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## The cytogenetics of a *Triticum turgidum* × *Psathyrostachys juncea* hybrid and its backcross derivatives

Received: 23 February 1994 / Accepted: 18 August 1994

**Abstract** *Psathyrostachys juncea* ( $2n = 2x = 14$ , NN), a source of barley yellow dwarf (BYDV) virus resistance with tolerance to drought and salinity, has been successfully hybridized in its autotetraploid form ( $2n = 4x = 28$ , NNNN) as the pollen parent to durum wheat (*Triticum turgidum* L.). The  $2n = 4x = 28$  (ABNN)  $F_1$  hybrid has a mean meiotic metaphase-I configuration of 20.29 univalents + 0.29 ring bivalents + 3.36 rod bivalents + 0.14 trivalents. Spike length, internode length, glume awn length and lemma awn length, as well as the general spike morphology of the  $F_1$  hybrid, are intermediate with those of the two parents. Pollinating the ABNN  $F_1$  hybrid has given backcross (BC)-I derivatives of an amphiploid (AABBNN) that expresses limited self-fertility. BC-2 derivatives have been obtained from these plants. Direct transfers of useful genes from *Ps. juncea* to wheat would require substantial genetic manipulation strategies. Both conventional and novel methodologies, which may complement each other, and so facilitate reaching an agricultural objective end point, are addressed.

**Key words** Wheat · *Psathyrostachys juncea*  
Intergeneric hybridization · Backcross amphiploids  
Genetic manipulation

### Introduction

Several new intergeneric hybrids in the Triticeae have been produced and cytogenetically described during the last decade (Sharma and Gill 1983; Dewey 1984; Mujeeb-Kazi et al. 1987, 1989; Plourde et al. 1989, 1990; Wang 1989; Limin and Fowler 1990; Pienaar 1990). Crossability barriers have been ingeniously circum-

vented leading to success in achieving extremely divergent cross combinations. In many cases fertilization was coupled with alien genome elimination, and subsequent plantlet regeneration resulted in wheat polyhaploids (wheat × maize: Laurie and Bennett 1988; wheat × pearl millet: Ahmad and Comeau 1990; wheat × sorghum: Ohkawa et al. 1992; wheat × *Tripsacum dactyloides*: Riera-Lizarazu and Mujeeb-Kazi 1993; wheat × teosinte: Ushiyama et al. 1991).

Despite the advances in intergeneric hybridization methodology some hybrid combinations have been difficult to obtain. These include wheat (*Triticum aestivum* L.) with *Elymus scabrus* (Ahmad and Comeau 1991), *Leymus innovatus* (Plourde et al. 1989), and *Psathyrostachys juncea* (Plourde et al. 1990). Direct hybridization of *Ps. juncea* ( $2n = 2x = 14$ , NN) with *T. aestivum* was unsuccessful (Mujeeb-Kazi et al. 1987). However, by using the *Ps. juncea* autotetraploid ( $2n = 4x = 28$ ) a hybrid was obtained (Mujeeb-Kazi and Asiedu 1990) and the options of additional genetic variability for wheat improvement were diversified. *Ps. juncea* (synonymous with *Elymus junceus*) is known to possess tolerance to salinity and drought (Dewey 1984) and also possesses resistance to barley yellow dwarf virus (Plourde et al. 1990). The species grows on rocky open slopes and has a demonstrated potential of re-vegetating depleted rangelands. These biotic and abiotic attributes of *Ps. juncea* make the species an invaluable source for use in wheat breeding. To diversify the working range for applied global agricultural objectives, we have embarked on utilizing *T. turgidum* in our intergeneric hybridization program which involves crosses with the annual and perennial Triticeae. Because of our earlier success with *T. aestivum* × *Ps. juncea* hybridization using the latter's autotetraploid (Mujeeb-Kazi and Asiedu 1990), the same *Ps. juncea* source was hybridized onto *T. turgidum*. In this paper we report the production, cytogenetics, and morphology, of the  $F_1$  hybrid, its backcross-I and -II derivatives obtained using *T. turgidum* as recurrent pollen parent, and derivatives from the  $F_1$  × *T. aestivum* cross.

Communicated by K. Tsunewaki

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## Materials and methods

### Germplasm

Seeds of *Ps. juncea* ( $2n = 4x = 28$ ; colchicine-induced autotetraploid) were obtained from the late Dr. D. R. Dewey, USDA/ARS Logan, Utah, and germinated in Jiffy-7 peat pots. After attaining a 6-week juvenile growth the seedlings were vernalized in a growth chamber under environmental regimes 8 h of diffuse light for 8 and 12 weeks at 8 °C. Following vernalization, the seedlings were transplanted into 20-cm plastic pots filled with a 2:1:1 (soil:sand:peat) steam-sterilized mix and maintained under greenhouse conditions of 16 h of natural day-light and 24°C/14°C day/light temperatures. In the same greenhouse three plantings in pots (four plants/pot) of *Triticum turgidum* cvs 'Laru', 'Chen', 'Altar 84', 'Memo/Mexicali' and 'Duergand' ('Cndo'/'R143', 'Ente'/'Mexi') were made, 15 days apart. The durum cultivar seeds were obtained from CIMMYT's wheat germplasm bank at El-Batan, Mexico, the location at which this study was conducted.

### Hybrid production

Spikes of the five durum wheat cultivars were emasculated, pollinated by *Ps. juncea* pollen 3–4 days after emasculation and treated with gibberellic acid. From the seeds set, the embryos were excised 16 days after pollination and cultured on a special medium for small embryos. These and subsequent procedures associated with embryo differentiation, plantlet growth, transfer to peat pots, and transplanting to a potted soil mix in the greenhouse, were similar to those reported by Mujeeb-Kazi et al. (1987, 1989). The environmental growth regimes were identical to those maintained for the growth of the parental germplasm in this study.

### F<sub>1</sub> somatic and meiotic sampling

After assuming vigorous growth, the F<sub>1</sub> hybrid was physically divided and the clones obtained were allowed to grow into vigorous plants. From each clone, root-tips were collected for somatic cytology and C-banding. The procedure of Mujeeb-Kazi and Miranda (1985) was followed for somatic cytology. The C-banding procedure was essentially similar to that described by Jahan et al. (1990).

Spikes for meiotic analysis were collected in early morning (8:00–9:00 a.m.), fixed in Carnoy's (6:3:1, absolute alcohol:chloroform:acetic acid) for 48 h, and stored under refrigeration (4 °C) in 70% alcohol until use. Anthers at metaphase-I were stained in alcoholic-acid-carmin for several days, squashed in 45% acetic acid with a drop of 2% aceto-carmin. Meiotic chromosome associations were analyzed at metaphase-I.

### Spike characterization and backcross-I seed production

Fully emerged spikes from the F<sub>1</sub> hybrid and the durum parent involved were characterized for spike morphology. F<sub>1</sub> self-sterile spikes were pollinated with *T. turgidum* or *T. aestivum* to produce the equivalent of backcross-I progeny. Embryo excision was routinely used to ensure progeny advance. These procedures were similar to those reported earlier for F<sub>1</sub> hybrids. The cytological and morphological analysis of the backcross plants also involved the techniques already outlined. The *T. turgidum*-based BC-1 plants were similarly advanced to BC-2 and cytologically analyzed.

## Results and discussion

### Hybrid production and spike morphology

Crossing between the two species was satisfactorily accomplished. Seed set was observed on almost all *T.*

**Table 1** Hybridization details between *T. turgidum* L. cultivars (female parents) and *Ps. juncea* ( $2n = 4x = 28$ ) under greenhouse conditions

<i>T. turgidum</i> cultivar	No. of florets pollinated	Seeds set	No. of embryos excised	No. of plants obtained
Altar 84	100	1	0	–
Chen	100	0	–	–
Duergand	100	2	1	1
Laru	100	1	0	–
Memo/Mexicali	100	1	1	0

*turgidum* cultivars, its overall frequency being 1% (Table 1), a level characteristic of a difficult cross. This is further substantiated by the poor embryo formation rate (2/500) and the 50% plant differentiation (1/2). Even though the two excised embryos were plated in a special medium for small embryos, the one which differentiated into a plantlet had a formative shape and substantially more fluid in the endosperm cavity. Though the hybridization frequencies here are poor (less than 1%), in essence a viable hybrid is all that is necessary for achieving the practical goals of an intergeneric hybridization program.

Among the spike characters observed, the spike length, internode length, spikelet length, number of spikelets per spike, glume body length, glume awn length and lemma awn length of the F<sub>1</sub> hybrid were intermediate to those of the two parents (Table 2, Fig. 1). A useful single descriptor is the presence of large awns in *T. turgidum*, awnlessness in *Ps. juncea*, and very reduced but positive awn presence in the F<sub>1</sub> hybrid. The BC<sub>1</sub> derivatives from pollinating the F<sub>1</sub> hybrid ( $2n = 4x = 28$ ; ABNN) with *T. turgidum* possessed 42 chromosomes (ABNN + AB = AABBN), and still expressed the intermediate phenotype with longer awns than the F<sub>1</sub> hybrid (Fig. 2 a, b). A similar expression prevailed in the offspring of the cross between the durum/*Ps. juncea* F<sub>1</sub> hybrid and *T. aestivum* (Fig. 2 c;  $2n = 7x = 49$ , AABBDNN). An intermediate phenotype has been a common observation for several intergeneric hybrids within the Triticeae (McFadden and Sears 1946; Mujeeb-Kazi and Asiedu 1990; Pienaar 1990) and may be considered a valid indicator of alien genetic expression in a wheat background.

### Cytology of the parents and the F<sub>1</sub> hybrid

The two satellited chromosomes, 1B and 6B (Fig. 3a), which are present in pairs in *T. turgidum*, were identified in the F<sub>1</sub> hybrid (Fig. 3b). Satellites of the autotetraploid *Ps. juncea* were not visible in the hybrid – a consequence of amphiplasty. The 28 C-banded chromosomes of the *Ps. juncea* autotetraploid and the 14 chromosomes of this species contributing to the hybrid were readily identified (Figs. 3c, d). The banding sites were essentially similar to those reported by William and Mujeeb-Kazi

**Table 2** Mean spike characteristics of an intergeneric hybrid of *T. turgidum* L. with an autotetraploid *Ps. juncea* accession, and its advance derivatives

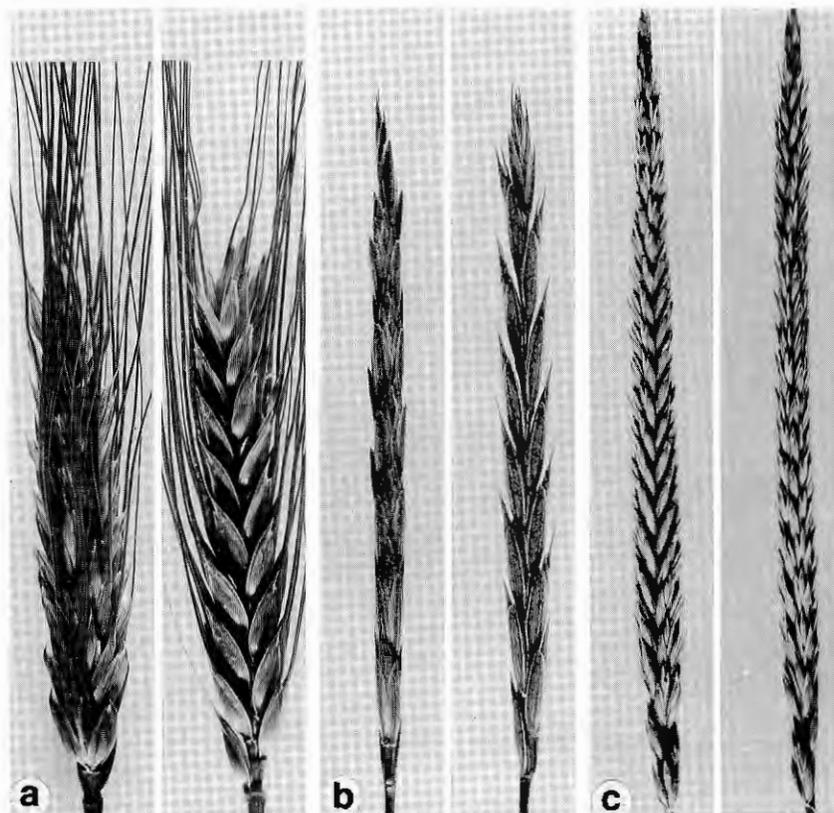
Material	2n	Spike character <sup>a</sup>												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Duergand	(28)	6.3	1.42	17.7	0.36	1.36	0.94	17.66	5.0	0.88	0.3	1.1	12.7	0.3
F <sub>1</sub> B87-3941	(28)	10.0	1.27	12.67	0.64	1.87	0.85	12.67	5.0	1.38	0.1	1.37	0.3	0.31
F <sub>1</sub> x <i>T. turgidum</i> cv 68112/Ward														
(1) B91-4161	(42)	9.1	0.7	17.0	0.5	1.5	0.6	17.0	4.0	1.1	0.1	1.3	1.5	0.3
(2) B91-4162	(42)	9.6	0.8	17.5	0.5	1.7	0.7	17.5	4.5	1.1	0.1	1.2	1.2	0.3
(3) B91-4163	(42)	9.3	0.6	16.3	0.5	1.0	0.5	16.3	3.0	1.1	0.1	1.1	1.8	0.2
(4) B91-4164	(42)	9.9	0.7	16.0	0.5	1.4	0.6	16.0	4.7	1.1	0.1	1.3	1.9	0.3
F <sub>1</sub> x <i>T. turgidum</i> cv 68111/RGB//Ward resel/3/Stil "S"														
(1) B92-4165	(41) <sup>b</sup>	10.7		15.0	0.6	2.0	0.8	15.0	5.3	1.2	0.1	1.4	2.2	0.3
F <sub>1</sub> x <i>T. aestivum</i> cv Pvn "S" Buc "S"														
(1) B91-4166	(48)	7.6	0.7	11.8	0.6	1.5	0.6	11.8	4.3	1.0	0.1	1.1	1.6	0.2
(2) B91-4167	(49)	7.3	0.6	11.0	0.6	1.4	0.5	11.0	4.5	1.0	0.1	1.1	0.8	0.2
(3) B91-4168	(47)	5.3	0.5	10.0	0.5	1.4	0.5	10.0	4.0	0.8	0.1	1.1	0.2	0.2

<sup>a</sup> 1 = spike length (cm); 2 = spike width (cm); 3 = nodes per spike; 4 = internode length (cm); 5 = spikelet length (cm); 6 = spikelet width (cm); 7 = spikelets per spike; 8 = florets per spikelet; 9 = glume body

length (cm); 10 = glume awn length (cm); 11 = lemma body length (cm); 12 = lemma awn length (cm); 13 = anther length (cm)

<sup>b</sup> This plant possessed a dicentric chromosome

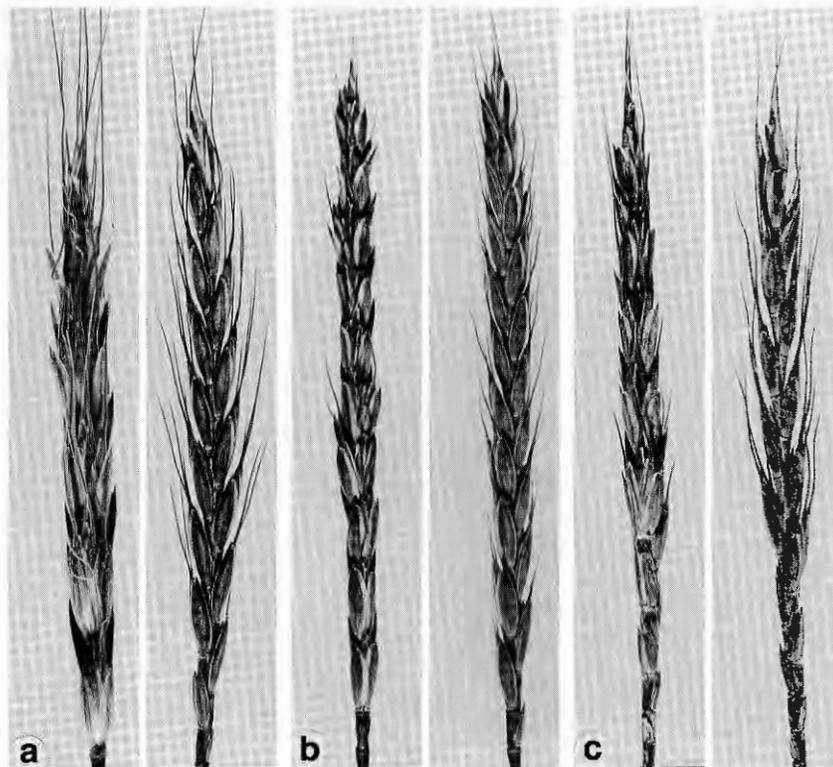
**Fig. 1a-c** Dorsal and ventral views of spikes of durum wheat, *Ps. juncea* and their F<sub>1</sub> hybrid. **a** *T. turgidum* cv 'Duergand', **b** *Turgidum* x *Ps. juncea* F<sub>1</sub> hybrid, **c** *Ps. juncea* (autotetraploid, 2n = 4x = 28)



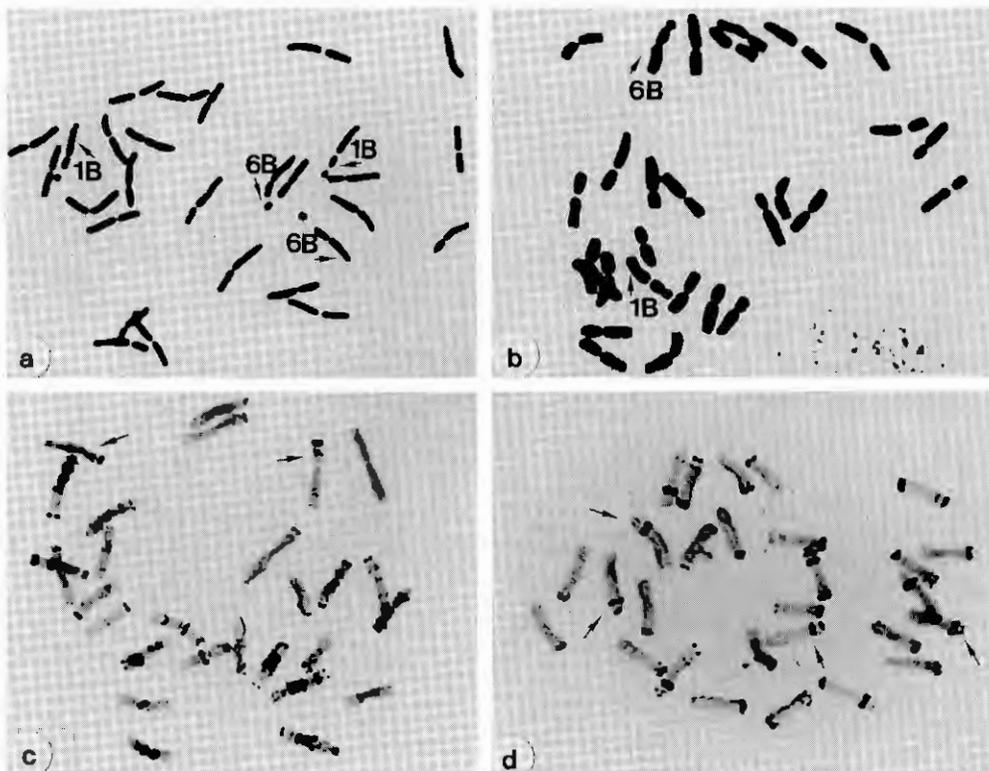
(1992). No aneuploidy was observed among either the mitotic or meiotic cells of the F<sub>1</sub> hybrid. The mean metaphase-I configuration over 349 meiocytes was 20.29 univalents + 0.29 ring bivalents + 3.36 rod bivalents + 0.14 trivalents (Table 3). The total bivalent association of 3.64 per meiocyte contrasts with the anticipated seven homologous pairs derived from

the NN genomes. The frequencies of the meiocytes showing a different number of bivalents were as follows: 5-7 bivalents = 14.04% (Fig. 4d); 4 bivalents = 24.35% (Fig. 4b,c); 3 bivalents = 27.79% (Fig. 4a); 1-2 bivalents = 32.38% and 0 bivalents = 1.43%. One or more ring bivalents were present in seven meiocytes (2.01%).

**Fig. 2a–c** Dorsal and ventral views of spikes of the backcross-I derivatives of *T. turgidum* × *Ps. juncea* F<sub>1</sub> hybrid with *T. turgidum* and *T. aestivum*. **a** *T. turgidum*/*Ps. juncea*//*T. turgidum*, B91-4161 with 42 chromosomes, **b** *T. turgidum*/*Ps. juncea*//*T. turgidum*, B91-4165 with 41 chromosomes, **c** *T. turgidum*/*Ps. juncea*//*T. aestivum*, B91-4166 with 49 chromosomes

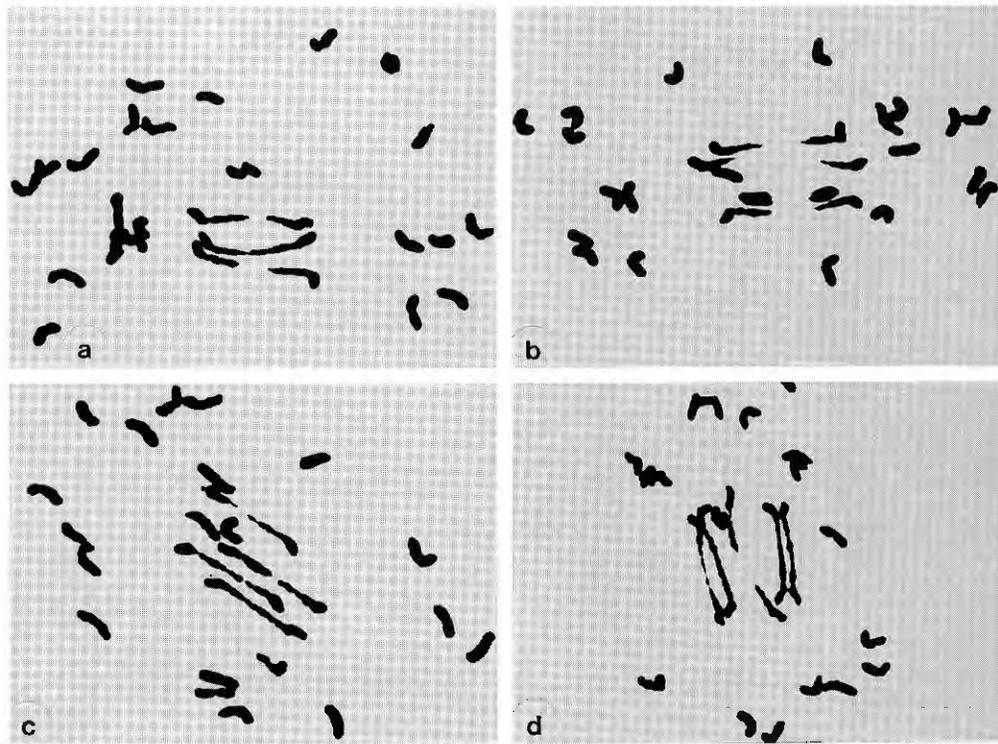


**Fig. 3a–d** Mitotic metaphase cells of *T. turgidum* and a *T. turgidum* × *Ps. juncea* F<sub>1</sub> hybrid. **a** 2n = 4x = 28 chromosomes of *T. turgidum*; satellited chromosome pairs arrowed, **b** 2n = 4x = 28 chromosomes of a hybrid cell of *T. turgidum* × *Ps. juncea*; each satellited chromosome is arrowed, **c** an F<sub>1</sub> somatic C-banded cell showing the 14 *Ps. juncea* chromosomes. Two of the four marked chromosomes of one set in **d** are identified (arrows), **d** a C-banded *Ps. juncea* somatic cell with 2n = 4x = 28 chromosomes. Each of the chromosome sets are represented four times of which one set of four is marked with arrows



**Table 3** Mean meiotic metaphase-I chromosome pairing relationships in a  $F_1$  hybrid of *T. turgidum*/*Ps. juncea* ( $2n = 4x = 28$ ); and its backcross derivatives with *T. turgidum* and *T. aestivum*.

Material	2n	Meiocytes analyzed	Mean metaphase-I chromosomal association					
			I	II rings	II rods	II total	III	Xta/cell
<i>T. turgidum</i> / <i>Ps. juncea</i> ( $F_1$ ) B87-3941	28	349	20.29 (14–28) <sup>a</sup>	0.29 (0–1)	3.36 (0–7)	3.64	0.14	4.36
$F_1$ / <i>T. turgidum</i> (BCI) B91-4162	41	20	7.40 (4–10)	12.90 (11–15)	4.40 (2–7)	17.30	–	30.20
$F_1$ / <i>T. turgidum</i> (BCI) B91-4164	42	20	9.20 (6–12)	11.80 (9–14)	4.60 (2–7)	16.40	–	28.20
$F_1/2^*$ <i>T. turgidum</i> (BCII)	33	20	5.10 (5–7)	11.50 (9–13)	2.45 (1–5)	13.95	–	25.45
$F_1$ / <i>T. aestivum</i> (BCI)	50	20	18.50 (14–24)	7.90 (4–12)	7.70 (4–11)	15.60	0.10	23.70

<sup>a</sup> Range observed**Fig. 4a–d** Meiotic metaphase-I cells of an  $F_1$  hybrid of *T. turgidum* × *Ps. juncea* ( $2n = 4x = 28$ , ABNN). **a** 22 univalents + 3 rod bivalents, **b** 20 univalents + 3 rod and + 1 ring bivalent, **c** 20 univalents + 4 rod bivalents, **d** 18 univalents + 5 rod bivalents

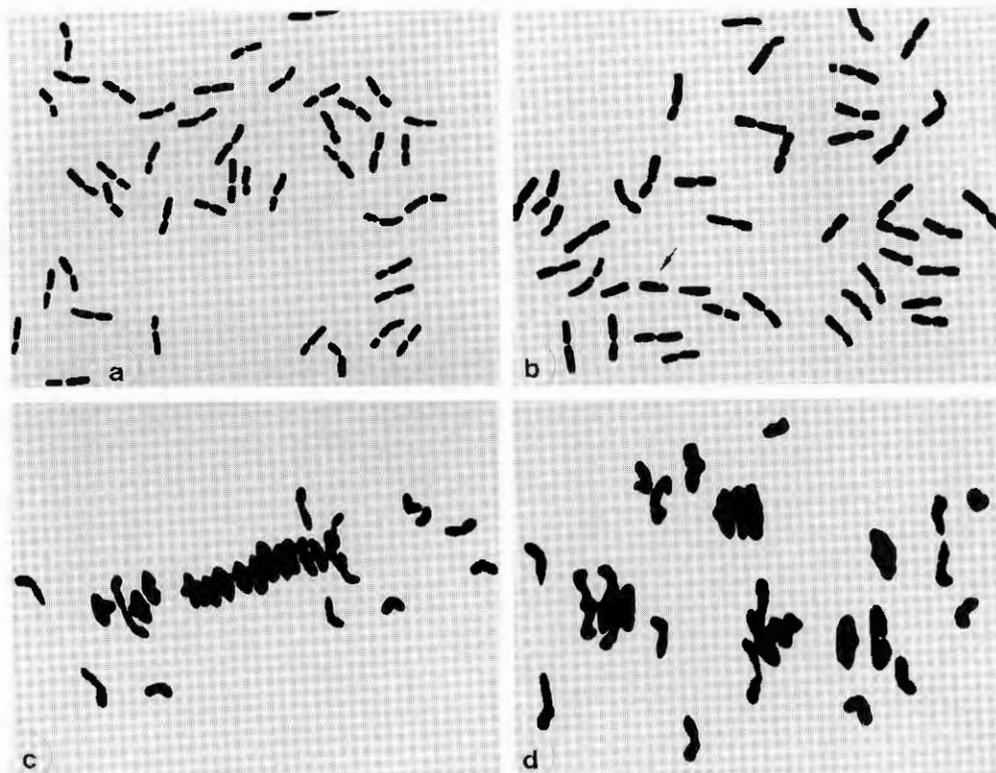
#### Production and cytogenetics of backcross-I derivatives

With the aim of developing a set of seven *Ps. juncea* disomic additions in the durum wheat background, and to screen the individual disomic additions for biotic/abiotic stresses, the *T. turgidum*/*Ps. juncea* hybrid was backcrossed with pollen from two *T. turgidum* cultivars, '68112/Ward' and '68111/RGB//Ward re-selection/3/Stil S'. Pollination of 320 florets set eight seeds, resulting in six excised embryos of which five developed into vigorous  $BC_1$  plants. Four  $BC_1$  plants possessed the normal chromosome composition of 42 chromosomes (Fig. 5a). The 14 *Ps. juncea* chromosome were

readily identified by C-banding. One  $BC_1$  plant possessed 41 instead of the normal 42 chromosomes and included a dicentric chromosome (Fig. 5b). Genomically, the normal  $BC_1$  plants would be AABBNN, equivalent to an amphiploid between *T. turgidum* and a diploid *Ps. juncea*, and are expected to show: (1) a high bivalent frequency with more ring bivalents, and (2) fertility upon selfing.

The meiocytes of two  $BC_1$  derivatives, possessing 42 chromosomes, showed a chromosome configuration of 17.3 bivalents + 7.4 univalents and 16.4 bivalents + 9.2 univalents respectively (Table 3; Fig. 5c, d). The AABB genomes of durum wheat would account for a maximum of 14 bivalents and the balance is attributed to the

**Fig. 5a-d** Mitotic metaphase and meiotic metaphase-I cells of some backcross-I derivatives of *T. turgidum*/*Ps. juncea*//*T. turgidum*. **a** somatic cell with normal 42 chromosomes; **b** somatic cell with 41 chromosomes, a dicentric chromosome is *arrowed*; **c** 6 univalents + 4 rod bivalents + 14 ring bivalents (42 chromosomes), **d** 6 univalents + 6 rod bivalents + 12 ring bivalents (42 chromosomes)



association or univalency of the *Ps. juncea* chromosomes. The BC<sub>1</sub> univalency pattern appears to be consistent with observations on the F<sub>1</sub> hybrids (ABNN). We feel that the fertility mode of *Ps. juncea* and its autotetraploid stock maintenance may have produced structural N-genome chromosome variations that have become manifested in greater than expected univalency. The high univalency in BC<sub>1</sub> derivatives has, presumably, led to self-sterility. Several cycles of cloning of the BC<sub>1</sub> perennial derivatives, and selfing of the spikes which emerged profusely, failed to produce abundant viable seeds. Twelve seeds produced by selfing were well formed, of which seven germinated and possessed a predominant normal somatic number of 42 chromosomes.

The advantages of having fertile amphiploids extend over a number of important features including germplasm distribution, the ease of biotic/abiotic stress screening, facilitating basic studies, and the development of genetic stocks. Though rapid advancement of an F<sub>1</sub> hybrid to the BC<sub>1</sub> derivatives is achieved by crossing the wheat parent or a related cultivar onto the hybrid, aneuploidy and structural alterations of significant magnitude may emerge, as reported in the *T. aestivum*/*Aegilops variabilis* and *T. aestivum*/*Thinopyrum ponticum* combinations (Jewell and Mujeeb-Kazi 1982). Use of alien autotetraploids alleviates the above constraint since pollinating wheat × autotetraploid species F<sub>1</sub> hybrids with wheat yields BC<sub>1</sub> derivatives that are genomically amphiploid, e.g., AABBNN in this study.

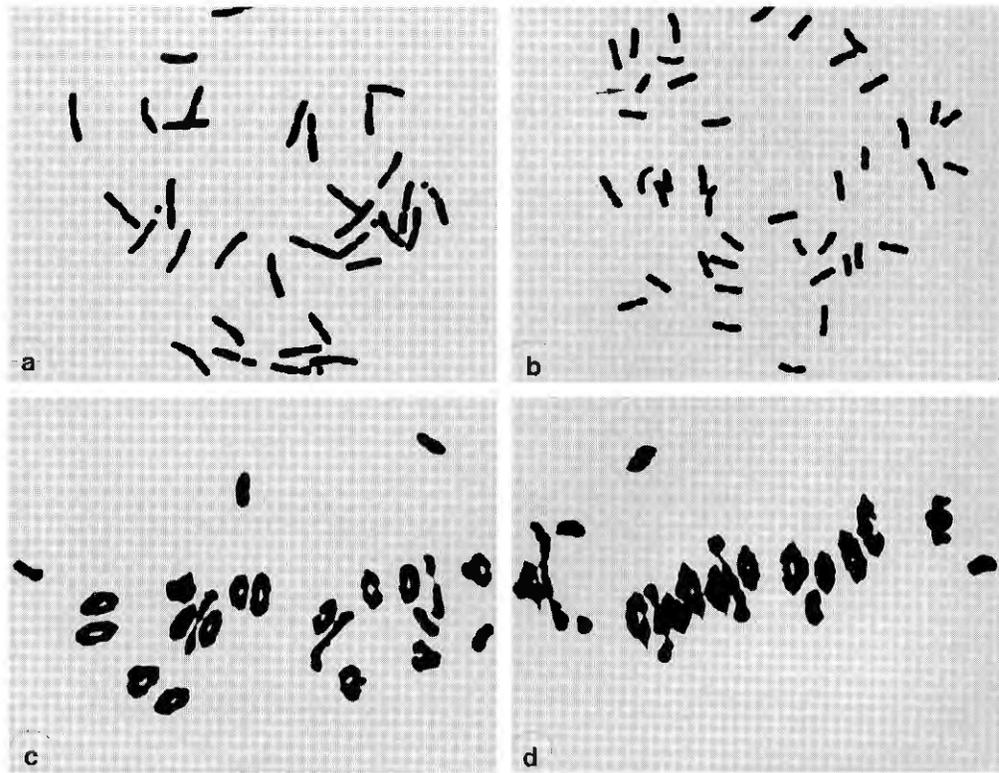
#### Practical applications after the BC<sub>1</sub> stage

The BC<sub>1</sub> derivatives (2n = 6x = 42; AABBNN) form a base for producing progeny that would potentially culminate in a durum wheat with seven unique disomic additions of *Ps. juncea* chromosomes. To achieve this goal, the BC<sub>1</sub> derivatives were pollinated with wheat, and BC<sub>2</sub> seeds were produced. Even though the BC<sub>2</sub> seeds were large, immature embryos were excised and cultured to speed up progress. They readily germinated and grew into healthy BC<sub>2</sub> plants. Somatic chromosome numbers of the BC<sub>2</sub> derivatives were 33 (Fig. 6a), 41 with an acrocentric (Fig. 6b), 42, or 45. The 41, 42 and 45 chromosome derivatives may presumably be ascribed to pseudogamo-apomixis, a phenomenon observed in barley/wheat backcrosses (Mujeeb-Kazi 1981). Meiotic association in the 2n = 41 plant is illustrated in Fig. 6c. Each of the four 2n = 33 plants meiotically analyzed had a preponderance of 14 bivalents (anticipated from AABB genomic pairing) plus five univalents (Fig. 6d). The rod and ring bivalents were variable in number. More BC<sub>2</sub> plants are being produced so that the entire *Ps. juncea* genome will be represented in the disomic addition lines.

#### Some manipulative strategies

We are concentrating on classical cytogenetic manipulation for introgressing *Ps. juncea* chromosomes into

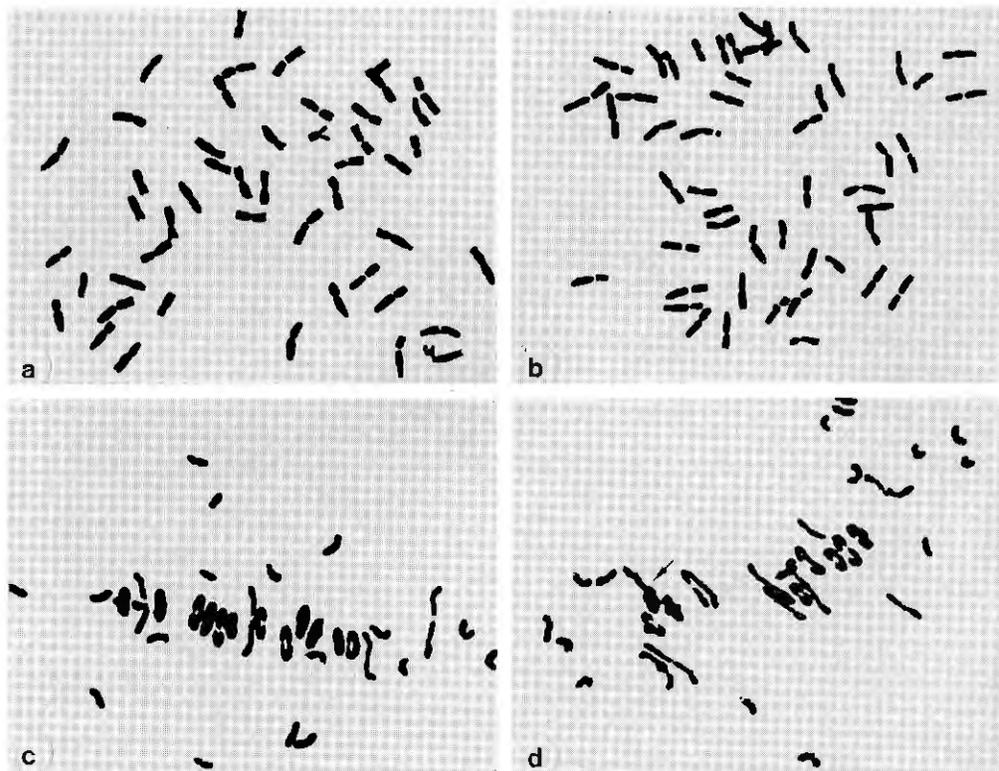
**Fig. 6a-d** Mitotic metaphase and meiotic metaphase-I cells of some backcross-II derivatives of *T. turgidum*/*Ps. juncea*//2\* *T. turgidum*. **a** A somatic cell with 33 chromosomes, **b** somatic cell with 41 chromosomes including an acrocentric (arrowed), **c** 5 univalents + 3 rod bivalents + 15 ring bivalents; a total of 41 chromosomes, **d** 5 univalents + 14 bivalents; a total of 33 chromosomes.



wheat. A few variations are, however, being explored. One is associated with induced translocations between the alien and the D-genome chromosomes. The base for intergeneric translocation is set when the ABNN F<sub>1</sub>

hybrid is pollinated with *T. aestivum*, yielding derivatives that possess 49, or near 49, chromosomes (AABBNNND; Fig. 7a, b). The bivalents observed in these derivatives (Fig. 7c, d) are inferred as associations be-

**Fig. 7a-d** Mitotic metaphase and meiotic metaphase-I cells of backcross-I derivatives of *T. turgidum* *Ps. juncea*//*T. aestivum*. **a** A somatic cell of a hypo-heptaploid ( $2n = 48$ ), **b** a somatic cell of a hyper-heptaploid ( $2n = 50$ ), **c** meiotic configuration of 18 univalents + 4 rod bivalents + 12 ring bivalents observed in a  $2n = 50$  plant, **d** meiotic configuration of 16 univalents + 6 rod bivalents + 8 ring bivalents + 1 open trivalent + 1 pan-handle trivalent (arrowed) observed in a  $2n = 50$  plant



tween the AA, BB and NN genomes. The univalents ascribed to seven chromosomes of the D genome others predominantly to the N-genome chromosomes that have not paired. These D- and N-univalents anticipated to yield centric-break fusion products eventually leading to alien translocation chromosomes. The above procedure has been applied in our program to a *T. aestivum./Th. bessarabicum* hybrid by crossing with a durum wheat cultivar and producing 42-chromosome AABB DJ derivatives. Extensive analyses are in hand and will be reported separately.

Another strategy to be utilized involves the use of maize-mediated polyploidy in wheat to fix alien segments and multiple chromosome additions (Mujeeb-Kazi et al. 1993). As yet, self-fertility of the BC<sub>2</sub> plants has not been observed since all spikes have either been utilized for BC<sub>3</sub> seed production or for meiotic analyses. When self-fertility is observed, the sexual process of achieving homozygosity will be interjected, as was successfully applied to BC derivatives from the *Th. elongatum./T. aestivum* combination (Mujeeb-Kazi et al. 1993). The polyploid approach is also intended to overcome paternal transmission constraints anticipated because of the recessive behavior (Jensen et al. 1990)