Biol. Zentralbl. 105 (1986) 361–368 VEB G. Thieme, Leipzig

M. Feldman

USDA-ARS, Crops Research Laboratory, Utah State University – UMC 63, Logan, UT 84322, U.S.A.

Diploid Perennial Intergeneric Hybrids in the Tribe Triticeae
II. Hybrids of *Thinopyrum elongatum* with *Pseudoroegneria spicata* and *Critesion violaceum**

By Richard R.-C. Wang

With 5 Figures

Abstract

Two new intergeneric hybrids involving diploid *Thinopyrum elongatum* were synthesized. The hybrid *T. elongatum* × *Pseudoroegneria spicata* ssp. *inermis* with the J^eS genome formula had spikes somewhat intermediate to those of the parents. Spikes of the *T. elongatum* × *Critesion violaceum hybrid*, which has the J^eH^v genome combination, did not resemble those of either parent. Meiotic metaphase I showed an average of $9.13^{I} + 2.35^{II} + 0.05^{III}$ for *T. elongatum* × *P. spicata* and $10.07^{I} + 1.86^{II} + 0.06^{III}$ for *T. elongatum* × *C. violaceum*. Both hybrids had many laggards at anaphase I and many micronuclei in the tetrads, and both hybrids were completely sterile. Karyotypes of root-tip cells of both hybrids fit the hypothetical ones, thus demonstrating the usefulness of karyotypes in identifying putative intergeneric hybrids. The meiotic chromosome pairing in these hybrids and the *P. spicata* × *C. violaceum* hybrids suggests that the S haplome is closer to H^v than to J^e and that J^e is farther diverged from H^v. Significance of these two new hybrids are discussed.

Introduction

Many important forage grasses in the tribe Triticeae are allotetraploids, such as *Elymus trachycaulus* (Link) Gould ex Shinners (slender wheatgrass), *E. lanceolatus* (Scribner & Smith) Gould (thickspike wheatgrass), *Leymus cinereus* (Scribner & Merr.) Á. Löve (Great basin wildrye), and *L. triticoides* (Buckl.) Pilger (beardless wildrye). The species in *Elymus* have the genome combinations of SH, SY, or SHY where S, H, and Y are designations for the haplomes, (basic genomes, Heilbronn and Kosswig, 1966) from *Pseudoroegneria*, *Critesion*, and an unknown source, respectively (Dewey, 1984). *Leymus* species are composed of the J haplome from *Thinopyrum* and the N haplome of *Psathyrostachys* (Dewey, 1984). Therefore, wide hybridization followed by amphiploidy has played an important role in the speciation of those perennial grasses. Certainly, plant breeders can attempt to mimic nature by synthesizing new genomic combinations to create new forage species.

Thinopyrum elongatum (Host) D. R. Dewey [= *Agropyron elongatum* (Host) P. Beauvois; *Elytrigia elongata* (Host) Nevski; and *Lophopyrum elongatum* (Host) Á. Löve] is a diploid species having high salt tolerance (McGuire and Dvořák, 1981). It has been hybridized with *Aegilops squarrosa* L. [= *Triticum tauschii* (Cosson) Schmalh.] (Dvořák, 1971) and *Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve (= *Agropyron bessarabicum* Savul. & Rayss) (Wang,

---

* Cooperative investigations of the USDA-ARS and the Utah Agricultural Experiment Station, Logan, Utah 84322. Approved as Journal Paper No. 3116.

1985b). Because of the similarity in karyotypes of *T. bessarabicum* and *T. elongatum* and the ability of their chromosomes to pair in meiosis, it was proposed that the haplome in *T. elongatum* be changed from E to J^e (WANG, 1985b).

Continuing the series presenting data on the newly synthesized diploid intergeneric hybrids involving perennial species (WANG, 1984b, 1986), this paper reports the successful hybridization of *T. elongatum* with *Pseudoroegneria spicata* (Pursh) Á. Löve ssp. *inermis* (Scribner & Smith) Á. Löve and *Critesion violaceum* (Boiss. & Hohenacker) Á. Löve. The spike morphology and cytology, both mitotic and meiotic, of the F_1 hybrids are presented. *Pseudoroegneria spicata* ssp. *inermis* has the S haplome and *C. violaceum* has the H^v haplome. Thus the two hybrids reported here represent the first synthetic J^eS and J^eH^v genomic combinations.

Material and Methods

The *Thinopyrum elongatum* accession (originally from the Mediterranean coast of France) was received from Dr. Y. CAUDERON of I.N.R.A., Versailles, France. *Pseudoroegneria spicata* ssp. *inermis* (PI 236670), from Alberta, Canada, and *Critesion violaceum* (PI 401390) from northcentral Iran, were provided by the USDA Regional Plant Introduction Station, Pullman, WA. Hereafter, the ssp. *inermis* will be referred to simply as *P. spicata*.

Plants of these species were grown from seeds and were vernalized in a cold chamber (5 °C) for various lengths of time. The vernalization requirement varied both among and within species. It ranged from 0–4, 0–2, and 2–12 weeks for *T. elongatum*, *T. spicata*, and *C. violaceum*, respectively. Vernalized plants were grown in a greenhouse under long days (18 hours of photoperiod).

Spikes of *T. elongatum* were emasculated and enclosed in glassine bags before anthesis. Twenty-four hours after hand pollination, a 75 ppm gibberellic acid solution was injected into the florets. Half seeds with embryo were aseptically plated on slanted orchid agar medium. Seedlings were transferred into pots at the two leaf stage and maintained in the greenhouse.

Spikes of the F_1 hybrids were fixed in Carnoy's (6 : 3 : 1) solution and stored in 70% ethanol. Pollen mother cells (PMCs) were squashed in acetocarmine for meiotic analysis. Mitotic root tip squashes were prepared according to the procedures of MUJEEB-KAZI and MIRANDA (1985). Karyotype analysis was performed with a microcomputer using the CHROMPAC III[1] software (GREEN et al., 1984).

Results

Thinopyrum elongatum × *Pseudoroegneria spicata* ssp. *inermis*

Ten caryopses varying in size and color were obtained and cultured from 56 florets pollinated by *P. spicata*. Three seedlings were obtained but only two were grown to maturity. Both of them are spring type, i.e., they headed in the greenhouse without being vernalized. The hybrid plants were completely sterile. Pollen grains were unstainable with the I_2-KI solution and anthers were nondehiscent.

The spikes of *T. elongatum* × *P. spicata* had close resemblance to those of *T. elongatum* although some attributes were intermediate to those of both parents (Fig. 1). The spikes of the hybrid resembled *T. elongatum* more than *P. spicata* in the number of spikelets per spike and in their size and spacing on the axis, but the morphology to its glumes was closest to *P. spicata*.

Meiosis in the hybrids revealed little chromosome pairing (Table 1). About 7% of the PMCs had 14 univalents at metaphase I (Fig. 2a). Some ring bivalents (Fig. 2b), occasional trivalents (Fig. 2c) and a few heteromorphic bivalents (Fig. 2d) were observed. Up to six bivalents were observed in the hybrids (Table 1). An average of 1.82 laggards per cell at anaphase I led to 1.67 micronuclei per tetrad.

Mitotic root tip cells showed 14 chromosomes (Fig. 3a) which gave an idiogram matching that constructed from standard idiograms of the parents (Fig. 4a). The S-2 chromosome (Fig. 3a) did not show a small satellite but the pointed short arm indicated the presence of a satellite.

---

[1] Mention of a trademark or vendor does not constitute a guarantee or warranty of the product by the USDA, and does not imply approval to the exclusion of other products or vendors that may also be suitable.

Fig. 1. Spikes of the parents and hybrids, (l to r): *Thinopyrum elongatum*, *T. elongatum* × *Pseudoroegneria spicata* ssp. *inermis*, *P. spicata* ssp. *inermis*, *T. elongatum*, *T. elongatum* × *Critesion violaceum*, and *C. violaceum*

Table 1. Meiotic behavior in *Thinopyrum elongatum*, *Pseudoroegneria spicata ssp. inermis*, *Critesion violaceum*, and their F_1 hybrids (range is given in the parentheses)

Species and hybrids	Genome	2n	Metaphase I						Anaphase I Laggards/ cell	Tetrad mn/ cell
			No. cells	I	ring II	rod II	Total II	III		
T. elongatum[a]	JJ	14	102	–	6.72 (5–7)	0.28 (0–2)	7.00 (7)	–	0.00	0.03 (0–2)
P. spicata ssp. *inermis*	SS	14	153	–	6.48 (4–7)	0.52 (0–3)	7.00 (7)	–	0.02 (0–1)	0.01 (0–1)
C. violaceum[b]	HH	14	102	0.43 (0–6)	5.25 (3–7)	1.53 (0–4)	6.78 (4–7)	–	0.25 (0–3)	0.29 (0–3)
T. elongatum × *P. spicata*	JS	14	204	9.13 (2–14)	0.08 (0–2)	2.27 (0–6)	2.35 (0–6)	0.05 (0–1)	1.82 (0–7)	1.67 (0–6)
T. elongatum × *C. violyceum*[c]	JH	14	206	10.07 (4–14)	0.13 (0–2)	1.73 (0–5).	1.86 (0–5)	0.06 (0–1)	2.63 (0–11)	2.46 (0–6)
P. spicata × *C. violaceum*[d]	SH	14	467	7.12 (0–14)	0.32 (0–3)	2.87 (0–7)	3.19 (0–7)	0.14 (0–3)	0.74 (0–3)	0.41 (0–3)

[a] Wang (1985b) [b] Wang (1984a) [c] Also had 0.01 quadrivalent

[d] Wang et al. (unpublished); also had 0.02 quadrivalent and 0.02 pentavalent.

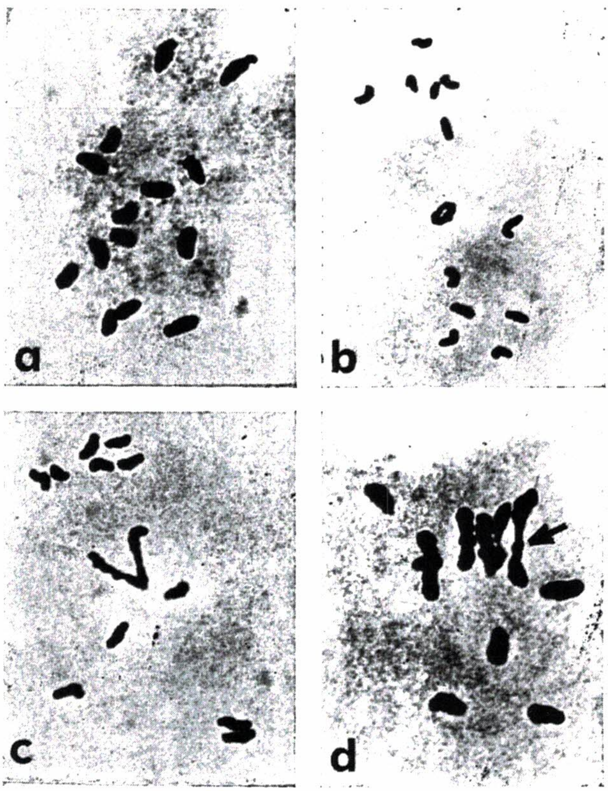

Fig. 2. Meiotic metaphase-I cells of the *Thinopyrum elongatum* × *Pseudoroegneria spicata* ssp. *inermis* hybrid. a. Fourteen univalents. b. Twelve univalents and one ring bivalent. c. Eleven univalents and one trivalent. d. Five univalents, three bivalents, and one trivalent; one heteromorphic bivalent is indicated by the arrow

Fig. 3. Mitotic chromosomes of the hybrids *Thinopyrum elongatum* × *Pseudoroegneria spicata* ssp. *inermis* (a) and *T. elongatum* × *Critesion violaceum* (b). Each chromosome is identified by its haplome symbol and number as in Fig. 4, except that J^e and H^v are abbreviated as J and H, respectively

Thinopyrum elongatum × *Critesion violaceum*

In the cross *T. elongatum* × *C. violaceum*, 23 brown and shriveled seeds were obtained from 46 florets. Only two of them germinated upon culturing and both survived. One of the hybrids is spring type. This hybrid combination was completely sterile with unstainable pollen grains and nondehiscent anthers.

The spikes of this hybrid were intermediate to those of the parents (Fig. 1) for the glume characteristics only. They were shorter than those of both parents and had fewer florets per spike. Being a short plant with narrow leaves, the overall morphology of the hybrids was closer to *C. violaceum* than *T. elongatum*.

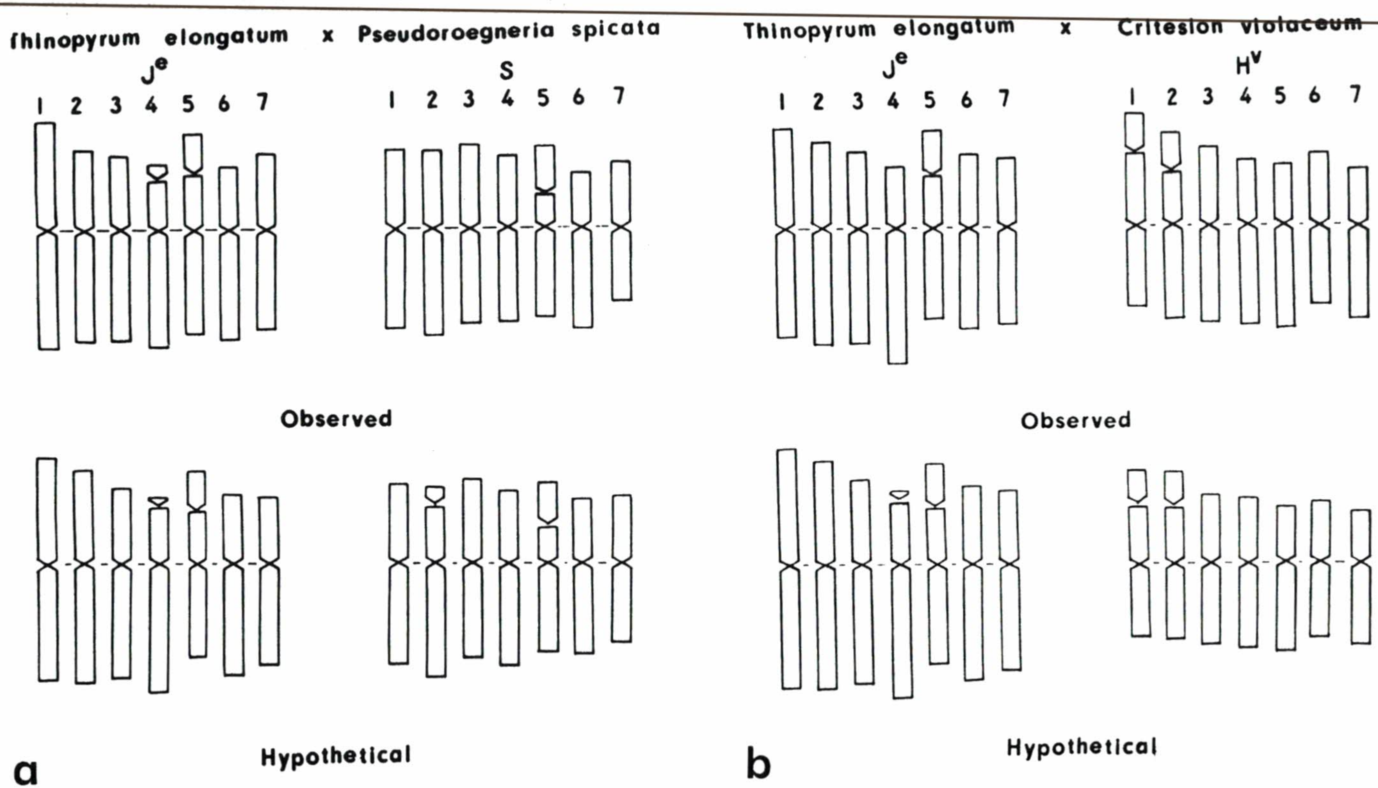

Fig. 4. Idiograms of the hybrids *Thinopyrum elongatum* × *Pseudoroegneria spicata* ssp. *inermis* (a) and *T. elongatum* × *Critesion violaceum* (b) compared to those constructed for hypothetical hybrids based on the standard idiograms of the parental species

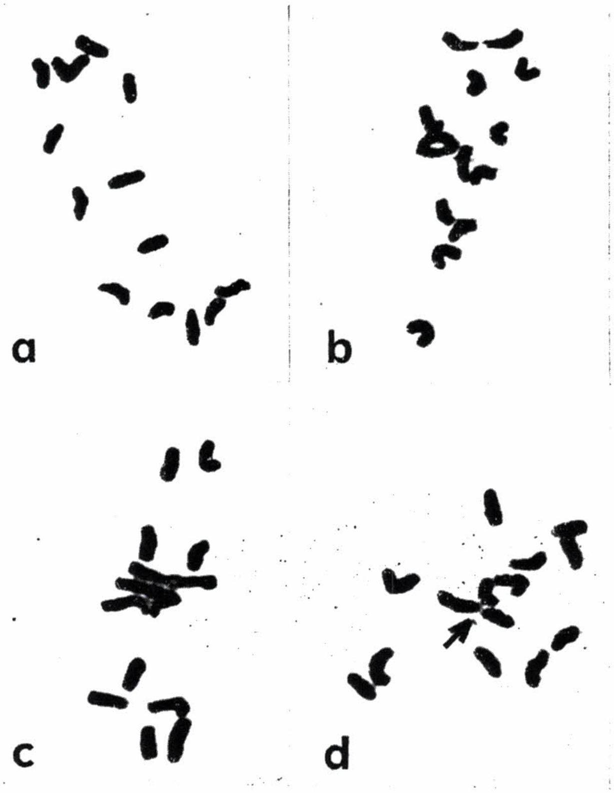

Fig. 5. Meiotic metaphase-I cells of the *Thinopyrum elongatum* × *Critesion violaceum* hybrid. a. Fourteen univalents. b. Ten univalents, one ring and one rod bivalent. c. Seven univalents, two rod bivalents, and one trivalent. d. Ten univalents, one ring bivalent, and one heteromorphic bivalent (arrowed)

A little less chromosome pairing was observed in the *T. elongatum* × *C. violaceum* hybrid than in the *T. elongatum* × *P. spicata* hybrid (Table 1). There was no difference in chromosome pairing between the spring- and winter-type plants. About 16% of its PMCs had 14 univalents at metaphase I (Fig. 5a). Again, ring bivalents (Fig. 5b), trivalents (Fig. 5c), heteromorphic bivalents (Fig. 5d) and one quadrivalent were observed occasionally. Up to five bivalents were formed at metaphase I (Table 1). Both anaphase-I laggards and micronuclei in tetrads were slightly higher in this hybrid than *T. elongatum* × *P. spicata*.

Mitotic cells had 14 chromosomes (Fig. 3b), seven from each parent. The idiogram of the hybrid matched the one developed for a hypothetical hybrid (Fig. 4b), except that the H^{v} chromosomes were longer than expected. The satellite of $\mathrm{J}^{e}4$ chromosome was not evident but suggested by the rounded tip of the short arm (Fig. 3b).

Discussion

Although it is possible, intergeneric hybridization among diploid species in the tribe Triticeae is still difficult. A reasonably high percentage of the pollinated florets, 18 and 50% for *T. elongatum* × *P. spicata* and *T. elongatum* × *C. violaceum*, respectively, set seeds. But not all of hybrid embryos germinated on the culture medium, and some of the hybrids died as young seedlings. Therefore, at the end only a few plants reached maturity. To evaluate the plant-breeding potential of a hybrid combination and its amphidiploids adequately, a larger population of F_1 hybrids is needed. Hundreds or even thousands of florets need to be emasculated and pollinated to obtain the needed hybrids. In an attempt to make diploid hybrids, certain plants of the female parent gave higher numbers of seeds, suggesting the presence of

favorable crossability gene(s). Selection and utilization of these desirable plants will make hybridization easier. However, it may lead to narrower genetic variation for performance evaluation. Additional research is needed to find a compromise.

Meiotic pairing in the hybrids was higher than expected, yet it was still lower than that expected for interspecific hybrids under the genomic system of classification (DEWEY, 1984; LÖVE, 1984). Therefore, it is evident that these species have basically different haplomes and belong to different genera. Haploids of *T. elongatum* had only one rod bivalent (WANG, 1985a). It may be assumed that one or two rod bivalent(s) occur in *P. spicata* and *C. violaceum* due to autosyndesis. Then, the excess number of bivalents observed in these intergeneric hybrids over the presumed sum of autosyndetic bivalents should be interpreted as allosyndesis or pairing between homoeologous chromosomes of the different haplomes.

If the average univalent frequency in the diploid hybrids is used as a measurement of phylogenetic distance (PHILLIPS, 1966), it can be concluded that the S haplome is closer to H^v than to J^e and that J^e is farther diverged from H^v (Table 1). The same conclusion is reached if a nuclear membrane map is constructed by the distance coefficient method (JACKSON, 1982).

With the standard idiograms developed for most of the perennial diploid species in the tribe Triticeae (HSIAO et al., 1986), it is now possible to construct an idiogram for a hypothetical intergeneric hybrid and then compare it with one from the actual synthetic hybrids. This study demonstrates that the technique is useful for early identification of putative intergeneric hybrids. Chromosome banding with stains would be required to identify interspecific hybrids because of the intrageneric similarity of karyotypes.

The hybrids reported here, *T. elongatum* × *P. spicata* ssp. *inermis* and *T. elongatum* × *C. violaceum*, have the J^eS and J^eH^v genomic formulas, respectively. The genomic combination EJS (= J^eJS) was proposed for *Elytrigia* (LÖVE, 1984), but genome analysis involving diploid species was not carried out to verify it. Therefore, the two hybrids in this study represent new synthetic genomic combinations. A cross of *T. elongatum* × *P. stipifolia* (Czern. ex Nevski) Á. Löve, also a J^eS combination, gave rise to four seedlings, but all died as seedlings (WANG, unpublished). Therefore, genotypic balance determines the survival and failure of plants having a given genomic combination.

Since the F_1 hybrids of these two crosses are relatively vigorous plants, the amphidiploids of these hybrids may be worth evaluating for use as new forage crops. Even if the amphidiploids cannot be used directly, the J^eJ^eSS plants would be useful for genome analysis of *Elytrigia* species. In addition, they can be backcrossed to the parental species to develop addition, substitution, and translocation lines for facilitating gene flow between genera. Because *T. bessarabicum* also has the J haplome and many species have S or H haplome, a large number of hybrids involving different species should be synthesized for a fair evaluation of the JS and JH genome combinations.

References

DEWEY, D. R.: The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: GUSTAFSON, J. P. (ed.), Gene manipulation in plant improvement. Plenum Publishing, New York–London (1984) 209–279.

DVORAK, J.: Hybrids between diploid *Agropyron elongatum* and *Aegilops squarrosa*. Can. J. Genet. Cytol. 13 (1971) 90–94.

GREEN, D. M., P. Z. MYERS, D. L. REYNA: Chrompac III: An improved package for microcomputer-assisted analysis of karyotypes. J. Hered. 75 (1984) 143.

HEILBRONN, A., C. KOSSWIG: Principia genetica. Grunderkenntnisse und Grundbegriffe der Vererbungswissenschaft. 2. neubearb. Aufl., Hamburg und Berlin 1966.

HSIAO, C., R. R.-C. WANG, D. R. DEWEY: Karyotype analysis and genome relationships of twenty-two diploid species in the tribe Triticeae. Can. J. Genet. Cytol. 28 (1986) 109–120.

JACKSON, R. C.: Polyploidy and diploidy: new perspectives on chromosome pairing and its evolutionary implications. Amer. J. Bot. 69 (1982) 1512–1523.

LÖVE, A.: Conspectus of the Triticeae. Feddes Repert. (Berlin) 95 (7–8) (1984) 425–521.

MCGUIRE, P. E., J. DVORAK: High salt-tolerance potential in wheatgrasses. Crop Sci. 21 (1981) 702–705.

MUJEEB-KAZI, A., J. L. MIRANDA: Enhanced resolution of somatic chromosome constrictions as an aid to identifying intergeneric hybrids among some Triticeae. Cytologia 50 (1985) 701–709.

PHILLIPS, L. L.: The cytology and phylogenetics of the diploid species of *Gossypium*. Am. J. Bot. 53 (1966) 328–335.

WANG, R. R.-C.: Genetically controlled desynapsis in diploid *Critesion violaceum* and its hybrids with autotetraploid *Psathyrostachys juncea*. Can. J. Genet. Cytol. 26 (1984a) 532–537.

WANG, R. R.-C.: Intergeneric and interspecific hybridization among perennial diploid species of the Triticeae tribe in greenhouse. Agron. Abst. (1984b) 94.

WANG, R. R.-C.: Monoploid of *Thinopyrum elongatum* derived from intergeneric hybridization with *Agropyron mongolicum*. First Can. Congr. Biol. Abst. GS2.2. (1985a).

WANG, R. R.-C.: Genome analysis of *Thinopyrum bessarabicum* and *T. elongatum*. Can. J. Genet. Cytol. 27 (1985b) 722–728.

WANG, R. R.-C.: Diploid perennial intergeneric hybrids in the tribe Triticeae. I. *Agropyron cristatum* × *Pseudoroegneria libanotica* and *C. violaceum* × *Psathyrostachys juncea*. Crop Sci. 26 (1986) 75–78.

Received October 2, accepted November 20, 1935

Author's address: Dr. RICHARD R.-C. WANG
USDA-ARS, Crops Research Laboratory
Utah State Universoty – UMC 63
Logan, UT 84322
U.S.A.