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Cytogenetic identification of *Aegilops squarrosa* chromosome additions in durum wheat *

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Summary. A set of four normal chromosomes (*1D, 2D, 3D,* and *6D*), and three translocation chromosomes (*4DS·5DS, 5DL·7DS,* and *7DL·4DL*) involving all 14 chromosome arms of the D-genome were obtained as monosomic additions from *Aegilops squarrosa* (genome D, n=7) in *Triticum durum* Desf. cv 'PBW114' (genome AB, n=14). The cyclical translocation occurred during the synthesis of the amphiploid probably as a result of misdivision and reunion of the univalents during meiosis of the F_1 hybrid *T. durum* × *A. squarrosa.* The amphiploid was backcrossed twice with the durum parent to obtain monosomic addition lines. The monosomic addition chromosomes were identified by C-banding and associated phenotypic traits. All monosomic addition lines were fertile. The development of disomic and ditelosomic addition lines is underway, which will be useful for cytogenetic analysis of individual D-genome chromosomes in the background of *T. durum.*

Key words: Amphiploid – *Triticum durum* – Alien additions – Aneuploidy – C-banding

Introduction

There had been several attempts to produce a set of disomic addition lines of D-genome chromosomes in *Triticum durum* Desf. In one approach, transfer of D-genome chromosomes was attempted from bread wheat (AABBDD) into durum wheat (AABB) via a pentaploid bridge (AABBD) (Matsumura 1952; Joppa and McNeal 1972; Makino 1981). Joppa and McNeal (1972) crossed Chinese Spring D-genome tetrasomics with durum wheat and recovered five disomic addition lines (*1D, 3D, 4D, 5D,* and *6D*) in BCF_3 generation. Only two of these lines (*4D* and *5D*) proved to be partially self-fertile, whereas the remaining lines (*1D, 3D,* and *6D*) were male sterile. Makino (1981) isolated seven monosomic additions from *T. spelta;* only four were identified as to their chromosome contribution.

In another approach, *Aegilops squarrosa* (syn. *T. tauschii*) was used to isolate D-genome additions in durum wheat (Alston 1970; Makino 1981). Monosomic additions were identified on the bases of morphological traits and/or pairing analysis with ditelosomics of Chinese Spring. Makino (1981) isolated six additions, four of which were identified (*1D, 2D, 3D,* and *7D*) and two unidentified.

In spite of these studies, a complete set of D-genome addition lines, where all seven D-genome chromosomes were conclusively identified, has not been recovered. This has been in part due to low transmission of D-genome chromosomes and lack of reliable techniques for chromosome identification. Moreover, most of the D-genome chromosome addition lines reported so far have been produced in a mixed background of several durum parents, or durum and hexaploid parents, and as such cannot be used in critical cytogenetic analyses. A set of D-genome disomic substitution lines in durum wheat has been produced, however (Joppa and Williams 1988). The usefulness of these stocks in cytogenetic mapping of disease and insect resistance genes has been demonstrated (Salazar and Joppa 1981; Joppa and Williams 1988;

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Amri et al. 1990). The present study is a first report on the isolation of a complete set of D-genome monosomic additions in a pure durum background. The added D-genome chromosomes were identified using C-banding and associated phenotypic traits.

Materials and methods

A synthetic amphiploid involving an advanced generation breeding line of spring durum (AABB) 'PBW114' (from Punjab Agricultural University, Ludhiana) and an *A. squarrosa* (DD) accession 3754 was obtained by spontaneous doubling of *T. durum* × *A. squarrosa* F_1 hybrid. The amphiploid (AABBDD) was backcrossed to the durum parent (PBW114). The pentaploid hybrid (AABBD) was again backgrossed to the durum parent, and chromosome numbers of backcross progeny were determined by the standard acetocarmine root squash technique (Endo and Gill 1984).

The C-band karyotypes of the durum parent, the synthetic amphiploid, and the D-genome monosomic addition (2n = 29) plants were made using the technique described by Giraldez et al. (1979). The data on contrasting characters between the durum parent and the synthetic amphiploid such as plant waxiness, seed color, head density and shape, and threshability were recorded on the monosomic addition plants. The data on fertility were recorded as seed set per spikelet on the greenhouse-grown plants.

Results

The frequency distribution of chromosome number of plants from the F_1 hybrid of pentaploid backcrossed with the durum parent 'PBW114' is given in Table 1. Of the 121 plants analyzed, 21 plants had 29 chromosomes each. The number of plants with two to seven additional D-genome chromosomes (2n = 30 to 2n = 35) decreased progressively. One of the monosomic addition plants was weak and died before heading. All other monosomic addition plants set a variable number of seeds (Table 2). Only one weak monosomic plant had poor seed set while others were as fertile as the durum parent or amphiploid. Many plants with 30 chromosomes (7/8 tested) were highly self-sterile, whereas a majority of the plants with 31, 34, or 35 chromosomes were partially fertile. Some of the seedlings with chromosome number 32 and 33 were not retained.

Table 1. Frequency distribution of chromosome number of F_1 plants from pentaploid (AABBD) × *T. durum* (AABB) 'PBW114' cross

Chromosome no.	No. of plants	%
28	48	39.7
29	21	17.4
30	16	13.2
31	15	12.4
32	10	8.3
33	7	5.8
34	3	2.5
35	1	0.8
Total	121	100.1

Giemsa C-banding identification of D-genome monosomic addition lines

C-banding karyotypes of the *T. durum* parent ('PBW114') and the *T. durum* × *A. squarrosa* amphiploid are shown in Fig. 1. It was possible to identify all A-, B-, and D-genome chromosomes of the parental material. No differences were detected between the C-banding pattern of the A- and B-genome chromosomes of the amphiploid and that of the durum parent. The comparison of the C-banding pattern of the D-genome chromosomes of the amphiploid with that of 'Chinese Spring' indicates that *1D, 2D, 3D,* and *6D* are present as intact chromosomes, whereas chromosomes *4D, 5D,* and *7D* were involved in translocations. Figure 2 shows a mitotic metaphase of

Table 2. Fertility and morphological characteristics of different monosomic addition lines of *Aegilops squarrosa* chromosomes in *Triticum durum*

Monosomic addition	No. of plants	Average seed set/spikelet	Waxiness	Glume color	Seed color	Head shape	Threshability
1D	4	2.2	Waxy	Red	Amber	Compact	Free
2D	2	1.4	Nonwaxy	White	Amber	Tapering, lax	Medium
3D	2	2.1	Waxy	White	Red	Compact	Free
4DS · 5DS	2	1.9	Waxy	White	Amber	Compact	Free
5DL · 7DS	2	1.5	Waxy	White	Amber	Lax	Free
7DL · 4DL	2	1.4	Waxy	White	Amber	Tapering, lax	Free
6D	3[a]	1.9	Waxy	White	Amber	Compact	Free
PBW114	–	1.6	Waxy	White	Amber	Compact	Free
Amphiploid	–	1.4	Nonwaxy	Red	Red	Tapering, lax	Hard

[a] One plant was very weak and only set 0.25 seeds per spikelet. The average seed set was recorded from the other two plants

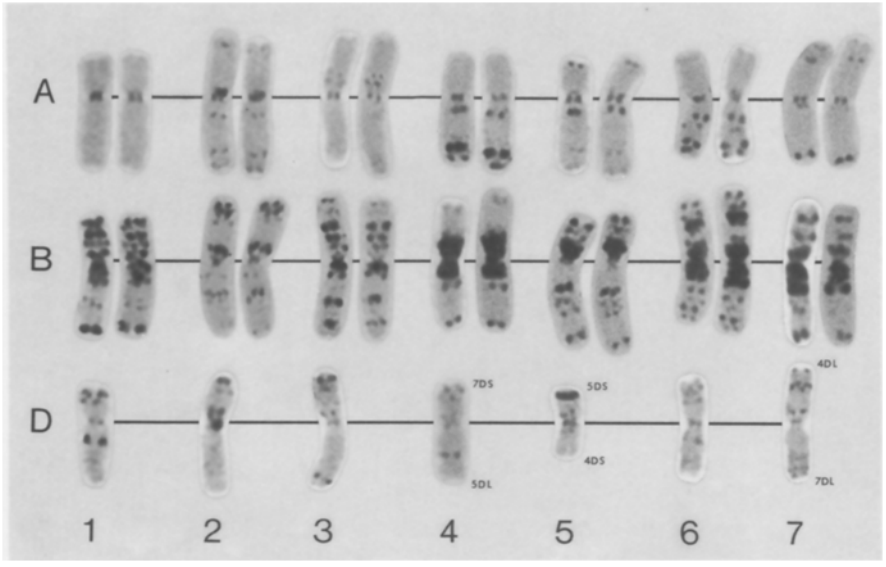

Fig. 1. C-banding karyotype of *Triticum durum* ('PBW114') × *Aegilops squarrosa* amphiploid (left), and *T. durum* parent cv 'PBW114' (right)

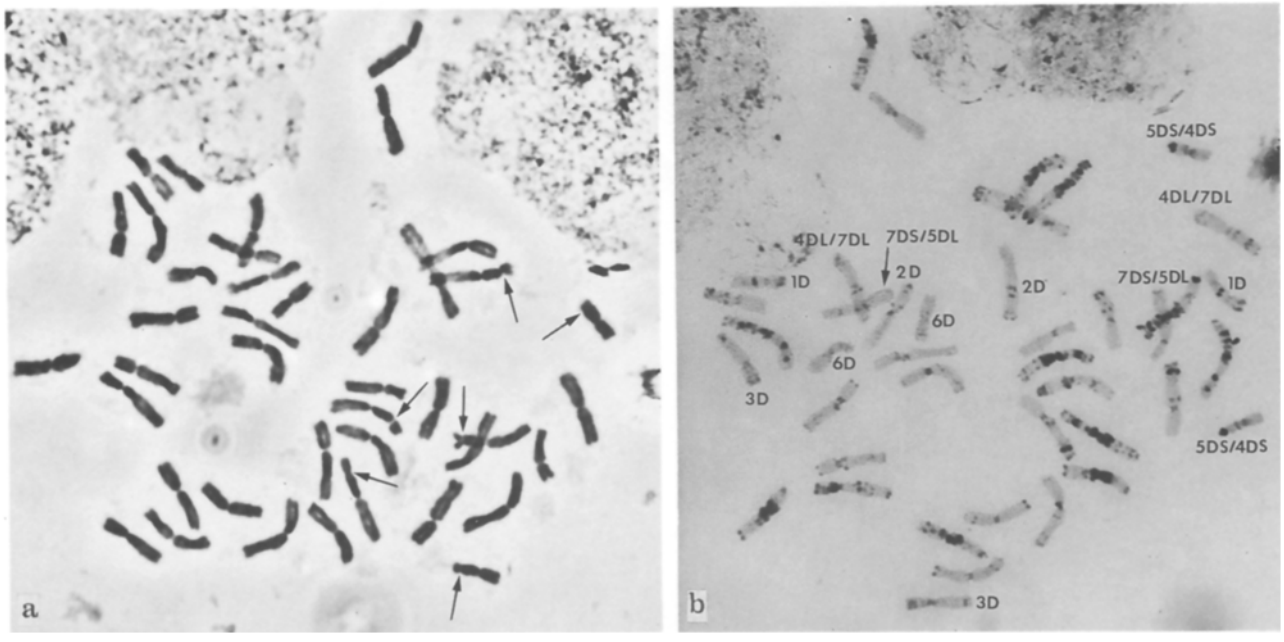

Fig. 2. **a** Phase contrast and **b** C-banding of the *Triticum durum* × *Aegilops squarrosa* amphiploid (the *arrows* point to the secondary constrictions)

the AABBDD amphiploid in phase contrast (a) and after C-banding (b). Three pairs of chromosomes show a secondary constriction and these were identified as being *1B*, *6B*, and *5D*. In hexaploid wheat three chromosome pairs – *1B*, *6B*, and *5D* – are known to possess nucleolar activity; however, usually only *1B* and *6B* show a secondary constriction (Cermeño et al. 1984). The presence of a secondary constriction in the short arm of chromosome *5D* in the amphiploid *T. durum*–*A. squarrosa* indicates a higher nucleolar activity of the *5D* chromosome of *A. squarrosa*.

Out of 20, 17 D-genome monosomic addition plants could be analzyed by C-banding: four plants were monosomic addition for *1D*, two for *2D*, two for *3D*, three for *6D*, two for *4DS* · *5DS* translocation, two for *5DL* · *7DS* translocation, and two for *7DL* · *4DL* translocation

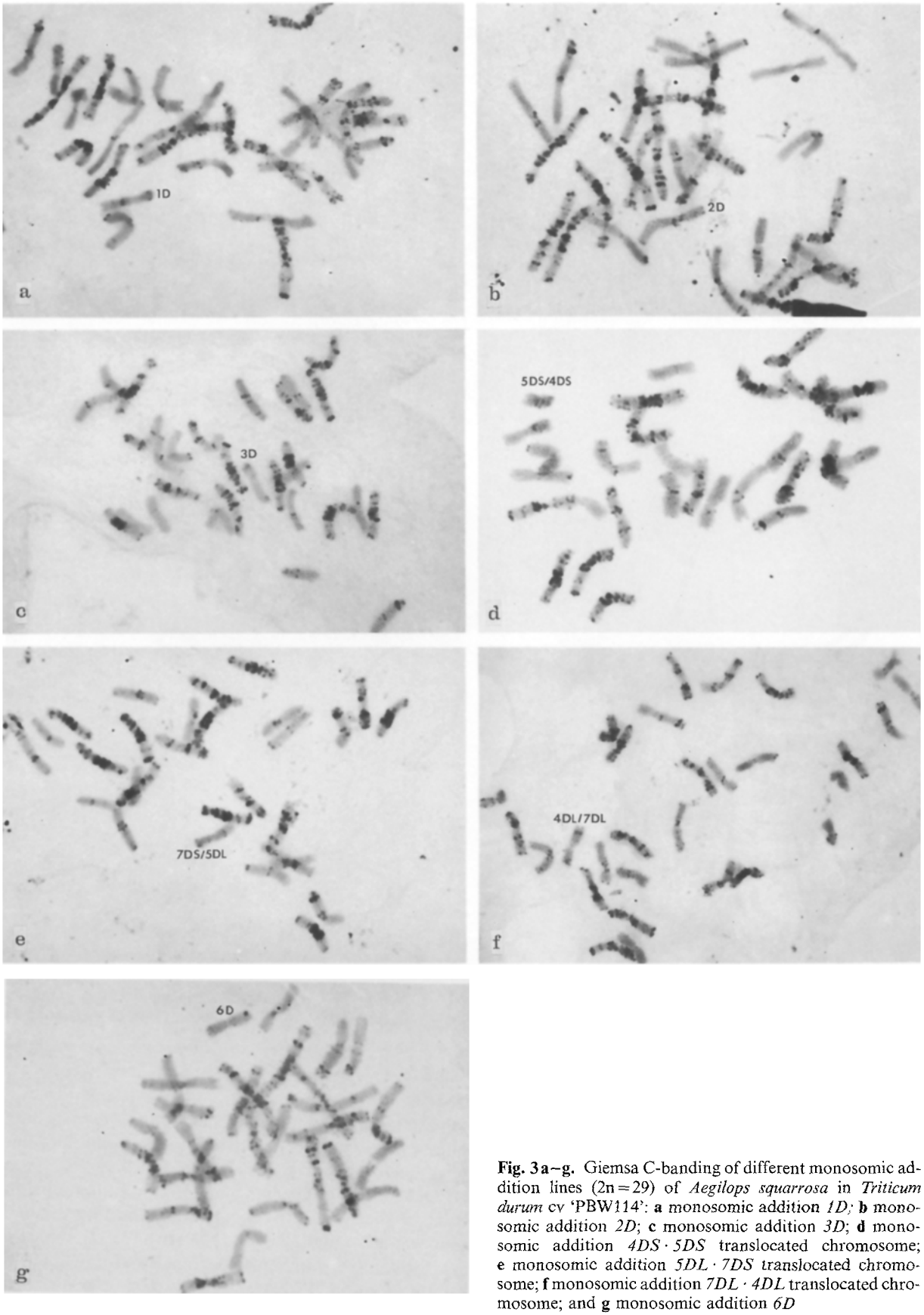

Fig. 3a–g. Giemsa C-banding of different monosomic addition lines (2n = 29) of *Aegilops squarrosa* in *Triticum durum* cv 'PBW114': **a** monosomic addition *1D*; **b** monosomic addition *2D*; **c** monosomic addition *3D*; **d** monosomic addition *4DS · 5DS* translocated chromosome; **e** monosomic addition *5DL · 7DS* translocated chromosome; **f** monosomic addition *7DL · 4DL* translocated chromosome; and **g** monosomic addition *6D*

(Fig. 3). Monosomic additions involving the complete complement of D-genome chromosomes either as intact or translocated chromosomes were obtained.

Analysis of phenotypic traits in D-genome chromosome addition lines in T. durum

The data on fertility, plant waxiness, glume color, seed color, head shape and size, and threshability of various groups of D-genome addition lines are given in Table 2. All monosomic *1D*-addition plants had red color glumes like that of the amphiploid, while other plants had white glumes. Monosomic *2D*-addition plants had nonwaxy plants like the amphiploid, while the remaining six groups of monosomic additions had waxy plants like the durum plant. The *3D*-monosomic addition plants had red seed color like that of the amphiploid. A plant with *4DS · 5DS* translocation addition also had red seed color. This plant had another translocation involving the long arm of chromosome *1B* and probably the arm of chromosome *3D* carrying the red seed color gene (Fig. 3d). Three monosomic addition groups could be easily associated with characteristic head type. Plants with the *2D* addition had tapering, lax, and narrow spikes with medium threshability. Plants with the *5DL · 7DS* translocation addition had lax, small but square spikes with lower rachis internodes longer than the upper ones. The most characteristic feature of this group was the long glume tooth, like a bristle, which was absent in other groups. The group of plants with the *7DL · 4DL* translocation had long lax and tapering head shape, which was virtually indistinguishable from the *T. aestivum* head shape.

Discussion

It has been possible for the first time to develop and identify monosomic addition lines of all the D-genome chromosomes of *A. squarrosa* in *T. durum*. In previous studies, Alston (1970) classified D-genome monosomic addition plants in 'Carleton' durum into eight groups on the basis of their morphological characteristics, but could not identify the added D-genome chromosomes. Joppa and McNeal (1972) obtained five D-genome disomic addition lines, out of which the disomic addition lines *1D, 3D,* and *6D* were male sterile and could not be maintained. Makino (1981) also could not identify all possible D-genome monosomic addition chromosomes in durum using chromosome pairing and morphological characteristics.

Out of seven possible D-genome addition lines, four had normal chromosomes (*1D, 2D, 3D,* and *6D*). The chromosomes *4D, 5D,* and *7D* were involved in a cyclical translocation. The translocated chromosomes were identified by C-banding to be *4DS · 5DS, 5DL · 7DS,* and *7DL · 4DL* (Fig. 1). In each case, the breakpoint of the translocation probably lies within the centromeric region. The translocated chromosomes will be useful in simultaneous mapping of chromosome and arm location of genetic markers.

The original *A. squarrosa* accession involved in the production of synthetic amphiploid was not available for C-banding analysis. It is most likely that the translocations occurred in the *T. durum* × *A. squarrosa* F_1 hybrid prior to amphiploidization. In the F_1 hybrid, most of the A-, B-, and D-genome chromosomes stay as univalents, which are prone to centromere breakage and fusion as shown in wheat-rye hybrids (Lukaszewski and Gustafson 1983; Friebe and Larter 1988). An additional evidence that translocations detected must have occurred during the synthesis of the amphiploid is that the translocations involving D-genome chromosomes were not detected in a survey of C-banding analysis of a large number of *A. squarrosa* accessions of diverse geographical origin (B. Friebe and B. S. Gill, unpublished results).

In addition to translocations involving D-genome chromosomes, one translocation involving *1B* and *3D* chromosomes was also detected. A *4DS · 5DS* monosomic addition plant also had red seed color, a marker of chromosome *3D* (Table 2), because of the presence of another translocation involving *1BL* and the arm of *3D* carrying the red seed color gene (Fig. 3d). Again, it is clear that this translocation probably arose during the production of monosomic addition lines.

All monosomic addition plants had medium to high fertility. Monosomic additions *1D, 3D,* and *6D* were more fertile than the other addition lines. Makino (1981) also reported very high fertility of all of his D-genome monosomic addition lines from *T. spelta* and *A. squarrosa* in *T. durum*. The high fertility of the monosomic addition plants indicates that it should be relatively easy to maintain these lines by selfing.

The plants with 29, 31, 34, and 35 chromosomes were fertile. However, a majority of the 30-chromosome plants (double monosomic addition) were highly sterile compared to the 29- and 31chromosome plants. The reason for the low fertility of 30-chromosome plants and high fertility of 29- and 31-chromosome plants is not known.

The association of certain morphological characteristics, e.g., red glume color in group *1D,* nonwaxiness in group *2D,* red grain color in group *3D,* and different head shape and size, suggests the possibility of identification of monosomic addition lines in the progeny of monosomic addition plants without resorting to detailed cytological analysis on all plants. Additional markers for isozymes, seed-storage proteins, genes for resistance to various diseases, and genes controlling certain quantitative characters differentiating the amphiploid and durum parents would further help to identify the additional D-genome chromosomes or telosomes more precisely.

Based on studies of Makino (1981), isolation of disomic addition lines of *A. squarrosa* in *T. durum* may not be feasible. Makino (1981) found frequency of transmission of *A. squarrosa* in monosomic addition lines to vary from 5.6%–22.7% through the female gametes and 1.8% through the male gametes. These transmission rates are too low for the recovery of disomic addition lines in the selfed progeny of monosomic additions. Moreover, even if disomic additions were obtained, they may be highly sterile. Thus, of the disomic additions that Joppa and McNeal (1972) isolated, three (*1D, 3D,* and *5D*) were male sterile.

It may be possible to isolate a set of ditelosomic addition lines, however. Makino (1981) found that certain of the *A. squarrosa* monosomic chromosomes misdivide to produce telocentric chromosomes, as earlier reported by Sears (1952). It is likely that telocentric monosomic chromosomes may show higher rates of transmission through male and female gametes, thereby enhancing probability for the recovery of ditelosomic addition lines in the selfed progenies of monotelosomic plants.

The set of monosomic additions reported here, and telosomic additions that may be isolated later, will add to the wealth of cytogenetic stocks in durum wheat (Joppa and Williams 1988). These stocks will be invaluable for the cytogenetic analysis of the D-genome of *A. squarrosa*.

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