Characterization of *T. aestivum-H. californicum* addition lines DA2H and MA5H

Wang XE, Kong F, Wang HY, Cao AZ, Qin B, Ji JH, Wang SI

State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

ABSTRACT

T. aestivum - H. californicum amphiploid was crossed to Chinese Spring, and the backcrossed and self-fertilized progenies were analyzed by morphological observation , cytological , biochemical and molecular marker techniques. Alien addition lines involved in different *H. californicum* chromosomes were identified and their genetic constitution was characterized. The STS-PCR analysis using chromosome 2B specific marker indicated that chromosome H3 of *H. californicum* belonged to homoeologous group 2, and hence could be designated as 2H. SDS-PAGE showed that chromosome H2 of *H. californicum* belonged to homoeologous group 5, and hence could be designated as 5H. The wheat×*H. californicum* amphiploid and the identified alien chromosome lines (DA2H and MA5H) were evaluated for powdery mildew resistance in the field. The results indicated that the amphiploid showed high powdery mildew resistance than "Chinese Spring". However, addition lines DA2H and MA5H were highly susceptible to powdery mildew, indicating that chromosome 2H and 5H might not carry major powdery mildew resistant genes.

INTRODUCTION

Hordeum californicum showed to have high levels of resistance to barley yellow dwarf virus and tolerance to freeze injury. When grew in the low-nitrogen conditions, it also showed a strong viability (Gupta and Fedak, 1985; Kolb et al., 2002). Gupta & Fedak had successfully obtained the hybrid F_1 and amphiploid between Chinese Spring and *H. californicum*. We observed that the amphiploid was moderately susceptible in seedling and has a kind of "slow powdery mildew" in adult stage performance. Sequential chromosome C–banding/Fluorescence *in situ* hybridization (FISH) was conducted on root-tip cell (RTC) chromosomes of *H. californicum* and *Triticum aestivum* c.v. Chinese Spring-*H. californicum* amphiploid and standard karyotype of *H. californicum* in the wheat background were established (Kong et al., 2007). However, up to now, the homoeologous relationship of *H. californicum* chromosomes and the collinearity of wheat and *H. californicum* chromosomes were still unknown. The development of a set of addition lines and other kind of alien chromosome lines would be helpful for both comparative research and for the utilization of useful genes of *H. californicum* in wheat improvement.

In the present research, integrated methods including morphology observation , sequential C-banding/FISH on root tip cell chromosomes , configuration analysis of pollen mother cell (PMC) at MI , biochemical and molecular marker analysis, will be used to enhance the authenticity of identified alien chromosomes or chromosome fragments and provide useful genetic resources for comparative genomics of *Triticeae* species.

MATERIALS AND METHODS

T. aestivum cv. Chinese Spring (CS)-*H. californicum* amphiploid was kindly supplied by WGRC, Kansas State University, Manhattan, Kansas, U.S.A. Diploid *H. brachyantherum* ssp *californicum* (Accession: CN28662) was introduced from PGRC.

C-banding followed the procedure described by Gill et al.(1991). FISH followed the procedure described by Jiang et al. (1993b). Images were captured using Olympus BX60, SPOT CCD.

2HS-specific primer ABC454 was synthesized according to published barley STS sequences in Genbank (http://www.ncbi.nlm.nih.gov; 2HL specific STS NAU/$STS_{BCD135-1}$ was designed by Ji et al. (2007). SDS-PAGE was performed according to the method of Payne et al. (1981).

RESULTS

Identification of CS-H. californicum disomic addition lines DA2H

Chromosome C-banding and sequential FISH that line KF6011-10-27 was a disomic addition, with two H3 chromosome added. (Fig. 1a, 1b, 1c). STS-PCR analysis using 2H specific markers ABC454 and NAU/$STS_{BCD135-1}$ indicated that the H3 chromosome has a relationship with homoeologous group 2 and could be referred as 2H (Fig 2a, 2b). Field powdery mildew resistance evaluation found that KF6011-10-27 was highly susceptible, indicating that chromosome 2H might not carry major powdery mildew resistant genes.

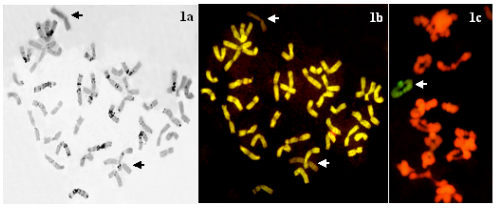

Figure 1 Sequential C-banding/FISH(Arrows show 2H) of line KF6011-10-27. 1a, 1b: Sequential C-banding/FISH (Fluoresceient-12-dUTP labeled Chinese Spring gDNA as probe, red signals show chromosome 2H) ; 1c: PMC MI FISH (Fluoresceient-12-dUTP labeled *H. californicum* gDNA as probe, green signals show bivalent of 2H)

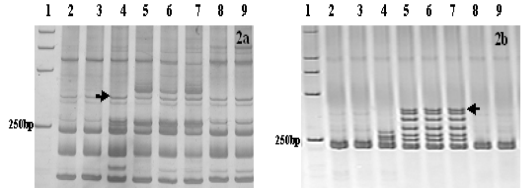

Figure 2 PCR using STS primers ABC454 (2a) and NAU/STSBCD135-1 (2b) (Arrows show specific bands of 2H) 1 : DNA ladder; 2-3 : Chinese Spring ; 4 : *T. aestivum-H. californicum* amphiploid ; 5 : 6010-13-49-1 ; 6 : 6011-10-27-10 ; 7 : 6011-10-67-6 ; 8 : 6128-9-5 ; 9 : 6128-14-1

Identification of CS-H. californicum monosomic addition lines MA5H

According to chromosome banding and FISH, a single H2 chromosome, which has high arm ratio, was added in line KF6128-9 (Fig. 3a, 3b). By the comparison of SDS-PAGE patterns of HMW and gliadin, same specific bands were observed only in line KF6128-9 and CS-*H. californicum* amphiploid. The same was observed for the gliadin γ region. The synthesis of HMW-GS is controlled by loci located in the long arm gene of homoeologous group 1 of wheat, and gliadin of the γ and ω region was controlled by loci located in the short arm. Previous study proved that barley chromosome 5H was homoeologous to the homoeologous group 1 of wheat. These indicated that the H2 chromosome in line KF6128-9 has a relationship with homoeologous group 5, and hence could be referred as 5H. MA5H was susceptible to powdery mildew in the field, indicating that chromosome 5H might not carry major powdery mildew resistant gene either.

DISCUSSION

Molecular markers, especially markers from the conserved coding region are useful for determine the homoeologous relationship of chromosomes from different species. Van Deynze et al. (1998) developed anchor markers which were useful for comparative mapping among grass chromosomes. Blake et al. (1996) converted RFLP markers of barley into STS markers. Using RFLP, EST or gene based markers located in different region along chromosomes, it will be also useful to clarify the synteny and reveal structural changes of chromosomes from different species during evolution history. The obtained information will be useful for comparative mapping and cloning of homologous genes and better utilization of wheat genetic resources. In the present study, we determined the relationship of two *H. californicum* chromosomes with that of wheat with the help of the developed addition lines. However, only two molecular markers located on different arms were used. It is necessary to confirm this result using more markers and to determine homoeologous relationship of the other 5 *H. californicum* chromosomes using a complete set of addition lines. The related research is in progress.

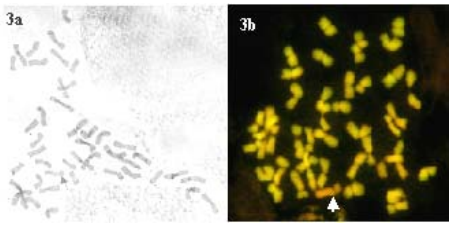

Figure 3 Chromosome C-banding (3a) and FISH (3b) of line KF6128-9 (Arrows show

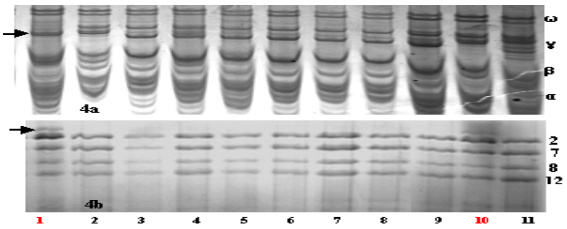

Figure 4 PAGE of gliadin (4a)and HMW-GS(4b) (Arrows show the specific bands) . 1 : 6128-9 ; 2 : 6010-14-25 ; 3 : 6010-13-47 ; 4: 6010-13-49 ; 5 : 6011-10-27 ; 6:6010-13-47 ; 7:6010-13-49 ; 8:6010-2-43 ; 9:6010-2-51 ; 10: amphiploid ; 11:Chinese Spring

ACKNOWLEDGEMENTS

The project was supported by the Chinese High Tech Program of China (Grant No. 2006AA10Z1F6, 2006AA100101) and the 111 Project (Grant No. B08025)

REFERENCES

Blake TK, Kadyrzhanova D, Shepherd KW, Islam, AKMR, Langridge PL, McDonald CL, Talbert LE (1996). STS-PCR markers appropriate for wheat-barley introgression. Theor Appl Genet 93: 826–832.

Gill BS, Friebe B, Endo TR (1991). Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat. Genome 34: 830–839.

Gupta PK, Fedak G(1985). Intergeneric hybrids between *H.californicum* and *T. aestivum*. Hered. 76: 365–368.

Ji JH, Cao AZ, Wang HY, Qin B, Wang SL, Kong F, Chen PD, Liu DJ, Wang XE (2007). Discrimination of the *Triticum aestivum-T. timopheevii* introgression lines using PCR-based molecular markers. Hereditas 29: 1256–1262. (in Chinese with an English abstract).

Jiang J, Gill BS (1993b). Sequential chromosome banding and *in situ* hybridization analysis. Genome 36: 792–795.

Kolb A, Alpert P, Enters D, Holzapfel C (2002). Pattern of invasion within a grassland community. J. Ecol. 90: 871–881.

Kong F, Wang HY, Zhao Y, Ji JH, Cao AZ, Wang SL, Zhou B, Wang XE (2007). Giemsa C-banding and karyotype analysis of California barley chromosomes. *Acta Agrestia Sinica*, 15:103–108.

Payne PI, Lawrence GJ. (1983). Catalogue of alleles for the complex gene loci Glu-A1, Glu-B1, and Glu-D1 which code for high molecular weight subunits of glutenin in hexaploid wheat. Cereal Res. Commun. 11: 29–35.

Van Deynze AE, Sorrells ME, Park WD, Ayres NM, Fu H, Cartinhour SW, Paul E, McCouch SR (1998). Anchor probes for comparative mapping of grass genera. Theor. Appl. Genet. 97: 356–369.