

Cytogenetics of a *Hordeum vulgare* × *Elymus patagonicus* hybrid ($n = 4x = 28$)

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Summary. *Hordeum vulgare* L. ($2n = 2x = 14$) was hybridized with *Elymus patagonicus* Speg. ($2n = 6x = 42$). The hybrid had 28 chromosomes, genomically represented as HSH₁H₂, and was perennial with a co-dominant phenotype. The chromosomes were meiotically associated as 19.6 univalents + 0.004 ring bivalents + 2.6 rod bivalents + 0.8 trivalents + 0.14 quadrivalents in 1,129 meiocytes, with a chiasma frequency of 4.77 per cell. The bivalent pairing presumably is an auto-syndetic but modified expression of the H₁H₂ genomes of *E. patagonicus*, since ring bivalents were rare. This does not preclude the association of the *H. vulgare* H genome chromosomes with either H₁ and/or H₂ genomes of *E. patagonicus* to form bivalent or multivalent associations. A further evaluation of the genome homologies of *H. vulgare*, *H. bogdanii*, *E. canadensis* and *E. patagonicus* is proposed.

Key words: *Hordeum vulgare* – *Elymus patagonicus* – Intergeneric hybridization – Genome analysis – Auto-syndetic pairing

Introduction

Progress in intergeneric hybridization involving some Triticeae has been substantial over the last decade (Mujeeb-Kazi and Kimber 1984; Mujeeb-Kazi and Rodriguez 1984; Mujeeb-Kazi et al. 1983, 1984; Sharma and Gill 1983). These efforts have shed light upon the mechanisms of utilizing alien genetic variation for cereal improvement by either (i) direct gene transfer, (ii) production of alien addition lines, (iii) development of alien substitution lines, or (iv) inducing alien/cereal chromosome arm translocations. Intergeneric hybrids

additionally aid in elucidating intergenomic and evolutionary relationships.

In hybrids of *Elymus canadensis*/*Hordeum vulgare* and those of the reciprocal cross, Mujeeb-Kazi and Rodriguez (1982) observed less than one open bivalent per cell, suggesting a lack of homology between the H genomes of the two genera. *E. patagonicus* Speg., $2n = 6x = 42$, exhibits *Hordeum*-like characteristics of three spikelets per node, a fragile rachis and self fertility indicative of its containing a *Hordeum* genome. Its genomic formula is S₁S₁H₁H₁H₂H₂ (Dewey 1972) where S is the basic *Agropyron spicatum* genome. Earlier Hunziker (1955) had also designated the H genomes of *E. patagonicus* to be of probable *Hordeum* derivation. Following the report by Mujeeb-Kazi and Rodriguez (1982), attempts were made to hybridize *H. vulgare* with *E. patagonicus*. These attempts had two objectives that form the content of the present paper:

i) The basic objective of determining the extent of the relationship of the H₁H₂ genomes in the two genera, and

ii) The applied objective of assessing the possibility of transferring genetic information for disease resistance from *E. patagonicus* to *H. vulgare* via direct chromosome recombination.

Materials and methods

Seeds of *Elymus patagonicus* Speg., PI 286201, were obtained from Dr. D. R. Dewey, USDA Crops Research Laboratory, Logan, Utah, USA. Plants were grown from these seeds and established in the greenhouse under conditions of 25 °C/15 °C (day/night), natural light duration of 14 to 16 h, and 45 to 50% relative humidity. Spikes from an F₂ field segregating population of barley were emasculated. The pollination, embryo excision, and embryo culture procedures were similar to those reported earlier for *H. vulgare*/*T. aestivum* hybrids (Mujeeb-

Kazi and Rodriguez 1984). The single differentiated plantlet was transferred to a sterilized potting soil mixture and maintained in the greenhouse under growth conditions similar to those described earlier for *E. patagonicus*.

The hybrid was identified on the basis of its somatic chromosome number of $2n=4x=28$, from the root-tips of the developing hybrid (Mujeeb-Kazi and Miranda 1984). The hybrid was vegetatively cloned for population increase. Spikes for meiotic analysis were fixed in 6:3:1 (absolute ethanol:chloroform:acetic acid) for 48 h. The 6:3:1 solution was replaced by 70% ethanol and samples were then refrigerated (4°C) until use. Anthers at metaphase I were stained with 2% alcoholic aceto-carmin and squashed in 45% acetic acid. Selected pollen mother cells were photographed from temporary slides on high contrast black and white film using a green plus yellowish orange filter combination. The film was developed in D-19 developer.

The sterile F_1 hybrid clones are maintained in the greenhouse at CIMMYT, El Batan, Mexico, as is the herbarium specimen.

Results and discussion

Genome relationships

Dewey (1971) hybridized *H. bogdanii* and *E. canadensis* to overcome the masking of chromosome homologies by autosyndetic pairing. He inferred that half of the *H. bogdanii* genome chromosomes were fully homologous with those of the *E. canadensis* genome, and the remaining chromosomes were partially homologous.

Mujeeb-Kazi and Rodriguez (1982), however, did not observe genome homoeology in hybrids of *E. canadensis* with *H. vulgare*. It seems logical to infer that the H genome may not have *H. bogdanii* as its sole donor source. This hypothesis receives further support from the data of Table 1. The 28 somatic chromosomes (Fig. 1) of the *H. vulgare*/*E. patagonicus* hybrid, with a modified plant and spike phenotype as compared to the maternal barley parent (Fig. 1), have associated meiotically as 19.6 univalents + 0.004 ring bivalents + 2.6 rod bivalents + 0.8 trivalents + 0.14 quadrivalents in 1,129 meiocytes yielding a 4.77 chiasma frequency per cell. Some meiotic associations are expressed in Fig. 2. Trivalent associations occurred in 61.5% of the cells, of which 44.21% possessed 1 trivalent, 17.2% had 2 trivalents, and 0.1% had 3 trivalents. Were the H H_1H_2 genomes of the two genera homologous, the hybrid should have indicated a higher trivalent frequency. This decreased chromosome association is also reflected in the bivalent pairing of 2.6 rod bivalents + 0.004 ring bivalents (Table 1).

In hybrids of *Agropyron spicatum* × *E. patagonicus*, Dewey (1972) observed 5.27 univalents + 10.75 bivalents (rods + rings) + 0.24 trivalents + 0.07 quadrivalents + 0.01 pentavalents + 0.03 hexavalents in 207 meiocytes. The range of bivalents was from 6 to 14, where in occasional cells with 14 bivalents it led to the inference that 7 bivalents were due to autosyndetic pairing

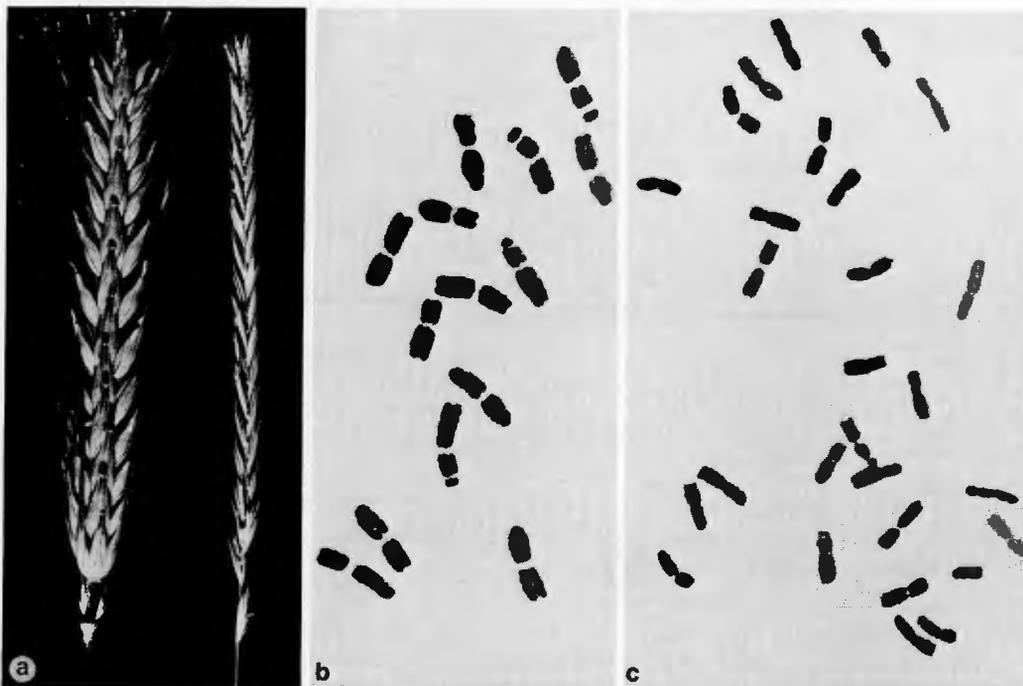


Fig. 1 a–c. Spike morphology and cytology of *H. vulgare* and its hybrid with *E. patagonicus*. a Spike of *H. vulgare* (left) and that of a *H. vulgare*/*E. patagonicus* F_1 expressing phenotypic variation from the maternal parent; b Somatic root tip cell of *H. vulgare* with $2n=2x=14$ chromosomes. Note the two satellited pairs; c Somatic root tip cell of *H. vulgare*/*E. patagonicus* with $n=4x=28$ chromosomes. The two satellited barley chromosomes are expressed in a single dosage in the F_1

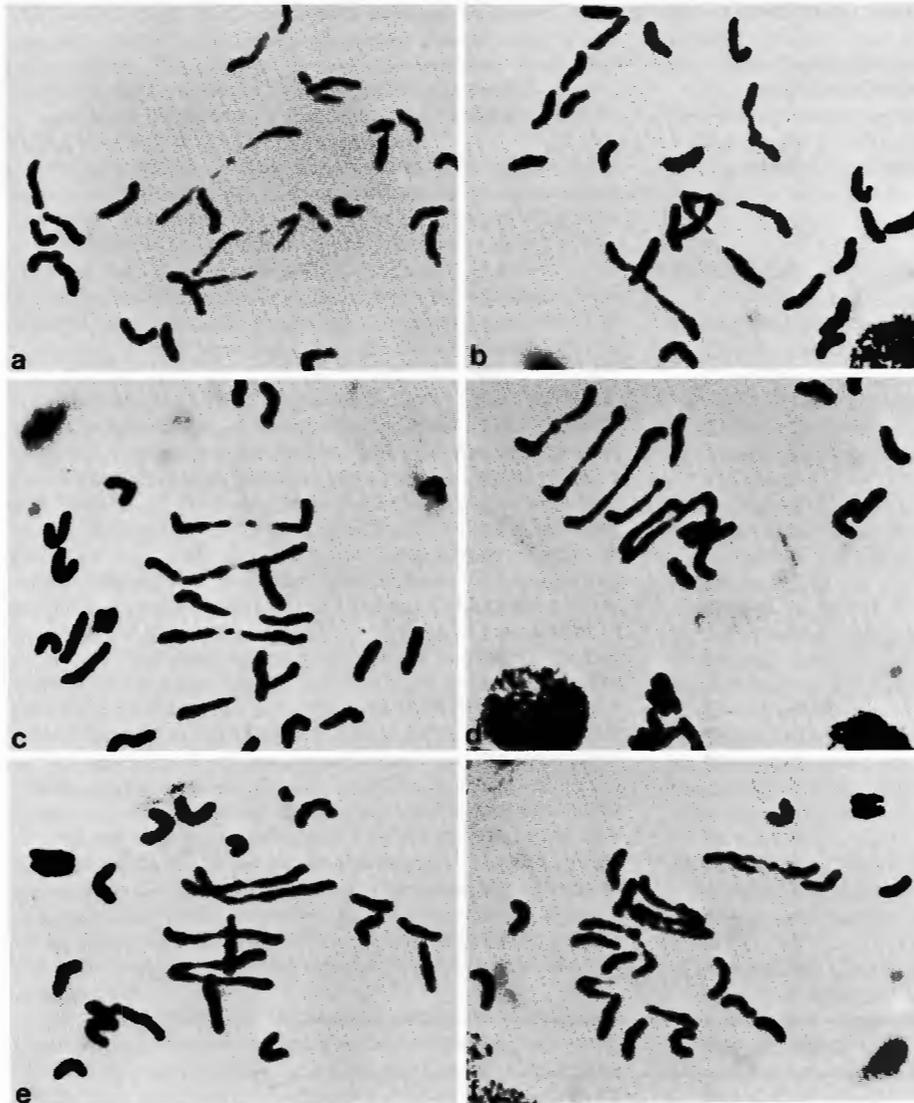


Fig. 2a-f. Chromosome associations at metaphase I in a *H. vulgare*/*E. patagonicus* F₁ hybrid. a 23 univalents, 1 bivalent (rod), 1 trivalent; b 21 univalents, 2 bivalents (rod), 1 trivalent; c 18 univalents, 5 bivalents (rod); d 5 bivalents (rod), 1 trivalent (2 univalents not in photograph frame); e 19 univalents, 3 bivalents (rod), 1 trivalent; f 16 univalents 2 bivalents (rod), 2 quadrivalents

among two of the three *E. patagonicus* genomes when their exact homologue was missing. The third *E. patagonicus* genome appeared to be more or less homologous with the *A. spicatum* genome forming 7 bivalents, thus accounting for the 14 observed.

The chiasma frequency of 4.77 per cell indicates that the *H. vulgare* genome differs from the H₁H₂ genomes of *E. patagonicus*. The high occurrence of rod bivalents in the *H. vulgare*/*E. patagonicus* suggests a weak autosyndetic relationship among the H₁H₂ genomes. It is also possible that the barley genome may have modified the autosyndetic associations of the H₁H₂ genomes, since observation of ring bivalents was

extremely rare (Table 1). In the *A. spicatum* × *E. patagonicus* hybrid, where autosyndesis was demonstrated for the H genomes (Dewey 1972), ring bivalents occurred more frequently even after allowing for the SS genome homology. In the present *H. vulgare*/*E. patagonicus* hybrid, close associations between 21 chromosomes were theoretically possible (H H₁H₂ genomes) but the relationship maxima was observed for 14 chromosomes in only 19 meiocytes as 4 rod bivalents + 2 trivalents. This leads to the inference that:

i) A close homology between the *H. vulgare* genome does not exist with the H₁ and H₂ genomes of *E. patagonicus*. There does appear to be enough residual

Table 1. Mean chromosome associations in a *Hordeum vulgare* L. × *Elymus patagonicus* hybrid (n = 4x = 28)

	No. of cells	Chromosome associations					% total	Mean chiasma frequency/cell
		I	II Rings	II Rods	III	IV		
	25	26		1			2.2	
	8	25			1		0.7	
	48	24		2			4.3	
	2	24	1	1			0.2	
	7	24				1	0.6	
	55	23		1	1		4.9	
	89	22		3			7.9	
	2	22		1		1	0.2	
	1	22			2		0.1	
	192	21		2	1		17.0	
	1	21	1	1	1		0.1	
	26	21			1	1	2.3	
	81	20		4			7.2	
	62	20		2		1	5.5	
	64	20		1	2		5.7	
	122	19		3	1		10.8	
	2	19	1	2	1		0.2	
	2	19		1	1	1	0.2	
	1	19			3		0.1	
	27	18		5			2.4	
	3	18		3		1	0.3	
	22	18		2	2		2.0	
	58	17		4	1		5.1	
	41	16		6			3.6	
	68	16		3	2		6.0	
	39	16		2		2	3.5	
	21	16		1	2	1	1.7	
	39	15		5	1		3.5	
	19	14		4	2		1.7	
	1	13		6	1		0.1	
	1	10		7		1	0.1	
Total	1,129	22,159	5	2,969	899	163		
Mean chromosome relationship		19.63	0.004	2.63	0.80	0.14	4.77	
Range		(10–26)	(0–1)	(0–7)	(0–3)	(0–2)		

homology present to allow for some bivalent associations (other than those due to H₁H₂ autosyndesis) and for trivalent formation.

ii) Structural chromosome re-arrangements (involving reciprocal translocation, segmental interchange) could be possible events leading to genome diversification. Cytogenetic evidence supports the H genome dissimilarities in *H. bogdanii* and *H. vulgare*. This is based upon meiotic data of the hybrids of these *Hordeum* species with *E. canadensis* (Dewey 1971; Mujeeb-Kazi and Rodríguez 1982). As yet, it needs to be determined how distant the H genomes of *H. bogdanii* and *H. vulgare* are, and it would also be interesting to

evaluate the H genome homology between the three genomes of such commonality in *E. canadensis* and *E. patagonicus*. Substantial information on the above genome homologies would be meaningfully obtained after hybrids are produced between *H. bogdanii*, *H. vulgare*, *E. canadensis* and *E. patagonicus* in pertinent combinations.

Practical utilization

As regards barley improvement, the desired resistance to pathogens and insect pests, as well as tolerance to environmental stresses, are being obtained currently from germplasm collections and from *H. spontaneum* (Moseman et al. 1983). In

the tertiary gene pool species of the tribe Triticeae, there resides a wealth of unexploited genetic resources for barley improvement. The *Elymus* species, for example, have been little used; and only two have been hybridized with *H. vulgare*, *E. canadensis* (Mujeeb-Kazi and Rodriguez 1982) and *E. mollis* (Schooler 1980).

In hybrids of *E. canadensis* with *H. vulgare*, the mean meiotic data indicated a lack of chromosome association with less than one open bivalent per cell (Mujeeb-Kazi and Rodriguez 1982), thus offsetting the possibility of direct gene transfers via recombination in F_1 .

Alien transfers into *H. vulgare* seem all the more complicated when the alien genera have unrelated genomes. This seemingly should have been the case for *H. vulgare* hybrids with *E. mollis* with the JJXX or the revised (Dewey, personal communication) JJNN genomes. Schooler and Anderson (1980), however, upon crossing autotetraploid *H. vulgare* with *E. mollis* reported that the hybrids indicated the occurrence of recombination events between the unrelated genomes (H, J and X or N). *E. mollis* possesses excellent resistance to several barley diseases (Schooler et al. 1982) and, from earlier work (Schooler and Anderson 1980), two lines highly resistant to barley yellow dwarf virus had been selected. The cytogenetic evidence supports pairing of the J and/or X (N) genome chromosomes of *E. mollis* with the H genome chromosomes of *H. vulgare*. This broadens the horizon for producing hybrids without total emphasis on assigned genomic designations prevalent in literature. Schooler and Anderson (1980) considered pairing in the F_1 hybrid ($n=4x=28$) to be a function of the association of the chromosomes of *Hordeum* and *Hordeum*, *Hordeum* and *Elymus*, and *Elymus* and *Elymus*. The latter association may not be the case, since in F_1 hybrids of *T. aestivum* with *E. giganteus* ($n=5x=35$, ABDJX) the J and X genomes do not associate autosyndetically. This also holds true for the SH genome species, *E. canadensis*. In hybrids of *E. canadensis* with *T. aestivum* (Mujeeb-Kazi and Bernard 1984), $n=5x=35$ (SHABD), there is less than 1 chiasma per cell indicating lack of pairing between the S and H genomes of *E. canadensis*.

Another example of the lack of autosyndetic pairing is found in data concerning chromosome associations in hybrids of *H. vulgare* and *E. canadensis* (Mujeeb-Kazi and Rodriguez 1982). With less than 1 chiasma per cell, there is reasonable justification for supporting the taxonomic separation of *Critesion* (H) and *Hordeum* (I). This does not preclude the fact that the prospects of attaining gene transfers in *H. vulgare*/*E. patagonicus* for barley improvement are still promising; these prospects will be conditioned by the pairing of chromosomes in F_1 as a consequence of the H genome homology of *E. patagonicus* with the *H. vulgare* genome (if the taxonomic separation is adopted) that yields multivalents. There is also the possibility that multivalents could arise exclusively due to autosyndesis within the *E. patagonicus* complement after undergoing some structural changes. Should the latter interpretation be the case, the chances of transferring genetic information from *E. patagonicus* to *H. vulgare* with recombination in F_1 would seem to be severely restricted. To resolve this, the F_1 hybrids should be advanced via backcrosses to *H. vul-*

gare and selecting plants in the backcross generations with characteristics that can be exclusively ascribed to *E. patagonicus*. These practical studies are to be initiated.

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