with numerous important functions in plants, but when in toxic concentration it can cause different physiological and morphological disorders (Yruela 2005). In wheat plants under excess Cu changes have been observed in: the content of nutrients, photosynthetic pigments and proteins; the activities of a number of enzymes; photosynthesis; chloroplast structure; growth and development (Lanaras et al. 1993, Ciscato et al. 1997, Sgherri et al. 2001, Bálint et al. 2002, Landjeva et al. 2004). One of the main molecular mechanisms of the development of Cu toxicity involves the production of reactive oxygen species (ROS) by autoxidation and Fenton reaction (Schützendübel and Polle (2002), Yruela 2005). The strict control by a number of enzymatic and non-enzymatic mechanisms, including peroxidase (PO), catalase, and phenolics over ROS tissue concentration enables plants to adapt to the variable environment.

Copper toxicity is a problem around copper-producing factories where in addition to industrial pollution there is a naturally high background of Cu. Agricultural production is another factor leading to soil pollution with Cu, as this element is a component of many widely used pesticides. An approach for more efficient use of polluted soils is the introduction of plant varieties, which can overcome the stress of Cu pollution. Heavy metals tolerance in plants, defined as the ability to survive in soils that are toxic to other plants, is manifested by an interaction between a genotype and its environment (MacNair 1993). In a broader sense this term is used for changes that may occur experimentally in the sensitive response to heavy metals (Yruela 2005). Genotypic variability in plant response to high heavy metals concentrations has been demonstrated by a number of authors (Manyowa and Miller 1991, MacNair 1993, Landjeva et al. 2002).

The wild *Aegilops* species are in close phylogenetic relationships with genus *Triticum*. Their wide adaptation

Seed conservation in *ex situ* genebanks—genetic studies on longevity in barley

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Abstract Recognizing the danger due to a permanent risk of loss of the genetic variability of cultivated plants and their wild relatives in response to changing environmental conditions and cultural practices, plant *ex situ* genebank collections were created since the beginning of the last century. World-wide more than 6 million accessions have been accumulated of which more than 90% are stored as seeds. Research on seed longevity was performed in barley maintained for up to 34 years in the seed store of the German *ex situ* genebank of the Leibniz-Institute of Plant Genetics and Crop Plant Research in Gatersleben. A high intraspecific variation was detected in those natural aged accessions. In addition three doubled haploid barley mapping populations being artificial aged were investigated to study the inheritance of seed longevity. Quantitative trait locus (QTL) mapping was based on

a transcript map. Major QTLs were identified on chromosomes 2H, 5H (two) and 7H explaining a phenotypic variation of up to 54%. A sequence homology search was performed to derive the putative function of the genes linked to the QTLs.

Keywords *Ex situ* collections · Germplasm · Long term seed storage · QTL mapping · Seed longevity

Introduction

World-wide existing germplasm collections contain more than 6 million accessions of which wheat represents the biggest group with about 800,000 samples followed by barley and rice comprising about 500,000 and 420,000 accessions, respectively. A list of the ten world-wide largest germplasm collections by crop is given in Table 1 (FAO 1998).

Plant *ex situ* genebank collections comprise seed genebanks, field genebanks and in vitro collections. Species, whose seed can be dried, without damage, down to low moisture contents, can be stored in seed banks. Field genebanks and in vitro storage are used primarily for species which are either vegetatively propagated or which have recalcitrant seeds that cannot be dried and stored for long periods. In addition, perennial species, for example certain forage species, which produce small quantities of seed, and

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Physiological Response of Wheat Seedlings to Mild and Severe Osmotic Stress

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In the present study the physiological status of two wheat (*Triticum aestivum* L.) cultivars subjected to polyethylene glycol-induced dehydration is evaluated. Wheat seedlings were exposed to either 8-d-long mild (15% PEG) or 24-h-long severe (30% PEG) osmotic stress by immersing their roots in PEG-supplemented Knop nutrient solution. Relative water content in the leaves and the levels of free proline, malondialdehyde, and hydrogen peroxide were chosen as indicative parameters corresponding to the degree of stress of the treated plants. Electrolyte leakage from leaf tissues of control and stressed plants was compared in terms of the common parameter Injury index used for characterizing cell membrane stability. In addition, a model test system was established for preliminary stress evaluation based on the kinetics of ion leakage. Short term exposure to higher concentration of PEG was considered to be more harmful than prolonged mild stress as judged by RWC, proline and hydrogen peroxide accumulation, and injury index. The two cultivars demonstrated more obvious dissimilarities under conditions of prolonged mild stress than under severe stress.

Keywords: electrolyte leakage, hydrogen peroxide, malondialdehyde, osmotic stress, polyethylene glycol, proline, wheat

Introduction

To maintain productivity while growing in unfavorable conditions is essential to all crop plants. Some physiological traits have been associated with yield potential (Richards et al. 2002; Hoffmann and Burucs 2005) suggesting that selection for such traits could be useful for screening physiologically superior genotypes to improve genetic yield gains. Drought is among the most damaging abiotic factors (Smirnov 1998). Besides its direct impact on water status osmotic stress often harmfully affects plant cell membranes. Symptoms of these adverse processes include oxidation of unsaturated fatty acids, protein degradation, and the resultant loss of selective permeability of membranes (Smirnov 1993; Hoekstra and Golovina 1999). To overcome or avoid lethal dehydration of their tissues plants react to low water potentials by increasing the intracellular concentration of 'compatible' solutes in a process known as osmotic adjustment (Serraj and Sinclair 2002; Larher et al.

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VARIABILITY AMONG FIVE BULGARIAN WHEAT CULTIVARS FOR SEEDLINGS RESPONSE TO IRON DEFICIENCY

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Summary. Iron-deficiency chlorosis brings about significant yield losses in numerous crops. The introduction of resistant genotypes is one possible solution to the problem. In this study the development of Fe deficiency symptoms (growth depression and yellowing of the youngest leaves) at early stages of growth in five Bulgarian cultivars of bread wheat grown hydroponically was compared. Chlorophyll fluorescence measurements were also carried out in order to assess the extent to which the stress had damaged the photosynthetic apparatus. Although at day 10 some effects of Fe deficiency on root length and chlorophyll content were observed, a differentiation among the cultivars could not be found. At day 17 the shoot growth was also affected and the chlorophyll as well as the carotenoid contents were lower. Sadovo 1, which is standard for the registration of new varieties in Bulgaria, was the most Fe-efficient among the studied cultivars, characterized by the smallest decrease in root length, shoot biomass, chlorophyll and carotenoid contents, and relative growth for the 7-day period. In Gladiator 113, Iskur 45 and Sadovska belija, together with a greater drop in the abovementioned parameters, lower actual PS II efficiency and higher amount of light, dissipated thermally by the PS II antenna were found, thus suggesting a greater susceptibility to Fe deficiency. Under optimum Fe supply Lozen 6 had the greatest biomass and relative growth, and high chlorophyll content. Under Fe deficiency it was characterized by the largest reduction in relative growth and in root length, but by a comparatively low decrease in chlorophyll content and by unchanged fluorescence parameters. The detected variability in the early responses of the studied cultivars to Fe deficiency might be important for the early establishment of seedlings and thus, it might be related to iron-efficiency in field conditions.

Key words: chlorophyll fluorescence, chlorosis, growth, iron deficiency, wheat cultivars

Abbreviations: Chl – chlorophyll; Fe – iron; PS II – photosystem II

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Genetic mapping within the wheat D genome reveals QTL for germination, seed vigour and longevity, and early seedling growth

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Abstract Quantitative trait loci (QTL) controlling germination, seed vigour and longevity, and early seedling growth were identified using a set of common wheat lines carrying known D genome introgression segments. Seed germination (capacity, timing, rate and synchronicity) was characterized by a standard germination test, based either on the 1 mm root protrusion (germination *sensu stricto*) or the development of normal seedlings. To quantify seed vigour, the same traits were measured from batches of seed exposed for 72 h at 43°C and high (ca. 100%) humidity. Seed longevity was evaluated from the relative trait values. Seedling growth was assessed both under non-stressed and under osmotic stress conditions. Twenty QTL were mapped to chromosomes 1D, 2D, 4D, 5D, and 7D. Most of the QTL for germination *sensu stricto* clustered on chromosome

1D in the region *Xgwm1291–Xgwm337*. A region on chromosome 7DS associated with *Xgwm1002* harboured loci controlling the development of normal seedlings. Seed vigour-related QTL were present in a region of chromosome 5DL linked to *Xgwm960*. QTL for seed longevity were coincident with those for germination or seed vigour on chromosomes 1D or 5D. QTL for seedling growth were identified on chromosomes 4D and 5D. A candidate homologues search suggested the putative functions of the genes within the respective regions. These results offer perspectives for the selection of favourable alleles to improve certain vigour traits in wheat, although the negative effects of the same chromosome regions on other traits may limit their practical use.

Keywords Accelerated ageing · *Aegilops tauschii* · Plant genetic resources · *Triticum aestivum*

Abbreviations

| | |
|------|--|
| AA | Accelerated ageing |
| CS | Chinese Spring |
| DILs | D genome introgression lines |
| IMA | Interval mapping analysis |
| ITMI | International Triticeae Mapping Initiative |
| LI | Longevity index |
| LOD | Logarithm of odds |
| PEG | Polyethylene glycol |
| SMA | Single marker analysis |
| QTL | Quantitative trait locus (loci) |
| SGT | Standard germination test |

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Genetic diversity assessment of Bulgarian durum wheat (*Triticum durum* Desf.) landraces and modern cultivars using microsatellite markers

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Abstract The genetic diversity in a *Triticum durum* Desf. collection, consisting of 102 Bulgarian landraces, nine Bulgarian and 25 introduced cultivars was studied using 14 highly polymorphic microsatellite markers. A total of 100 alleles were identified, with an average of 7.14 alleles per marker. The gene diversity values (H_e) of the markers for the total samples ranged from 0.23 (WMS357 and WMS631) to 0.77 (WMS46), with an average of 0.52. Within the landraces that were collected from 18 sites in Southern Bulgaria showed 2–11 alleles per locus with an average of 6.07. The microsatellite analysis suggests that the genetic diversity among landraces is lower compared to the diversity levels for durum wheat in countries close to the main centers of wheat domestication. Breeding activities have caused significant reduction of the

allelic polymorphism, elimination of rare alleles, and increase in the number of common alleles and the frequency of dominating alleles.

Keywords Genetic diversity · Landrace · Microsatellites · *Triticum durum*

Introduction

Triticum durum Desf. is the second widespread *Triticum* species constituting 10–11% of the world wheat crop and accounting for about 8% of the total wheat production. The countries in the areas where the initial wheat domestication and cultivation took place (The Mediterranean and Southern Europe, The Balkans, North Africa, and South-Western Asia) are still among the leaders of durum wheat production (Ivanov 1927; Bozzini 1988; Srivastava et al. 1988; Wang et al. 2007). The durum wheat growing area is restricted because of the insufficient cold resistance and spring growth habits of most of the traditional and modern cultivars (Zhukovsky 1964). One of the most important sources of genetic material for improving plant adaptability and grain quality are the local populations, a product of the natural selection but also a result of the thorough domestication and artificial selection carried out by generations of farmers throughout the years.

In Bulgaria, the durum wheat has been known since ancient times (Ivanov 1927). Seeds of durum wheat were present in archaeo-botanical materials dated to

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Gliadins of Bulgarian durum wheat (*Triticum durum* Desf.) landraces: genetic diversity and geographical distribution

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Abstract The polymorphism of gliadins was studied in 98 Bulgarian durum wheat (*Triticum durum* Desf.) landraces and classified according to the existing catalogues of blocks of gliadin components. In total, 31 alleles, including 12 new ones, were revealed for five gliadin-coding loci. Nine allele families, which included several alleles coding similar blocks differed only by minor components, were found. The gliadin loci had a high genetic diversity ($H = 0.70$), and *Gli-A2^d* was the most polymorphic locus. Significant differences in allele distribution were observed through the Bulgarian region under study. The results made it possible to explain the distribution by historical factors. Presumably, the genetic material flew into the country via two different ways and different durum wheat subgroups contributed to the formation of Bulgarian landraces. The landraces were a result of long-term selection and, probably, had a close association with the history of the human populations of the region.

Keywords Durum wheat · Genetic diversity · Gliadins · Landraces · *Triticum durum*

Introduction

Wheat is one of the most ancient and widespread crops. Several millennia of its natural and artificial selection have led to its huge genetic diversity, which is essential for current and future human well-being. Plant diversity can be characterized with the use of molecular genetic markers (Schulman 2007; Spooner et al. 2005; Landjeva et al. 2007). In the case of wheat, the alleles of gliadin-coding genes are highly efficient as genetic markers, because they have a codominant inheritance, are stable through many generations, are independent of the growth conditions, and are cheap to analyze (Kudryavtsev and Metakovsky 1993).

Gliadins are seed storage proteins that are extractable with 70% ethanol (Osborne 1907) as a mixture of protein molecules, which can be separated by one-dimensional electrophoresis (Bushuk and Zillman 1978). Gliadins are controlled by gliadin-coding genes, which are located on the short arms of the homeologous chromosomes of groups 1 and 6 (Payne et al. 1984a; Kudryavtsev 1994). It has been demonstrated that all components of the electrophoretic spectra of gliadin controlled by the same locus are inherited together as a

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ALLELE VARIATION IN LOCI FOR ADAPTIVE RESPONSE AND PLANT HEIGHT AND ITS EFFECT ON GRAIN YIELD IN WHEAT

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ABSTRACT

In the present study the microsatellite marker Xgwm261, linked to Rht 8 gene as well as allele specific markers for Ppd-D1 (Ppd1), Vrn-1A and Vrn-1D loci, and the gibberellic acid (GA) test were employed to examine the distribution of semi-dwarf and photoperiod response and vernalization genes in 21 old and modern Bulgarian, 4 introduced cultivars and 11 advanced breeding lines from the wheat germplasm collections of DAI, General Toshevo, Institute of Genetics, Sofia and IPGR, Sadovo. Several allele variants were identified at locus Xgwm261, among which the 192 bp allele can serve as a diagnostic marker for the presence of Rht 8 gene. The Ppd-D1a, vrn-A1 and vrn-D1 alleles were found in almost all wheat genotypes illustrating the relationship between the photoperiod and vernalization response and the adaptability to the regional environments. The photoperiod insensitivity allele (Ppd-D1a) was found in 93.3% of analyzed modern cultivars released during 1960-2003 and has been most probably introduced from the Ukrainian cultivar Bezostaya1 (192 bp allele at locus Xgwm261), which is the main donor of Rht 8 gene in Bulgarian wheat. All studied modern Bulgarian wheat cultivars excluding cv. Laska (vrn-A1, Vrn-D1) and cv. Gladiator 113 (Vrn-A1a, vrn-D1) were of winter type (vrn-A1 and vrn-D1). Allele Vrn-A1c determining also spring type was not found in the studied set of the old and modern Bulgarian wheat cultivars.

The association between specific alleles at the Rht-B1, Xgwm261, Ppd-D1, Vrn1 loci with plant height, heading time and some yield related traits was also examined. The results suggested that allele combination Ppd-D1a/vrn-A1, vrn-D1 led to earlier heading than the allele combination Ppd-D1b/vrn-A1, vrn-D1.

The recombinant genotypes obtained from the crosses between photoperiod sensitive spring and photoperiod insensitive winter type wheat behave differently under autumn and spring sowing. Under early spring sowing the earliest heading dates and yield per m² were observed in lines with Ppd-D1a/Vrn-A1, vrn-D1 and Ppd-D1b/Vrn-A1, vrn-D1 allele combinations.

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Keywords: adaptation, allele variation, vernalization genes (*Vrn*), photoperiod response (*Ppd*) and plant stature (*Rht*), polymerase chain reaction (PCR), wheat (*T. aestivum* L.)

Abbreviations: DAI - Dobroudja Agricultural Institute, General Toshevo, Bulgaria; IPGR - Institute of Plant Genetic Resources, Sadovo, Bulgaria;

Introduction

The adaptation of wheat (*T. aestivum* L.) to diverse environmental conditions is greatly influenced by variation in flowering time (30) that is mainly controlled by few major genes. These include genes involved in vernalization response (*Vrn* genes), photoperiod response (*Ppd* genes) and developmental rate genes (earliness per se, *Eps* genes). The expression of the first two groups of genes is environmentally dependant while the third one is not influenced by the environment conditions.

Cultivated wheat (*T. aestivum* L.) is usually classified as spring or winter growth habit according to the effect of low temperature on flowering time. Winter wheat requires a cold

period to induce flowering and is normally planted in the autumn for harvesting in the next year while spring wheat flowers without cold treatment and is typically sown and harvested in the same year. Winter and spring habit are controlled by three major loci, referred as *Vrn-A1*, *Vrn-B1* and *Vrn-D1* located on chromosomes 5A, 5B and 5D, respectively (24, 36). The molecular genetics mechanism of vernalization requirement in cereal crops including wheat is well understood and reviewed by Cockram et al. and Trevaskis et al. (3, 27). The spring habit alleles at these loci are dominant while recessive alleles at all three loci determine winter growth habit. The spring *Vrn-A1* allele provides complete insensitivity to vernalization whereas spring *Vrn-B1* and *Vrn-D1* alleles provide a reduced vernalization requirement relative to winter alleles.

Photoperiod response in wheat is described as sensitive in the case when wheat genotypes require long days for induction of flowering, while the insensitive genotypes flower independently of the day length. Photoperiod response is closely associated with adaptability and grain yield in wheat varieties (11, 31, 32, 33). Photoperiod insensitive wheat varieties are widespread worldwide. However such varieties

Seedling growth under osmotic stress and agronomic traits in Bulgarian semi-dwarf wheat: comparison of genotypes with *Rht8* and/or *Rht-B1* genes

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Abstract. Early-season drought is an adverse environmental factor affecting seedling emergence and crop establishment in winter wheat grown in continental climates. The different semi-dwarfing (*Rht*, reduced height) genes exert various effects on seedling growth, plant height and productivity. The occurrence of the major *Rht* genes in 69 Bulgarian bread wheat cultivars was postulated by molecular markers. Cultivars carrying *Rht-D1b+Rht8* (1), *Rht-B1b/d* (6), *Rht-B1b/d+Rht8* (19), or *Rht8* (43) were identified. The three latter groups were compared in respect to germination and seedling growth in response to polyethylene glycol-induced osmotic stress, plant height and main yield components. Cultivars carrying only *Rht8* produced longer roots, coleoptiles and shoots, and had smaller root-to-shoot length ratio in non-stress and in osmotic stress conditions compared with those carrying *Rht-B1b/d* or *Rht-B1b/d+Rht8*. The same cultivars produced shorter culms, shorter spikes with fewer spikelets, reduced number and mass of grain in the main spike, and reduced 50-grain mass. The *Rht* gene effects were confirmed on *Rht* near-isogenic lines. The preferential semi-dwarfing allele selection aiming to combine the advantages of *Rht8* and *Rht-B1b/d* alleles in specific eco-climatic conditions and to improve breeding efficiency in wheat is discussed.

Additional keywords: genetic variation, gibberellin-insensitive *Rht* genes, gibberellin-responsive *Rht* genes, near-isogenic lines.

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Introduction

In continental climates with temporary early-season drought, the capacity of germinating seed to withstand water deficiency near the soil surface and develop adequate roots, coleoptiles and shoots is critical for seedling emergence and early growth (Richards *et al.* 2000). Although germination and seedling growth are strongly environment dependent, many studies relate these characteristics with genetic factors (Cui *et al.* 2002; Edney and Mather 2004; Landjeva *et al.* 2008a, 2010). The effects of genotype and the contribution of wheat semi-dwarfing genes (*Rht* genes) to seedling growth variation in non-stress and osmotic stress conditions were presented by Landjeva *et al.* (2008b). Wojciechowski *et al.* (2009) demonstrated the direct effect of wheat *Rht* alleles on root growth during seedling establishment.

Since their introduction in the 1960s, wheat semi-dwarfing genes have been adopted worldwide and are currently present in more than 80% of cultivars registered globally (Hedden 2003). One of the merits of these genes is their positive pleiotropic effect on productivity associated with redirecting of assimilates to grain (Flintham *et al.* 1997; Rebetzke and Richards 2000; Chapman *et al.* 2007). The effects of *Rht* genes on height and yield are modulated by the environment and

genetic background (Börner *et al.* 1993; Blum *et al.* 1997; Chapman *et al.* 2007).

The major *Rht* genes are classified into two groups depending on their response to gibberellic acid (GA). The two agronomically prevailing GA-insensitive genes, *Rht-B1* (on chromosome 4B, with two alleles, *b* and *d*, derived from the old Japanese cultivars ‘Norin 10’ and ‘Saitama 27’, respectively), and *Rht-D1b* (on chromosome 4D, derived from ‘Norin 10’), generally produce reduced coleoptiles, leaves and stem internodes. That is why in cultivars carrying either gene, coleoptile emergence might be hampered in environments with limited water availability where greater sowing depth is required (Schillinger *et al.* 1998). In contrast, the GA-responsive gene *Rht8*, introduced into European wheat germplasm by the Italian breeder N. Strampelli from the Japanese landrace Akakomughi, reduces plant height without affecting coleoptile length and seedling vigour, thus allowing deeper sowing and more efficient moisture utilisation (Rebetzke *et al.* 1999; Rebetzke and Richards 2000; Botwright *et al.* 2001; Ellis *et al.* 2004). The latter gene is more widely distributed in warmer climate zones with frequent periods of drought, and this includes the countries in South-Eastern Europe (Chebotar *et al.* 2001; Ganeva *et al.* 2005; Tošović-Marić *et al.* 2008). Identification of the major

Molecular cytogenetic identification of a wheat-*Aegilops geniculata* Roth spontaneous chromosome substitution and its effects on the growth and physiological responses of seedlings to osmotic stress

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With 6 figures and 1 table

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Abstract

The karyotypic constitution of a wheat-*Aegilops geniculata* 2M^g(2A) disomic substitution line, isolated from backcross progenies of a wheat-*Ae. geniculata* amphiploid, was determined using multicolour fluorescence *in situ* hybridization and genomic *in situ* hybridization. The ability of the 2M^g chromosome to compensate for wheat chromosome 2A was studied in relation to phenotypic traits and the growth and physiological responses of seedlings to polyethylene glycol (PEG)-induced osmotic stress. Plants of the substitution line had altered spike and seed morphologies, increased tillering, moderately reduced productivity and maintained better seedling growth in stress conditions. PEG treatment caused severe stress, resulting in considerable water loss from the leaves. Under this stress, the substitution line retained better leaf water status and suffered less oxidative damage than the wheat parent, as indicated by higher relative water content and lower malondialdehyde accumulation. These changes were consistent with more sustained membrane stability in the substitution line, evidenced by the lower value of the membrane injury index and the less-prominent increase in ion efflux from the apoplast.

Key words: drought — goat-grass — membrane stability — seedling growth — stress tolerance — *Triticum aestivum*

The *Aegilops* genus, which is closely related to *Triticum*, represents a large reservoir of valuable genes that could be exploited for wheat improvement (Schneider et al. 2008). Within the genus, *Aegilops geniculata* Roth (2n = 4x = 28, M^gM^gU^gU^g) has useful agronomic traits, such as good adaptability to extreme climatic conditions, especially to drought (Molnár et al. 2004, Schneider et al. 2008).

An essential step in the transfer of alien chromatin into wheat is the development of chromosome addition and substitution lines (Gale and Miller 1987). Up to now, one complete set of wheat-*Ae. geniculata* addition lines was produced by Friebe et al. (1999), and a few other addition lines were isolated by Landjeva and Ganeva (1999) and Stoilova and Spetsov (2006). Only the 5M^g(5D) wheat-*Ae. geniculata* substitution line has been reported (Dhaliwal et al. 2002).

Detection and identification of alien chromatin introgressed into the wheat genome can be effectively accomplished using the molecular cytogenetic techniques fluorescence *in situ* hybridization (FISH) and genomic *in situ* hybridization (GISH). Multicolour FISH, using repetitive sequences such as pTa71, pSc119 and pAs1 or pAs1-like (Afa family) (described in Sepsi et al. 2008), allowed individual chromo-

somes of both wheat and *Aegilops* species to be distinguished (Mukai et al. 1993, Schneider et al. 2003, 2005, Badaeva et al. 2004, Molnár et al. 2011). With the aid of the GISH technique using differentially labelled total genomic DNA, it was possible to discriminate the U- and M-genome chromosomes in the allotetraploid species *Ae. geniculata* and *Ae. biuncialis* and to visualize the *Aegilops* chromatin in wheat-*Aegilops* introgression lines (Schneider et al. 2005, Molnár et al. 2011).

Wheat/alien substitution lines were used to study homoeologous relationships between wheat and related species and the ability of alien chromosomes to compensate for phenotypic and physiological traits (Molnár et al. 2007). The 5M^g(5D) wheat-*Ae. geniculata* substitution line was used to transfer leaf rust resistance into bread wheat (Dhaliwal et al. 2002). However, no information is available on the ability of any *Ae. geniculata* chromosome to compensate for a wheat chromosome regarding drought tolerance traits.

Water deficit is one of the most widespread abiotic stresses, limiting wheat productivity in drought-prone environments. In continental climates with temporary early-season drought, the capacity of germinating seed to withstand water shortage near the soil surface and to develop adequate roots, coleoptiles and shoots for emergence is critical to early seedling growth (Richards et al. 2000). Much of the injury to plants experiencing drought stress, especially at early developmental stages, is associated with osmotic stress. At the cellular and subcellular levels, osmotic stress triggers various physiological responses, including excessive production of reactive oxygen species (ROS), leading to dysfunction of cell membranes (Smirnov 1993). Oxidative stress is indicated by the accumulation of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) and is manifested as a loss of membrane selective permeability accompanied by ion leakage from the cells (Blum and Ebercon 1981). The ability to maintain cell membrane stability is one of the most characteristic physiological components of stress tolerance (Blum and Ebercon 1981). The degree of cell membrane disturbance is usually expressed as an injury index (Bandurska and Gniazdowska 1995) or is estimated by studying the kinetics of ion efflux (Koicheva et al. 2005).

The treatment of plant roots with solutions of high molecular weight polyethylene glycol (PEG) has been widely used to induce osmotic stress in hydroponic culture systems

**ЕФЕКТ НА ГЕНИТЕ ЗА НИСКО СЪБЛО 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 гиберелин-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 гиберелин-чувствителния ген *Rht8*, шест сорта носят гиберелин-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

Conclusion

The development of new wheat/barley addition lines with two-rowed and six-rowed winter barley cultivars (Igri, Manas) makes it possible to increase the allelic variation in wheat/barley introgression lines. The selection of ditelosomic additions from these lines is in progress, and those can be subjected to chromosome sorting by flow cytometry, thus allowing barley chromosome arms with an allele composition different from Betzes to be sequenced. The wheat/barley introgression lines made it possible to study the effect of the introgressed barley chromosomes (regions) on various morphological and agronomic characters of wheat.

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***Aegilops geniculata* chromosome introgressions into bread wheat and their effects on plant physiological responses to abiotic stress**

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The wild relative of wheat *Aegilops geniculata* Roth (syn. *Ae. ovata* L.) is an allo-tetraploid species (2n=4x=28, genome formula M^sM^sU^sU^s) native to the Mediterranean region,

BRIEF COMMUNICATION

Frost tolerance in winter wheat cultivars: different effects of chromosome 5A and association with microsatellite alleles

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Abstract

Frost tolerance of ten Bulgarian winter wheat (*Triticum aestivum* L.) cultivars (Milena, Pobeda, Sadovo-1, Enola, Kristal, Laska, Svilena, Russalka, No301, and Lozen) and five foreign cultivars (Mironovskaya 808, Bezostaya-1, Rannaya-12, Skorospelka-35, and Chinese Spring) was studied in two experimental seasons following natural cold acclimation and in one experiment carried out in controlled acclimation conditions. Considerable intercultivar variability in plant survival was observed after freezing at -21 °C following sufficient cold acclimation, or at -18 °C following insufficient or controlled acclimation. In seven cultivars, the effects of chromosome 5A on frost tolerance were investigated in their F₂ hybrids with chromosome 5A monosomic lines of cultivars with high, intermediate, and low frost tolerance. The effects of chromosome 5A depended on the stress severity and the genetic background of the hybrids and varied even in cultivars of similar frost tolerance and vernalization requirements. Effects of other chromosomes besides 5A on frost tolerance were assumed. The analysis of six microsatellite loci located in the interval from centromere to *Vrn-1* on chromosomes 5AL, 5BL, and 5DL showed that the major loci determining frost tolerance in Bulgarian winter wheats were *Fr-A2* on chromosome 5AL and, to a lesser extent, *Fr-B1* on chromosome 5BL. A strong association of the 176 bp allele at locus *wmc327* tightly linked to *Fr-A2* with the elevated frost tolerance of cvs. Milena, Pobeda, Sadovo-1, Mironovskaya-808, and Bezostaya-1 was revealed. Relatively weaker association between frost tolerance and the presence of the 172 bp allele at locus *Xgwm639* tightly linked to *Fr-B1* was also observed.

Additional key words: cold acclimation, genetic variability, PCR, QTL, *Triticum aestivum*.

In hexaploid wheat, almost all chromosomes contribute to plant response to low temperature stress (Sutka 2001). Frost tolerance is determined by major loci (*Fr*) and genes controlling plant response to acclimation and vernalization (*Vrn*), both located on the long arms of homoeologous group 5 of chromosomes (Sutka 2001, Tóth *et al.* 2003). The most potent loci, *Fr-A1* and *Fr-A2*, were mapped on chromosome 5AL in close proximity (2 and 30 cM, respectively) to the gene *Vrn-A1* (Galiba *et al.* 1995, Vágújfalvi *et al.* 2003). Orthologous frost

tolerance loci were identified on chromosomes 5B and 5D (Tóth *et al.* 2003). The evaluation of plant genetic resources for variation in frost tolerance and the examination of the association between phenotypic variation and variation at frost tolerance loci could accelerate modern wheat breeding. The presented study aimed at 1) assessment of frost tolerance of Bulgarian bread wheat cultivars under different cold acclimation conditions and freezing temperatures; 2) estimation of the effects of chromosome 5A on frost tolerance, and

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Abbreviations: CBF - C-repeat binding factor; PCR - polymerase chain reaction; QTL - quantitative trait locus.

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Characterization of Two Wheat Doubled Haploid Populations for Resistance to Common Bunt and Its Association with Agronomic Traits

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Two segregating populations of doubled haploid (DH) wheat lines derived androgenetically from crosses ‘Svilena’ (susceptible) × A-38b-4-5-3-3 (highly resistant) and ‘Svilena’ × WWRN (moderately resistant to moderately susceptible) were characterized for resistance to common bunt. Disease incidence was evaluated after inoculation of seeds with a mixture of *Tilletia foetida* teliospores in two autumn sown field experiments. Two-gene model of inheritance of resistance in line A-38b-4-5-3-3 was suggested. The transgressive segregation in the latter population was indicative for a quantitative mode of inheritance. The DH lines were assessed for plant height, heading time and important yield components in a three-year field experiment without bunt infection. In both populations, transgressive segregation was observed for all agronomic characteristics. Although the disease incidence was positively correlated with most of the agronomic traits, genotypes combining bunt resistance with good yield potential were isolated from ‘Svilena’ × A-38b set of lines. These genotypes are valuable for breeding varieties designed for growing in low-input and organic farming systems. The two DH populations are suitable to be used for further studies on the genetic basis of bunt resistance.

Keywords: common bunt, doubled haploid population, genetic control, *Tilletia* sp., wheat

Abbreviations: DH – doubled haploid; QTL – quantitative trait locus; TKW – thousand-kernel weight

Introduction

Common bunt is among the most destructive fungal diseases in wheat worldwide and probably has existed since wheat domestication (Fischer and Holton 1957). Even contamination levels as low as 0.1% can cause dramatic losses in grain yield and quality (Liatukas and Ruzgas 2005). The disease is caused by two soil borne heterobasidiomycete fungi – *Tilletia caries* (DC) Tul. & C. Tul. [syn. *T. tritici* (Bjerk.) G. Winter] and *T. foetida*

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DROUGHT STRESS

Changes in Water Status, Membrane Stability and Antioxidant Capacity of Wheat Seedlings Carrying Different *Rht-B1* Dwarfing Alleles under Drought StressK. Kocheva¹, V. Nenova¹, T. Karceva¹, P. Petrov¹, G. I. Georgiev¹, A. Börner² & S. Landjeva¹¹ Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences Sofia, Bulgaria² Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany**Keywords**

antioxidant enzymes; cell membrane stability; DELLA proteins; drought; osmoregulation; reduced height genes; wheat

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Abstract

Water deficiency is a major constraint to wheat productivity in drought prone regions. The wheat DELLA-encoding height-reducing genes (*Rht*) are associated with significant increase in grain yield. However, the knowledge of their benefit in dry environments is insufficient. The objective of the study was to examine the effect of induced drought on leaf water content, level of oxidative stress, cell membrane stability, accumulation of osmoprotectants and activity of some antioxidant enzymes in wheat near-isogenic lines carrying the alleles *Rht-B1b* (semi-dwarfing) and *Rht-B1c* (dwarfing) in comparison with the tall control *Rht-B1a*. Six-day-long water deprivation was imposed at seedling stage. Plants carrying *Rht-B1c* and, to a lesser extent, those carrying *Rht-B1b* performed better under stress compared with *Rht-B1a* in terms of more sustained membrane integrity, enhanced osmoregulation and better antioxidant defence. These differential responses could reflect pleiotropic effects of the *Rht-B1* gene associated with the accumulation of the mutant gene product, that is, altered DELLA proteins, or might be related to allelic variations at neighbouring loci carrying candidate genes for proteins with a major role in plant water regulations and stress adaptation. These findings might be of importance to breeders when introducing *Rht-B1* alleles into wheat cultivars designed to be grown in drought liable regions.

Introduction

Water deficiency is a major stress factor affecting plants at different developmental stages and at various levels of their organization. In continental climate environments, autumn-sown wheat frequently experiences early season drought stress during seedling establishment. Such early setbacks can constrain the subsequent growth and development and may finally result in a substantial yield reduction (Blum 2011). At the organ and tissue level, the harmful consequences of drought include disturbed balance between water uptake by roots and loss of shoot water through transpiration flow, causing tissue water deficiency and associated osmotic stress. Generation of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂) (Sairam and Saxena 2000, Apel and Hirt 2004, Singh et al. 2012), and accumulation of malondialdehyde (MDA), a secondary by-product of the oxidation of polyunsaturated

fatty acids in cell membranes, are typical symptoms of the development of the accompanying oxidative stress (Selote and Khanna-Chopra 2006). Subsequent lipid peroxidation may disturb membrane integrity and thus lead to loss of selective permeability causing ion leakage from damaged tissues (Blum and Ebercon 1981).

The plant adaptive responses to drought are mainly focused to maintain water homeostasis and membrane stability, and limit the consequences of the developing oxidative stress. A general biochemical adaptation at the cellular level is the accumulation of organic solutes such as amino acids, sugars and polyols which improve osmotic potential and water uptake ability (Chen and Jing 2010, Sperdoui and Moustakas 2012). Besides acting as osmoprotectants, these low molecular weight and highly soluble substances may contribute for maintaining membrane integrity, stabilizing enzymes or proteins and minimizing oxidative damage (Matysik et al. 2002, Chen and Jing 2010). In addition,

DROUGHT STRESS

Wheat *Rht-B1* Dwarfs Exhibit Better Photosynthetic Response to Water Deficit at Seedling Stage Compared to the Wild TypeV. R. Nenova¹, K. V. Kocheva¹, P. I. Petrov¹, G. I. Georgiev¹, T. V. Karceva¹, A. Börner² & S. P. Landjeva¹¹ Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria² Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), OT Gatersleben, Stadt Seeland, Germany**Keywords**DELLA proteins; drought stress; dwarfing genes; leaf anatomy; *Triticum aestivum***Correspondence**V. R. Nenova
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Abstract

Wheat reduced height (*Rht*) genes encode modified DELLA proteins, which are gibberellin insensitive, accumulate under stress, restrain growth and affect plant stress response. The seedling reaction to soil water deficit regarding leaf gas exchange and chlorophyll fluorescence was compared in near-isogenic lines carrying the alleles *Rht-B1a* (tall), *Rht-B1b* (semi-dwarfing) and *Rht-B1c* (dwarfing) and was related to leaf water content and anatomy. Under drought, *Rht-B1c* line was characterized by less decreased CO₂ assimilation, delayed non-stomatal limitation of photosynthesis and higher instantaneous water use efficiency. The functional state of its photosynthetic apparatus was better preserved as evidenced by the less decreased actual quantum yield (Φ_{PSII}) and potential maximum quantum yield (F_v/F_m) of PSII, and the less increased quantum yield of non-regulated energy dissipation (Φ_{NO}). *Rht-B1b* line also tended to perform better than *Rht-B1a*, but differences were less pronounced. Although the leaves of both dwarf lines were smaller, thicker and more pubescent, their water content was not higher in comparison with the tall line. Nevertheless, in *Rht-B1c*, leaf thickness was less decreased and mesophyll cells were less shrunk under drought. The more effective performance of the photosynthetic machinery of dwarf lines under water deficit could be explained by a combination of morpho-anatomical and metabolic characteristics.

Introduction

Soil water deficit is a leading environmental challenge for the developing plant. It triggers various physiological responses among which changes in photosynthesis (Lawlor and Tezara 2009) and modifications in leaf anatomy (Vasileva et al. 2012). The adverse effects of this stress on photosynthesis lead to plant growth inhibition and eventually to substantial loss in yield. Wheat is rather sensitive to drought and genetically determined differences in resistance to water stress among genotypes exist (Molnár et al. 2004, Wu and Bao 2011a,b, Marcińska et al. 2013). One of the mechanisms for adaptive modulation of plant growth is mediated by DELLA proteins. They are nuclear transcriptional regulators with central role in gibberellin (GA) signalling. Under stress, these proteins accumulate and

both restrain growth and increase plant survival (Achard et al. 2008). As DELLAs do not have a clearly identified DNA-binding domain, they are likely to regulate the expression of their target genes by interacting with other transcription factors. DELLAs are rapidly subjected to proteolysis after becoming part of a complex with GA and the GA-GID1 receptor (GA-INSENSITIVE DWARF1) (Sun 2010). In wheat, DELLAs are encoded by the wild counterparts of the reduced height (*Rht*) genes. Dwarfs with a broad range of plant height have been achieved through the introduction of mutant alleles at the *Rht-B1* and *Rht-D1* locus on chromosomes 4B and 4D, respectively. Mutations in these genes affect DELLAs binding to GID1, but not their transcriptional regulatory activity (Pearce et al. 2011). The result is size reduction at cellular, organ and whole plant level due to hampered GA-induced degradation of

*Brief communication***Variation in ion leakage parameters of two wheat genotypes with different *Rht-B1* alleles in response to drought**

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The reaction to soil drying was evaluated in two *Triticum aestivum* near-isogenic lines carrying different alleles of the height-reducing gene *Rht-B1* based on an improved method for assessment of electrolyte leakage. The two lines were previously shown to differ in their physiological responses to induced water deficit stress. Drought was imposed for 6 days on 10-day-old seedlings. Ion efflux from leaves was measured conductometrically in multiple time points during the 24 h incubation period, and the obtained biphasic kinetics was interpreted according to a previously developed theoretical model proposing different leakage rates through the apoplast and the symplast. Most of the model parameters were able to properly differentiate the two closely related genotypes. The mutant *Rht-B1c* displayed lower and slower electrolyte leakage in comparison with the wild-type *Rht-B1a*. It was speculated that the *Rht* genes expressing defective DELLA proteins might be involved in water stress response through modulation of cell wall stiffness, which influences its capacity for ions retention, and also by their contribution to ROS detoxification, thus indirectly stabilizing cellular membranes. The presented analytical approach relating processes of ion and water flow in and out of the cell could be used for characterization of membrane and cell wall properties of different genotypes under normal and stress conditions.

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1. Introduction

Development of water deficit stress in plants is the common consequence of the unavailability of water to cells in conditions such as drought, high salinity and extreme temperatures (Hare *et al.* 1998; Sperdouli and Moustakas 2012). Cell membranes are the main targets of damage, which is generally associated with metabolic disturbances, leading to generation of reactive oxygen species (ROS) (Hare *et al.* 1998; Thapa *et al.* 2011; Kocheva *et al.* 2014b).

The most widely employed technique for assessment of membrane integrity is by estimating the Injury Index based on ion leakage from damaged plant tissues (Blum *et al.* 1997; Prášil and Zámečník 1998; Farooq and Azam 2006; Chipilski *et al.* 2012). Electrolyte leakage was used as a selection marker for differentiation of drought-tolerant

genotypes, which tended to leak at lower rate compared to sensitive ones (Whitlow *et al.* 1992; Pelah *et al.* 1997; Bajii *et al.* 2002; Roy *et al.* 2009). However, inability to distinguish ion fluxes through different cellular compartments is one disadvantage of the method. This problem was recently solved by establishing a kinetic approach based on measurement of ion leakage from plant tissues at multiple time points (Kocheva *et al.* 2005). Diffusion processes between the apoplast and the symplast have been elucidated lately by introducing the rates of ion fluxes through cell walls and membranes, respectively, as promising parameters for evaluation of leaf water status (Kocheva *et al.* 2014a). The proposed improved model suggested that not only membrane permeability but also diffusion through the cell wall (apoplast) were influenced by dehydration.

Keywords. Cell wall; drought; electrolyte leakage; membranes; semi-dwarfing genes; wheat

THE GENETIC APPROACH TO PHYSIOLOGICAL STUDIES IN BREAD WHEAT

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Summary: Genetics has long been applied as a tool to dissect biological processes in plants. Using genetic approaches, the phenotypic variation for many traits has received physiological and molecular genetic characterization. The natural variation for most physiological traits such as developmental processes, vegetative growth, nutrient uptake and utilization, stress tolerance and adaptation, etc., is continuous and determined by multiple genes. In addition, these traits are environmentally dependent and, therefore, their genetic analysis requires a quantitative trait locus (QTL)-based approach in which linkage maps are combined with phenotypic data. The large genome and the allopolyploid nature of bread wheat (*Triticum aestivum* L.) have long been substantial barriers to genetic dissection of quantitative traits. The boost in molecular marker technologies, the generation of dense linkage maps, coupled with improved statistical methods and development of precise genetic stocks have immensely increased the power of genetic analysis of complex physiological traits in wheat. In this paper, recent applications to two important phenology processes in bread wheat – seed germination and flowering time, are presented. These are examples of using QTL approach and novel segregating populations to map responsible loci followed by the use of the homology-based understanding of plant gene functionality to suggest the putative function of the candidate genes within the regions harboring the detected QTL. The gained knowledge could aid the fine tuning of seasonal phenology in wheat cultivars tailored for growing in specific environments and in response to climatic changes.

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Key words: Flowering time; genetic mapping; plant physiology; QTL analysis; seed germination; wheat.

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PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE (PGRFA) – MAINTENANCE AND RESEARCH

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Summary: Plant genetic resources play a major role for global food security. The most significant and widespread means of conserving plant genetic resources is *ex situ* conservation. Most conserved accessions are kept in specialized facilities known as genebanks. World-wide 7.4 million accessions are stored in 1 750 *ex situ* genebanks. One of the ten largest *ex situ* collections of our globe is located at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, safeguarding about 150 000 accessions from 3 212 plant species and 776 genera comprising wild and primitive forms, landraces as well as old and more recent cultivars.

Since the majority of genebank holdings globally are stored as seed, seed storability is of exceptional importance for germplasm preservation. At IPK, research on seed longevity was initiated for a range of crops maintained over decades. Variation between and within crop species was detected and genetic analyses were initiated. Results obtained for several crop species are presented.

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Keywords: *Ex situ* conservation; Genetic mapping; Germplasm; Long term seed storage; Seed longevity.

Plant Genetic Resources – The global view

The *ex situ* conservation of plant genetic resources for food and agriculture (PGRFA) is a global concern and plays a central role for food security in the future. The germplasm is conserved in genebanks

supported by public or private institutions. *Ex situ* genebank collections comprise seed genebanks, field genebanks and collections maintained via *in vitro* or cryo preservation. Most species native to temperate climate

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Genetic diversity of old bread wheat germplasm from the Black Sea region evaluated by microsatellites and agronomic traits

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Abstract

Old germplasm is an important genetic resource for enhancing modern crops with new alleles. In the present study, the genetic diversity of 52 historic varieties and landraces of bread wheat originated from the Western (Bulgaria) and Northeastern (Ukraine, Russia and Georgia) regions of the Black Sea basin was assessed based on microsatellite markers and agronomic characteristics. A set of 24 markers detected a total of 263 alleles at 25 microsatellite loci, with an average number of 10.5 alleles per locus and an average polymorphic information content (PIC) of 0.74. A total of 63 alleles at 22 loci were unique, being specific to a particular accession. Half of the alleles (132) were regionally specific, and the rest were common between the Western and Northeastern accessions. The latter group was characterized with greater total and private allelic richness, a higher number of unique alleles and a higher average PIC. The population was found to be very heterogeneous (average heterogeneity 41%), with the Northeastern pool (52.8%) being more diverse than the Western pool (30.9%). Most of the accessions of the Western group clustered together, and the rest were distributed among the subclusters of the Northeastern germplasm. Large inter-group differences in the frequencies of alleles ranging from 3.1 at *Xgwm294-2A* to 16.7 at *Xgwm333-7B* were observed. This variation might partly account for the differences in certain yield-related traits. The Northeastern accessions had significantly longer spikes with more number of spikelets. Some issues related to germplasm preservation in seed genebanks are discussed herein. The large molecular variation observed could be utilized by breeders for the selection of diverse parents, or by researchers for the production of mapping populations.

Keywords: agronomic traits; historic/traditional variety; landraces; molecular diversity; *Triticum aestivum* L.

Introduction

Landraces and historic varieties are a rich portion of the bread wheat gene pool (Newton *et al.*, 2010). On the one hand, while modern varieties are a result of intended

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Genetic dissection of earliness by analysis of a recombinant chromosome substitution double haploid mapping population of bread wheat (*Triticum aestivum* L.) in different geographic regions

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Abstract The synchronization of flowering with the time of most favorable temperatures and light has substantial effects on grain yield and quality. In bread wheat, the major developmental genes determining vernalization requirement (*Vrn-1*) and photoperiod response are used in breeding cultivars adapted to different latitudes and climates. Fine regulation of flowering date is provided by minor genes whose allelic variation is not well studied. Although spring cultivars Saratovskaya 29 (S29) and Yanetzki Probat (YP) carry the same two dominant *Vrn-1* alleles, YP is several days later in flowering compared to S29. The aim of the current study was to establish the chromosomal and map locations of loci determining this difference. Inter-cultivar single chromosome substitution lines S29(YP) and a set of recombinant chromosome substitution double haploid lines grown

at three contrasting climatic and geographic locations in Western Siberia and Europe. The substitution line S29(YP 4D*7A) carrying the entire donor chromosome 4D and an additional fragment of chromosome 7A showed the largest delay in flowering at all sites. A quantitative trait locus (QTL) between microsatellite markers *Xgwm0089* and *Xgwm4736* on chromosome 4D was detected only in Europe following substantially earlier sowing. It was manifested under increasing day length, and, therefore, was regarded as a photoperiod response locus. Another QTL associated with (TG)₈ or 9-(CG)₃ polymorphism of the *TaFTA* gene on chromosome 7A was effective under both long and short days thus representing an intrinsic earliness per se gene. This knowledge could aid the fine regulation of flowering in cultivars tailored for growing in specific agro-climatic conditions.

Electronic supplementary material The online version of this article (doi:10.1007/s10681-015-1500-6) contains supplementary material, which is available to authorized users.

Keywords Flowering date · Substitution lines · Recombinant lines · QTL · Gene mapping

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Diversity within Bulgarian old bread wheat germplasm

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‘Old’, or historic varieties are defined as “relatively homogeneous selections made within existing landraces or in the early stages of formal plant breeding programmes that was once on a variety registration list but are no longer registered” (Serpalay et al., 2011). Being a product of natural and purposeful selection carried out by generations of farmers through the years this old germplasm is highly adaptive to particular ecological conditions. Therefore, it represents a rich source of traits and alleles that should be characterized, evaluated and appropriately utilized to widen the diversity within modern crops.

Wheat (*Triticum aestivum* L.) is the most important crop in Bulgaria. The old Bulgarian germplasm grown to the west of the Black Sea was noted for its stable yield, drought tolerance, high protein content, or good bread-making quality (Majdrakov, 1945).

A collection of 60 historic bread wheat varieties released in Bulgaria from the beginning of last century up to the early 1970s was gradually assembled during the period 2005-2012 (Table 1). It comprises both selections within local forms, and varieties having a local material in their pedigree. The majority of accessions (28) has come from the IPK genebank, followed by the entries kindly provided from the Czech genebank at the Crop Research Institute, Prague (25), the Bulgarian genebank at Sadovo (6), and the Dobrudzha Agricultural Institute (1).

Brief characterization of the collection

For 42 out of 60 accessions, the origin - either collection site or release breeding station, is known based on the information listed in the genebanks’ databases. Most of the old varieties have come from local breeding stations or nearby farmers’ selections from North-Eastern territories and from the central Southern part of the country. Few entries have come from breeding centres near Black Sea coast and Sofia valley.

The botanical composition of the collection was determined on the basis of 5 highly heritable traits: presence of awns, colour of awns, colour of glumes, colour of grain, pubescence. The *erythrosperrum* (27) and *ferrugineum* (20) varieties were prevailing, the rest of accessions were of *lutescens* (7), *graecum* (2), *sardoum* (1), *milturum* (1), *hostianum* (1), and *pseudohostianum* (1) type.

The information about the pedigree of the historic varieties was scarce. Eight of the accessions have varieties Noé and/or Mentana as parents that have been crossed with local varieties (Martynov et al., 2006). Noé itself is a selection from a local wheat from Odessa region (Martynov et al., 2006). The Italian varieties Fortunato and San Pastore are parents in the pedigree of three other varieties, contributing shorter stem and earliness. One variety (Burgas-1) has received the wheat-rye 1B.1R translocation from the German variety Neuzucht.

**ПРОДУКТИВНОСТ И АЗОТНА ЕФЕКТИВНОСТ
ПРИ ОБИКНОВЕНАТА ПШЕНИЦА – СРАВНИТЕЛЕН АНАЛИЗ
НА СТАРОДАВНИ И СЪВРЕМЕННИ БЪЛГАРСКИ СОРТОВЕ**

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Резюме

Значителното нарастване на световните добиви при пшеницата след Зелената революция се дължи до голяма степен на глобалното интродуциране на ниски сортове и на 7-кратното увеличение на употребата на азотни торове. В България всички съвременни сортове обикновена пшеница, създадени след 1960-те години, носят гени за ниско стъбло. Стародавните високостъблени сортове са създадени в условия на слабо обезпечаване с хранителни елементи и следователно, представляват източник на генетично вариране за селекция на генотипи, подходящи за отглеждане в условия на по-слабо торене и за нуждите на биологичното земеделие. В настоящето проучване се прави сравнение между 79 съвременни и 21 стародавни български сортове пшеница по отношение на тяхната продуктивност и азотна ефективност при отглеждане на два варианта на азотно торене в две местонахождения (София и Генерал Тошево). Установени са значими ефекти на факторите „Тип сорт“ (стародавни или съвременни), „Торене“ и „Място“, както и на взаимоотношенията „Тип сорт x Място“ и „Торене x Място“. При екстензивните условия на експерименталното поле в София, характеризиращо се с по-слаба почва и предшественик царевица, по-голяма част от стародавните сортове (18) бяха определени като ефективни и отзивчиви на азотно торене, а повечето съвременни сортове (45) – като неефективни и неотзивчиви на азот. Обратна тенденция беше наблюдавана в Генерал Тошево в условия на излужен чернозем и бобов предшественик.

Abstract

Landjeva S., V. Koutev, N. Tsenov, P. Chamurlijski, T. Trifonova, V. Nenova, T. Kartseva, K. Kocheva, P. Petrov, G. Georgiev, 2014. Productivity and nitrogen use efficiency in bread wheat – comparative analysis of old and modern Bulgarian cultivars

The worldwide yield outbreak in wheat following the Green Revolution has been associated with the introduction of semi-dwarf nitrogen (N)-responsive high-yielding cultivars, as well as with substantial increase in N fertilizers consumption. In Bulgaria, all modern cultivars released after 1960s are semi-dwarf. The old tall-stature cultivars have been developed mostly in environments with low nutrient availability and, therefore, represent a source of genetic variation for selection of genotypes suitable for growing in low-input or organic farming



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Research article

The wheat mutant DELLA-encoding gene (*Rht-B1c*) affects plant photosynthetic responses to cadmium stressAnelia G. Dobrikova^{a,*}, Ekaterina K. Yotsova^a, Andreas Börner^b, Svetlana P. Landjeva^c, Emilia L. Apostolova^a^a Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria^b Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), D-06466 Stadt Seeland, OT Gatersleben, Germany^c Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

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Rht-B1c mutant

ABSTRACT

The sensitivity to cadmium (Cd) stress of two near-isogenic wheat lines with differences at the *Rht-B1* locus, *Rht-B1a* (tall wild type, encoding DELLA proteins) and *Rht-B1c* (dwarf mutant, encoding modified DELLA proteins), was investigated. The effects of 100 μM CdCl_2 on plant growth, pigment content and functional activity of the photosynthetic apparatus of wheat seedlings grown on a nutrient solution were evaluated through a combination of PAM chlorophyll fluorescence, oxygen evolution, oxidation-reduction kinetics of P700 and 77 K fluorescence. The results showed that the wheat mutant (*Rht-B1c*) was more tolerant to Cd stress compared to the wild type (*Rht-B1a*), as evidenced by the lower reductions in plant growth and pigment content, lower inhibition of photosystem I (PSI) and photosystem II (PSII) photochemistry and of the oxygen evolution measured with Clark-type and Joliot-type electrodes. Furthermore, the enhanced Cd tolerance was accompanied by increased Cd accumulation within mutant plant tissues.

The molecular mechanisms through which the *Rht-B1c* mutation improves plant tolerance to Cd stress involve structural alterations in the mutant photosynthetic membranes leading to better protection of the Mn cluster of oxygen-evolving complex and increased capacity for PSI cyclic electron transport, protecting photochemical activity of the photosynthetic apparatus under stress. This study suggests a role for the *Rht-B1c*-encoded DELLA proteins in protective mechanisms and tolerance of the photosynthetic apparatus in wheat plants exposed to heavy metals stress.

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1. Introduction

Cadmium (Cd) is a major heavy-metal pollutant. Its bioaccumulation in plants represents a serious health risk to animals and humans. In soil, Cd is usually found naturally at low concentrations and can affect the normal growth and development of many plant species (for review, see [Tran and Popova, 2013](#)). Plants grown in hydroponic media accumulate Cd in their organs (roots, stems and leaves) and in chloroplasts ([Lysenko et al., 2015](#)). Changes in leaves include alterations in chloroplast ultrastructure, low pigment (chlorophyll and carotenoids) content and inhibition of photosynthesis ([Hakmaoui et al., 2007](#); [Moussa and El-Gamal, 2010](#); [Parmar et al., 2013](#)). A number of studies have shown that

Cd toxicity first affects pigment content, then results in inhibition of the photosynthetic activity ([Maurya et al., 2008](#); [Mohamed et al., 2012](#); [Lysenko et al., 2015](#)). Moreover, the effects of Cd on the photosynthetic apparatus and the degree of damage vary depending on the genotypic and ecotypic differences among plant species (see [Parmar et al., 2013](#)).

Photosynthesis is one of the most important targets of Cd action, as the main effect of Cd is inhibition of the photochemical activity of both photosystem I (PSI) and photosystem II (PSII) ([Atal et al., 1991](#); [Chugh and Sawhney, 1999](#); [Wodala et al., 2012](#); [Parmar et al., 2013](#)). However, during the initial stages of Cd toxicity, PSII electron transport and especially the oxygen-evolving complex (OEC) are more susceptible than PSI to the toxic effects of Cd ([Clijsters and Assche, 1985](#); [Chugh and Sawhney, 1999](#); [Sigfridsson et al., 2004](#); [Wang et al., 2013](#)). Previous results have indicated that Cd ions have multiple effects on both the donor and the acceptor side of PSII ([Sigfridsson et al., 2004](#)).

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Photosynthetic Responses of a Wheat Mutant (*Rht-B1c*) with Altered DELLA Proteins to Salt Stress

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Abstract Salinity increases in the world's land area and significantly affects the rate of photosynthesis and corresponding plant growth. In this study, the impact of salt stress (200 mM NaCl equivalent to an electrical conductivity of 18.6 mS cm⁻¹) on the photosynthetic apparatus and some growth parameters were investigated in wheat DELLA mutant (*Rht-B1c*) and wild-type (*Rht-B1a*) seedlings grown on a half-strength Hoagland solution. Results revealed that salt toxicity was alleviated in the *Rht-B1c* mutant compared to the *Rht-B1a* wild type, as manifested by less-reduced leaf pigment content, relative water content, and photochemical activity of photosystem II (PSII) and photosystem I (PSI) after a 9-day salt exposure of plants. Compared to the wild-type wheat, a higher capacity for PSI-dependent cyclic electron flow, preventing the photosynthetic apparatus from oxidative damage, was observed in the mutant plants before and after salt treatment. In addition, an increase of PsaB proteins

was detected in the mutant plants after long-term salt stress unlike the wild type. The observed higher oxidation level of P700 (P700⁺) in the mutant was consistent with higher abundance of PSI-related protein complexes. The data demonstrated that alterations in thylakoid membrane proteins and/or their structural reorganization in wheat DELLA mutant (*Rht-B1c*) significantly contribute to the alleviation of salt-induced damage of the photosynthetic apparatus. Molecular mechanisms involved in the photosynthetic responses of wheat DELLA mutants to salt stress are discussed.

Keywords Chlorophyll fluorescence · Cyclic electron flow · DELLA proteins · Photochemical activity · Pigments · *Rht-B1c* mutant · Salt stress tolerance

Introduction

Salinity is one of the most severe environmental factors limiting productivity of crop plants (Parida and Das 2005; Munns and Tester 2008). High concentrations of salts (especially the accumulation of toxic ions, such as Na⁺ and Cl⁻) have various effects on plants, including ionic imbalance, dehydration, and inactivation of enzymes, that influence the metabolic processes, photosynthetic efficiency, plant growth, and thus plant yields (Tester and Davenport 2003; Sudhir and Murthy 2004). Depending on the degree of salinity, its duration, and the plant species employed, the increased amount of salts may also cause destruction of the chloroplast envelope and disintegration of the granal thylakoid systems (Shu and others 2012), accompanied by a decrease in the amount of photosynthetic pigments in leaves. Some reports have suggested that salt-induced phytotoxicity can be prompted by the formation of reactive oxygen species (ROS)

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Relationships between leaf morpho-anatomy, water status and cell membrane stability in leaves of wheat seedlings subjected to severe soil drought

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Abstract

The impact of the genotype-specific leaf morphological and anatomical characteristics on the ability of wheat plants to preserve leaf water balance and cell membranes stability under drought stress was investigated. Seedlings of six modern semi-dwarf (carriers of *Rht*, Reduced height genes) and six old tall bread wheat varieties were subjected to soil drought by withholding watering for 6 days. Morpho-anatomical traits (leaf area, perimeter, thickness, stomata and trichome density) of daily watered (control) plants were characterized by light microscopy, scanning and image analyses. The leaf water status in both control and stressed plants was determined by measuring the relative water content (RWC). The leaf cell membranes stability in stressed plants was estimated by conductometric determination of the membranes injury index. On average, the modern semi-dwarf varieties had less leaf area and leaf perimeter, and less dissection index, a parameter characterizing the leaf shape. Under drought stress, the modern genotypes maintained better water balance evidenced by significantly higher leaf RWC and better-preserved the cell membranes stability supported by significantly lower Injury index. The correlations between morpho-anatomical traits in control plants and drought tolerance-related traits showed that the higher the leaf dissection index (i.e. more oblong leaves), the greater the water loss and the leaf membrane damages after desiccation were. The effect of shape of the evaporating surface on the water loss was modelled using wet filter paper. Similar to plant leaves, the evaporation and, respectively, water loss from paper pieces of more oblong shape (i.e. higher dissection index) was more intensive. The elucidation of the impact of the leaf shape on transpiration might contribute to better understanding of the mechanisms used by plants to maintain water reserves during drought stress and could be a basis for developing of simple and fast screening methods aiding the selection of drought tolerant genotypes.

KEYWORDS

leaf dissection index, leaf shape, membrane injury index, *Rht* genes, transpiration, water deficit

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