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Short Communication

Genetic variation among laboratory accessions of Chinese Spring wheat (*Triticum aestivum* L.)

I. W. Mott* and R. R.-C. Wang

USDA-Agricultural Research Service, Forage and Range Research Laboratory, Logan, UT 84322-6300, USA

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Abstract

Chinese Spring (CS) wheat (*Triticum aestivum* L.) is commonly used in genetic research including cytogenetic analysis, molecular mapping and germplasm development. Aneuploid lines of alien chromosomes in the CS background have been used in studies with diverse objectives. Thousands of genomic and complementary DNA sequences from expressed sequence tag (EST) libraries of biotic- and abiotic-stressed tissues are publicly available from CS. Gene expression analysis of salt-tolerant wheat lines, W4909 and W4910, compared with the CS common wheat background led to the discovery of several expressed sequences that were absent in the CS accession held in our laboratory. A survey of 20 CS accessions from 13 laboratories using the polymerase chain reaction with gene-specific primers for eight salt-responsive genes resulted in amplification success ranging from 15 to 100%. Amplified fragment length polymorphism analysis showed that 99% of the genetic variation was among the accessions while the remaining 1% was within the accessions. A neighbour-joining phylogram showed that four of the five CS accessions from the International Maize and Wheat Improvement Center (CIMMYT) grouped with the salt-tolerant wheat cultivar Yecora Rojo while the remaining 16 CS accessions had limited genetic differences. Thus, variation exists among these highly self-pollinating CS sources, suggesting that appropriate consideration should be taken when using CS accessions to conduct molecular and genetic analyses.

Keywords: AFLP; Chinese Spring; salt tolerance; wheat

Experimental

Chinese Spring (CS) wheat seeds were acquired from various laboratories around the world and assigned an accession number (Table 1). The accession held in our laboratory is CS22. Seedlings from each source were grown in the greenhouse and harvested for DNA extraction using DNeasy extraction kits (Qiagen, Inc., Valencia, CA, USA) as described by the manufacturer. Quantity and quality of DNA were assessed by spectrophotometry and agarose gel electrophoresis. Polymerase chain reaction (PCR) primers (Supplementary Table S1, available online only at http://journals.cambridge.org) were designed to amplify selected targets as identified in salt tolerance subtraction [4909D4 (AY924304) and 4910D4 (AY924305); Mott, unpublished data] and microarray studies (Ta13485, Ta25111, Ta3109, Ta25815 and Ta10194; Mott and Wang, 2007).

* Corresponding author. E-mail: ivan.mott@ars.usda.gov

Table 1. Results of polymerase chain reaction (PCR) amplification in Chinese Spring wheat (*Triticum aestivum* L.) accessions with primers designed from gene sequences identified in salt-responsive expression studies

	PCR primers								
Line	Actin	Ta13485	Ta25111	4910D4	Ta3109	Ta5503	4909D4	Ta10194	Ta25815
W4909[a]	+	+	+	+	+	+	+	+	−
W4910[a]	+	+	+	+	+	+	−	+	+
AJDAj5[a]	+	+	+	+	+	+	+	+	−
Ph[l a]	+	+	+	+	+	+	+	+	+
CS01[b]	+	+	+	+	+	+	+	+	−
CS02[c]	+	+	+	+	−	+	+	+	−
CS03[d]	+	+	+	+	+	+	+	+	−
CS04[d]	+	+	+	+	+	+	+	+	−
CS05[d]	+	+	+	+	+	+	+	+	−
CS06[e]	+	+	+	+	+	+	+	+	−
CS07[f]	+	−	+	+	−	+	+	+	−
CS08[g]	+	+	+	+	+	+	+	+	−
CS09[h]	+	+	+	+	−	+	+	+	−
CS10[i]	+	+	+	+	+	+	+	+	−
CS11[i]	+	+	+	+	+	+	+	+	−
CS12[j]	+	+	+	+	+	+	+	+	−
CS13[k]	+	+	+	−	+	−	−	+	+
CS16[k]	+	+	+	−	+	−	−	+	+
CS17[k]	+	+	+	−	+	−	−	+	+
CS18[k]	+	+	+	−	+	−	−	+	−
CS19[k]	+	+	+	+	+	+	+	+	−
CS20[l]	+	+	+	+	+	+	+	+	−
CS21[m]	+	+	+	+	+	+	+	+	−
CS22[a]	+	+	+	−	−	−	−	−	−
YR[a]	+	+	+	−	−	−	−	+	+

[a] R. Wang, USDA-ARS, Logan, UT, USA. [b] A. Lukaszewski, UC-Riverside, Riverside, CA, USA. [c] H. Ohm, Purdue University, West Lafayette, IN, USA. [d] P. Gustafson, University of Missouri, Columbia, MO, USA. [e] W. Cao, Agriculture and Agri-Food Canada, Ottawa, ON, Canada. [f] J. Koenig, INRA, France. [g] M. Sorrells, Cornell University, Ithaca, NY, USA. [h] A. Martin, CSIG, Cordoba, Spain. [i] T. Koniarov, Dobroudja Agricultural Institute, Bulgaria. [j] A. Graner, IPK Genebank, Germany. [k] T. Payne, CIMMYT, Mexico. [l] M. Mackay, AWCC, Tamworth, Australia. [m] J. Raupp, Kansas State University, Manhattan, KS, USA.

PCR [25 μl, containing 40 ng genomic DNA, 1 × reaction buffer containing 15 mM $MgCl_2$, 0.2 μM gene-specific primers, 0.25 mM dNTP, 1.25 U Taq DNA polymerase (Promega, Madison, WI, USA)] were incubated as follows: 95°C for 2 min, then 36 cycles of 95°C for 15 s, 55°C for 15 s, 72°C for 30 s in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). PCR products were visualized by electrophoresis in 2% agarose gels followed by staining with ethidium bromide and illumination on a UV lightbox. The PCR results, shown in Table 1, varied from bands that amplified in all CS accessions (actin and Ta25111), bands that amplified in all accessions except one (Ta13485 and Ta10194), bands that did not amplify in several accessions (4910D4, Ta3109, Ta5503 and 4909D4), to bands that failed to amplify in most CS accessions (Ta25815).

For amplified fragment length polymorphism (AFLP) analysis, two plants from each source were analysed. The AFLP procedure followed the protocol of Vos *et al.* (1995), using the selective primers E.ACA/M.CAT, E.ACG/M.CAA, E.AGG/M.CTG, E.ACG/M.CTC, E.AGG/M.CTA and E.ACT/M.CTC. Amplicons were separated on a capillary ABI 3730 instrument with the GS-500 LIZ size standard and GeneScan software (Applied Biosystems). Individual profiles were manually scored for the presence (1) or absence (0) of fragments with Genographer software (Benham, 2001). Raw binary data were converted to Euclidian distance, with the resulting distance matrix used to calculate the population genetic distance using GeneAlEx (Peakall and Smouse, 2006). The mean genetic distance matrix was the input for the assembly of a neighbour-joining (NJ) cladogram using PAUP* version 4.0b (Swofford, 2002). The six selective AFLP primer pairs generated 764 bands, of which 66% (501) had no polymorphisms while 34% were polymorphic. As would be expected for a highly self-pollinating species, 99% of the genetic variation was among the CS collections, while only 1% was within each CS source. Four accessions, all from the CIMMYT, Mexico collection, were grouped

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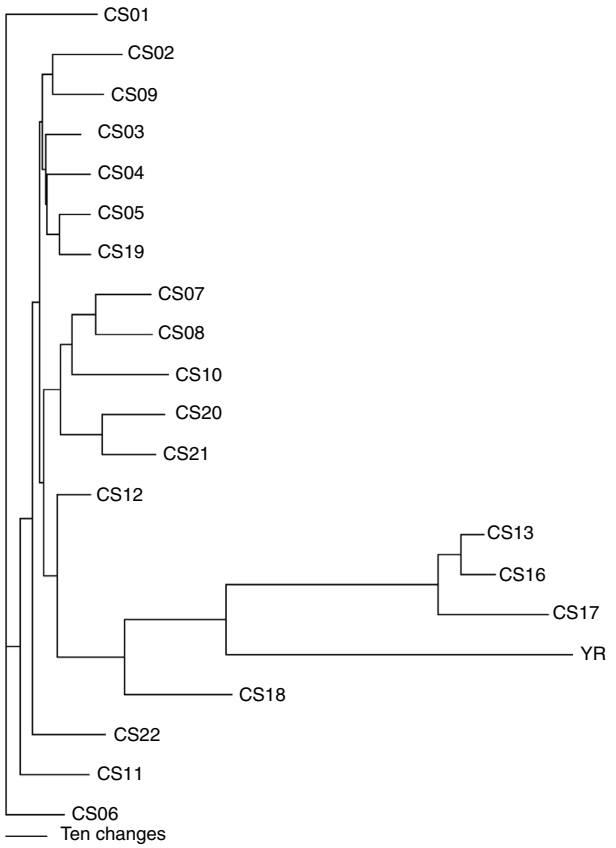

Fig. 1. NJ phylogram. The tree was based on the pairwise matrix of the mean binary genetic distance generated from 42 plants from 21 CS and YR wheat accessions using 764 AFLP bands.

with Yecora Rojo (YR) and were distinct from all the other CS accessions (Fig. 1).

Discussion

CS wheat (*Triticum aestivum* L.) is commonly used for genetic and molecular analyses, including genome sequencing, cytogenetics and germplasm development. Two salinity-tolerant wheat recombinant lines, W4909 and W4910 (Wang *et al.*, 2003b), were generated by crossing the disomic addition line AJDAj5 with the *Ph* inhibitor line Ph^I. AJDAj5 was developed in France and harbours a pair of *Thinopyrum junceum* chromosomes (Forster et al., 1988; Charpentier, 1992; Wang *et al.*, 2010). Ph^I was developed in Kansas and harbours the wheat *Ph* inhibitor gene derived from *Aegilops speltoides* that promotes homoeologous recombination (Chen *et al.*, 1994). Both the AJDAj5 and Ph^I lines were developed in CS wheat. W4909 and W4910 have been shown to contain non-CS22 DNA markers and both lines have greater salt tolerance than either parent (AJDAj5 and Ph^I), which, in turn, have greater salt tolerance than the CS22 background (Wang *et al.*, 2003a; Mott and Wang, 2007). Using subtraction suppression hybridisation, we isolated two genes, 4910D4 and 4909D4, in AJDAj5 and Ph^I that were not expressed in CS22 (Mott, unpublished data). Furthermore, microarray analysis (Mott and Wang, 2007) identified additional genes not expressed in CS22, but expressed in both the AJDAj5 and Ph^I parental lines, which suggests that the source of these genes was CS, not the alien chromatin in AJDAj5 and Ph^I. Given that AJDAj5, Ph^I, W4909, and W4910 all share CS background, it seemed likely that our CS22 accession might have appreciable genetic differences with the very CS accessions used to generate Ph^I and AJDAj5, and, possibly, with CS accessions held at other laboratories.

CS seeds from 13 laboratories were screened for the presence or absence of PCR amplified bands from eight primer pairs designed from differentially expressed genes identified in the microarray (Mott and Wang, 2007) or subtraction experiments that did not amplify in CS22 (Table 1). The results of these PCR assays ranged from primers that amplified from all CS accessions (actin and Ta25111) to primers that only amplified from four of the CS accessions. In every case that CS22 lacked amplification, at least one other CS accession did amplify with that primer pair. This supports our hypothesis that some genes not expressed in CS22, but expressed in W4909 or W4910, have CS origins, and did not originate from the alien chromatin present in Ph^I or AJDAj5.

AFLP data were used to further assess the genetic diversity among the accessions of CS wheat. The most prominent structure in the cladogram was four CIMMYT accessions grouped with YR that were distinct from all the other CS accessions (Fig. 1). Because YR has been used extensively in the CIMMYT breeding programme, some of its CS accessions might have been contaminated by outcrossing with YR. Interestingly, the salt-responsive gene Ta25815 is only present in W4910, Ph^I YR, and the three CS lines most similar to YR (CS13, CS16 and CS17) (Table 1 and Fig. 1). Only one accession (CS19) of the CIMMYT collection is similar to the other CS accessions from 12 different laboratories. Variations among these CS accessions could have resulted from seed contamination, outcrossing and a variety of mutations involving transposable elements in the largely repetitive DNA. Because variation exists among the CS accessions maintained in different laboratories, appropriate actions should be exercised when using CS plants to conduct any research. The exact parental plant used in making hybrids needs to be properly identified and its seeds preserved for subsequent studies to act as historic

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controls. The genetic background of past and subsequent generations that resulted from different breeding programmes needs to be carefully documented to ensure valid collection and interpretation of experimental data.

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References

Benham JJ (2001) *Genographer, Version 1.6.1*. Bozeman: Montana State University.

Charpentier A (1992) Production of disomic addition lines and partial amphiploids of *Thinopyrum junceum* on wheat. *Comptes Rendus de l'Academie des Sciences* 315: 551–557.

Chen PD, Tsujimoto H and Gill BS (1994) Transfer of *Ph*[1] gene promoting homoeologous pairing from *Triticum speltoides* into common wheat and their utilization in alien genetic introgression. *Theoretical and Applied Genetics*. 88: 97–101.

Forster BP, Miller TE and Law CN (1988) Salt tolerance of two wheat *Agropyron junceum* disomic addition lines. *Genome* 30: 559–564.

Mott IW and Wang RR-C (2007) Comparative transcriptome analysis of salt-tolerant wheat germplasm using wheat genome arrays. *Plant Science* 173: 327–339.

Peakall R and Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

Swofford DL (2002) *PAUP*: Phylogenetic Analysis using Parsimony (*and Other Methods), Version 4.0b10*. Sunderland, MA: Sinauer Associates.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J and Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407–4414.

Wang RR-C, Li X-M, Hu Z-M, Zhang J-Y, Larson SR, Zhang X-Y, Grieve CM and Shannon MC (2003*a*) Development of salinity-tolerant wheat recombinant lines from a wheat disomic addition line carrying a *Thinopyrum junceum* chromosome. *International Journal of Plant Sciences* 164: 25–33.

Wang RR-C, Larson SR, Horton WH and Chatterton NJ (2003*b*) Registration of W4909 and W4910 bread wheat germplasm lines with high salinity tolerance. *Crop Science* 43: 746.

Wang RR-C, Larson SR and Jensen KB (2010) Analyses of *Thinopyrum bessarabicum*, *Th. elongatum* and *Th. junceum* chromosomes using EST-SSR markers. *Genome* 53: 1083–1089.