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Evidence of Homoeologous Relationship Between Chromosomes of Wheat and of *Aegilops geniculata*

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Abstract: Intergeneric hybrids were produced between hexaploid wheat variety Chinese Spring and five different accessions of *Aegilops geniculata*; 2n=4x=28 $C^UC^UM^OM^O$ (*Aegilops ovata*). The objective was to detect differences in chromosomal associations at meiotic metaphase-1 in the F_1 hybrids using squash preparations of prefixed immature anthers. Chromosome pairing between all the hybrids raged between 35 univalents to 3 rod bivalents except for hybrid between Chinese Spring x *Ae. geniculata* accession 361881. This hybrid exhibited chromosome pairing up to 4 rod bivalents, 1 ring bivalent and 2 trivalents with the chiasma frequency of 4.10 per cell. This indicated possibilities of homoeologous chromosome pairing and genetic exchange between chromosomes of wheat and of *Ae. geniculata*. Significance of this pairing and the potential of *Ae. geniculata* for improvement of stress tolerance in wheat would be discussed in detail.

Key words: Intergeneric hybrids, *Triticum aestivum, Aegilops geniculata,* Homoeologous pairing, Chiasma frequency

Introduction

Meiotic chromosome pairing in hexaploid wheat is under strict control of pairing promoter genes located on homoeologous group 3 and 5 (Feldman, 1966) while its suppressers are located on the long arm of chromosome 5B (Sears and Okamoto, 1958) and short arm of chromosome 3D (Sears, 1983). Restriction of meiotic pairing to fully homologous chromosomes is mainly due to the effect of a strong homoeologous pairing suppresser gene located on the long arm of chromosome 5B (5B L) later designated as *Ph* (pairing homoeologous) by Wall *et al.* (1971) and presently *Ph*-1 by McIntosh (1979). When this gene is present even hemizygously, only homologous chromosome can pair. However, meiotic studies made in different intergeneric hybrids (Charpentier *et al.*, 1988 and Farooq *et al.*, 1990a) indicated that if the alien species possesses the genes that are epistatic to wheat pairing control system, homoeologous pairing is expected to occur even in the presence of *Ph* genes. In one of such studies made between hexaploid wheat and different accessions of *Ae. variabilis* (2n=4x=28 $C^UC^US^VS^V$) accessions A, B, and E (Farooq *et al.*, 1990b), higher chiasma frequency was exhibited by hybrids involving accession E than that of A and B.

Ae. geniculata; 2n=4x=28 $C^UC^UM^OM^O$ (*Aegilops ovata*) is another tetraploid species which shares one genome (C^U) with *Ae. variabilis.* It possesses resistance (Warham *et al.*, 1986) against Karnal bunt (*Neovosia indica*) which is one of the serious wheat diseases. Some of its accessions also possess salt tolerance (Farooq *et al.*, 1989; Farooq, 1997; Farooq *et al.*, 1997). Whether accessions of *Ae. geniculata* also possesses pairing promoter genes is not yet known and can be found at meiotic metaphase-I in the F_1 hybrids of wheat with accessions of *Ae. geniculata*. The objective of the present study is therefore, to produce hybrids between different accessions of *Ae. geniculata* with wheat variety Chinese Spring and to study the chromosomal associations between the two species at meiotic metaphase-I.

Materials and Methods

Material used in this study comprised wheat variety Chinese Spring and five different accessions of *Ae. geniculata* L. viz. F, Clae-65, P1-276978, P1-330487, and P1-361881. Accession F was obtained from Plant Breeding Institute, Cambridge, UK (now Cereal Research Department, Jhon Innes Center, Norwich, UK), while the other 4 accessions were obtained from USDA, ARS, Beltsville, Maryland, USA. The origin of accession F and Clae-65 is unknown while that of accessions P1-276978, P1-330487 and P1-361881 is Japan, U.K, and Romania respectively.

Intergeneric hybrids were produced using hand emasculation of wheat variety Chinese Spring used as female parent and pollination with different accessions of *Ae. geniculata.* Post pollination treatment with growth hormones was also applied (Kruse, 1973). Seeds were harvested at maturity after the crossability data was recorded. The hybrid seeds were germinated and root tips were collected from two weeks old seedlings for chromosomes counting. The root tips were pretreated with a solution of 0.05% colchicine and 0.025% 8-hydroxyquinoline for 3 hours and then transferred to 1% aceto-orcein and stored in refrigerator. Mitotic preparations were made by squashing the root tips in 45% acetic acid. The chromosomes were examined under microscope and were photographed using 5x photo lens of Olympus Vanox-S research microscope. All F_1 plants that showed 35 chromosomes were transferred into 25cm diameter plastic pots filled with soil and were placed in the net house in the month of November. At flowering, immature spikes were fixed in Carnoy's solution. After fixation for two days at room temperature, spikes were transferred to 70% ethanol and were stored in freezer. Anthers with dividing PMCs at metaphase-1 were extracted and fixed in alcoholic hydrochloric acid carmine (Snow, 1963). Meiotic preparations were made by squashing the anthers in a drop of 45% acetic acid on a glass slide. Cover slips were removed by freezing the material at -70^{0}C in ultra-cold freezer. Photographs were taken in a manner similar to that of mitotic preparations. The present study was carried out at Nuclear Institute for Agriculture & Biology (NIAB), Faisalabad, Pakistan as a part of Plant Molecular Breeding work and completed in two years.

Results

Mitotic analysis of all the hybrids showed 35 chromosomes (Fig. 1a) including 21 of wheat and 14 of *Ae. geniculata* chromosomes. The hybridity was additionally confirmed through plant morphology, which indicated co-dominant phenotype.

Meiotic analysis made at metaphase-1 in the hybrids of Chinese Spring with accession 276978 of *Ae. geniculata*

Table 1: Mean meiotic chromosomal associations at metaphase-1 in the F_1 intergeneric hybrids of wheat variety Chinese Spring (C.S.) with *Aegilops geniculata*.

Hybrid combination	No. of cells analyzed	Mean meiotic associations				Chiasmata/cell
		I	(II)	II	III	
C.S. x *Ae. geniculata* acc: 361881	30	28.16	2.24	0.43	0.50	4.10 A
C.S. x *Ae. geniculata* acc: 65	30	30.96	0.17	1.40	0.30	2.34 B
C.S. x *Ae. geniculata* acc: 330487	30	31.37	0.27	1.50	0.03	2.10 B
C.S. x *Ae. geniculata* acc: F	30	33.06	0.10	0.57	0.20	1.17 C
C.S. x *Ae. geniculata* acc: 276978	30	33.12	0.06	0.88	-	1.00 C

Figures followed by the same letter are non-significant at 5% level of significance according to DMRT

Fig. 1: Cytological behaviour in Chinese Spring x *Ae. geniculata* F_1 hybrids;
a) Mitotic chromosomes (2n=5x=35). Meiotic chromosomal association at metaphase-1 in Chinese Spring with *Ae. geniculata;*
b) acc. 276978; 35 univalents,
c) acc. 330487; 31 univalents, 2 rod bivalents.

indicated 35 univalents as dominant meiotic configuration (Fig. 1b). In other hybrids, meiotic configuration ranged between 35 univalents to 31 univalents and 2 rod bivalents in Chinese Spring x *Ae. geniculata* acc. 330487 (Fig. 1c) and Chinese Spring x *Ae. geniculata* acc. F (Fig. 2a), 35 univalents to 29 univalents and 3 rod bivalents in Chinese Spring x *Ae. geniculata* acc. 65 (Fig. 2b) and between 27 univalents and 4

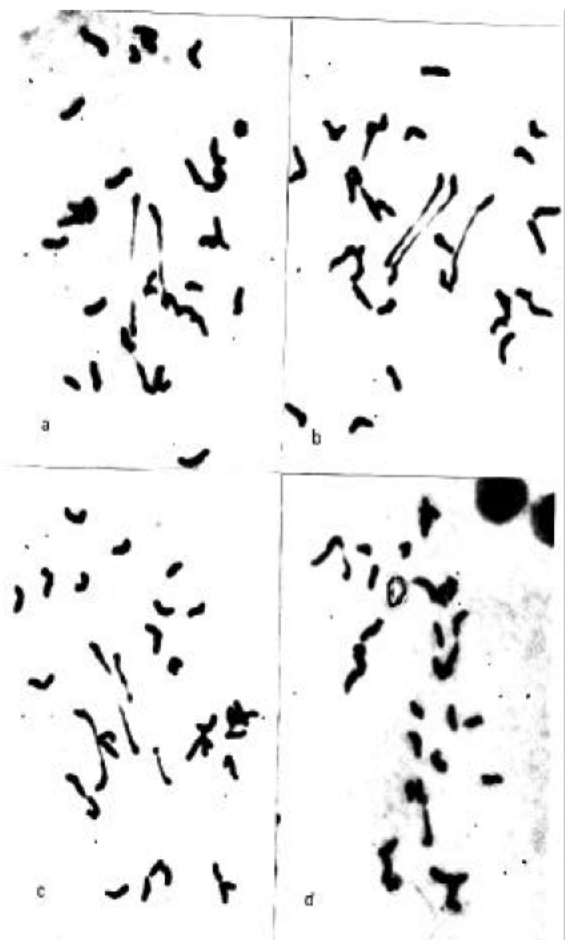

Fig. 2: Meiotic chromosomal association at metaphase-1 in Chinese Spring with different accessions of *Ae. geniculata*.
a) acc. F; 31 univalents, 2 rod bivalents,
b) acc. 65; 29 univalents, 3 rod bivalents,
c) acc. 361881; 27 univalents, 4 rod bivalents,
d) acc. 361881; 27 univalents, 1 rod bivalent, 2 trivalents.

rod bivalents (Fig. 2c) to 27 univalents, 1 rod bivalent and 2 trivalents (Fig. 2d) in Chinese Spring x *Ae. geniculata* acc. 361881. Significant difference in mean chiasma frequencies was also observed among the hybrids of different accessions of *Ae. geniculata* with Chinese Spring (Table 1). The highest chiasma frequency (4.10) was observed in the hybrid of Chinese Spring and *Ae. geniculata* accession 361881. There was no significant difference in the chiasma frequencies observed in the hybrids of Chinese Spring and *Ae. geniculata* accessions 65 and 330487. Similarly, difference in chaisma frequencies observed in the hybrids of Chinese Spring with *Ae. geniculata* accessions F and 276978 was also not significant.

Discussion

The frequency of different meiotic figures observed in the present study particularly in Chinese Spring x *Ae. geniculata* accession 361881 is significantly higher than the frequency generally expected in intergeneric hybrids. Normally, such hybrids reveal chromosomal associations of 1-3 rod bivalents except in the hybrids of wheat with alien species where *Ph* genes are not functional. In the wheat variety Chinese Spring used in the present study, *Ph* genes were fully active yet, the frequency of rod bivalents is significantly high. This pairing which may or may not be between the chromosomes of wheat and of *Ae. geniculata* reflects the presence of some additional pairing promoter genes particularly on *Ae. geniculata*. Such effect have already been observed by Farooq *et al.* (1989 and 1990b) in different accessions of *Ae. variabilis*. Since *Ae. geniculata* shares C^UC^U genome with *Ae. variabilis*, it is possible that the gene(s) affecting meiotic pairing in intergeneric hybrid could also be present in *Ae. geniculata* accession 361881. This was also indicated in the hybrids of wheat cv LU-26 with *Ae. geniculata* accession 361881 where chiasma frequency as high as 10 per cell was observed (Farooq *et al.*, 1996). The significance of this pairing is that the economically important genes like genes for karnal bunt resistance and for salt tolerance residing in *Ae. geniculata* could be transferred to cultivated wheat through crossing over between chromosomes of wheat and of *Ae. geniculata* which is a stable transfer compared to the transfer made through production of addition and substitution lines which are beset with chromosomal and yield instability.

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