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Genlou Sun
Sichuan Agricultural University

Chi Yen
Sichuan Agricultural University

Junliang Yang
Sichuan Agricultural University

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Genlou Sun* Chi Yen and Junliang Yang
Triticeae Research Institute, Sichuan Agricultural University,
Dujiangyan City 611830, Sichuan, China

ABSTRACT

Leymus Hochst. is a perennial genus of Triticeae. All species in *Leymus* have the genomes NX. The genome N is from the genus *Psathyrostachys*. Two *Psathyrostachys* species, diploid *P. huashanica* Keng ex Kuo and *P. juncea* (Fische.) Nevski (2n=14), were hybridized with allotetraploid, *Leymus secalinus* (Georgi.) Tzvelev and *L. multicaulis* (Kar. & Kir.) Tzvelev. Meiotic behavior of the synthetic hybrids was studied. The chromosome pairings indicated that one *L. secalinus* genome and one *L. multicaulis* genome were closely homologous with both *P. huashanica* and *P. juncea* genomes. The data of genomic analysis in the hybrids of *P. huashanica* crossed with *L. secalinus* and *L. multicaulis* are so similar to those in the hybrids of *P. juncea* crossed with *L. secalinus* and *L. multicaulis*, there is no significant difference between them. Both *P. huashanica* and *P. juncea* are possible donors of the N genome of *L. secalinus* and *L. multicaulis*.

INTRODUCTION

Leymus Hochst., a perennial genus of Triticeae, includes about 30 species. They are distributed in the temperate regions of Eurasia, North and South America and extend to the subtropic and the tropic alpine regions. All species in *Leymus* have the genomes N and X. Here the N genome is donated by *Psathyrostachys*.

Psathyrostachys is a small genus with no more than 10 species, about half of which have been determined to be diploid (2*n*=14) containing the N genome (Dewey, 1984). Interspecific hybrids were made among the three diploid species *P. juncea* (Fisch.) Nevski, *P. fragilis* (Boiss.) Nevski, and *P. huashanica* Keng ex Kuo. Chromosome pairing in the hybrids indicated that each species has a modified form of the N genome (Dewey and Hsiao, 1983; Bothmer *et al.*, 1987; Wang, 1987; Lu *et al.*, 1990). Therefore, the symbols N^j, N^f, and N^h are used for these species, respectively.

The desirability of a classification based on relationships is obvious. Cytogenetic data from species and generic hybrids are effective measures of biological relationships. Intergeneric hybrids of *Psathyrostachys juncea* with *Leymus* species have been reported. The cytological data showed that *P. juncea* was one of the original diploid parents of *Leymus* species (Dewey 1970, 1972a, 1972b; Wang *et al.*, 1984). But all these studies only involved *P. juncea* as a parent. None has involved other *Psathyrostachys* species. Because each *Psathyrostachys* species has a modified form of N genome, it is worthwhile to extend the investigation to other species of *Psathyrostachys*. This paper reports successful hybridization of *P. huashanica* and *P. juncea* with *L. secalinus* and *L. multicaulis*. The genomic relationships are analyzed. The major objective was to determine whether the *P. huashanica* genome is found in *Leymus secalinus* and *L. multicaulis*.

MATERIALS AND METHODS

Leymus secalinus (Georgi.) Tzvelev (6040) was collected from Fuhai county, Xinjiang, *L. multicaulis* (Kar. & Kir.) Tzvelev (Y094) from Habahe, Xinjiang, and *Psathyrostachys juncea* (Fisch.) Nevski (Y136) fromTeilike, Xinjiang, China. *Psathyrostachys huashanica* Keng ex Kuo is an endemic species of the Huashan mountains of Shaanxi, China. All materials were grown in the field at the Triticeae Research Institute, Sichuan Agricultural University.

The *L. secalinus* and *L. multicaulis* accessions were used as female parents. The spikes of *L. secalinus* and *L. multicaulis* were emasculated and covered by cellulose bags. Several days later, artificial pollinations were made by putting newly mature anther powder into maternal florets. The 15-16-day old hybrid embryos were cultured. When the hybrid seedlings had three leaves, they were transplanted into sand pots and kept in an air conditioned room to survive the hot summer.

Spikes for cytological analysis were fixed in Carnoy's (6:3:1) solution for 24hr, then transferred into 70% ethanol and stored in a refrigerator. Slides were prepared by acetocarmine smear for cytological observation.

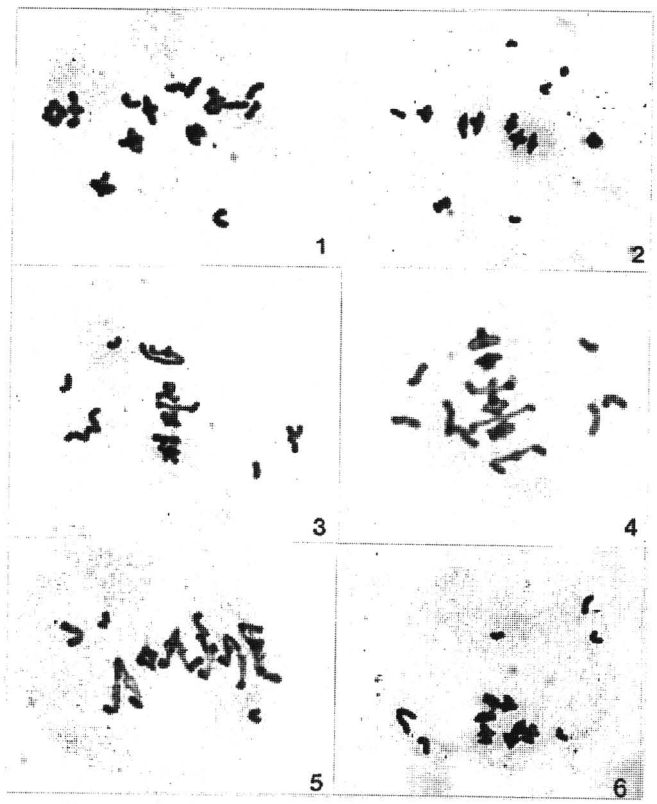
Results

The chromosome pairings at metaphase-I of pollen mother cells in both the parental species and hybrids are

listed in Table 1, and the meiotic configurations are shown in Fig. 1-6. Chromosome pairings of the parents in meiosis were very high (Table 1). Univalent and multivalents were only occasionally observed in *Leymus secalinus* and *L. multicaulis* (Table 1).

Table 1. Meiotic behaviour in parental species and hybrids; The range is given in the parentheses

Species or hybrids	I	II			III	IV	Chiasmata per cell
		ring	rod	Total			
L. secalinus	0.15 (0-2)	13.01 (8-14)	0.81 (0-4)	13.82 (13-14)		0.04 (0-1)	26.95
L. multicaulis	0.58 (0-4)	11.2 (8-14)	2.5 (0-6)	13.7 (12-14)			24.9
P. juncea		6.12 (4-7)	0.88 (0-7)	7 (7-7)			13.12
P. huashanica		3.81 (2-7)	3.19 (0-5)	7 (7-7)			10.18
L. secalinus x *P. huashanica*	7.03 (5-9)	5.91 (4-8)	1.08 (0-4)	6.99 (6-8)			12.9
L. secalinus x *P. juncea*	7.1 (5-11)	5.99 (3-7)	0.93 (0-4)	6.92 (5-8)	0.01 (0-1)		12.93
L. multicaulis x *P. huashanica*	7.3 (3-13)	4.15 (1-7)	2.54 (0-5)	6.69 (4-7)	0.086 (0-3)		12.02
L. multicaulis x *P. juncea*	7.48 (5-11)	5.26 (2-7)	1.49 (0-6)	6.75 (5-8)	0.01 (0-1)		12.03



Figs. 1-6 Chromosome pairing at metaphase-I in hybrids of *Leymus* species crossed with *Psathyrostachys* species. (1) *L. secalinus x P. huashanica*, 7 bivalents + 7 univalents. (2-3) *L. secalinus x P. juncea* : 2. 7 bivalents + 7 univalents, 3. 8 univalents + 5 bivalents + 1 trivalent. (4-5) *L. multicaulis x P. huashanica* : 4. 7 bivalents + 7 univalents, 5. 5 univalents + 5 bivalents + 2 trivalents. (6) *L. multicaulis x P. juncea*, 7 bivalents + 7 univalents.

Table 2. Chromosome pairing at the MI of the PMCs in intergeneric hybrids

Chromosome pairing IV	III	II	I	No. of cells observed	%
L. secalinus x P. huashanica					
		7	7	57	86.36
		6	9	5	7.58
		8	5	4	6.06
		Total		66	100.00
L. secalinus x P. juncea					
		7	7	59	86.76
		6	9	4	5.88
		5	11	1	1.47
	1	5	8	1	1.47
		8	5	3	4.11
		Total		68	99.99
L. multicaulis x P. huashanica					
		7	7	57	54.81
		6	9	19	18.27
		5	11	7	6.73
		4	13	1	0.96
		8	5	12	11.54
		9	3	1	0.96
	3	4	4	1	0.96
	1	6	6	5	4.81
	1	7	4	1	0.96
		Total		104	100.00
L. multicaulis x P. juncea					
		7	7	57	67.06
		6	9	19	22.35
		5	11	3	3.58
	1	6	8	1	1.18
		8	6	5	5.88
		Total		85	100.00

Leymus secalinus x *P. huashanica* had 21 chromosomes. The metaphase-I (MI) pollen mother cells of this hybrid gave a mean pairing configuration of 7.03 univalents + 5.91 ring bivalents + 1.08 rod bivalents (Table 1, Fig. 1). Chromosome pairing was examined in 66 metaphase-I cells, 57 cells, or 86.36% of total, had 7 bivalents and 7 univalents (Table 1). The metaphase-I pollen mother cells of *L. secalinus x P. juncea* gave a mean pairing configuration of 7.10 univalents + 5.99 ring bivalents + 0.93 rod bivalents + 0.01 trivalents. Of 68 metaphase I cells examined, 59 cells, or 86.76% had 7 bivalents and 7 univalents (Table 2, Fig 2). The ring bivalents were predominant. Trivalents were observed in one of the 68 cells (Fig. 3).

The metaphase-I pollen mother cells of *L. multicaulis x P. huashanica* gave a mean pairing configuration of 7.30 univalents + 4.15 ring bivalents + 2.54 rod bivalents + 0.086 trivalents (Table 1, Fig. 4-5). Chromosome pairing was examined in 104 metaphase I cells, the configuration of 7 bivalents and 7 univalents was observed in 54.81% of total cells (Table 2, Fig. 4). Chromosome pairing in the *L. multicaulis x P. juncea* hybrid averaged 7.48 univalents + 6.75 bivalents + 0.01 trivalents (Table 1). 85 metaphase-I cells examined, 57 cells, or 67.06% of the total had 7 bivalents and 7 univalents (Table 2, Fig. 6). Chromosome bridges at anaphase I and II were observed in this hybrid. PMCs with less than seven univalents were observed in all four intergeneric hybrids (Table 2, Fig. 5), which indicated that autosyndetic or homoeologous pairing occurred.

Discussion

The two diploid *Psathyrostachys* species, *P. huashanica* and *P. juncea* were crossed with *Leymus secalinus* and *L. multicaulis* to identify the N genome in *L. secalinus* and *L. multicaulis*. The average number of bivalents were 6.99 per cell in *L. secalinus x P. huashanica*, 6.92 per cell in *L. secalinus x P. juncea*, 6.69 bivalents per cell in *L. multicaulis x P. huashanica*, and 6.75 bivalents per cell in *L. multicaulis x P. juncea*, which indicated that one *L. secalinus* and *L. multicaulis* genome was closely homologous with the *P. huashanica* and *P. juncea* genome. Fewer than seven univalents were observed in these four cross combinations, which showed either autosyndetic or homoeologous pairing occurred.

Genomic and phylogenetic relationships of species can be supported by observation on F_1 hybrids whose parents include one common diploid tester. Therefore, phylogenetic studies of a genus with a higher ploidy level often commence with the establishment of the genomic relationships to diploid species. Many cytogenetic investigations have been carried out on intergeneric hybrids between species of *Leymus* and *P. juncea* (Dewey 1970, 1972a, 1972b; Wang & Hsiao 1984). Meiotic pairing in the hybrid *L. mollis x P. juncea* demonstrated that an N genome is present in *L. mollis* (Wang & Hsiao 1984). Cytological data have further shown that the North American *L. ambiguus* (syn: *Elymus ambiguus*) is an allotetraploid species with one N genome (Dewey 1976 used to designate N as J) closely homologous with the genome of *P. juncea* (syn: *Elymus junceus*), suggesting that *P. juncea*, or a precursor of *P. juncea*, was one of the original diploid parents of *L. ambiguus*. Dewey (1976) concluded that the N genomes in *P. juncea* and *L. ambiguus* are so nearly alike that one need not look beyond *P. juncea* for the source of the *L. ambiguus*

N genome. Chromosome pairing in the synthetic triploid hybrid *P. juncea x L. innovatus* leaves little doubt that one of the *L. innovatus* genomes came from *P. juncea*, either directly of indirectly (Dewey 1970). Cytological data on some *Leymus* species crossed with *P. juncea* led to the conclusion that *P. juncea*, or a precursor of *P. juncea*, was one of the original diploid parents of *Leymus* species (Dewey 1970, 1976; Wang & Hsiao 1984. However, each *Psathyrostachys* species with a modified form of N genome has been identified, at least in *P. huashanica, P. juncea* and *P. fragilis* (Dewey and Hsiao 1983; Bothmer it al. 1987; Wang 1987; Lu *et al.* 1990). Zhang & Dvorak (1991) examined variation in 26 repeated nucleotide sequence families isolated from four species of the Triticeae to investigate the origin of the tetraploid species of *Leymus*. Their results leave no doubt that the N genome of *Psathyrostachys* is in *Leymus*, and suggesting that it is currently unknown which *Psathyrostachys* species were involved in the hybridization that gave rise to *Leymus*. In the present study, data of meiotic pairing in the hybrids of *P. huashanica* crossed with *L. secalinus* and *L. multicaulis* are so similar to those in the hybrids of *P. juncea* crossed with *L. secalinus* and *L. multicaulis* that one cannot determine if the N genome in *L. secalinus* and *L. multicaulis* originated from *P. huashanica* or from *P. juncea*. It is not excluded that *P. huashanica* or another *Psathyrostachys* species also are possible donors of the *Leymus* N genome. Polyploid species may have complex origins derived from a single or multiple genomic donors. In phylogenetic studies, it is important to use a broad set of *Psathyrostachys* and *Leymus* species. With more species involved, more detailed information can be obtained, and consequently, a greater resolution of the relationships between *Psathyrostachys* and *Leymus* may be provided.

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