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MEIOTIC STUDIES IN AN INTERGENERIC HYBRID BETWEEN *TRITICUM AESTIVUM* L. AND *THINOPYRUM BESSARABICUM* AND THEIR BACKCROSS PROGENIES

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ABSTRACT

In order to transfer desirable traits from diploid wheatgrass, an intergeneric hybrid (*Triticum aestivum* cv. Chinese Spring monosomic 5B x *Thinopyrum bessarabicum* with 2n = 2x = 14, JJ genome) was backcrossed to two breadwheat cultivars. Pollination of the F_1 hybrid (2n = 27, ABDJ) with the pollen of wheat cultivars resulted in one BC_1 hybrid (2n = 48) plant. Subsequent backcrossing with common wheat produced about 60 BC_2 plants with the chromosome number ranging from 2n = 38 to 48. Plants having 2n = 48 chromosomes were also observed in BC_3 generation. The phenomenon of meiotic nonreduction was observed in BC_1, BC_2 and BC_3 progenies. Analysis of these progenies led to identification of the supposed wheat *Th. bessarabicum* addition lines.

Key words: *Thinopyrum bessarabicum*, *Triticum aestivum*, intergeneric hybrid, meiotic analysis, meiotic nonreduction.

The wild relatives of wheat have been successfully utilized for wheat improvement, particularly for disease resistance [1, 2]. Among the distantly related species, *Agropyron* species possess potentially valuable traits for wheat improvement including tolerance to drought and salt [3, 4]. The diploid wheatgrass *Thinopyrum bessarabicum* has been identified as a source of salt tolerance [5]. Hybrids of wheat with *Th. bessarabicum* have been reported by many workers [6–8] but successful incorporation of genes imparting salt tolerance in *T. aestivum* background has not been established yet either due to the failure in obtaining fertile F_1 hybrids or due to difficulty in getting direct gene introgression by homoeologous recombination in early generations. In the present study, the 5B mechanism of *T. aestivum* has been exploited to get alien recombination between the hexaploid *T. aestivum* and diploid wheatgrass *Th. bessarabicum*. Results on production of the hybrids and subsequent

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cytological analysis of backcross derivatives obtained from the F_1 hybrid x breadwheat cultivars (BC_2), and BC_2 progenies are presented here.

MATERIALS AND METHODS

Monosomic 5B of *Triticum aestivum* L. cv. Chinese Spring (2n = 6x = 42, AABBDD genome) was crossed to *Thinopyrum bessarabicum* (Savul & Rayss) A Love (2n = 2x = 14, JJ genome) (=*Agropyron bessarabicum* S & R = *Agropyron junceum* ssp. *boreoatlanticum* Simonet & Guinochet). Chromosome number in the F_1 hybrid plants were determined at meiotic metaphase I. A few plants in BC_2 and BC_3 generations were scored randomly for chromosome number and the spikes were covered with butter paper bags in order to obtain self seeds. The seed set per main spike was calculated in per cent. All cytological observations were made from temporary slides. PMCs were stained with 1% leucobasic fuchsin.

RESULTS AND DISCUSSION

Two F_1 hybrid plants obtained were deficient for 5B chromosome of *T. aestivum* as their chromosome number was 2n = 27 (2n = 4x = 27, ABDJ). Observations in early metaphase of meiosis in this nulli 5B hybrid showed that bivalents and multivalents up to hexavalents were present (Table 1). The high frequency of bivalents (6.6%) and multivalents suggests that some of the chromosomes of *Th. bessarabicum* have paired with wheat chromosomes. This is evident from the fact that the frequency of various associations observed in the present study was much higher than that reported earlier in the nulli haploid of Chinese Spring by Forster and Miller [9]. They observed 3.5 bivalents in nulli 5B haploid which accounts for autosynthetic pairing between A, B and D genomes in the presence of active Ph gene. If this situation is considered then in the present hybrid the remaining 3.1 bivalents should account for the pairing between *T. aestivum* and *Th. bessarabicum* chromosomes. In the 2n=28 chromosome hybrid *T. aestivum* x *Th. bessarabicum*, mostly cells with 28 bivalents were [7, 8] observed. Although we did not observe any PMCs with less than 7 univalents, the level of multivalents formation and bivalent frequency was far greater than those expected in a nulli 5B haploid which strongly suggested pairing between wheat and *Th. bessarabicum* chromosomes. Cauderon [11] reported weak homology between wheat and *Agropyron* species as suggested by the production of substitution lines.

Both F_1 hybrid plants were self sterile and resembled more Chinese Spring in growth and vigour. The spikes were long and lax, the leaves narrow and dark green. These hybrid plants were pollinated with the pollen from ten breadwheat cultivars. Out of about 3000 florets pollinated, only two seeds were obtained. The extremely poor seed set in these plants could be due to nontransmission of fertile gametes from the female side. The seeds were

Table 1. Meiotic pairing and mean number of chiasmata per cell in the cv. Chinese Spring mono 5B x *Th. bessarabicum* hybrid and its backcross derivatives

Cross/Progeny	2n	Total cells obser-ed	Chromosomal associations per cell					Mean chias-mata per cell	Selfed seed set (%)
			univa-lents	biva-lents	triva-lents	quadri-valents	penta-hexa valents		
T. aestivum CS mono 5B x *Th. bessarabicum* (F_1)	27	15	12.2 (9–18)	6.6 (4–10)	0.6 (0–2)	0.09 (0–1)	0.01 (0–1)	9.02 (8–11)	0
F_1 x *T. aestivum* cv. HD 2009 (BC_1)	48	28	11.2 (9–13)	15.7 (11–19)	1.3 (0–3)	0.3 (0–1)	— —	28.0 (21–30)	1.4
BC_1 x *T. aestivum* cv. C 306 (BC_2)	43	35	3.9 (3–5)	18.6 (17–20)	0.7 (0–1)	— —	— —	33.0 (24–36)	0
	39	20	5.6 (4–9)	13.5 (11–17)	1.9 (0–3)	0.1 (0–1)	— —	26 (23–28)	0.5
	48	36	7.8 (6–9)	14.8 (11–17)	2.4 (1–4)	0.7 (0–1)	— —	27 (23–29)	0
BC_2 (2n = 48) x cv. C 306 progenies (BC_3)	42	48	2.3 (2–3)	18.3 (18–19)	1.3 (0–2)	— —	— —	36.8 (34–37)	9.8
	43	40	3.8 (3–7)	19.4 (18–20)	— (0–1)	— —	— —	28 (25–30)	1.2
	44	35	3.1 (2–4)	19.0 (18–20)	0.2 (0–1)	0.5 (0–1)	— —	36.5 (34–38)	2.5
	46	36	4.6 (4–6)	18.9 (17–20)	0.3 (0–1)	0.8 (0–1)	— —	37.6 (34–40)	1.5
	46	25	4.8 (2–6)	19.4 (16–21)	0.1 (0–1)	0.5 (0–1)	0.09 (0–1)	34.2 (30–39)	0.8
	48	40	7.0 (5–10)	18.8 (17–20)	0.8 (0–1)	0.1 (0–1)	— —	30.3 (28–33)	0

Note. Range in parentheses.

germinated in nutrient medium where only one survived. Twenty-day-old seedling was transplanted to pot. Chromosome count at MI of meiosis showed 2n=48 chromosomes in this BC_1 progeny, indicating that meiotic nonreduction has operated in the F_1 hybrid. Consequently, the genomic constitution of the BC_1 plant should be 41 W + 7 J (W-wheat chromosomes; J-*Th. bessarabicum* chromosomes) and was expected to show a maximum chromosome association of wo^{II} W + 1^{I} W + J^{I} J. But on an average, 15.7 bivalents and 11.2 univalents were observed. More than 90% of the cells had one trivalent or quadrivalent per cell. The bivalents were either ring or rod shaped. Morphologically, the plant was vigorous with 16 effective tillers, with the culm length 120 cm, and was deeply pigmented. The leaves were narrow, stiff and dark green. The main spike was 13 cm long and lax with 25 spikelets. Pollen fertility was 0.1%. Of the 16 ear bearing tillers, 15 spikes were emasculated and

hand-pollinated with pollen from different wheat cultivars. The female fertility of the BC_1 plant had considerably increased as compared to the F_1 hybrid (1.4% seed set on selfing) and about 60 crossed seeds were obtained.

Random cytological observations in the BC_2 progenies revealed that their 2n chromosome number varied from 38 to 48. Chromosome configurations of all the BC_2 plants are not being represented in Table 1. The frequency of plants with the chromosome number near or around 48 was higher. Interestingly, nonreduction during megasporogenesis had also occurred in BC_1 resulting in the production of a few plants with 48 chromosomes. Detailed analyses of large population in subsequent generations should confirm whether this is due to the presence of a single chromosome, a pair of chromosomes, or the entire genome of *Th. bessarabicum*. If it is due to only one chromosome, this trait of nonreduction could be used as a chromosome marker. Phenotypically, the plants were closer to *T. aestivum* but large variation existed for individual morphological characters in the BC_2 progeny. Cytological observation on one of the 2n=48 plants showed that the number of univalents per cell in BC_2 had reduced to 16 from 23 per cent in BC_2. The bivalent frequency remained more or less same (15.7 in BC_1 and 14.8 in BC_2) but the number of trivalents and quadrivalents increased (1.3^{III}, 0.3^{IV} in BC_1 to 2.4^{III}, 0.7^{IV} per cell in BC_2). In the 2n=43 chromosome plant, PMCs with $20^{II} + 3^{I}$ were quite frequent (27% cells) suggesting that it could be a wheat–*Th. bessarabicum* addition line.

The BC_2 plants were selfed as well as backcrossed to the common wheat cultivars. Cytological observations were recorded only in the progenies derived from the cross BC_2 (2n = 48) x *T. aestivum* cv. C 306. Chromosome count on 16 randomly chosen BC_3 plants revealed that their number varied from 2n = 41 to 48, the frequency of cells with 2n = 46 chromosomes was highest (19%). In one of the 2n = 46 plants, the PMCs had higher associations in about 50% cells and in one cell 11 bivalents and a chain of 14 chromosomes were observed. The pollen fertility in the BC_3 population (observed in 1% acetocarmine) ranged from 0 to 2.5%. Even in the plants with 2n = 42 chromosomes, where bivalent frequency was higher (18.3/cell) pollen fertility did not show much improvement, resulting in poor seed set on selfing. It is worth mention that the plant with 43 and 44 chromosomes having good bivalent frequency could presumably be monosomic or double monosomic addition lines. If fertility of such plants is increased by selfing or backcrossing with common wheat they will be a new source of addition lines involving *Thinopyrum bessarabicum* chromosomes. The identification of 2n = 48 plants in BC_3 generation is an indication that *Th. bessarabicum* chromosomes are well adapted to wheat cytoplasm. Nonreduction at MI of meiosis in BC_2 seems to be controlled by the J genome. The frequency of plants having more than 43 chromosomes in BC_3 generation shows that the chromosomes belonging to *Th. bessarabicum* have selective advantage in transmission through the female gamete. Chen et al. [12] recorded a relatively high frequency of nonreduced egg gametes in BC_1 and BC_2 generation of the *T. aestivum* x *Agropyron cristatum* (4x) cross and suggested that this could

be due to the presence of a pair(s) of *Agropyron* chromosomes or the entire P genome of *A. cristatum*. A similar phenomenon in Wheat x Barley crosses has been demonstrated by Islam and Shepherd [13]. Although population analysed cytologically in BC_1, BC_2 and BC_3 generations was relatively smaller in the present study, our observations nevertheless support above view and the phenomenon of gametic nonreduction during megasporogenesis seems to be controlled by entire J genome or by any individual chromosome of *Thinopyrum bessarabicum*.

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