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The addition of *Dasypyrum villosum* (L.) Candargy chromosomes to *durum* wheat (*Triticum durum* Desf.)*

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Summary. Six monosomic addition lines were produced in which different *Dasypyrum villosum* (L.) Candargy chromosomes were added to the chromosome complement of *Triticum durum* Desf. cv. 'Creso'. Each added alien chromosome was found to have a specific effect on plant morphology and fertility. Transmission rate varied widely (from 7.5 to 27.7%) among the six univalent chromosomes. Different monotelosomic addition plants derived by a relatively high frequency of chromosome misdivision were isolated. The addition lines should be useful for studying *Dasypyrum* chromosome homoeology and the introduction of alien variation into *durum* and common wheats.

Key words: *Triticum durum* – *Dasypyrum villosum* – Alien addition lines – Interspecific gene transfer

Introduction

Alien chromosome addition or substitution series to wheat have proved to be extremely valuable to geneticists and breeders for the investigation of chromosome homoeology and for transferring desirable characters from wild species of *Triticinae* to cultivated wheats (Sears 1968; Riley et al. 1968). A systematic production of complete sets of such lines has been carried out mainly in the genetic background of *Triticum aestivum* L. cv. 'Chinese Spring' (for a complete list see Driscoll 1984). Similar investigation in *durum* wheat has been limited because the tetraploid wheats are of less economic importance and the aneuploids are in general less vigorous, lacking in fertility and more difficult to maintain. Nevertheless, the production of some sets of monosomic and/or disomic addition lines have been reported. Sadanaga (1957) produced the addition lines of *Secale cereale* L. chromosomes to a *durum* wheat variety. Mochizuki (1960, 1962) obtained six disomic additions of *Agropyrum elongatum* (Host) P.B. chromosomes, and Alston (1970) attempted to add *Aegilops squarrosa* L. chromosomes to *T. durum*. The addition of the D genome chromosomes of common wheat to *T. polonicum* L. was attempted by Yamashita (1947) and Matsumura (1952), and to *T. durum* by Joppa and McNeal (1972). Makino (1976) produced the seven monosomic additions of *Aegilops umbellulata* Zhuk. chromosomes to var. 'melanopus' of *durum* wheat. Joppa and Williams (1977) and Joppa et al. (1978) obtained the D-genome substitution monosomic and disomic lines of *durum* wheat in the cultivar 'Langdon'.

Dasypyrum villosum (L.) Candargy, a wild species synonymous with *Haynaldia villosa* (L.) Schur., is an allogamous annual grass of the subtribe *Triticinae*, native to the Mediterranean region and South-West Asia. It is a diploid (2n = 14, genome designated by the letters VV) with one or more genes for disease resistance (powdery mildew, stem and leaf rust, *Gaeumannomyces graminis*) tolerance to drought, increased protein content and other desirable morpho-physiological characteristics (Pasquini et al. 1978; Leske 1979; Scarascia et al. 1982; Heun and Mielke 1983; Stefani and Onnis 1983; Della Gatta et al. 1984).

The hybridization of *D. villosum* with diploid, tetraploid and hexaploid species of *Triticum* has been investigated, and different F_1 hybrids and amphiploids have been obtained (for review see Blanco et al. 1983, 1984). Hyde (1953) obtained an incomplete set of disomic additions of *D. villosum* chromosomes to *T.*

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aestivum by using the amphiploid *T. dicoccoides – D. villosum* as a bridge.

In the present paper the development of six monosomic addition lines of *D. villosum* chromosomes to *T. durum* Desf. and some of their morphological and cytological characteristics will be reported.

Materials and methods

The *Triticum durum* Desf. cv. 'Creso' (2n = 4x = 28, AABB) and *Dasypyrum villosum* (L.) Candargy (2n = 2x = 14, VV) parents and primary hybrids (2n = 21, ABV) have been described previously (Blanco et al. 1983).

Triploid F_1 hybrid spikes were backcrossed with pollen from *durum* wheat cv. 'Creso', and pentaploid seeds (2n = 35, AABBV) were obtained by the fertilization of unreduced female gametes (ABV) with the normal male gametes (AB). The pentaploid plants were again backcrossed with wheat pollen and produced BC_2 progeny. Monosomic addition plants (2n = 29) were selected at the seedling stage by counting chromosome numbers in root tip cells and further confirmed by observing chromosome pairing at metaphase I in pollen mother cells (PMCs).

Plant morphology and spike characteristics were recorded on each monosomic plant and on ten *durum* wheat plants grown in the field 10 cm apart in rows spaced 30 cm apart. As an index of fertility, the seed set on three spikes of each plant was measured and expressed as the ratio between the number of kernels per spike and the number of spikelets per spike, and as the number of kernels per spikelet in a sample of 6 spikelets from the middle part of the spikes.

The transmission rate of the univalent chromosomes was determined in the progeny derived from some selfed plants of each monosomic addition line.

The chromosome constitution of hybrids and the derived plants was determined at both mitosis and meiosis. Root tips were pre-treated for 4 h with 0.05% colchicine and then fixed in Carnoy's 3 : 1 fixative for mitotic studies. Individual anthers containing pollen mother cells at metaphase I were fixed in Carnoy's 6 : 3 : 1 fixative for meiotic studies. Standard Feulgen staining and squash procedures were used with root tips and PMCs to prepare material for cytological examination.

Results and discussion

The *T. durum* × *D. villosum* hybrids were pollinated with the *durum* wheat parent, and 22 backcross seeds (BC_1) developed to maturity on the hybrid spikes without requiring special treatment. The BC_1 plants resembled the *durum* wheat parent in morphology, but exhibited some evident characters of the *D. villosum*, such as tall culms, short awns on the glumes (1.5–2.0 cm) and hairy and fragile rachis at maturity. The chromosome number of eight BC_1 plants was determined from root tip cells, and 6 of them possessed 35 chromosomes, whereas the other 2 had 34 chromosomes. These observations suggested that the majority of the functional egg cells in the F_1 hybrids possessed a 21 chromosome restitution nucleus, which produced pentaploid seeds when fertilized with *durum* wheat gametes. Meiotic studies confirmed that the BC_1 plants were pentaploid since they exhibited an average chromosome pairing of $13.6^{II} + 7.8^{I}$ and a maximum chromosome association of $14^{II} + 7^{I}$ in 80% of PMCs. The pentaploid plants were only partly fertiles, but enough BC_2 seeds were obtained by backcrossing again with wheat pollen. The chromosome numbers of the BC_2 plants were determined from root tips for the selection of monosomic addition plants (28 wheat + 1 *Dasypyrum* chromosomes). As expected, the chromosome numbers ranged from 28 to 35 among the 210 BC_2 plants examined, including 37 plants with 29 complete chromosomes. Twenty-three monosomic addition plants showing $14^{II} + 1^{I}$ at metaphase I were unambiguously classified into six groups on the basis of the several morphological and physiological characteristics examined (Table 1). Karyological analysis was not useful in this classification because of the lack of distinct differences in size and morphology between *durum* wheat and *Dasypyrum* chromosomes (Bisignano et al. 1984). The *Dasypyrum* satellited chromosome was not observed in any addition line, probably because the satellite is inconspicuous and may not be easily observable in contracted mitotic or meiotic chromosomes (Fig. 1). It is also possible that the secondary constriction was not well expressed or was suppressed in the genetic background of *durum* wheat as already observed in analogous material (Lacadena et al. 1984). Indeed, the *Dasypyrum* satellited chromosome was never observed in mitotic or meiotic cells of the *durum* wheat – *D. villosum* amphiploid or in its pentaploid hybrids with *durum* wheat. Unfortunately, the remaining 14 plants, all having 29 chromosomes, could not be classified with certainty into groups due to their poor

Fig. 1. Somatic chromosomes of *Dasypyrum villosum* (2n = 14)

Table 1. Mean of characteristics in six monosomic addition lines of *T. durum* Desf. cv. 'Creso' for *D. villosum* (L.) Candargy chromosomes and in the *T. durum* parent

Character	Line						
	'Creso'	A	B	C	D	E	F
Plants examined (n)	10	7	4	2	3	5	2
Flag leaf:							
length (cm)	29.9	27.4	33.0	29.5	34.7	–	28.0
width (cm)	2.2	1.9	2.6	2.6	1.8	–	2.3
Spike:							
length (cm)	8.5	9.6	10.3	8.2	8.0	7.3	8.5
spikelets (n)	23.1	24.0	23.4	21.8	19.4	22.9	21.6
density [a] (n)	27.1	24.9	22.8	26.5	24.4	31.4	25.5
glume awn length (cm)	0.2	0.2	0.4	0.2	0.2	0.2	0.4
lemma awn length (cm)	15.7	15.8	15.9	15.3	12.2	12.2	19.5
rachis [b]	T	R	T	T	T	T	T
Fertility:							
kernels/spike (n)	66.2	51.8	59.5	30.6	41.6	45.2	19.2
kernels/spikelets [c] (n)	2.9	2.2	2.6	1.4	2.1	2.0	0.9
kernels/spikelets [d] (n)	3.5	2.6	3.2	1.9	2.9	2.5	0.7
Seeds:							
length (mm)	7.9	8.8	8.3	7.9	8.6	7.4	8.3
width (mm)	3.4	3.3	3.7	3.6	3.3	2.8	3.5
color [e]	L	D	L	L	L	D	L
Powdery mildew [f]	S	S	S	R	S	S	S

[a] N. spikelets/10 cm rachis

[b] T = tough and F = fragile

[c] N. kernels per spike/n. spikelets per spike

[d] N. kernels per spikelet in a sample of 6 spikelets of the middle part of spike

[e] L = light amber and D = dark amber

[f] S = susceptible and R = resistant

development and low vigour or due to the presence of deleted chromosomes, isochromosomes, occasional asynapsis of wheat chromosomes and other chromosome abnormalities. Studies are now in progress on the progenies of these plants mainly for isolating the missing seventh addition line.

Table 1 reports means for characteristics of the monosomic addition plants and their *durum* wheat parent. Spikes are shown in Fig. 2. As suggested by Kimber (1967), the addition lines were designated by letters to avoid confusion with the numbers of the wheat homoeologous groups. Each line will be renumbered when it is possible to incorporate *Dasypyrum* chromosomes into the wheat homoeologous grouping by isozyme analysis or interspecific substitution studies. Phenotypically the monosomic additions differ from 'Creso' in several respects. Line A was distinct from the others in having fragile rachis at maturity and dark amber seeds like *D. villosum*. Of the two addition lines (B and F) possessing short awns on the glumes, B had longer and lax spikes and was as fertile as 'Creso', whereas F had reduced fertility. Moreover, gel electrophoresis of the endosperm storage proteins of line B showed a distinct band at the α-gliadin region that was not present in *durum* wheat (unpublished data). Line C carries on the short arm of the chromosome a gene or genes conferring resistance to powdery mildew (*Erysiphe graminis* f.s. *tritici*). Line D was taller and with a longer flag leaf than 'Creso'. Line E, characterized by smaller and dark amber seeds, was also distinguishable from the others by its short and dense spikes.

Meiotic chromosome pairing at metaphase I was examined and all the six monosomic addition lines showed $14^{II}+1^{I}$ in the majority of PMCs (Fig. 3). The *D. villosum* univalent was not observed to pair with a wheat chromosome in any of the six lines examined, neither was there a noticeable decrease of synapsis.

The transmission rate of each univalent in the six addition lines was determined in the progenies derived from selfed plants (Table 2). The occurrence of telocentric chromosomes was also scored. Out of 605 plants examined, an average of 17.9% of monotelosomic or monosomic addition plants was observed. The transmission rates varied widely from a minimum of 7.5% for chromosome A to a maximum of 27.7% for chromosome B. A total of 14 monotelodisomic or disomic

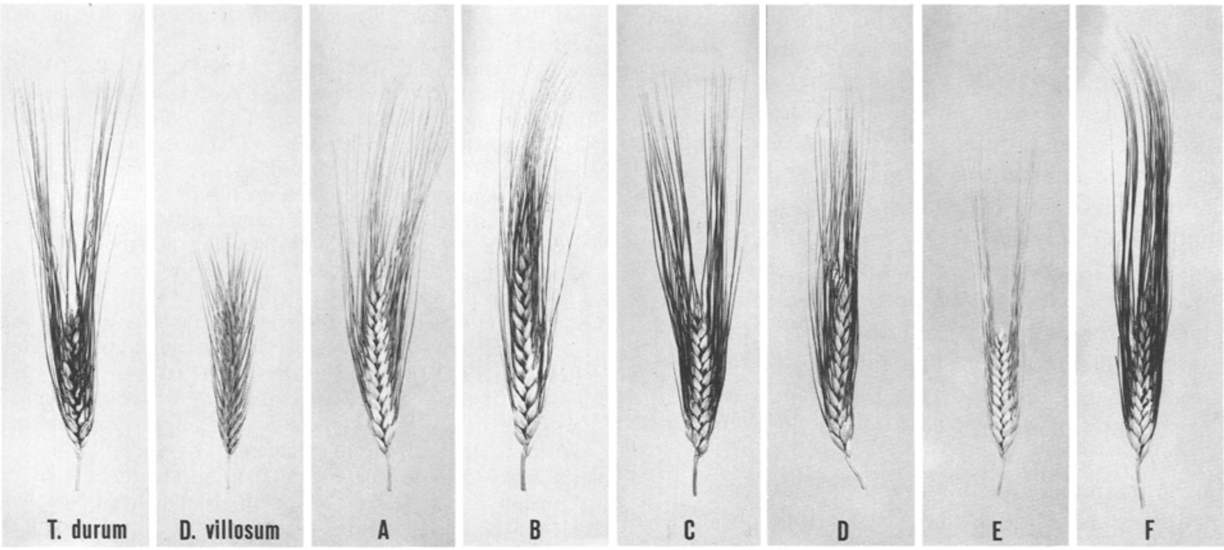

Fig. 2. From *left to right.* Spikes of *T. durum, D. villosum* and monosomic addition lines *A, B, C, D, E, F* of *D. villosum* to *durum* wheat

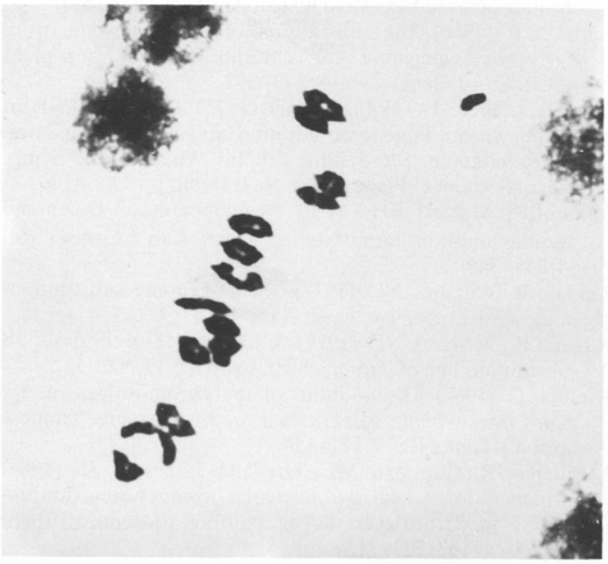

Fig. 3. Metaphase I showing $14^{II} + 1^{I}$ of the monosomic addition line of chromosome B of *D. villosum* to *durum* wheat

Table 2. Breeding behaviour of the selfed monosomic addition lines of *D. villosum* in *T. durum*

Line	No. plants examined	% frequency (2n)				
		28	28+ teloc.	29	29+ teloc.	30
A	119	91.6	2.5	5.0	0.9	0.0
B	159	67.9	12.0	15.7	1.9	2.5
C	130	89.2	1.5	8.5	0.0	0.8
D	31	77.4	9.7	9.7	0.0	3.2
E	137	76.6	11.0	11.0	0.7	0.7
F	29	72.4	6.9	13.8	3.4	3.4
Total	605	79.8	7.3	10.6	1.0	1.3

addition plants was also scored, the average frequency being 2.3%. This percentage indicates that the transmission rate of the extra chromosome through the pollen grains is very low in comparison to the transmission rate through the ovules. Similar results with transmission rates of the added chromosomes were also obtained by Simeone et al. (1985) in the primary trisomics of *durum* wheat, and by Mochizuki (1962) and Makino (1976) for the monosomic addition lines of *T. durum* for *Agropyron elongatum* and *Aegilops umbellulata* chromosomes, respectively.

The frequency of monotelosomic addition plants in the offspring varied from 1.5% for chromosome C and 12.0% for chromosome B with an average frequency of 7.3%. This indicates that in the present material the univalent misdivision occurred at higher frequency than in other aneuploid stocks (Sears 1952; Mochizuki 1962; Makino 1976), and that all the telosomic additions can be produced with relative ease.

Although the *Dasypyrum* addition lines, like other alien additions, appear to have no direct agronomic value because of their low fertility and cytological instability, they are, however, interesting in several respects. Because the same recurrent homozygous cv. 'Creso' was used and no other genetic variability was introduced, the alien addition lines are useful, for example, for determining the gene content and the behaviour of each *Dasypyrum* chromosome in the wheat background. Moreover, the addition lines are a prerequisite for an understanding of the evolutionary relationships between wheat and alien chromosomes. After Sears (1965) showed how the wheat chromosomes could be classified, both by origin and function, into seven homoeologous groups of three, numerous attempts to incorporate related (alien) chromosomes into this homoeologous grouping were made (Riley 1964; Johnson 1966; Sears 1968), assuming that a specific genetic relationship between the chromosomes of wheats and the chromosomes of related genera depends on their genetic equivalence. It was generally found that in most cases a single alien chromosome will substitute specifically for the 3 chromosomes of one, and only one, homoeologous group in wheat (Riley 1966; Riley et al. 1966). Attempts to substitute the *Dasypyrum* chromosomes for all the wheat chromosomes, so that their compensating ability can be evaluated, are being made by using the D-genome substitution disomic lines of *durum* wheat developed by Joppa et al. (1978).

Finally, the present alien addition lines can play an important role as a bridge material for incorporating *Dasypyrum* chromosome segments in homoeologous or non-homologous wheat chromosomes by meiotic recombination, as a result of allosyndetic pairing, or by translocation induced with radiations (Sears 1972; Driscoll 1965). Because the induction of translocation between wheat and alien chromosomes by ionizing radiations has not been entirely satisfactory, the 5B genetic system (Okamoto 1957; Riley and Chapman 1958) is now generally preferred because most *ph*-mediated exchanges involve homoeologous chromosomes. The procedures for *durum* wheat, based largely on Sears' proposal (1981), involve removal of the *Ph* gene or the whole chromosome 5B from the alien addition lines by a series of crosses involving the *ph*-mutant line (Giorgi 1978) or the disomic 5D-nullisomic 5B substitution line (Joppa and Williams 1979; Ono et al. 1980) of *durum* wheat. Selection of those progeny plants which have both homoeologous pairing and the character being transferred is made. In order to increase the homoeologous pairing frequency and to be sure that most or all transfers will involve a particular wheat chromosome (instead of being distributed among the homoeologues), one could also develop a particular *Dasypyrum* chromosome substitution line. This line could be used in the breeding program to have plants monosomic for both desired chromosomes, and at the same time, presenting homoeologous pairing.

References

Alston FH (1970) The addition of individual chromosomes of *Aegilops squarrosa* to *Triticum aestivum*. Cytologia 35: 402–408

Bisignano V, Simeone R, Blanco A (1984) Analisi cariotipica di *Haynaldia villosa* (L.) Schur. Ann Fac Agric Univ Bari 33: 65–70

Blanco A, Orecchia C, Simeone R (1984) Cytology, morphology and fertility of the amphiploid *Triticum durum* Desf.× *Haynaldia villosa* (L.) Schur. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Kyoto University, Kyoto, pp 205–211

Blanco A, Simeone R, Tanzarella OA, Greco B (1983) Morphology and chromosome pairing of a hybrid between *Triticum durum* Desf. and *Haynaldia villosa* (L.) Schur. Theor Appl Genet 64:333–337

Della Gatta C, Tanzarella OA, Resta P, Blanco A (1984) Protein content in a population of *Haynaldia villosa* and electrophoretic pattern of the amphiploid *T. durum*× *H. villosa*. In: Porceddu E (ed) Breeding methodologies in *durum* wheat and triticale. University of Tuscia, Viterbo (Italy), pp 39–43

Driscoll CJ (1965) Induced intergeneric transfers of chromosomes segments. In: The use of induced mutation in plant breeding, FAO-IAEA, Rome, pp 727–739

Driscoll CJ (1984) Third compendium of wheat-alien chromosome lines. In: Sakamoto S (ed) Suppl Proc 6th Int Wheat Genet Symp. Kyoto University, Kyoto, pp 1–34

Giorgi B (1978) A homoeologous pairing mutant isolated in *Triticum durum* Desf. cv. 'Cappelli'. Mutat Breed Newslett 11:4–5

Heun M, Mielke H (1983) Breeding for resistance to *Pseudocercosporella herpotrichoides* and *Gaeumannomyces graminis* in wheat. Plant Breed Abstr 2998

Hyde BB (1953) Addition of individual *Haynaldia villosa* chromosomes to hexaploid wheat. Am J Bot 40: 174–182

Johnson R (1966) The substitution of a chromosome from *Agropyron elongatum* for chromosomes of hexaploid wheat. Can J Genet Cytol 8:279–292

Joppa LR, Bietz JA, Williams ND (1978) The aneuploids of *durum* wheat: D-genome addition and substitution lines. In: Ramanujam S (ed) Proc 5th Int Wheat Genet Symp. Indian Soc Genet Plant Breed, New Delhi, pp 420–426

Joppa LR, McNeal FH (1972) Development of D-genome disomic addition lines of *durum* wheat. Can J Genet Cytol 14:335–340

Joppa LR, Williams ND (1977) The D-genome substitution-monosomics of *durum* wheat. Crop Sci 17:772–776

Joppa LR, Williams ND (1979) A disomic 5D-nullisomic 5B substitution line of *durum* wheat. Crop Sci 19:509–511

Kimber G (1967) The addition of the chromosomes of *Aegilops umbellulata* to *Triticum aestivum* (var. 'Chinese Spring'). Genet Res 9: 111–114

Lacadena JR, Cermeno MC, Orellana J, Santos JL (1984) Evidence for wheat-rye nucleolar competition (amphiplasty) in Triticale by silver staining procedure. Theor Appl Genet 67:207–213

Leske R (1979) Resistance to *Gaeumannomyces graminis* and the possible incorporation of this resistance into wheat. Aust Plant Breed Genet Newslett 29:54–55

Makino T (1976) Genetic studies on alien chromosome addition to a *durum* wheat. I. Some characteristics of seven monosomic addition lines of *Aegilops umbellulata* chromosomes. Can J Genet Cytol 18:455–462

Matsumura S (1952) Chromosome analysis of the Dinkel genome in the offspring of a pentaploid wheat hybrid. III. 29 chromosome D-haplosomics and their relations to nullisomics. Cytologia 17:35–49

Mochizuki A (1960) Addition of individual chromosomes of *Agropyron* to *durum* wheat. Wheat Inf Serv 11:22–23

Mochizuki A (1962) *Agropyron* addition lines of *durum* wheat. Seiken Ziho. 13:133–138

Okamoto M (1957) Asynaptic effect of chromosome V. Wheat Inf Serv 5:6

Ono H, Mochizuki A, Nagayoshi T (1980) A disomic substitution line of a common wheat chromosome 5D for 5B of *durum* wheat. Jpn J Genet 55:251–257

Pasquini M, De Los Angeles Gicas M, Vallega J (1978) *Haynaldia villosa* (L.) Schur. come fonte di resistenza alle ruggini e all'oidio, da incorporare nelle specie di frumento coltivate. Atti Giornate Fitopat: 349–353

Riley R (1964) Cytogenetics and plant breeding. Genetics Today. Proc 11th Int Congr Genet 3: 681–688

Riley R (1966) Cytogenetics and wheat breeding. In: Proc 5th Yugoslav Symp Res Wheat. Novi Sad, pp 107–117

Riley R, Chapman V (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182: 713–715

Riley R, Chapman V, Johnson R (1968) Introduction of yellow rust resistance of *Aegilops comosa* into wheat by genetically induced homeologous recombination. Nature 217: 383–384

Riley R, Chapman V, Macer RCF (1966) The homoeology of an *Aegilops* chromosome causing stripe rust resistance. Can J Genet Cytol 8: 616–630

Sadanaga K (1957) Cytological studies of hybrids involving *Triticum durum* and *Secale cereale*. I. Alien addition races in tetraploid wheat. Cytologia 22: 312–321

Scarascia Mugnozza GT, De Pace C, Tanzarella OA (1982) *Haynaldia villosa* (L.) Schur: una specie di potenziale valore per il miglioramento genetico del frumento. I. Analisi di alcuni caratteri morfologici. Genet Agrar 36: 76–77

Sears ER (1952) Misdivision of univalents in common wheat. Chromosoma 4: 535–550

Sears ER (1965) Nullisomic-tetrasomic combinations in hexaploid wheat. In "Chromosome manipulations and plant genetics". Heredity (Suppl) 20: 29–45

Sears ER (1968) Relationships of chromosomes 2A, 2B and 2D with their rye homoeologue. In: Finlay KW, Shepherd KW (eds) Proc 3rd Int Wheat Genet. Symp Canberra Aust Acad Sci, Canberra, pp 53–61

Sears ER (1972) Chromosome engineering in wheat. Stadler Symp 4: 23–38

Sears ER (1981) Transfer of alien genetic material to wheat. In: Evans LT, Peacock WJ (eds) Wheat science: today and tomorrow. Cambridge University Press, Cambridge London, pp 75–89

Simeone R, Giorgi B, Blanco A (1985) Chromosome transmission in primary trisomics of *durum* wheat (*T. durum* Desf.). Cereal Res Commun 13: 27–31

Stefani A, Onnis A (1983) Contributo alla conoscenza delle caratteristiche fenologiche di popolazioni naturali di *Dasypyrum villosum* (L.) Candargy di diverse provenienze. Inf Bot Ital 15: 213–224

Yamashita K (1947) Cytogenetic studies on some trisomic plants obtained from the backcrosses of a pentaploid *Triticum* hybrid (*T. polonicum* × *T. spelta* × *T. polonicum*). Rep Kihara Inst Biol Res 3: 40–53