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***Aegilops* as a source of dietary fiber and drought stress tolerance**

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ABSTRACT

Ae. geniculata and *Ae. biuncialis* are tetraploid species with the U and M genomes. While they are known to have resistance to biotic and abiotic stresses, they are also known for their high nutritional value. The aim of our work was to determine the effects of the addition of *Ae. geniculata* and *Ae. biuncialis* chromosomes on the content and composition of protein and dietary fiber in Chinese Spring and Mv9kr1 wheat lines, as well as the effects of drought stress on these components. The thousand kernel weight (TKW), Kjeldahl protein content, gluten protein composition (glutenin/gliadin ratio and unextractable polymeric proteins Glu/Gli and UPP% by SE-HPLC) and contents of dietary fibers (β -glucan, total (TOT) and water-extractable (WE) pentosans by spectrophotometric assays) were measured on whole meal samples. The structures of the β -glucan and arabinoxylan (AX) fractions were also compared by enzyme fingerprinting of the released oligosaccharides by HP-AEC. The chromosomal positions of putative orthologs of the key genes determining these components were also identified using *Ae. umbellulata* sequences. Seeds of both *Aegilops* species had higher proportions of β -glucan compared to AX (determined as pentosans) than wheat lines; elevated β -glucan contents were also observed in wheat chromosome addition lines 5U, 7U and 7M. The pentosan content in wheat was increased by the addition of chromosomes 5U^e, 7U^e and 1U^b while WE-pentosan was primarily increased by the addition of 5U, 5M and 7M. Chromosomes 5U^e and 7M^b also affected the structure and solubility of wheat pentosans. In general, drought stress resulted in increased contents of protein, TOT- and WE-pentosans in the addition lines, while the TKW and the β -glucan content decreased. The differences found between the wheat/*Aegilops* addition lines and wheat parents under well-watered conditions were also found in drought stress conditions. Furthermore, increased UPP% was found in addition lines 2U^e, 3U^e, 7U^e, 2M^e, 5M^e and 6M^e, while the β -glucan content was higher in lines 1-4U^e, 6U^e, 2M^e, 6M^e and 1U/6U^b under drought stress. The grain composition of the *Aegilops* accessions was more stable under drought stress than that of wheat, and the negative effects of drought stress on both the physical and compositional properties of wheat were reduced especially by the addition of *Aegilops* chromosomes 5U^e and 7M. These results will contribute to the efficient transfer of wild alleles in introgression breeding programs to develop wheat varieties with improved health benefits and drought stress tolerance.

INTRODUCTION

Because of its central role in the human diet, wheat is one of the major sources of dietary fiber (DF): arabinoxylan (AX) and (1-3)(1-4)- β -D-glucan (β -glucan). The health benefits of DF have led to a search for variation in DF content and composition in wild species that can be exploited to improve the DF content of wheat, mainly species in the genus *Triticum*. However, these efforts have neglected wild relatives of wheat from the genus *Aegilops*, even though these species have been important donors of new genes and alleles for wheat breeding.

The genus *Aegilops* consists of eleven diploid, ten tetraploid and two hexaploid species with six different genomes (D, S, U, C, N and M), indicating the great genetic diversity of the genus. Biochemical analysis of the grain indicated that the U- and M-genomes of *Aegilops* species are rich reservoirs of genes for improving the nutritional quality of wheat, including micro- and macronutrients. High variation in the

composition of the HMW subunits of glutenin which determine breadmaking quality has also been reported in *Ae. geniculata* by several authors.

Several environmental factors affect the yield and composition of the grain, with drought stress reducing the yield by up to 50%. Generally, drought stress is known to reduce the carbohydrate content of the grain and increase the relative protein content. However, the effects are highly dependent on the degree and timing of the drought and on interactions with other environmental stresses (Rakszegi et al. 2014).

Improving the stability of grain quality and quantity under drought conditions is an important breeding target, as major research programmes exist to develop drought-resistant wheat cultivars. Wild relatives have been proposed as genetic resources for improving the stress tolerance of wheat, including *Aegilops*. Several useful agronomic traits have already been transferred from wild *Aegilops* species to wheat by developing wheat-*Aegilops* hybrids, disomic addition and translocation lines. However, less information is available on the grain quality of wild *Aegilops* species, and on the effects of transferring chromosomes from these species on the grain composition and quality of wheat (Rakszegi et al. 2017).

The main goals of the present study were therefore to determine the effects of added *Ae. biuncialis* and *Ae. geniculata* chromosomes on the content and composition of the grain storage protein and dietary fiber components of hexaploid wheat under well-watered and drought stress conditions. The chromosomal positions of putative orthologs of the key genes determining these components were also identified using *Ae. umbellulata* chromosome sequences.

MATERIALS AND METHODS

Plant materials and growing conditions. A set of bread wheat (cv. Chinese Spring) / *Ae. geniculata* (TA2899) chromosome addition lines and a set of bread wheat (line Mv9kr1) / *Ae. biuncialis* (MvGB642) lines were used in experiments together with their wheat and *Aegilops* parental genotypes. The plants were grown for 12 weeks as described by Rakszegi et al. (2017). The growth conditions and water status of plants were similar for all plants (Z41 stage of Zadoks's scale) (Zadoks et al. 1974). Drought stress was then applied by withholding water, keeping the average soil water content (SWC) between 10-15% until the ripening period (for 3 weeks). The control plants were irrigated regularly keeping the SWC values between 30-35% (Rakszegi et al. 2018).

Measurements. The measurements were performed on 3 biological replicates for each genotype and treatment. TKW was measured using the standard MSZ 6367/4-86 (1986) method. The crude protein content of the wholemeal samples was determined by the Kjeldahl method (ICC method 105/2). Size Exclusion-High Performance Liquid Chromatography (SE-HPLC) was used to determine the protein composition according to Batey et al. (1991). The total amount of mixed-linkage β -glucan was determined using a Megazyme assay kit (Megazyme, Bray, Ireland) (ICC Standard No 166). Total and water-extractable pentosans were determined by the method of Douglas (1981). Enzyme fingerprinting of AX and β -glucan was carried out as described by Ordaz-Ortiz et al. (2005). Statistical analyses were as described by Rakszegi et al (2017).

RESULTS AND DISCUSSION

Both *Aegilops* parents have higher contents of protein and β -glucan and higher proportions of WE-arabinoxylans (determined as pentosans) when grown under both well-watered and drought stress conditions.

The addition of chromosomes 2U^e, 4U^e, 5U^e, 7U^e, 2M^e, 5M^e and 7M^e of *Ae. geniculata* and 3U^b, 2M^b, 3M^b and 7M^b of *Ae. biuncialis* into bread wheat increased the seed protein content, while chromosomes 1U^e and 1M^e also increased the proportion of polymeric glutenin proteins. Increased β -glucan content was observed in wheat chromosome addition lines 5U, 7U and 7M. The AX content was increased by the addition of chromosomes 5U^e, 7U^e and 1U^b while WE-AX was increased by the addition of chromosomes

5U, 5M and 7M, and to a lesser extent by chromosomes 3, 4, 6U^e and 2M^b. The addition of chromosomes 5U^e and 7M^b also affected the structure of wheat AX, as shown by the pattern of oligosaccharides released by digestion with endoxylanase (Rakszegi et al. 2017).

In general, the compositions of the *Aegilops* accession were less affected by drought stress than those of the wheat parents and the addition of a number of *Aegilops* chromosomes, such as 5U^e, 5M^e and 7M^e also resulted in greater stability in protein content and composition under drought stress. Furthermore, the addition of chromosomes 5-7U^e, 1-2M^e, 5-7M^e, 1U^b, 3U^b and chromosomes 3U^e, 6U^e, 1-2M^e, 1U^b and 3M^b resulted in the stabilization in the contents of total and WE-pentosans, respectively, while chromosomes 5U^e, 5M^e and 7M^e contributed to greater stabilization of both protein and pentosan contents under drought stress. Since the addition of chromosomes 1-2U^e, 5-7U^e, 2-3M^e, 3M^b and 7M^b resulted in greater stability in kernel size, it can be concluded that chromosomes 5U^e and, probably also chromosomes 7M, contributed to the stabilization of both kernel size and composition (Fig. 1) (Rakszegi et al. 2018).

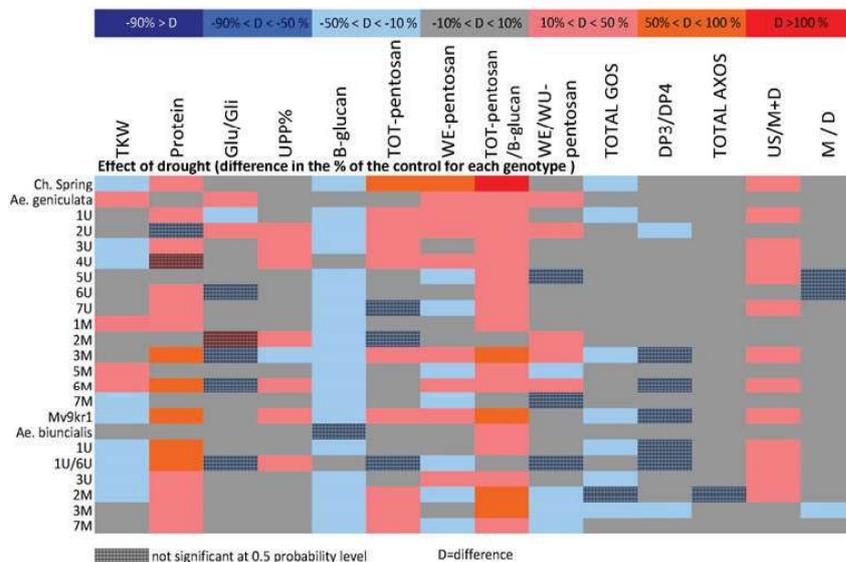


Figure 1. Effect of drought on the compositional properties of the addition lines. Reddish and blue blocks indicate values higher and lower than those of well-watered control, respectively. (Rakszegi et al. 2018).

A BLASTn search on the cDNA sequences of the key genes responsible for wheat grain storage proteins showed that most of the genes of interest (HMW glutenins, LMW glutenins, γ -gliadins, β -glucan, arabinoxylan) are located on the same homeologous group chromosomes of the U-genome of *Ae. umbellulata* as in bread wheat (Rakszegi et al. 2017).

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Variability in phenolic acid composition and content in CIMMYT durum wheat cultivars and Mexican landraces

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ABSTRACT

The genetic variation for phenolic acid concentration was extensively studied in bread and durum wheat collections in Europe and in some areas of the Mediterranean Region, but a comprehensive survey in Mexican wheat materials was lacking. The aim of the present research was to evaluate the variability for individual and total phenolic acids of six durum wheat varieties developed at CIMMYT. Environmental effects, including full and reduced irrigation, mild and severe heat stress were considered to estimate potential association between phenolic acid content and different level of water-deficit and heat stress conditions. The principal component analysis of the whole dataset for the cultivars showed that different environmental conditions had a clear impact on genotypes clustering. A different response to drought and heat stress was observed among the cultivars, though a general increase of phenolic acids was observed in some varieties. A collection of Mexican durum wheat landraces available at CIMMYT was also characterized for the phenolic acid profile. A large variation for total phenolic acid content was observed among the landraces with a mean value of about 600 µg/g d.m. Results could help durum wheat breeding for phenolic acid content to improve the antioxidant properties of durum products.

INTRODUCTION

Phenolic acids are the most common phenolic compounds found in whole wheat grains (Moore et al. 2005). These components are particularly concentrated in the external coating of mature caryopsis in which they are present as mostly bound to cell wall polymers. Food processing, such as thermal processing or fermentation, contributes to the release of bound phenolic acids making them more bioavailable (Laddomada et al. 2015). The interest in phenolic acids is due to their ability to protect against degenerative diseases in which reactive oxygen species are involved, such as cardiovascular disease and colon cancer. The content of phenolic acids may depend on genotype but also on several environmental conditions, including biotic and abiotic stimuli (Laddomada et al. 2016; Menga et al. 2010; Shewry et al. 2010). The influence of heat and drought stresses on phenolic acids has been studied more in roots and leaves, as well as during grain ripening, but their impact on mature caryopsis has not been fully investigated yet.

In the present work, our focus was to evaluate the effects of water-deficit and heat stress on individual and total phenolic acid content in six durum varieties that are some of the most important in 50 years of breeding at CIMMYT. We also assessed the variation for individual and total phenolic acids in a collection of durum wheat landraces available at CIMMYT.

MATERIALS AND METHODS

Plant material and grain characteristics. Six durum varieties, some of which are the most important in 50 years of breeding at CIMMYT, were considered in the study: Mexicali C75, Yavaros C79, Altar C84, Atil C2000, Jupare C2001 and Cirno C2008. The cultivars were sown in the 2015-16 crop season in Ciudad Obregon, Sonora, in northwestern Mexico. The trials were planted with two replicates in a randomized complete Block Design under seven different environmental conditions: full drip irrigation (OPT), full