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PRODUCTION AND CYTOGENETICS OF A NEW *THINOPYRUM ELONGATUM* / *TRITICUM AESTIVUM* HYBRID, ITS AMPHIPLOID AND BACKCROSS DERIVATIVES

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Abstract

Genetic diversity is crucial for crop improvement. In wheat this resource is distributed within the three gene pools of the tribe Triticeae and priority usage over the last decade has been with the D genome diploid progenitor of the primary gene pool. Potent variability that contributes towards resistance/ tolerance to key biotic/abiotic stresses limiting wheat production is also available in the tertiary pool where *Thinopyrum elongatum* (2n=2x=14, EE) possesses usable diversity for improving wheat. It has been hybridized with a top quality commercial hexaploid wheat cultivar as the maternal parent in order to capture its cytoplasmic advantage if any and for developing genetic stocks in terms of *Th. elongatum* disomic chromosome addition lines for identifying positive individual chromosomal contributions towards some key stresses that limit wheat productivity. The F_1 hybrid possessed a 2n=4x=28 (ABDE) composition and exhibited predominantly 28 univalents at meiotic metaphase 1. The 28 chromosome F_1 hybrid upon pollination with hexaploid wheat gave 2n=7x=49, AABBDDE backcross 1 progeny that upon further backcrossing produced various monosomic A to G addition lines (2n=6x=42 + 1=43) generating monosomic haploids (n=3x=21 + 1=22) by the wheat / maize protocol which were stabilized by colchicine treatment as their respective disomic additions (2n=6x=42 + 2=44). Giemsa C- banding coupled with biochemical and fluorescent *in situ* hybridization (FISH) were the validation diagnostics for categorization of the disomic addition lines produced. The biochemical applications were to cover one marker per homoeologous group for high molecular glutenins (HMW; Group 1), superoxide dismutase (SOD; group 2), esterase (EST; group 3), alcohol dehydrogenase (ADH, group 4), β-amylase (β-AMY; group 5), glutamate oxaloacetate transaminase (GOT, group 6) and α-amylase (α-AMY, group 7). *Th. elongatum* possesses diversity for salinity tolerance and for *Fusarium* head scab resistance. The four disomic addition lines produced (biochemically partial for groups 1, 3, 5 and 7) have rendered this diversity to be used in a targeted fashion around user friendly germplasm after they are categorized for their stress attributes.

Introduction

Current globally recognized major bread wheat improvement priorities are associated with genetic security for three rusts, *Fusarium* head scab resistance, drought, heat and salinity tolerance. Alien grass species of the tribe Triticeae are a rich reservoir for providing diverse genes that can enrich cultivated wheats independently or cumulatively; the latter being the preference. Such resources with multiple stress traits reside in the primary gene pool species like *Aegilops tauschii* (2n=2x=14, DD), (Mujeeb-Kazi, 2003, 2006). Potent variation is also existent in tertiary gene pool species from which

exploitation of trait positive diploid donors for wheat improvement holds an advantage over tetraploid and higher polyploidy sources (Mujeeb-Kazi, 2005). One such diploid is *Thinopyrum elongatum* (2n=2x=14, EE) that fortuitously also possesses resistance to head scab (Jauhar & Peterson, 1996) and is salt tolerant (Dvorak *et al*., 1988). Hence its candidacy as a contributor of resistance/tolerance gene/s to wheat is currently high in the area of intergeneric hybridization.

This diploid was initially hybridized with bread wheat (*Triticum aestivum* L. cv Chinese Spring) by Dvorak & Knott (1974) from which disomic and ditelosomic addition lines were developed. Subsequently, Dvorak *et al*., (1988) evaluated the salt tolerance potential of the addition lines and reported the positive contribution of three addition lines associated with homoeologous groups 3, 4 and 7.

We decided to remake this hybrid combination with two significant modifications:

(a) Attempt the reciprocal cross in order to capture any practically beneficial cytoplasmic factors that may be present in *Th. elongatum* and

(b) Use a commercially high yielding bread wheat cultivar instead of Chinese Spring in order to obtain end product derivatives that are agronomically superior and better adapted to global field testing conditions.

In this paper is documented the production, cytogenetics and morphology of the F_1 hybrid, its amphiploid and backcross progenies using *T. aestivum* as the recurrent pollen parent. Also are reported observations of the *Th. elongatum* diploid for Giemsa C-banding, plus some biochemical profiles and fluorescent *in situ* hybridization details of the F_1 hybrid, the amphiploid and advanced derivatives that are the source of production of alien chromosome addition lines and/or stabilizing them by the wheat x maize doubled haploid protocol (Mujeeb-Kazi *et al*., 2006).

Material and Methods

Germplasm: Seeds of *Thinopyrum elongatum* (2n=2x=14, EE) used in this study were obtained from late Dr. D. R. Dewey, USDA/ARS Logan, Utah, and germinated in Jiffy-7 peat pellets. After 6-weeks of juvenile growth, the seedlings were vernalized in a growth chamber under environmental regimes of 8h diffuse light for 8 weeks at 8C. Following vernalization, the seedlings were transplanted into 20 cm plastic pots filled with a 2:1:1 (soil: sand: peat) steam sterilized mix, and maintained under greenhouse conditions of 16h natural light and 24/14C day/night temperatures. In the same greenhouse, four plantings of a bread wheat (*Triticum aestivum* L) cultivar (cv. Goshawk 'H') were made at 15day intervals. The wheat cultivar was obtained from CIMMYT's wheat germplasm bank at El Batan, Mexico, the location where this study was undertaken

Hybrid production: Bread wheat cultivar spikes were emasculated, pollinated by fresh pollen from *Th. elongatum* 1 to 3 days after emasculation and treated once daily for three days with 75 ppm gibberellic acid and 2, 4-di-chlorophenoxyacetic acid. From the seed set, embryos were excised 13 to 15 days after pollination, and cultured on a special medium for small embryos (Taira & Larter, 1978). These and subsequent procedures associated with embryo differentiation, plantlet growth, transfer to Jiffy-7 pots and then to a soil mix in the greenhouse were similar to those reported earlier (Mujeeb-Kazi *et al*., 1987). The environmental growth regimes were identical to those maintained for the growth of the parental germplasm in this study.

F_1 cloning, somatic/meiotic sampling and colchicine treatment: After assuming vigorous growth, each F_1 hybrid was physically divided into 3 to 5 clones. The clones were allowed to grow into vigorous plants. From each clone of each hybrid, root-tips were collected for somatic cytology, Giemsa C-banding and fluorescent *in situ* (FISH) hybridization. The cytological procedures were essentially similar to those described by Mujeeb-Kazi *et al*., 1994, Jahan *et al*., 1990, Islam-Faridi & Mujeeb-Kazi, 1995. For FISH, wheat DNA was used for blocking, while the J or E genome biotin labeled DNA from *Th. bessarabicum* or *Th. elongatum* served as the detection probe.

Spikes for meiotic analyses were collected in the early morning hours (8:00 to 9:00AM), fixed in Carnoy's fixative (6:3:1; absolute alcohol: chloroform: acetic acid) for 72 hours and stored under refrigeration (4C) in 70% alcohol until use. Anthers at metaphase-1 were stained in alcoholic-acid-carmine for several days, and squashed in 45% acetic acid with a drop of 2% aceto-carmine to enhance coloration. Cytological photography was done of quality representative cells on a black and white high contrast Kodak 2415 film using a special green/ yellow filter combination and a two second exposure.

One clone of each original plant was treated with colchicine (0.05%) and 2.0% di-methyl-sulfoxide (DMSO) solution using the aerated treatment protocol (Mujeeb-Kazi *et al*., 1987) in order to induce amphiploidy.

Spike categorization and backcross seed production: Ten fully emerged spikes from the F_1 clones were characterized with the wheat parent for spike morphology. For backcross seed production 15 self-sterile but female-fertile spikes were pollinated with fresh pollen from cv. Goshawk 'GH' and the seed set allowed to mature on the hybrid plants. This constituted the backcross 1 (BC_1) seed and anticipated to be 2n=7x-49, AABBDDE.

Biochemical and molecular diagnostics: The amphiploid (*Th. elongatum* / *T. aestivum*, 2n=86=56, AABBDDEE) was subjected to biochemical analyses that would attempt to partially cover at least one marker for each of the seven homoeologous wheat chromosome groups. These were high molecular weight glutenins (HMW-Glu, Group 1), superoxide dismutase (SOD, Group 2), esterase (EST, Group 3), alcohol dehydrogenase (ADH, Group 4), β-amylase (β-AMY, Group 5), glutamate oxaloacetate transaminase (GOT, Group 6) and α-amylase-2 (α-AMY, Group 7).

Mature grains were used for SOD, EST-5, ADH and β-AMY assays. Endosperms of mature grains were used for HMW-glutenin test. α-AMY was extracted using six day old germinating seeds, while young fresh leaf tissue was used for GOT analysis. Analytical protocols relative to enzyme extraction, gel running conditions, buffer solutions and staining procedures were similar to those used by William & Mujeeb-Kazi (1993).

After the respective homoeologous groups were identified, two samples of each group were subjected to cytological characterization by the fluorescent *In situ* hybridization (FISH) and C-banding procedures.

Results and Discussion

Hybrid production, cloning, validation, backcrossing and amphiploidy: The cross combination between *Th. elongatum* and *T. aestivum* gave a low frequency output of 1.2% embryos from which three embryos differentiated into F_1 seedlings. The embryos were minute, translucent and globular in shape recovered from seed devoid of endosperm. All

hybrids grew vigorously and were cloned to increase the number of plantlets per hybrid. The hybrid phenotype was intermediate as compared with their parents. This has been a common observation for most intergeneric hybrids within the Triticeae (Mujeeb-Kazi & Rajaram, 2002), and has been considered a valid morphological indicator of alien genetic expressivity in a wheat back-ground. The phenotypic parameters generally affected included spike length/size, spike width, reduced awn length, lax head structure due to increased internodal distance and presence of little pubescence.

Somatically the hybrid seedlings were all normal with 2n=4x=28, ABDE chromosomes and each hybrid possessed the C-banded *Th. elongatum* component (ABDE) characteristic of the maternal detail. Even though somatic evidence was adequate to validate the hybrid status, meiotic information set the clarity for exploiting this hybrid combination for practical usage. Meiocytes of the F_1 hybrid exhibited univalency suggesting that recombinational products would not be obtained *via* normal homoeologous exchanges. The univalency observation also indicated that production of the seven disomic addition lines would proceed with relative ease since chromosomal integrity was well maintained. In order to proceed on this aspect we used two strategies:

(a) Hybridize the self-sterile F_1 but female-fertile plants with bread wheat to directly obtain backcross 1 (BC_1) derivative seed with a 2n=7x=49, AABBDDE composition and
(b) Obtain the amphiploid (2n=8x=56, AABBDDEE) *via* colchicine doubling on some F_1 plants to be used as a continuing stable source in addition line development.

Pollinating F_1 spikes gave from 3 to 7 BC_1 seed per spike whereas colchicine treatment of 3 F1 plants led to doubled products on two plants, yielding 1 and 4 seed respectively. The C-0 amphiploid seed were all 56 (2n=8x=56, AABBDDEE) in chromosome number and upon seed increase were to be of use for stress screening and biochemical/molecular analyses.

Backcross 1 plants (2n=7x=49, AABBDDE) became the source of yielding BC_2 seed when they were pollinated again with wheat. These BC_2 seed would then have derivatives with 42 chromosomes of wheat (AABBDD) plus from either none to a maximum of 7 chromosomes of *Th. elongatum.* Such derivatives then become the potential source of producing disomic *Th. elongatum* chromosome addition lines upon further backcrossing, cytological validation and selfing of distinctly different alien monosomic addition chromosomes (2n=6x=42 +1 = 43) according to the conventional procedures that have been utilized by researchers (Friebe *et al*., 1995, Littlejohn & Pienaar 1995, Lukaszewski, 1988). Alternatively, integration of polyhaploidy offers a new dimension and was exploited by us here to produce such addition lines. This procedure was used by Islam *et al*., 1978, 1981, when they produced one barley disomic chromosome addition line in their wheat/barley program. Since then the haploid procedure has been extremely simplified and hence we put it to routine use at the BC2 level where one to three monosomic alien chromosome additions were involved.

Polyhaploid plant production and doubled haploids: Individual BC_2 derivatives from *Th. elongatum*/3* *T. aestivum* with somatic chromosome numbers in the progeny of 2n=43 to 45 led to production of 587 seeds in total when 3040 florets were pollinated with maize. From the 524 embryos excised, 406 haploid seedlings emerged ranging in mitotic counts from n=21 to 24 (Table 1). Majority of the 21 chromosome polyhaploids were discarded and 342 plants remained. Of those, 218 vigorous plants were treated with colchicine (Table 1).

Table 1. Production of polyhaploids from *Thinopyrum elongatum* / 3* *Triticum aestivum* derivatives with 43 to 45 chromosomes crossed with *Zea mays*.

Polyhaploid category	No. of seedlings differentiated	Colchicine treatment	
		Treated	Setting seed
21	64	12	7
22	273	168	96
23	57	29	12
24	12	9	2
Total	406	218	117

Seven double haploid (DH) plants from the 21 chromosome polyhaploids were to serve as homozygous alloplasmic germplasm. Numbers of DH plants from n=22 (or 22 + a telosome), 23 and 24 polyhaploids were 96, 12 and 2 respectively. A total of 54 DH plants (C-0 generation) were selected and analyzed in detail for somatic chromosome number, number of spikes and advanced to the C-1 generation (Table 2).

The selected DH plants grew satisfactorily under controlled glasshouse conditions, producing four to 15 spikes per plant except for B92-106. The number of seeds set per plant ranged from one to 110. C-1 plants from the low seed setting C-0 plants (one to three seeds), yielded large number of seeds (31 to 164 seeds) when advanced to C-1.

Cytogenetic analyses of a representative from each disomic addition group provided evidence of normalcy of meiotic metaphase I chromosome associations (Table 3) as reflected through the high frequency of ring bivalents. Their fertility was also high, setting 98 to 165 seed per plant. This selection sieve is advantageous for meeting applied agricultural objectives where seed quality, fertility and germplasm stability contribute towards alleviating constraints associated with biotic/ biotic screening for complex traits.

In wheat/barley hybrids two complexities existed as to self-fertility. In barley/wheat backcross derivatives the barley cytoplasm prevented self-fertility to be obtained; a constraint alleviated when wheat was subsequently used as the maternal parent (Islam *et al*., 1978). However, barley chromosome 5 induced self-sterility still prevailed preventing the production of this chromosomes disomic addition (Islam *et al*., 1981).

Th. elongatum was used as the maternal parent in our study to salvage possible positive cytoplasmic effects associated with salinity tolerance that characterizes this species. Self-sterility was not encountered in our *Th. elongatum/T. aestivum* combination as occurred for barley / wheat derivatives. However, the low alien chromosome paternal transmission was a constraint in obtaining high frequencies of 44 chromosome disomic addition lines after selfing of the BC derivatives. Use of the polyhaploid methodology resolved this alien chromosome paternal transmission constraint leading to double haploid (DH) production. Doubling polyhaploid plantlets yielded derivatives with varied chromosomal numbers in their progeny. Polyhaploids with 22, 23 or 24 chromosomes are anticipated to yield normal progeny upon doubling of their chromosomal compositions from 22 to 44, 23 to 46, or 24 to 48 chromosomes. Table 2 illustrates the variation present where 22 chromosome polyhaploids yielded 19 DH derivatives with stable 44 chromosomes and 11 possessed between 43 to 44 chromosomes. As a consequence of meiotic irregularities present frequently in wide hybrids, some 22 chromosome polyhaploids produced DH progeny with 45, 45 (telocentric) or 46 (telocentric) chromosomes. A similar trend also existed for the 23 chromosome polyhaploids (Table 2). Our cytological screening pressure focused on selecting 44 chromosome plants only within each DH derivative, that after further population advance combined with cytology allowed for maintenance of stable 44 chromosome compositions. The variation for the C-0 somatic scores (Table 2) is

presumably based upon variable chromosomal and chromatid separations during meiotic metaphase I and/or II. Such anomalous separation; using a 22 chromosome DH tiller as an example, could produce egg cells with 21 to 23 chromosomes. Additional chromosomal abnormalities would be expressed around these base maternal chromosome numbers. The pollen trend could theoretically also possess the same wheat and alien chromosome composition with a low transmission frequency of the male gametes. The maternally buffered alien chromosomal transmission together with the higher frequency of normal 21 chromosome wheat pollen gametes would allow for permutations around the base 21 to 23 egg cell chromosome compositions with other cell division abnormalities as explained by the varied somatic DH progeny obtained where telocentrics were present (Table 2). Where cytological evidence supported a product with both a male and female alien chromosome transmission trend, we speculate that polyhaploidy may have been more conducive for such chromosome transmissions due to nucleo-cytoplasmic uniformity. This contention is supported by the observation where 23 chromosome polyhaploid plants with two alien monosomic additions yielded 46 double disomic alien chromosome addition line derivatives. Such additions are anticipated to be more stable because of the DH induced allelic homozygosity. Other alternatives cannot be discounted, with anomalous chromosome separations coupled with meiotic restitution being plausible options. Despite these variations, the DH methodology and cytological screening have enabled substantial purification of the BC derivatives to give a wide array of 44 chromosome plants. Within each 44 chromosome DH population, individual plants with a high seed number were selected to facilitate biochemical analyses. We envision the DH approach in alien transfer areas as a means of producing alien disomic addition lines and genetic stocks of greater stability.

Characterization of disomic addition lines by protein/isozyme markers: Some 44 chromosome derivatives, the *Th. elongatum/T. aestivum* amphiploid (2n=8x=56; AABBDDEE) and both parents involved in the amphiploid were tested for protein and isozyme patterns. Positive markers were identified for HMW glutenins (Group 1), esterase-5 (Group 3), β-amylase (Group 5) and α-amylase-2 (Group 7) in the alien species, the amphiploid and some disomic chromosome addition lines.

Th. elongatum had a HMW-GLU subunit that migrated somewhat slower than the fastest moving subunit of 'GH' (Fig. 1). This HMW-GLU subunit was expressed in the amphiploid as well as in two disomic addition lines, B92-6698 and 6704, establishing partial chromosome homoeology with the group 1 chromosomes of wheat. Association of HMW glutenins with group 1 chromosomes has been earlier reported (Beitz *et al*., 1975, Lawrence & Shepherd, 1981).

A positive group 3 marker was identified for EST-5, of which a relation to the homoeologous group 3 chromosomes was earlier established by Ainsworth *et al*., (1984). In the cathodal region, *Th. elongatum* possesses a band not present in 'GH' bread wheat, but present in the *Th. elongatum* x 'GH' amphiploid (Fig. 2). The disomic addition lines, B92-6871 and 6881 also possessed this band.

Some polymorphism was observed between two *Th. elongatum* accessions tested for the β-AMY patterns (Fig. 3). This polymorphism does not limit its use as a marker in characterizing the *Th. elongatum* chromosome with β-AMY genes, since no overlap existed between the wheat and *Th. elongatum* patterns (Fig. 3). The amphiploid showed the distinctive wheat and *Th. elongatum* accession (Th-26) patterns. This marker was also observed in the disomic addition lines, B92-6899 and 6911, indicating partial arm homoeology of the added chromosomes to group 4 / 5 wheat chromosomes.

Table 2. Cytological and seed set status of some C-0 doubled haploid plants [a] from *Thinopyrum elongatum / Triticum aestivum* BC derivatives advanced to C-1[b].

Polyhaploid		C-0 generation			C-1 generation	
No	n[t]	No. of spikes	No. of seeds	2n[d]	No. offspring observed	No. seeds/plant (range)
B92-77	22	6	13	44	8	67-130
B92-83	22	7	46	44	7	39-141
B92-91	22	6	24	43-44	8	53-130
B92-94	22	6	33	44	8	36-119
B92-95	22	7	2	42-44	2	1-163
B92-98	22	4	12	43-44	8	65-206
B92-105	22	6	2	44	1	131
B92-106	22	1	1	44	1	50
B92-109	22	5	21	43-44	8	40-118
B92-111	22	7	21	43-44	8	44-146
B92-120	22	11	13	43-44	8	72-128
B92-121	22	1	50	44	7	24-102
B92-134	22	6	54	43-44	7	79-118
B92-135	22	5	1	44	1	31
B92-136	22	7	38	44	8	20-115
B92-154	22	7	10	43-44	7	30-134
B92-156	22	9	39	44	7	77-135
B92-159	22	6	3	44	1	114
B92-164	22	7	50	44	5	97-156
B92-169	22	7	74	44-45t	6	29-121
B92-170	22	6	10	43-44	6	19-165
B92-171	22	8	93	43-44	8	2-86
B92-172	22	11	30	44	5	22-183
B92-177	22	7	70	44-46t	7	40-141
B92-178	22	7	5	44-45	5	28-191
B92-179	22	6	10	43-44(t)	5	21-101
B92-184	22	10	110	44-45t	8	108-197
B92-185	22	12	72	44	8	116-178

Table 2. (Cont'd.).

Polyhaploid		C-0 generation			C-1 generation	
No	**n[t]**	**No. of spikes**	**No. of seeds**	**2n[d]**	**No. offspring observed**	**No. seeds/plant (range)**
B92-202	22	14	3	44	2	25-30
B92-207	22	6	36	44(2t-44)	8	97-191
B92-208	22	4	20	43-44	8	75-153
B92-213	22	13	5	44	2	47-102
B92-214	22	12	1	44	1	164
B92-231	22	10	6	43-44	5	72-138
B92-233	22	11	4	44-45	4	34-122
B92-238	22	4	35	44-44t	8	68-176
B92-241	22	15	52	44	7	81-155
B92-250	22	6	7	44	6	46-169
B92-251	22	4	6	44	4	35-115
B92-166	22t	8	3	46(2t)	3	40-81
B92-173	22t	10	37	43t-46(2t)	8	1-192
B92-181	22t	9	63	44-46(2t)	7	54-124
B92-198	22t	15	21	45-46(2t)	8	33-111
B92-211	22t	7	19	44-46(2t)	7	43-156
B92-226	22t	11	4	46(2t)	3	42-198
B92-141	23	10	13	44-46	8	22-117
B92-149	23	7	4	45t-46	3	25-66
B92-155	23	6	10	45-47	6	2-42
B92-182	23	9	23	43-46	7	17-83
B92-188	23	7	3	44-46	6	1-39
B92-221	23	6	19	46	7	49-150
B92-245	23	8	39	45-47	10	18-151
B92-146	24	7	7	44-47	4	8-76
B92-243	24	6	17	46	12	16-138

[a]C-0: Colchicine treated polyhaploid plant
[b]C-1: Selfed offspring of C-0 plants
[c]t: telosome
[d]Thirty one plants were chimeric for chromosome constitution as indicated

Table 3. Mean meiotic metaphase I chromosome association and fertility of one disomic addition DH line for each homoeologous group.

Homoeologous group	Addition line (ID no.)	Metaphase I association (range)			No. seeds/ plant
		Univalents	Rings bivalents	Rod bivalents	
1	B92-6698	0.3	20.0 (17-22)	1.85 (0-5)	112
3	B92-6871	0	21.1 (20-22)	0.9 (0.2)	98
5	B92-6899	0	20.1 (18-22)	1.9 (0-4)	108
7	B92-6840	0	20.6 (18-22)	1.4 (0-4)	165

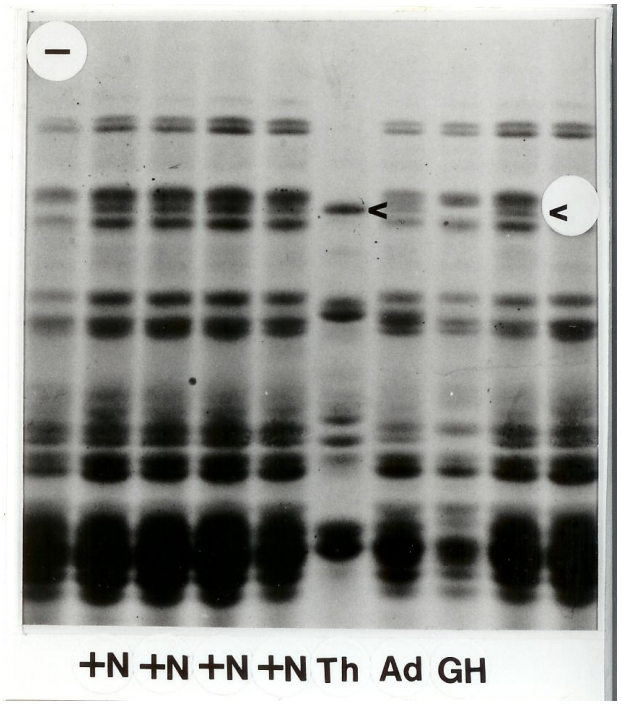

Fig. 1. High molecular-weight subunits of glutenin on SDS-PAGE using 8.5% polyacrylamide gels showing the band that serves as a marker for detecting the homoeologous group 1 disomic addition of *Thinopyrum elongatum* to wheat. From left to right: +N = 4 addition line tracks (2 each of 92-6698 and 6704), *Th* = *Th. elongatum* with marker band, Ad =*Th. elongatum / T. aestivum* cv. 'Goshawk' amphiploid with the marker band, and 'GH' = *T. aestivum* cv. 'Goshawk' missing the marker band. Marker band arrowed

The homoeologous group 7 chromosomes of wheat carry the α-AMY-2 genes (Gale *et al*., 1983). *Th. elongatum* possesses one band in the α-AMY-2 region, not present in the wheat cultivar 'GH' (Fig. 4). The *Th. elongatum*/'GH' amphiploid expressed both the wheat and *Th. elongatum* isozymes (Fig. 4) as did the disomic chromosome addition lines, B92-6840 and 6854. The *Th. elongatum* band therefore serves as a diagnostic for characterizing its chromosome pair in the addition lines with homoeology to group 7 wheat chromosomes.

AD, GOT and SOD isozymes did not provide positive information since no polymorphism existed for their patterns between wheat and *Th. elongatum* under our analytical conditions. Other isozyme marker systems were not utilized.

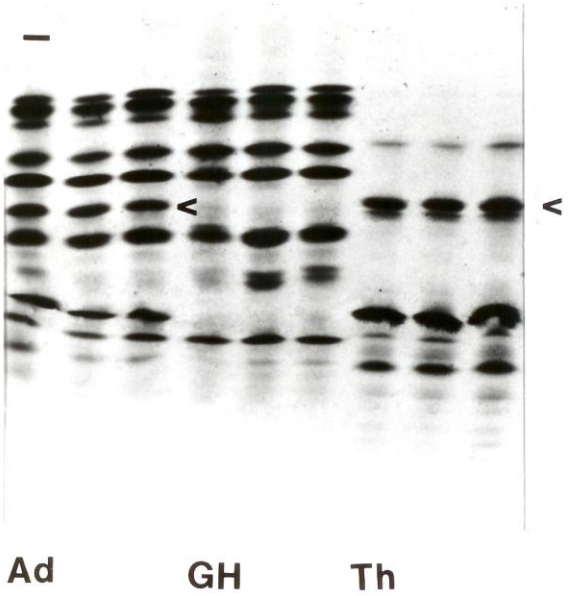

Fig. 2. Separation of EST-5 isozymes on IEF (pH 3.5-9.5) polyacrylamide gels showing from left to right: Ad = *Th. elongatum* / *T. aestivum* cv. 'Goshawk' ('GH') amphiploid with the arrowed marker band in each of the three tracks, 'GH' = 'Goshawk' wheat devoid of the marker in its three tracks, Th = *Thinopyrum elongatum* tracks with the arrowed marker band.

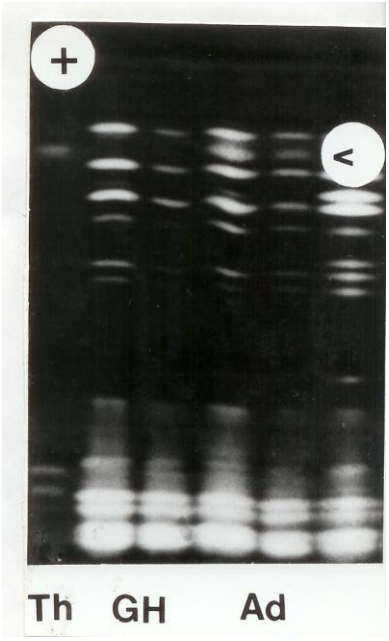

Fig. 3. β-Amylase isozymes separated on Native-PAGE (8.5%) gels. The diagnostic marker band of *Thinopyrum elongatum* (Th) is present in both tracks of its amphiploid (Ad) with *T. aestivum* cv. 'Goshawk' ('GH') and is arrowed. It does not appear in the 'Goshawk' ('GH') wheat cultivar tracks.

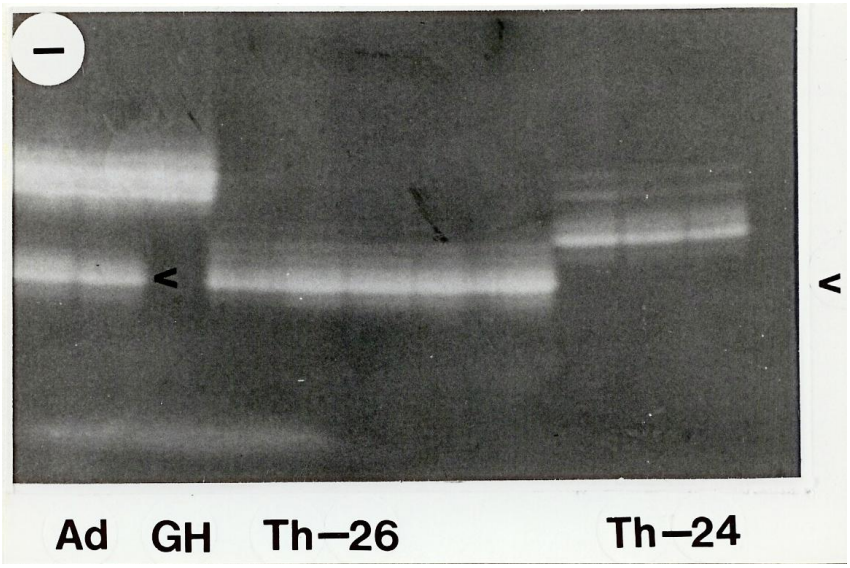

Fig. 4. α-Amylase 2 separation on IEF (pH 4.0-6.5) polyacrylamide gels. From left to right: 2 tracks of the *Thinopyrum elongatum / Triticum aestivum* cv 'Goshawk' ('GH') amphiploid (Ad), 'Goshawk' ('GH'), five tracks of the *Th. elongatum* accession 26 (Th-26) with its positive marker location specified, and three tracks of the *Th. elongatum* accession 24 (Th-24) that does not contribute to the diagnostic Ad band site.

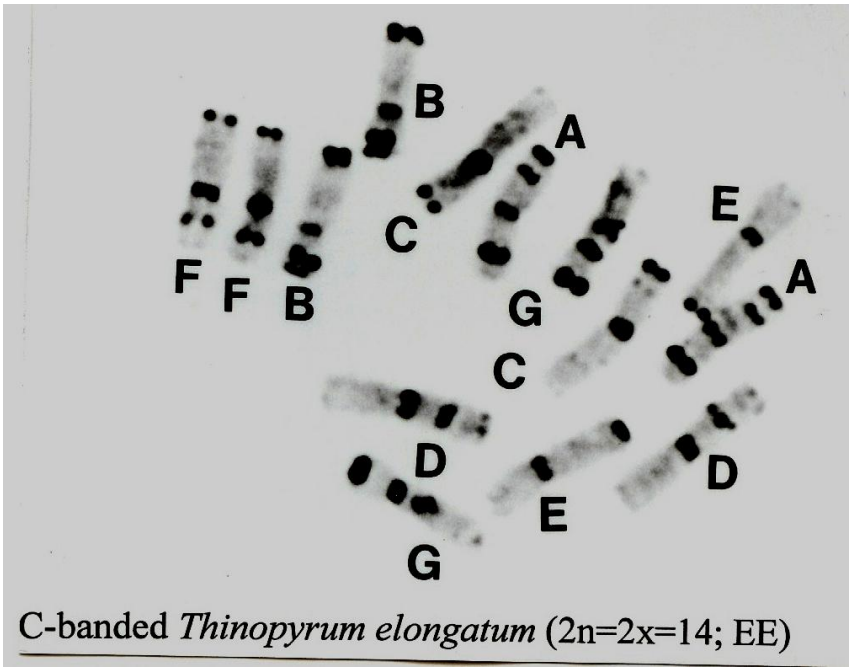

Fig. 5. A Giemsa C - banded root-tip mitotic cell of *Thinopyrum elongatum* (2n=2x=14, EE) with homologous chromosomes identified as A to G.

The four addition lines have associated a single marker related with the corresponding wheat homoeologous group. This approach makes two assumptions:

(1) There is perfect correspondence between the wheat homoeologous group and the added *Th. elongatum* chromosome pair and

(2) That the alien chromosomes do not become structurally re-arranged during the production of the disomic set. Such re-arrangements cannot be detected by single marker analyses or by *in situ* hybridization, throwing emphasis upon use of Giemsa differential staining for authenticating the respective addition lines.

The C-banding pattern of *Th. elongatum* enables identification of each of the 7 chromosomes (Fig. 5). The banding details in our investigation generally conform to those published earlier (Endo & Gill, 1984) with maintenance of similar alphabetical designations. Absent so far are positive biochemical markers for 3 homoeologous groups. Banding distinctiveness is present for each of the seven *Th. elongatum* chromosome pairs and would enable characterization of the three missing groups, after which other isozyme markers could focus on these new addition sets if necessary for association. This aspect is currently under study.

Fluorescent *In situ* hybridization: In backcrossed (BC) intergeneric hybrid derivatives, structural chromosomal modifications are frequent, especially when the self-sterile F_1 is the source for obtaining BC progeny (Jewell & Mujeeb-Kazi, 1982). This variability would be reduced if an amphiploid is first produced, and then utilized for BC progeny leading to production of disomic addition lines. Nevertheless, it is essential to check the structural validity of an addition line in order to identify the presence of only two paired alien chromosomes in a disomic addition. For this inference to be made, we used fluorescent *in situ* hybridization (FISH) diagnostic analyses on two samples within each biochemically ascertained group, and detected in each sample only one pair of alien *Th. elongatum* chromosomes (Fig. 6). None of the 42 wheat chromosomes gave visible evidence of alien introgression detectable by FISH analyses (Islam-Faridi & Mujeeb-Kazi, 1995). Thus the nine DH disomic lines corresponding to four homoeologous groups, will serve as genetic stocks. The integrity of the four addition lines associated with a single biochemical marker needs validation by banding information and will be extended to further complete the set of *Th. elongatum* disomic addition lines by isolating the remaining three chromosome pairs.

Conclusions

The new *Th. elongatum* / *T. aestivum* intergeneric hybrid has utilized a high yielding wheat cultivar in the cross and hence the derived amphiploid, backcross progenies and some disomic addition lines exhibit good agronomic plant phenology. The addition line production strategy based upon crossing of the individual single to triple monosomic addition derivatives has used the wheat/maize protocol for haploid generation leading to homozygosity upon induced doubling. Use of biochemical markers have led to partial detection of disomic additions associated with four wheat homoeologous groups. Flourescent *in situ* hybridization has authenticated that in each such mono-marker addition the entire chromosome in each of the four cases is of *Th. elongatum*. Conclusive validation of the alien additions through Giemsa C-banding has identified four alien chromosomes. Efforts are underway to isolate the remaining three chromosome additions for completing the set of 7 possible and conducting subsequent trait analysis plus targeting genetic manipulation aspects. The results of the C-banding validation have been deferred for another communication.

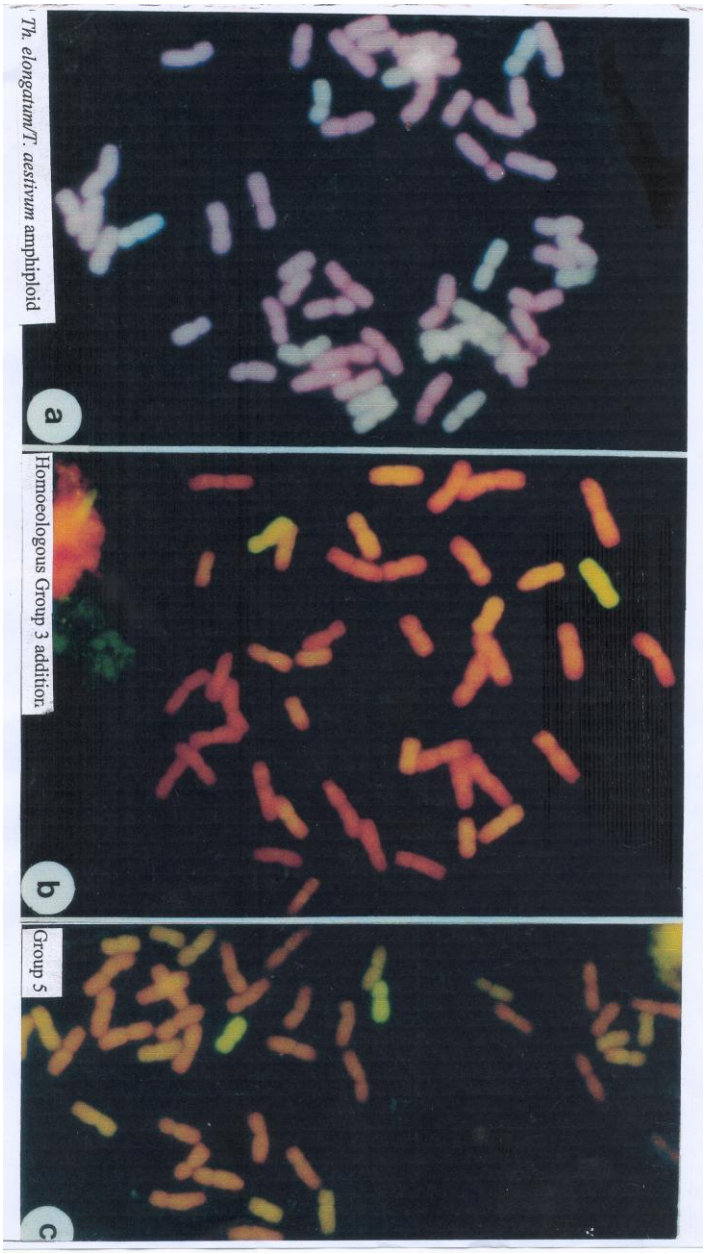

Fig. 6. Fluorescence micrograph of an amphiploid of *Thinopyrum elongatum / Triticum aestivum* amphiploid (2n=8x=56, AABBDDEE) and two disomic addition lines. Wheat chromosomes purple in color were blocked with sheared unlabeled *T. aestivum* DNA. Biotin labeled *Th. elongatum* DNA used as a probe enabled detection of *Th. elongatum* chromosomes (whitish in color) with Zeiss filter 02 (a) or (yellowish in color) filter 09 (b and c). Shown are in: a. amphiploid; b, c, disomic additions (2n=6x=42+2) homoeologous to groups 3 and 5 respectively.

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