Three new monosomic chromosome 5B genetic stocks of bread wheat

A. Mujeeb-Kazi[1], A. Cortes[1], V. Rosas[1], R. Delgado[1], F. A. Shafiq[2], and A.A. Vahidy[2]

1.   International Maize and Wheat Improvement Center (CIMMYT), Apartado Postal 6-641, 06600 Mexico, D. F., Mexico, and
2.   Genetics Department, Karachi University, Karachi, Pakistan.

Summary

One way to promote chromosome pairing between wheat and alien genomes is by using chromosome 5B deficient-genetic stocks (monosomic 5B) as female parents in hybridization. Such a stock is available in the cultivar Chinese Spring that has superior crossability but is a poor agronomic wheat. We have developed monosomic 5B stocks in three elite bread wheat cultivars (Ciano 79, Nacozari 75, Pavon 76) using Chinese Spring as the monosomic 5B donor source through a backcrossing protocol. The three new monosomic 5B stocks produced were validated by C-banding and by meiotic analyses of the 5B deficient F_1 hybrids (2n=5x=34) of each cultivar with *Aegilops variabilis*.

Key words: *Triticum aestivum*, Monosomic 5B, Intergeneric hybrids, Chromosome pairing.

Introduction

One aspect that facilitates alien genetic introgression from distant gene pools of the annual and perennial Triticeae into *Triticum aestivum* is genetic recombination through homoeologous chromosomal pairing. In general, the dominant gene *Ph1* (Okamoto 1957; Riley and Chapman 1958; Sears and Okamoto 1958) precludes such events from occurring unless its action is suppressed (Chen et al. 1994, Dvorak 1972, Kimber and Athwal 1972; Riley et al. 1968), or its recessive form is involved in the hybrid combination (Islam and Shepherd 1988, 1992; Rogowsky et al. 1991), or the gene is absent through the lack of the chromosome 5B that possesses the *Ph1* gene on the long arm (5BL), (Riley and Chapman 1958; Sears and Okamoto 1958). The recessive *ph1b* gene in Chinese Spring (Sears 1977) is another alternative for enhancing recombination. So far Chinese Spring has been a female parental choice in making wide intergeneric hybrids with the perennial Triticeae species. More recently an agronomically superior cultivar "Fukuhokomugi" with good crossability has been used in hybrid production (Jauhar 1995) and options of using new highly crossable wheat genotypes from Sichuan (China) also exist. However, crossability and hybrid production address only the initial hurdles in the alien introduction process with little resolve to counter the recombinational constraints present in a vast majority of intergeneric wheat/alien species hybrids. Within our spring wheat germplasm, three cultivars have consistently demonstrated satisfactory crossability (Mujeeb-Kazi and Kimber 1985) with the perennial Triticeae species including the tester species (Riley and Chapman 1967) for the *kr* gene action; *Secale cereale* L. These *T. aestivum* cultivars are Ciano 79, Nacozari 75, and Pavon 76. All

three cultivars are superior spring bread wheats, with better agronomic traits that Chinese Spring lacks.   Hence, these cultivars became our choice of developing monosomic chromosome 5B stocks into, using monosomic 5B Chinese Spring as the donor monosomic parent for each F_1 hybrid. The monosomic 5B production steps, cytological validation and cytogenetic confirmation of each cultivars monosomic 5B status are described to form the basis of this paper.

Materials and methods.
Seeds of *Triticum aestivum* L. cvs. Ciano 79, Nacozari 75, and Pavon 76 were obtained from the CIMMYT bread wheat breeding program. Seeds of the complete Chinese Spring monosomic series originated from late Dr. E.R. Sears, University of Missouri, Columbia, Missouri, USA, of which the monosomic 5B (2n=6x=42-1) stock was used in this study.

F₁ hybrid production.
Five cytologically identified *Triticum aestivum* cv. Chinese Spring monosomic 5B (2n=6x=41) plants were emasculated and pollinated by pollen from the wheat cultivars Ciano 79, Nacozari 75, and Pavon 76 to yield F_1 seed. F_1 seeds were germinated, mitotically counted and five plants of each F_1 combination with 41 chromosomes transplanted in a potted soil mix.   These plants were maintained under greenhouse conditions of 24°C/14°C, 15h/9h natural day/night regimes, and approximately 55 to 60% RH. Three F_1 plants with 41 chromosomes from each combination were Giemsa C-banded to unequivocally ascertain the monosomic 5B status in these plants. Cytological procedures of Mujeeb-Kazi et al. (1994) and Jahan et al. (1990) were followed for somatic cytology and Giemsa C-banding. Cytologically identified monosomic 5B F_1 plants were emasculated, and each F_1 monosome combination plant was pollinated by pollen from its corresponding wheat cultivar i.e. Ciano 79, Nacozari 75, Pavon 76 to yield BC_1 seed set. BC_1 seed were germinated, 41 chromosome monosomics cytologically validated and used as females to produce BC_2 progeny. This procedure was continued for six additional generations when BC_8 derivative seed were harvested.

Increase of BC₈ seed stock and validation .
Five BC_8 plants with 41 chromosomes were cytologically selected and their monosomic 5B status validated by C-banding. Three plants of each BC_8 selfed combination were emasculated   (one spike per plant) and pollinated by pollen from *Aegilops variabilis* (2n=4x=28, UUSS). The remaining spikes on each of the three plants of each combination were glassine bagged to ensure pure selfed seed production.

Fifteen days after pollination by *Aegilops variabilis,* embryos were excised and plated on artificial media. The hybrid seedlings were transplanted to pots, kept in the greenhouse and cytologically selected for 2n=5x=35 or 2n=5x=35-1 =34 chromosome plants. The 34 chromosome plants were C-banded for ascertaining the '- 5B' status, and eventually meiotically analyzed. Representative 35 chromosome hybrids served as controls within each cross to delineate the 5B *Ph* meiotic influence. The intergeneric crossing, embryo

rescue and cytology procedures including meiotic protocols were similar to those earlier reported (Mujeeb-Kazi et al. 1987; Jahan et al. 1990; Mujeeb-Kazi et al. 1994).

Results and discussion

In intergeneric or interspecific hybridization programs where alien genetic diversity of the primary, secondary or tertiary gene pools is to be introgressed into wheat, chromosomal recombination is essential. Hence, interspecific programs; where the closely related A, B, or D genome diploid species are donors for desired traits; have a priority —more so in applied agriculture. Since these three sources reside in the primary and secondary gene pools (Jiang et al. 1994) the genetic diversity within their several accessions can be readily incorporated through genetic recombination.

It is where the tertiary gene pool sources offer promise for wheat improvement that recombination constraints exist, and genetic transfers become difficult to achieve due to the genetic distance that leads to a lack of intergenomic pairing. This pairing restriction is due to the activity of the pairing homoeologous gene, *Ph1*. The *Ph1* gene not only suppresses chromosome pairing between the three (A, B, D) wheat genomes, but also influences pairing between the genomes of wheat and alien species in their intergeneric hybrids. One of the various ways to promote pairing between wheat and alien genomes is by using chromosome 5B deficient genetic stocks (monosomic 5B) as female parents. When a monosomic 5B stock is crossed by an alien species, two hybrid types result of which; one lacks chromosome 5B (i.e. *Ph1* gene), thereby facilitating recombination between wheat and alien species chromosomes.

The above contentions are supported by the mitotic (Figs. 1a, b) and meiotic (Figs. 2a, b) details. The Giemsa C-banded metaphase cell with 41 chromosomes (Fig. 1b) shows the presence of only one 5B chromosome. The meiotic validation of the monosomic 5B stocks produced in Ciano 79, Nacozari 75, Pavon 76 comes from the high metaphase I chromosome pairing seen in 34 chromosome F_1 hybrids (Fig. 2b) where chromosome 5B is deficient. These tester hybrids were produced by crossing each of the three monosomic 5B wheat stocks after their BC_8 selfing stage by *Ae. variabilis*. F_1 hybrids with 35 chromosomes (2n=5x=35, ABDUS) showed negligible metaphase I chromosome pairing (Fig. 2a) due to the presence of the 5B chromosome and the functional activity of the *Ph1* gene. When the F_1 hybrid was produced between Chinese Spring wheat with the recessive *ph* gene and *Ae. variabilis*, high meiotic pairing was observed that resembled chromosome associations of Chinese Spring mono 5B/*Ae. variabilis* hybrids with 34 chromosomes. The F_1 high chromosome pairing in −5B hybrids is crucial for affecting alien genetic transfers and is encouraging. These transfers can now be achieved in a superior wheat background represented by the cultivars Ciano 79, Nacozari 75, and Pavon 76. Use of *ph* genetic stock maintains its role in wheat improvement (Sears 1977). This area is not elaborated in detail here, since we have addressed the development of monosomic 5B genetic stocks in elite readily crossable spring wheat cultivars. Usage of mono 5B germplasm has been rather limited, presumably due to the difficulty of advancing monosomic 5B based F_1 hybrids to the backcross I stage. Some concern about the effectiveness of this approach (5B deficient)
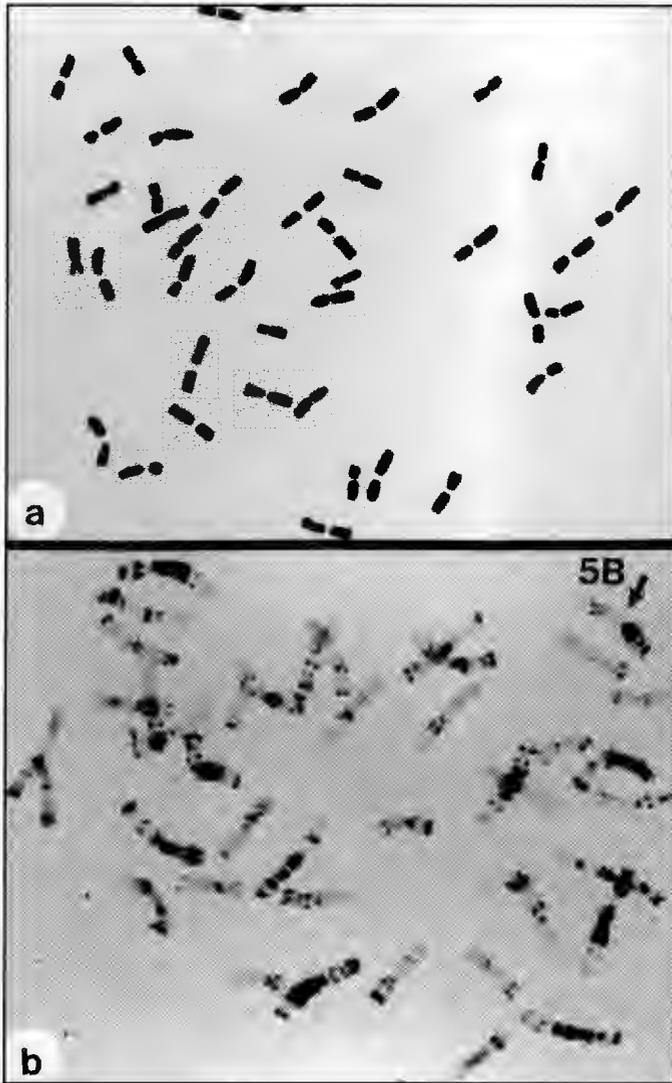
Figure 1. A bread wheat Ciano 79 monosomic 5B mitotic cell showing 41 orcein stained chromosomes in (a) and Giemsa C-banded single 5B chromosome in (b).
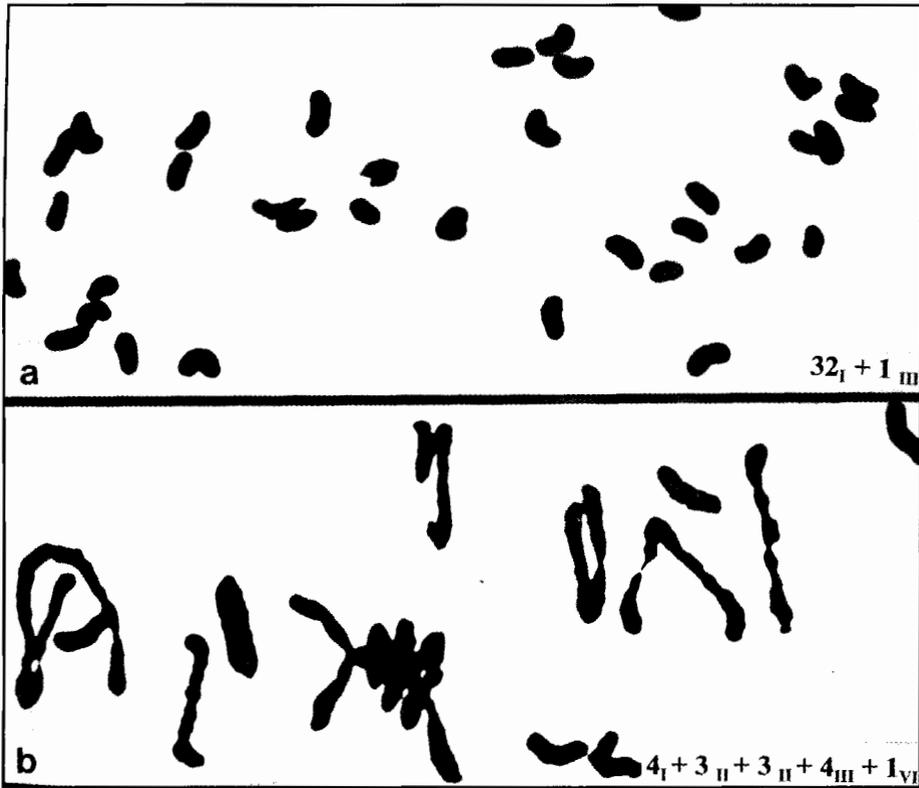
Figure 2. Meiotic associations of a *Triticum aestivum* cv. Ciano 79/*Aegilops variabilis* F_1 hybrid with and without chromosome 5B showing in (a) 35 chromosomes as $32_I + 1_{III}$ and in (b) 34 chromosomes as $4_I + 3_{II}$ (rings)+3_{II} (rods) + 4_{III} (trivalents) + 1_{VI} (hexavalent)

has been expressed. Further, Miller et al. (1998) also considered that introgressing genes from wild relatives by utilizing the absence of *Ph1* has been largely misguided and less effective. They felt the need to have an improved technique, especially for widely diverged genomes, and suggested transformation to be a more realistic option. The technique is indeed promising. However, for it to have positive practical impact on global wheat stress constraints remains futuristic and is faced with complex research plus crop field deployment issues. *Ph* manipulation needs to be exploited more aggressively in wheat/alien species crosses than has been done so far before it is under-played. Use of CS *ph* in intergeneric hybrids was reported by Sharma and Gill (1986). They observed high F_1 pairing in several hybrid combinations but BC_1 production was a constraint. Since then, novel protocols to overcome such limitations have been reported (Mujeeb-Kazi et al. 1999), and hence the use of our new crossable monosomic 5B bread wheat cultivars in intergeneric programs is encouraged. Fedak (1998) cites several examples of the techniques contributions and promise. Mujeeb-Kazi (1998) further proposed novel modifications that combine *ph* manipulation protocols, with *in situ* and PCR diagnostics in conjunction with doubled haploidy. Our new 5B monosomic stocks thus hold a significant place for promoting alien genetic transfers into wheat from distant secondary and tertiary gene pool species.

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