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APPLICATION OF A SOMATIC AND MEIOTIC CYTOLOGICAL TECHNIQUE TO DIVERSE PLANT GENERA AND SPECIES IN THE TRITICEAE

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Abstract

Within the Triticeae, cereal crop improvement alternatively exploits use of alien genetic variation emanating from several annual and perennial species. Somatic and meiotic cytological procedures are used to diagnose the presence of alien chromosomes forms, an essential component in derived progenies. Quality somatic analytical products are achieved through a high cellular mitotic index, chromosome definition, primary and secondary constriction detail, intense nuclear staining with minimum to none cytoplasmic staining and virtually no chromosome stickiness. Meiotic preparations with similar attributes are considered to be representative of high quality, where in addition separation of multivalent associations and minimum chromosome stretching are more crucial. Such cytological results described in this technique, are anticipated to serve as the basis to further build upon with other differential heterochromatin staining and molecular cytology aspects.

Introduction

Somatic chromosomal counts remain significant as taxonomic descriptors whenever such investigations are necessary. Generally root-tips from germinating seeds or from young actively growing plants facilitate cytological analysis, though other plant parts may also be utilized. Apart from obtaining a mere chromosome number information, structural details are prerequisites for karyotypic determinations, ascertaining hybridization validity or elucidating chromosome structure uniqueness associated with primary, secondary or tertiary constrictions. Furthermore, ideal chromosome contractions and high mitotic frequency metaphase spreads are almost universally regarded as base essentials for applying techniques of chromosome banding and *in situ* hybridization. Recent advances in automated microscopy may preclude the need for exceptionally high somatic preparation quality but such expensive microscopes may be out of reach of a majority of the plant cytogenetical research laboratories for quite sometime, placing a continued emphasis on quality related cytology techniques.

Cytological techniques have been noted for restrictions across taxonomic domains, but apparently some conformity exists among the monocotyledonous genera, particularly within the Triticeae (Mujeeb-Kazi & Miranda, 1985; Hsiao *et al.*, 1986; Liu & Wang, 1988). For the dicotyledonous genera however, a techniques application across various families has been problematic as a consequence of chromosomal size minuteness, lack of quality separation of primary constrictions, darkly stained cyto-

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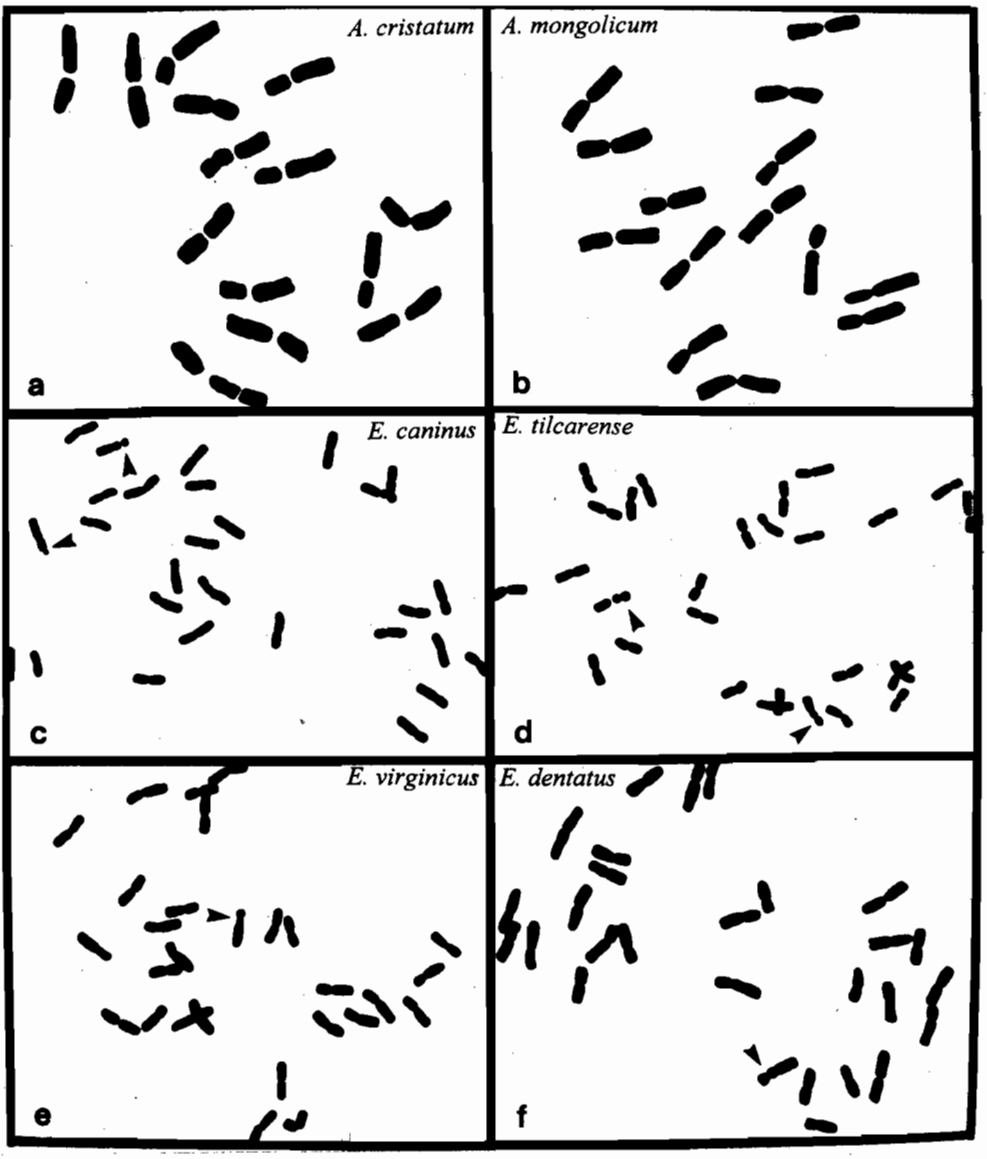

Fig.1. Mitotic chromosomal details at metaphase of some diploid *Agropyron* and tetraploid *Elymus* species: a) *A. cristatum* (2n=2x=14), b) *A. mongolicum* (2n=2x=14), c) *E. caninus* (2n=4x=28), d) *E. tilcarense* (2n=4x=28), e) *E. virginicus* (2n=4x=28), f) *E. dentatus* (2n=4x=28)

plasm rendering poor nuclear content contrast, complicated plus highly variable pre-treatment schedules for chromosome contraction and amongst others a total lack of/or inadequate resolution of secondary chromosome constrictions.

In this study on the Triticeae, in order to ascertain the versatility of a slightly modified technique from that of Mujeeb-Kazi & Miranda (1985) some species of *Aegilops, Secale cereale, Triticum monococcum, T. urartu, T. boeoticum, T. turgidum, T. dicoccum, T. dicoccoides, T. aestivum* (1B homozygous and 1B/1R homozygous), *Agropyron* sp., *Thinopyrum* sp., *Psathyrostachys juncea, Pseudoroegneria spicata* and *Elymus* species of diverse polyploidy levels were evaluated. The application and quality of a meiotic procedure was also demonstrated with some hybrid progeny derivatives involving the above species.

Materials and Methods

***Germplasm for the Study*:** Germplasm from various genera, species, accessions, cultivars, origin and polyploidy levels ($2n=2x=14$ to $2n=12x=84$) have been utilized to demonstrate the wide application range of the presented somatic cytological technique. The germplasm types are identified within the various figures that are included in this paper.

***Mitotic Procedure*:**

(i) Root tip collection: Except for *Hordeum, Secale* and *Triticum*, other seeds were germinated in small 3-inch pots filled with a sterilized soil:sand:dry leaf (2:1:1) blended mixture in which the plantlets were allowed to grow for 3 weeks. The root-tips were collected from these growing plants by tipping the plants out after inverting the potted pots, then pre-treated for 3h with colchicine: 8-hydroxyquinoline: dimethylsulfoxide (Mujeeb-Kazi & Miranda 1985) and fixed plus stained in a 0.2% solution of aceto-orcein prepared in 45% acetic acid. The samples were refrigerated (4°C) until two days prior to mitotic preparations being made, when the 0.2% stain was replaced with 2.0% aceto-orcein for intensifying the staining. Seeds of *Hordeum, Secale* and *Triticum* species were germinated in jiffy-7 peat pellets. The pellets were kept in a container lined with vermiculite. Once the root-tips penetrated the vermiculjte they were collected and processed identically as were the other samples from pots.

(ii) Root-tip squashing: The process deviated from Mujeeb-Kazi & Miranda's (1985) procedure only in extraction of meristematic cells from the root-tip apices. The apex was cut off (about 0.05 mm) and the tip longitudinally slit by a fine needle, while the root was held by a forcep on a clean glass slide. With the aid of an arrow-head needle the cells were excised onto the slide, a drop of 45% acetic acid added, cover glass placed and cells flattened using direct vertical pressure mediated by an absorbent paper.

(iii) Permanent preparations: The procedure of cover glass removal, dehydration, drying, xylene treatment and mounting for permanency were similar to those of Mujeeb-Kazi & Miranda (1985).

(iv) Meiotic analyses: Young spikes collected for meiotic analyses were fixed in 6:3:1 (absolute ethanol:chloroform: glacial acetic acid) for 48 h or longer under room conditions until all chlorophyll had eluted into the fixative. The fixative was then decanted,

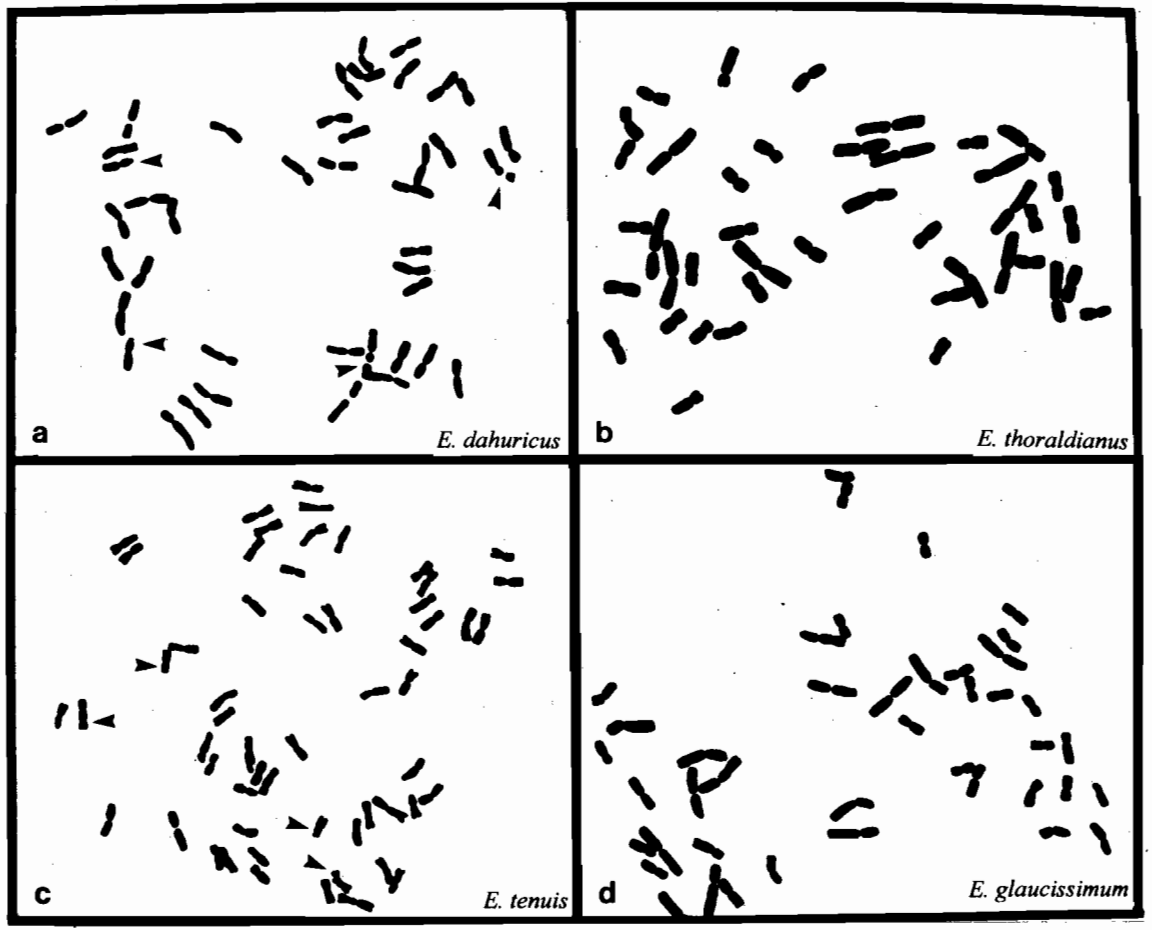

Fig. 2. Mitotic chromosomal details at metaphase of some hexaploid and octoploid *Elymus* species:
a) *E. dahuricus* (2n=6x=42), b) *E. thoraldianus* (2n=6x=42), c) *E. tenuis* (2n=8x=56), d) *E. glaucissimum* (2n=6x=42)

replaced with 70% ethanol and samples stored in the freezer (-5 to -10°C) until their processing.

From each spike, anthers identified to be at metaphase I (based upon a 1/2 anther check from the three in each floret) were extracted, immersed in vials containing alcoholic carmine (Snow, 1963) and stored in the refrigerator (4°C) for intense staining until slide preparation. Slide preparation phase involved removing the anthers from the alcoholic carmine and squashing each on a glass slide in a drop of 45% acetic acid. The cover glass sides were sealed by wax or cement and meiotic analysis of the sample subsequently followed.

(v) Photography: Representative cells (mitotic and meiotic) were photographed on a Zeiss photo-microscope using a special green and 53 barrier yellow filter on a black and white Kodak technical pan film 2415 (Estar-AH base) at 18 Din. Neutral filters were used to allow for a two second exposure. Individual prints were cropped, composited and rephotographed for obtaining the plates used as Figures 1 to 8 in this study.

Results and Discussion

Mitotic Preparations: The pre-treatment duration for all samples of the Triticeae genera analyzed at 3 h seems to be at an optimum (Figs. 1 to 7). This is evidenced by the chromosome size at metaphase that is conducive for karyotype analysis. In general, the diploid species poorly expressed the definitive secondary constriction region over the number of cells analyzed, though their presence in each root-tip was not a constraint. One of the satellite paired chromosomes is shown for *A. cristatum* (Fig. 1a), two are visible for *E. caninus* (Fig. 1c), a pair is marked for *E. tilcarense* (Fig. 1d), while one satellited chromosome is prevalent for *E. virginicus* and *E. dentatus* respectively (Figs. 1e and f). From the tetraploid polyploidy level and higher the secondary constrictions were more readily resolved in more cells of a root-tip. The technique allowed perfect primary constriction detail in each metaphase cell analyzed; an asset for karyotypic detail.

Genomic chromosome size variations earlier observed for a *Thinopyrum elongatum x Secale cereale* hybrid (Mujeeb-Kazi & Miranda, 1985) were also present in the hexaploid *E. thoraldianus* and *E. glaucissimum* (2n=6x=42) where 14 chromosomes are significantly larger than those of the remaining genomes (Figs. 2b and d). In some species the secondary constriction was almost obscure for a chromosome pair and photographic resolution was difficult. This technique however, does allow such minute details to be observed and accurate inferences to be made even though superb photographic documentation remains elusive. Such chromosomes are seen in Fig. 2a for *E. dahuricus* and 2c for *E. tenuis* where one pair of satellites are clear and the second almost obscure in the figure, but were clear when microscopic observations were facilitated by fine focus adjustment. In *Triticum aestivum* the 5D satellite pair is a similar kind of chromosome pair where the secondary constriction site more than often does not get resolved, and if resolved is difficult to photograph. It is our contention that if these details are to be studied critically using aceto- orcein or aceto-carmine staining, a reduced pretreatment time may alleviate the constraint. This may however, not be

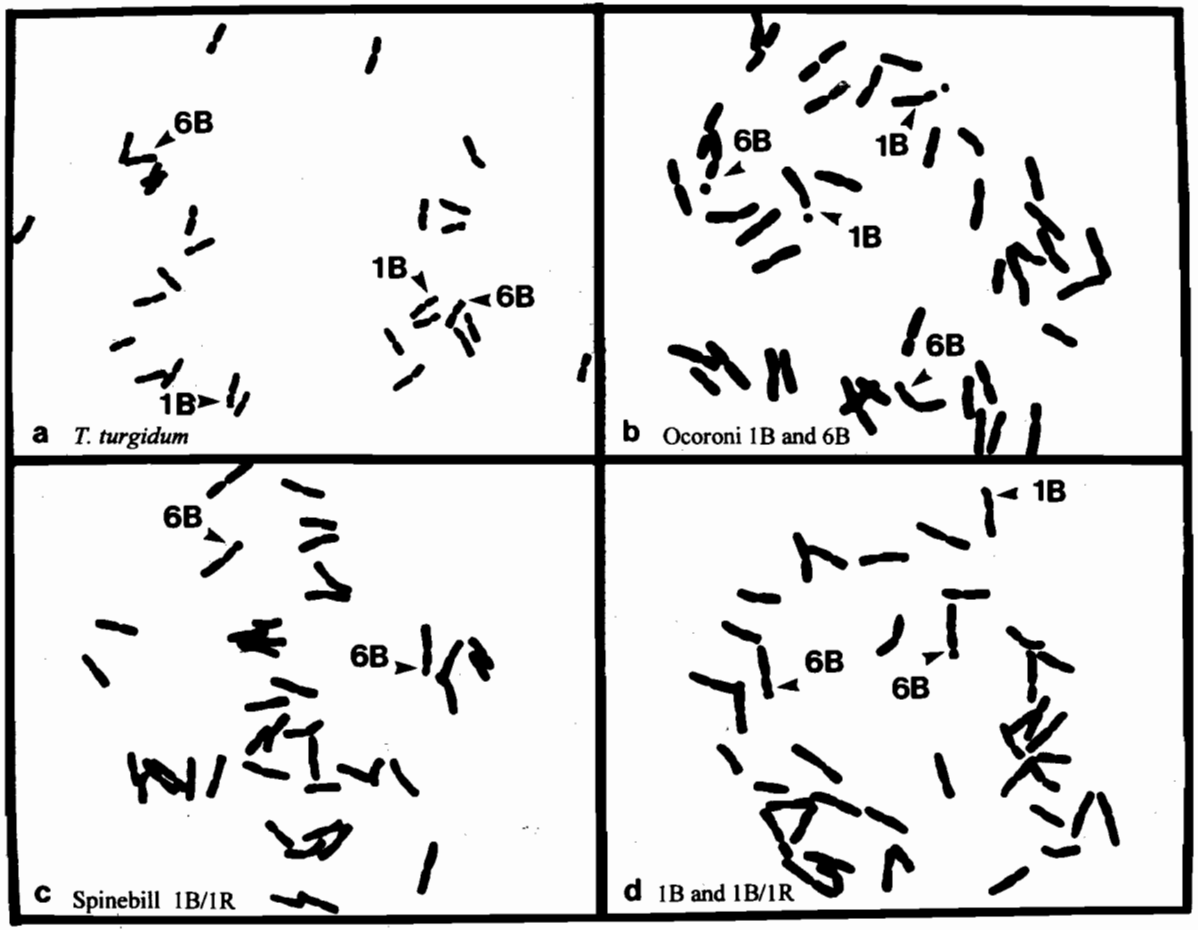

Fig.3. Mitotic chromosomal details at metaphase of some *Triticum* species and an F_1 hybrid:

a) *T. turgidum* (2n=2x=28;AABB), b) *T. aestivum* cv. Ocoroni (2n=6x=42;AABBDD); 4 satellites (1B and 6B) arrowed and marked,

c) *T. aestivum* cv. Spinebill (2n=6x=42;AABBDD); being a 1B/1R wheat, only the 6B satellite pair is marked, d) *T. aestivum*; a 1B and 1B/1R F_1 hybrid with two 6B satellited and one 1B satellited chromosomes.

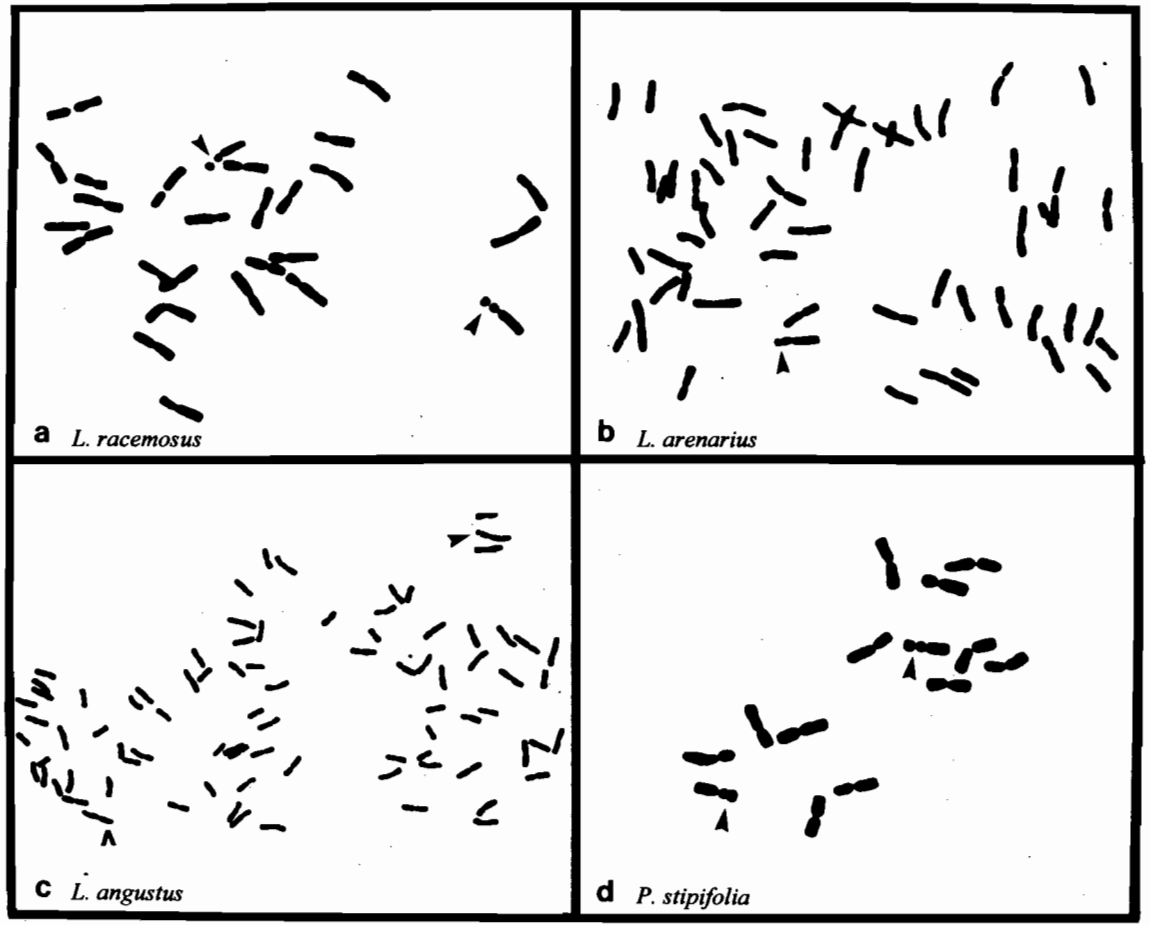

Fig.4. Mitotic chromosomal details at metaphase of some *Leymus* and *Pseudoroegneria* species:
a) *L. racemosus* (2n=4x=28), b) *L. arenarius* (2n=8x=56-1), c) *L. angustus* (2n=12x=84), d) *P. stipifolia* (2n=2x=14)

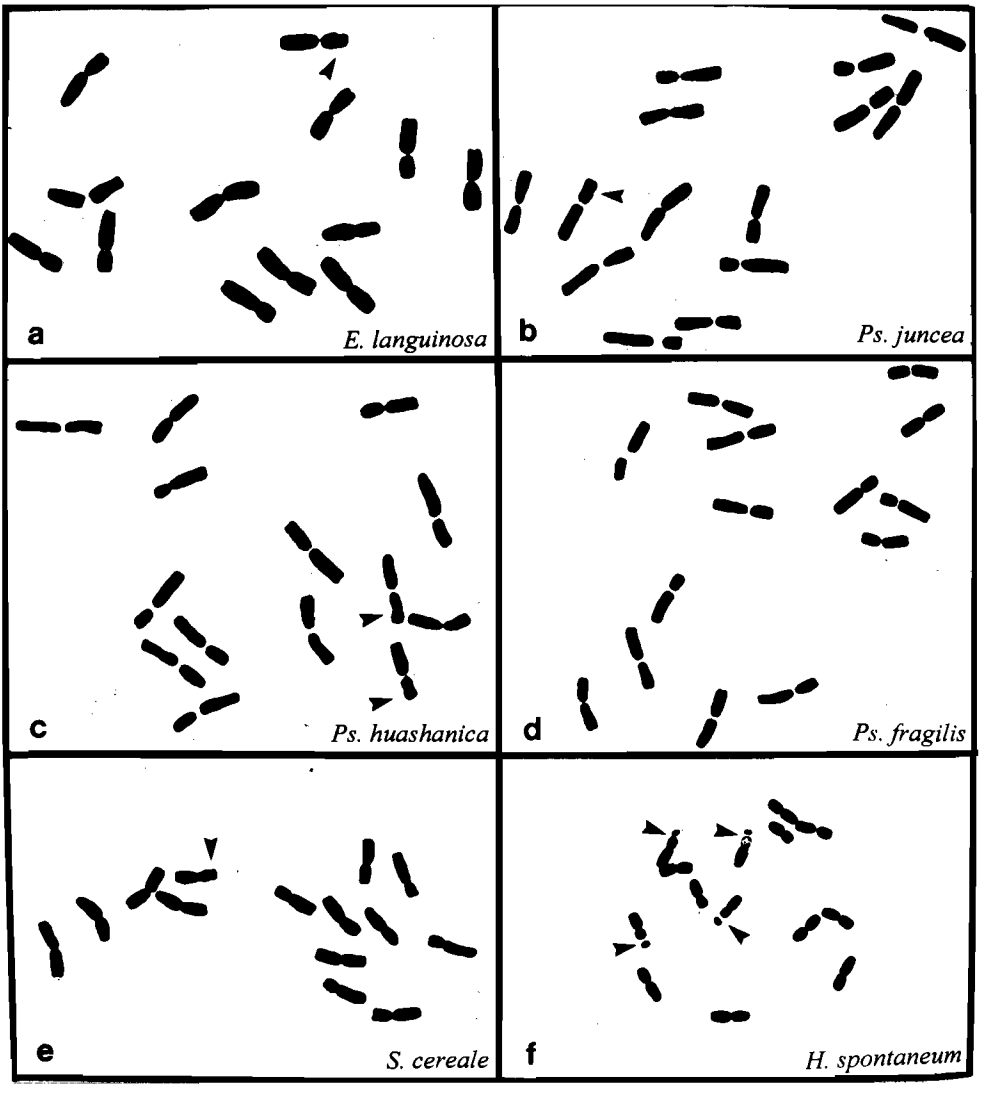

Fig.5. Mitotic chromosomal details at metaphase of some diploid (2n=2x=14) Triticeae; *Elymus*, *Psathyrostachys*, *Secale* and *Hordeum*:
a) *E. languinosa*, b) *Ps. juncea*, c) *Ps. huashanica*, d) *Ps. fragilis*, e) *S. cereale*, f) *H. spontaneum*

readily applied to all intergeneric hybrids where secondary constriction suppression due to amphiplasty or other ill-defined mechanisms are functional. Such mechanisms are common in wheat x barley hybrids, may be operative in polyploids like *E. thoraldianus* or *glaucissimum* and are consistently found in 1B/1R translocated wheats where the 1RS satellite does not express in cytological preparations (Figs. 3a to d). In durum wheats (Fig. 3a) 4 satellites appear (1B, 1B; 6B, 6B), in bread wheats homozygous for 1B chromosome normally 4 satellites (1B and 6B pairs) are present (Fig.3b) coupled rarely with a 5D pair, in bread wheat cultivars that are chromosome 1B/1R homozygous normally a 6B pair of satellites (Fig. 3c) coupled rarely with a 5D pair are visible and an F_1 between a 1B and a 1B/1R wheat the satellites seen are of chromosomes 1B, 6B, 6B (Fig. 3d) or rarely 1B, 6B, 6B, 5D, 5D.

Secondary constriction resolutions were restricted to a pair in the tetraploid, hexaploid and octoploid *Leymus* species (Figs. 4a to c). Sharp resolution existed for *L. racemosus*, and average for *L. arenarius* and *L. angustus*. For *L. angustus* the pair of satellites were well resolved but quality separation of an 84 chromosome cell did not correlate with one possessing 2 quality secondary constrictions; hence one is shown in Fig.4c. In *P. stipifolia* the satellites were ideally resolved (Fig. 4d).

Diploid species generally do not show superior secondary constriction details and of the 6 species cells shown in Fig. 5 (a to f) this trend does prevail except for *H. spontaneum* (Fig. 5f). Secondary constrictions observed on chromosomes when viewed microscopically are arrowed but these did not manifest into superior photographic quality. However, for each species metaphase separation was ideal, primary constriction explicit, rendering karyotyping relatively simplistic. This trend also extended to *Th. bessarabicum* (Fig. 6a) where of the secondary constriction heterobrachial chromosomal pair only one was resolved with a detail that resembles chromosome 5D of *T. aestivum*. A pair each was variably resolved for *Th. caespitosum*, *Th. scirpeum* and *Th. intermedium* (Figs. 6 b to d). These 4 species have tremendous use in practical agriculture and currently *Th. bessarabicum* is being utilized for transferring salt tolerance to wheat (Mujeeb-Kazi, 1993) with its wide range of markers being an asset (William & Mujeeb-Kazi, 1993) of which the standard chromosome karyotype may be another, for which a quality somatic preparation (like the one in Fig. 6a) may be a source. *Th. intermedium* has become presumably the most potent source for BYDV resistance in wheat in that advanced wheat/*Th. intermedium* tissue culture derivatives have been identified by FISH to possess resistance (Bertschinger *et al.*, 1994; Mujeeb-Kazi *et al.*, 1994a). Observing satellites in the diploid *Triticum* species *monococcum* and *boeoticum* was generally elusive (Figs. 7a, b), highly irregular in *T. dicoccoides* (Fig.7c) and very consistent for 1B, 1B and 6B, 6B in *T. dicoccum* (Fig. 7d). The use of these species has been largely directed towards a swift means of wheat improvement because of their A and B genomic similarities to the genomes of wheat. *T. dicoccoides* has been more exploited particularly for its high protein aspects. More recently *T. dicoccum* accessions have surfaced as resistant sources to the Russian wheat aphid constraint. The A genome diploids (Fig. 7a, b) of which *T. urartu* also is a group member, offer unique diversity that may provide novel alleles for durum and bread wheat improvement. Our interest is looking at the novel HMW glutenin sub-units that these diploids may provide.

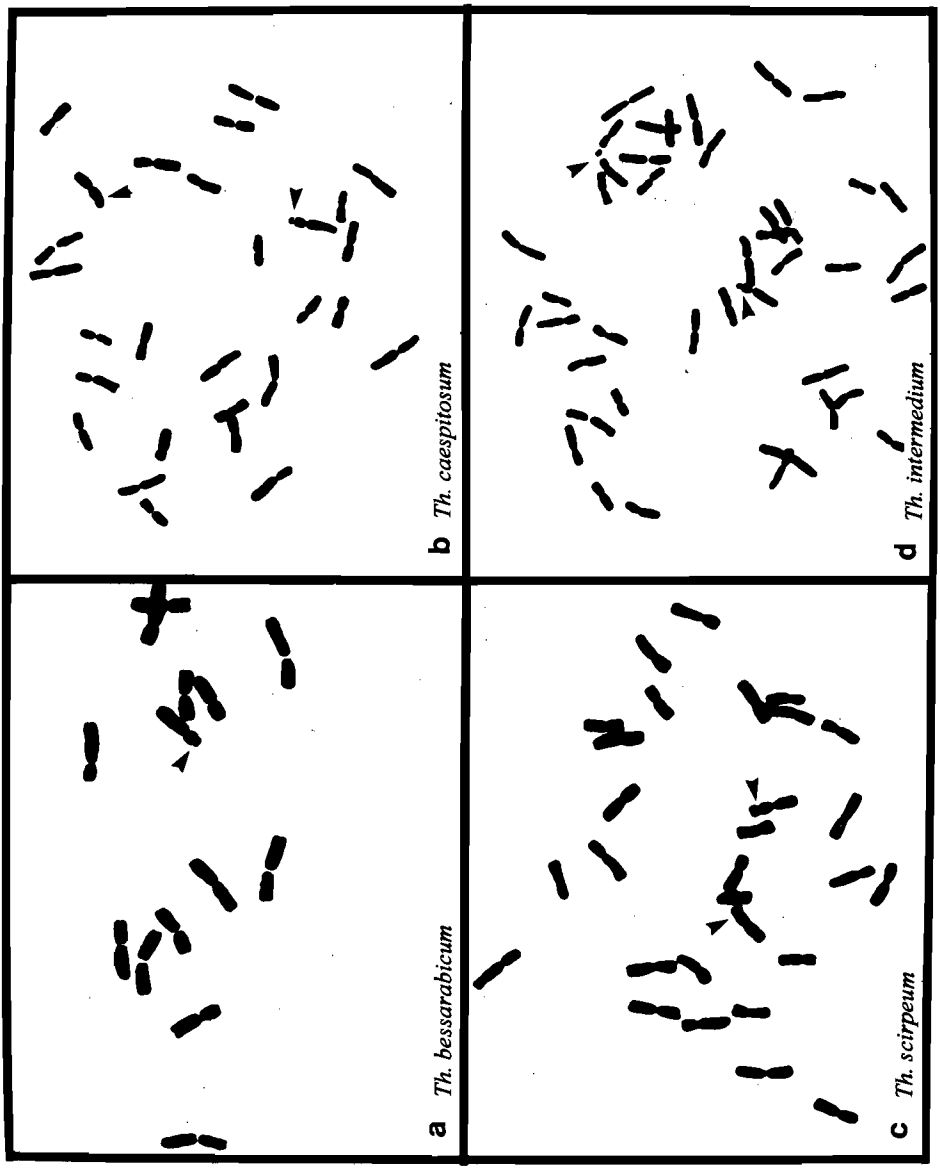

Fig.6. Mitotic chromosomal detail at metaphase of some *Thinopyrum* species:
a) *Th. bessarabicum* (2n=2x=14), b) *Th. caespitosum* (2n=4x=28), c) *Th. scirpeum* (2n=4x=28), d) *Th. intermedium* (2n=6x=42)

The extent of germplasm diversity that we have utilized for elucidating the application of a mitotic techniques variation is an attempt towards suggesting that for applied alien transfer research areas, quality cytological procedures that are easily adopted do exist. These techniques are also advantageous for taxonomic studies based crucially upon karyotypic analyses. The high mitotic index achieved and chromosome separation at metaphase with a standard chromosome contraction duration are advantageous in preparation of cytological preparations of use in chromosome banding studies. In such cases the aceto-orcein fixation step alone is replaced by a 0.1% aceto-carmine fixation and preparations are made within 2 to 3 days to avoid intense cellular staining by 0.1% aceto- carmine that hinders with giemsa staining during banding. The current molecular cytogenetic area of *in situ* hybridization to facilitate alien DNA detection; both enzymatic and fluorescent; also requires superior metaphase (generally) chromosomal preparations and we have utilized this technique to an advantage (William & Mujeeb-Kazi, 1994; Islam- Faridi & Mujeeb-Kazi, 1994). The technique variation used by us has been treating the root tips after fixation with an enzyme mixture (cellulase + pectolyase) that facilitates softening and spreading of the chromosomes with little cytoplasmic debris (Islam-Faridi & Mujeeb-Kazi, 1994). Such techniques shall become even more essential when alien transfer programs emphasize a focus on F_1 based transfer (Mujeeb-Kazi *et al.*, 1994b) and genome relationships in the Triticeae utilize the molecular cytological inputs.

Meiotic analyses in wide cross germplasm: In order to obtain a critical assessment relative to wheat/alien genome relationships, meiotic analyses are essential for making interpretations that direct the research programs advance in order to achieve alien introgressions. Generally meiotic preparations do provide this information for almost all cytogeneticists through preparations that exhibit a wide array of analytical quality. Three major constraints that occur in the Triticeae germplasm are: (i) Chromosome stickiness, (ii) Stretched and poorly stained chromosomes and (iii) Intense background staining rendering poor contrast; more vividly expressed in several photographic records. In genetic transfers wide hybrids often express meiotic associations where alien exchanges are impractical since recombination does not occur. One option (Kimber, 1993; Mujeeb-Kazi & Kimber, 1985) is to utilize the recessive *ph* or use mono 5B system of Chinese Spring wheat to enhance recombination. In hybrids thus produced, meiotic preparations exhibit enormous stickiness that may lead to difficulty in interpretation of multivalent associations. The methodology described by us uses a 6:3:1 (ethanol:chloroform:acetic acid) fixative, 70% ethanol storage, excision of 2 ½ anthers at metaphase I, their storing and staining in alcoholic carmine for about a week or longer (refrigerated), bench top storage for 2 days to intensify tissue staining and normal squashes in 45% acetic acid with slight warming of the slide plus minimal thumb pressure. The quantity of acetic acid, heat and pressure determines how much chromosomal spread will occur where the latter also will control chromosome stretching. Sealing the slides with wax or rubber cement before which a 'small' drop of 2% aceto-carmine is added by a dropper to the cover-glass side allows to intensify the stain over-night for photography the next day. After the aceto-carmine drop is added and it moves under the cover-glass, waxing of the slide is done by keeping the thumb rigidly on top of the cover-glass so that excess stain oozes out. This ensures all cells to be in one plane when photography is done.

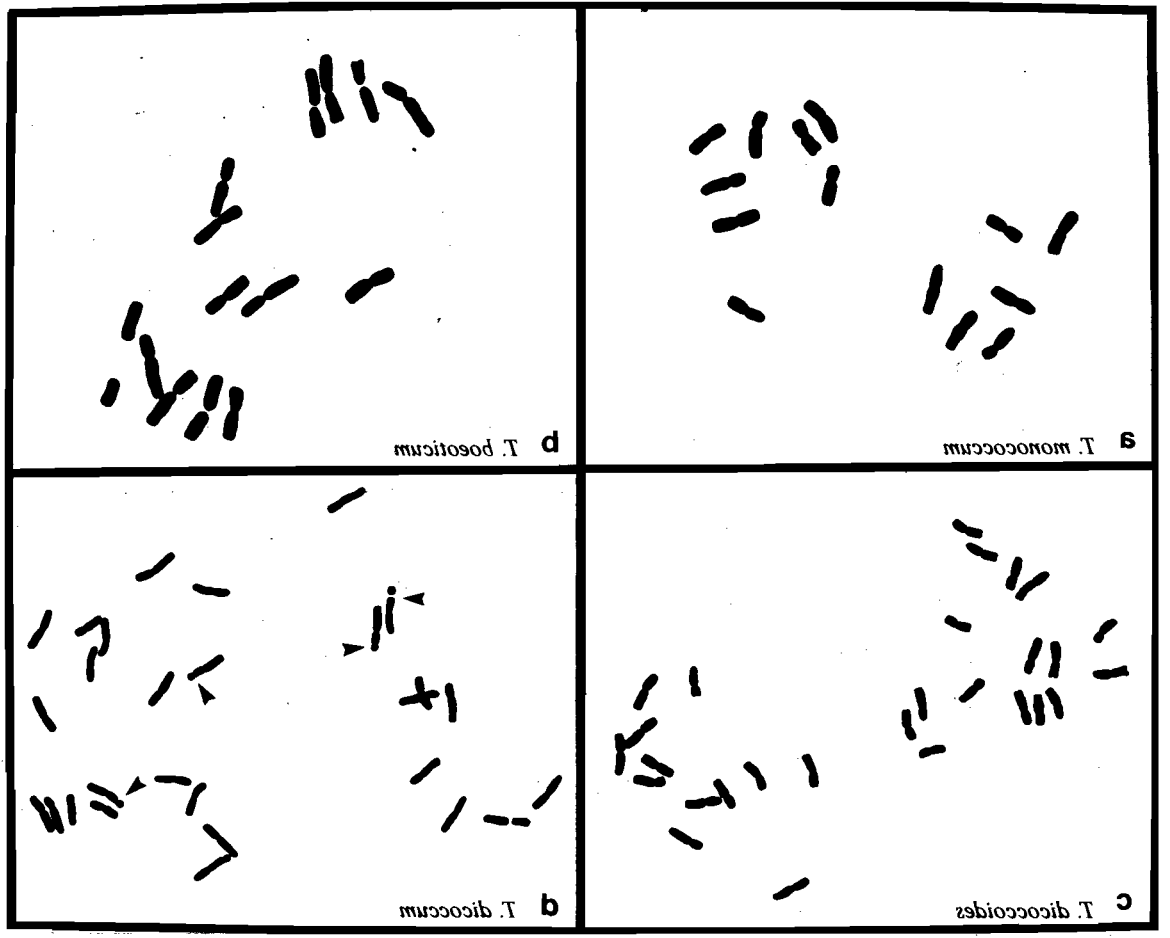

Fig.7. Mitotic chromosomal detail at metaphase of some AA and AABB genome *Triticum* species:

a) *T. monococcum* (2n=2x=14;AA), b) *T. boeoticum* (2n=2x=14;AA), c) *T. dicoccoides* (2n=4x=28;AABB), d) *T. dicoccum* (2n=4x=28;AABB)

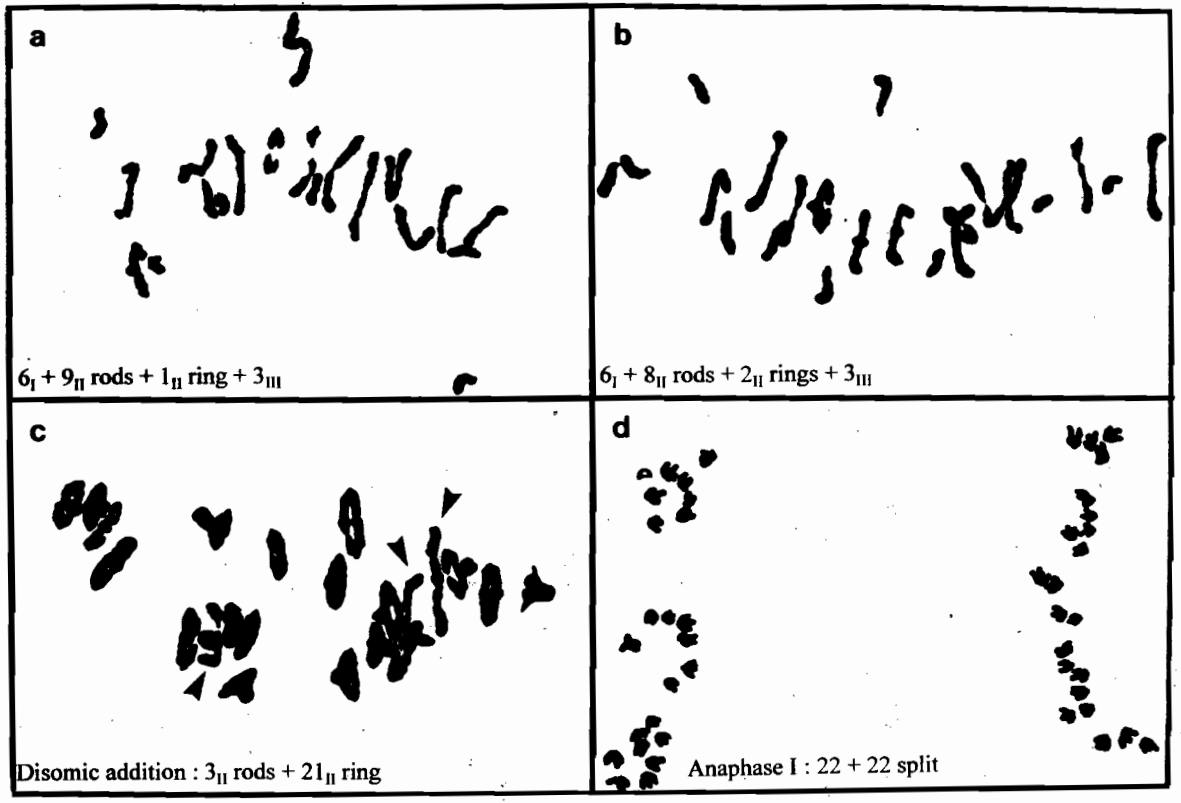

Fig.8. Meiotic metaphase I and anaphase I relationships in *Triticum aestivum* x alien genera:
a) *T. aestivum* cv. Chinese Spring (ph1b) x *Aegilops variabilis*: 35 chromosomes associated as 6 univalents + 9 rod bivalents + 1 ring bivalent + 3 trivalents, b) Same as (a) with associations of 6 univalents + 8 rod bivalents + 2 ring bivalents + 3 trivalents.c) A disomic addition line of *Thinopyrum bessarabicum* in *T. aestivum* (2n=6x=42 + 2) associated as 19 ring bivalents + 3 rod bivalents (arrowed), d) A 22+22 anaphase separation of the disomic addition in (c).

The ph based F_1 hybrid meiosis (Fig. 8 a, b) illustrates our contention where chromosome separation, structure, staining and clear cytoplasm are apparent. The *Th. bessarabicum* disomic line with 22 bivalents at metaphase I and their 22+22 split at anaphase I (Fig. 8 c, d) further substantiate the above observations.

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