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

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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) F₁ 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 F₁ hybrid, are intermediate with those of the two parents. Pollinating the ABNN F₁ 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.

Keywords 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 circumvented 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 F₁ hybrid, its backcross-I and -II derivatives obtained using *T. turgidum* as recurrent pollen parent, and derivatives from the F₁ × *T. aestivum* cross.
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/night 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-carmine for several days, squashed in 45% acetic acid with a drop of 2% aceto-carmine. 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** hybrids. The cytological and morphological analysis of the backcross plants also involved the techniques already outlined. The *T. turgidum*-based BC-I 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. 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** 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** hybrid. The BC<sub>1</sub> derivatives from pollinating the **F**<sub>1** hybrid (2n = 4x = 28; ABNN) with *T. turgidum* possessed 42 chromosomes (ABNN + AB = AABBNN), and still expressed the intermediate phenotype with longer awns than the **F**<sub>1** hybrid (Fig. 2 a, b). A similar expression prevailed in the offspring of the cross between the durum/*Ps. juncea* **F**<sub>1** 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** 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** 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

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

<table>
<thead>
<tr>
<th><em>T. turgidum</em> cultivar</th>
<th>No. of florets pollinated</th>
<th>Seeds set</th>
<th>No. of embryos excised</th>
<th>No. of plants obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altar 84</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chen</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duergand</td>
<td>100</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Laru</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Memo/Mexicali</td>
<td>100</td>
<td>1</td>
<td>1</td>
<td>0</td>
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