

PRODUCTION AND CYTOGENETICS OF INTERGENERIC HYBRIDS OF *TRITICUM AESTIVUM* WITH *AEGILOPS* *VARIABILIS* AND *AE. VAVILOVI*

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Abstract

Intergeneric hybrids between *Triticum aestivum* L., and Karnal bunt (*Neovossia indica*) resistant accessions of two *Aegilops* species were produced in different frequencies. *Aegilops variabilis* was hybridized with Genaro 81, Mrl-Buc"S", Buc-Pvn"S" and Chinese Spring at frequencies of 5.0 to 29.0%. The endosperm formation was normal, mature seeds were slightly shrivelled but germinated normally. The *T. aestivum* x *Ae. variabilis* hybrids, genomically represented as ABDUS, were cytologically normal, with $2n=5x=35$ chromosomes. The mean meiotic associations at metaphase-I were categorized as low pairing and expressed as 33.96 univalents + 0.52 rod bivalents over 56 meiocytes analyzed with a 0.52 mean chiasmata frequency. Hybrid production frequencies of *T. aestivum* cultivars Sind 81, ZA-77, Buc"S", Chinese Spring with *Ae. vavilovi* ranged between 1.7 to 31.3%. Meiotic associations were 33.31 univalents + 0.19 ring bivalents + 2.94 rod bivalents + 0.81 trivalents over 32 meiocytes characteristic of $2n=6x=42$ chromosomes that genomically were ABDDMS with a 4.94 mean chiasmata frequency. The germplasm offers avenues of alien addition line development or *Ph locus* manipulation; events currently lacking for applied objective exploitation of these two species.

Introduction

Intergeneric hybridization amongst the Triticeae has the potential of providing unique alien genetic variability for resistances or tolerances to biotic and abiotic conditions. The critical initial phase in such gene acquisition is hybrid production accompanied by cytogenetic observations that determine the subsequent breeding strategies where hybridization goals may involve practical agricultural objectives.

Aegilops species have a wealth of desired resistances for disease and stress situations, are relatively easy to hybridize with *Triticum* species and accordingly a preferred choice for effecting alien gene transfers. Presumably in the intergeneric category *Secale cereale* may be the top priority for *Triticum* spp. x alien genera hybridization, with others like *Agropyron*, *Elymus*, *Haynaldia*, *Hordeum* etc., being considerably removed because of several complexities of hybrid production, genomic remoteness and gene transfer constraints (Mujeeb-Kazi & Asiedu, 1989).

Several *Aegilops* species and accessions of each species were screened for Karnal bunt resistance (Warham *et al.*, 1986). Accessions were identified with infection

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ranging from 0 to 60 percent over a severity scale of 0 (zero infection) to 5 (completely bunted grain). Two resistant *Aegilops* species (*Ae. variabilis* and *Ae. vavilovi*) from the above screening of Warham *et al.*, (1986) were incorporated for hybridization with *T. aestivum*. This paper reports on hybrid production and cytogenetics of *T. aestivum* x *Ae. variabilis* plus *T. aestivum* x *Ae. vavilovi* hybrids.

Materials and Methods

Germplasm: Seeds of *Aegilops variabilis* (13E) and *Ae. vavilovi* (41A) were obtained from CIMMYT, Mexico. The accession numbers are of CIMMYT and the alphabets of PBI (now AFRC-IPSR). These are consistent with the number and origin categories as listed in Table 1 of Warham *et al.*, (1986). The seeds were germinated in jiffy peat pots and vernalized for 10 weeks at 4°C in a refrigerator. The slightly etiolated seedlings were conditioned for 7 days, transplanted to clay pots with a soil: leaf manure mix and kept in a screenhouse. Several *Triticum aestivum* cultivars were also grown in similar pots and maintained in the screenhouse. Staggered *T. aestivum* plantings were made to ensure a niche for hybridization between the wheat cultivars and the *Aegilops* accessions. A similar planting was maintained in CIMMYT, Mexico, under controlled greenhouse conditions of 22°C/12°C (day/night) with 15 hours natural light and approximately 55% relative humidity.

Hybrid production: *T. aestivum* spikes were emasculated and pollinated by pollen from the *Aegilops* species according to conventional wheat breeding procedures. In Pakistan, potential hybrid seed development on the wheat spikes was observed regularly from 10 days post-pollination to determine whether embryo culture would be necessary; which was not. Mature but slightly shrivelled seeds obtained from Pakistan were germinated in Petri dishes in CIMMYT, Mexico, transplanted to a steam sterilized 2:1:1 (soil: peat: sand) planting mix in plastic pots and maintained in a naturally lighted glasshouse at 22°C day/12°C night temperatures with approximately 55% relative humidity. Despite a well formed endosperm in Mexico, all embryos from seeds set on crossed spikes were cultured in MS (Murashige & Skoog, 1962) media to speed up the hybrid population growth.

Cytology: Root-tips were collected from seedlings in Petri dishes or from plants in pots, pretreated for 3.5 hours with colchicine + 8-hydroxy-quinoline + dimethylsulfoxide, fixed in 2 percent aceto-orcein and stored at 4°C in the refrigerator according to the procedure of Mujeeb-Kazi & Miranda (1985). Meristem cells were squashed in 45% acetic acid and preparations made permanent. These were photographed on a Zeiss photo-microscope using a Kodak high contrast 2415 black and white film with a green base plus 53 yellow barrier filter at 18 Din. Spikes for meiotic analyses were fixed for 48 hours in a 6:3:1 (ethanol: chloroform: acetic acid) fixative, then transferred to 70% ethanol and stored in a freezer until use. Excised anthers at metaphase-I (MI) were stained with alcoholic carmine and analyzed for mean chromosomal pairing relationships over at least 30 meiocytes per hybrid plant. Preserved cytological samples transferred from Mexico to Pakistan were of root-tips fixed in 2% aceto-orcein plus two and a half anthers in alcoholic carmine. The metaphase-I meiotic stage was predetermined on a 1/2 anther temporary preparation. Spike morphology was documented through photography.

Table 1. *Triticum aestivum* x *Aegilops variabilis* (13E) and *T. aestivum* x *Ae. vavilovi* (41A) hybrid production frequencies at two locations (Pakistan and Mexico).

Alien Species	Wheat cultivar	Florets Crossed	Total Seeds	Seeds with Embryos	Hybrid Plants	Hybrid chromosome Number and Genomic status
<i>Ae. variabilis</i>	Chinese Spring	248	72	72	72	2n=5x=35, ABDUS
	Genaro 81	40	2	2	2	2n=5x=35, ABDUS
	Buc"S"/Pvn"S"*	180	44	43	43	2n=5x=35, ABDUS
	Mr1"S"/Buc"S"*	108	25	23	23	2n=5x=35, ABDUS
<i>Ae. vavilovi</i>	Chinese Spring*	96	30	30	30	2n=6x=42, ABDDMS
	Buc"S"	54	11	11	11	2n=6x=42, ABDDMS
	Sind 8 ₁ **	60	1	1	1	2n=6x=42, ABDDMS
	ZA 77**	80	4	4	4	2n=6x=42, ABDDMS

*Crosses done under controlled conditions in Mexico with embryo rescue 16 days after pollination.

**Crosses done in Karachi, Pakistan, under field conditions.

Results and Discussion

Triticum aestivum x alien species crossability is markedly influenced by the *T. aestivum* crossability genes located on chromosomes 5A (Kr2), 5B (Kr1), 5D (Kr3) of the homoeologous group 5 (Falk & Kasha, 1981; Fedak & Jui, 1982; Riley & Chapman, 1967). Though the genetic basis is derived through inferences drawn from *T. aestivum* x *Secale cereale* crosses, the Kr genetic control has been extrapolated to other wheat x alien crosses (Mujeeb-Kazi *et al.*, 1987, 1989; Sharma & Gill, 1983 a,b). An exception to this extrapolation; that suggests that wheats like Chinese Spring with recessive Kr genes are highly crossable (Table 1); are the findings of Laurie & Bennett (1987) where crossability of wheat x maize was reportedly independent of the Kr gene status.

In intergeneric hybrids of *T. aestivum* L. cv. Chinese Spring x *Thinopyrum* (*Th.*) *intermedium* the hybrid seeds had well developed endosperm, consequently embryo rescue was not essential (Mujeeb-Kazi *et al.*, 1989). The alien species was also hybridized with some other commercial wheat varieties that were not homozygous recessive for the Kr genes. This prompts us to infer that where hybrid seed is produced in a high frequency with Chinese Spring and embryo rescue is not essential the alien genera may be hybridized more readily with commercial wheat cultivars. This trend was apparent in the crossability data of this study involving *T. aestivum* x *Ae. variabilis* and *T. aestivum* x *Ae. vavilovi* (Table 1) and is seemingly consistent with earlier reports of Mujeeb-Kazi *et al.*, (1987, 1989) for F₂ hybrid production of Chinese Spring plus some commercial *T. aestivum* cultivars with the *Th. intermedium* group of sub-species i.e., *acutum*, *pulcherrimum*, *trichophorum* and *vavilovense*.

In general, the hybrid production frequencies of this study have been lower than anticipated for what is seemingly a relatively simple cross combination. One must

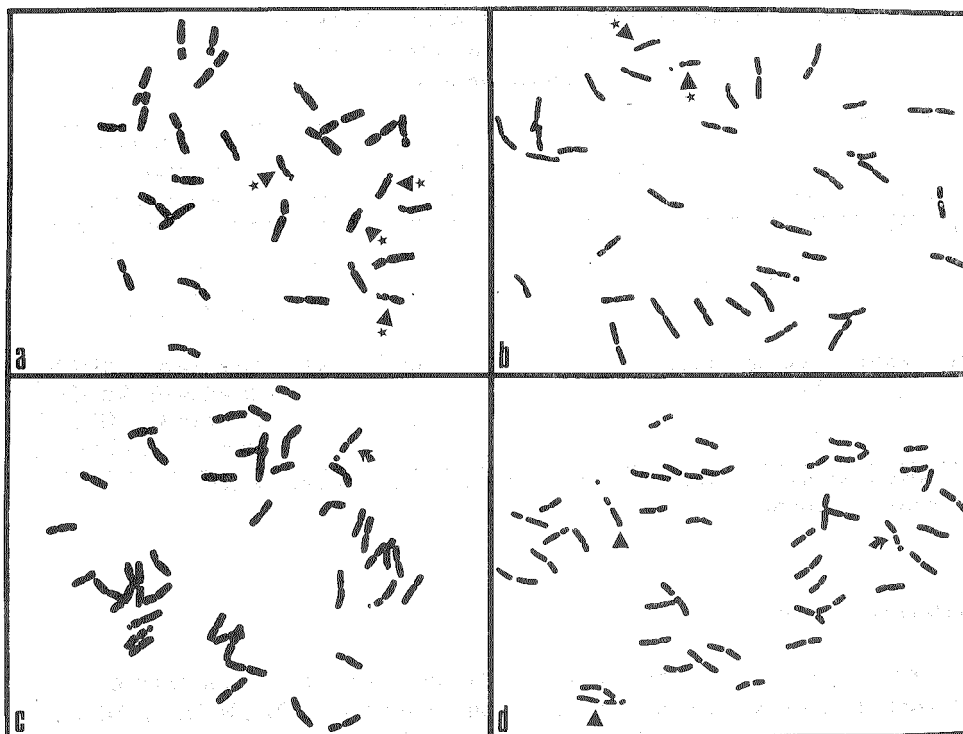


Fig.1. Metaphase somatic cytology of *Aegilops variabilis* ($2n=4x=28$), *Ae. vavilovi* ($2n=6x=42$) and their F_1 hybrids with *Triticum aestivum* ($2n=6x=42$):

(a) *Ae. variabilis* ($2n=4x=28$) chromosomes with 2 diagnostic chromosome marker pairs arrowed. (b) *T. aestivum* x *Ae. variabilis* F_1 hybrid with 35 chromosomes (See single dosage of marker chromosomes shown in a identified together with the 1B, 6B satellited *T. aestivum* chromosomes). (c) *Ae. vavilovi* ($2n=6x=42$) chromosomes with diagnostic chromosomes arrowed. (d) *T. aestivum* x *Ae. vavilovi* F_1 hybrid with 42 chromosomes (See single 1B and 6B of *T. aestivum* and single *Ae. vavilovi* chromosome markers).

recognize that for this study the emphasis was for (i) obtaining F_1 hybrids with Chinese Spring, (ii) attempt to have these advanced by crossing them with elite commercial *T. aestivum* cultivars (modified BCI), and (iii) have enough plants to produce advanced BC derivatives. We are cognizant that meticulous crossing procedures, environmental manipulations and culture variations do exist that could augment the embryo recovery frequencies if such would have been our emphasis. The above inference gains endorsement from the recent observations of Riera-Lizarazu & Mujeeb-Kazi (1990) where remarkably high wheat polyhaploid frequencies have been obtained by crossing wheat x *Zea mays*. Their modified procedure using detached spikes integrated with the post-pollination use of 2,4-Dichloro-phenoxy acetic acid has been a significant improvement over frequencies obtained earlier by Laurie & Bennett (1988).

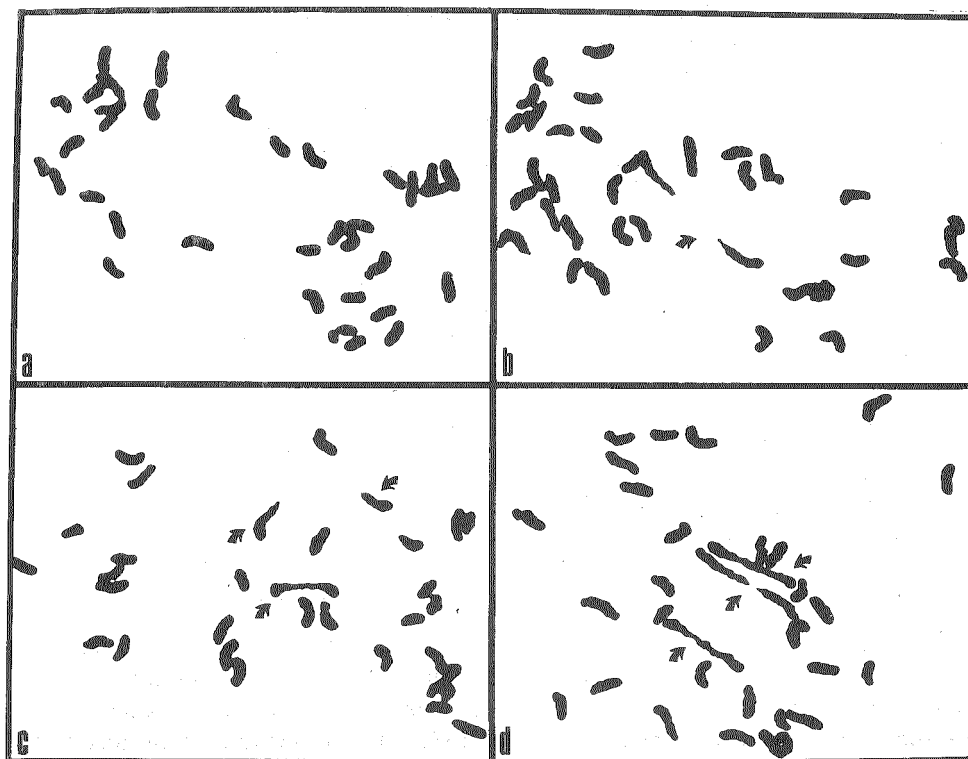


Fig.2. Metaphase-I meiotic chromosomal associations in an F_1 hybrid of *Triticum aestivum* L. cv. Chinese Spring (CS) x *Aegilops variabilis* with 35 chromosomes.

(a) 35 univalents. (b) 33 univalents + 1 rod bivalent (arrowed). (c) 31 univalents + 2 rod bivalents (arrowed). (d) 29 univalents + 3 rod bivalents (arrowed).

Hybrid production frequencies over all *T. aestivum* cultivars x *Ae. variabilis* ranged between 5% (Genaro 81) to 31% (Chinese Spring), with hybrid somatic chromosome numbers and constitutions characteristic of the hybrid combinations, that is:

Hybrids of *T. aestivum* cv. Chinese Spring, Buc"S"/Pvn"S", Mr1"S"/Buc"S" and Genaro 81 with *Ae. variabilis*, possessed 35 chromosomes constituted by 21 chromosomes of *T. aestivum* + 14 of *Ae. variabilis* (Figs. 1a & 1b) genomically represented as ABDUS. The mean meiotic associations indicate a range of chromosomal relationships at metaphase-I characteristic of low pairing predominantly as a consequence of the 5B *Ph locus*. The 35 chromosomes were meiotically associated as 34.05 univalents + 0.36 rod bivalents, (Figs. 2a to d, & Table 2).

The role of the 5B system in enhancing F_1 chromosome pairing and effecting gene transfers has been actively considered (Darvey, 1984; Mujeeb-Kazi & Asiedu, 1989, 1990; Mujeeb-Kazi & Rodriguez, 1981; Mujeeb-Kazi *et al.*, 1987, 1989; Sharma

Table 2. Mean metaphase-I meiotic chromosomal associations in an F₁ hybrid of *Triticum aestivum* L. cv. Chinese Spring x *Aegilops variabilis* (2n = 5x = 35, ABDUS).

Number of Cells	Metaphase-I Chromosomal Associations				% Cells	Chiasmata/Cell
	Rings		Rods			
	I	II	II	III		
1	29	0	3	0	1.8	
6	31	0	2	0	10.7	
14	33	0	1	0	25.0	
35	35	0	0	0	62.5	
Total:	56	1902	0	29	0	100
Mean	33.96		0.52			0.52
Range	29-35		0-3			

& Gill, 1983a,b). There does however, exist a constraint in advancing a high pairing F₁ hybrid derived from the chromosome 5B manipulation system to yield backcross derivatives (Sharma & Gill, 1986). This constraint has recently been successfully overcome (Ter-Kuile *et al.*, 1987; Rosas *et al.*, 1988). It is consequently envisaged that chromosome 5B mediated F₁ recombination manipulation may prove to be highly significant in advancing hybrid progeny for agricultural practicality; in this case for transferring Karnal bunt resistance from *Ae. variabilis* to *T. aestivum*. Recent developments however, have shed light (Gill, 1990) that the visually high recombination based upon *Ph1b* manipulation may not facilitate genetic cross-overs of traits when the physical location of genes is considered. Molecular evidence is supportive that only a restricted distal portion of the chromosomes gets involved in metaphase-I associations characteristic of the *ph1b* locus. Consequently irradiation approaches or callus culture incorporation either with F₁ hybrids at their initial stages or at the ultimate phase after desirable alien disomic addition lines have been produced are apparently in the offing.

Low pairing hybrids are generally ideal sources of alien addition line production. Pairing in a Chinese Spring x *Ae. variabilis* F₁ hybrid was reportedly less than one chiasmata per cell (Driscoll & Quinn, 1968). Later, the findings of Jewell & Driscoll (1983); substantiating the above observation; indicated a lack of homology between the N-banded B genome chromosomes of *T. aestivum* and those of *Ae. variabilis*. This study led to the production of nine *Ae. variabilis* addition lines that unfortunately were not conserved adequately and are thus unavailable for exploitation in wheat improvement. The current attempt of reproducing hybrids of *T. aestivum* x a Karnal bunt resistant accession of *Ae. variabilis* is definitely warranted for an objective of increasing concern in Pakistan and international wheat agriculture.

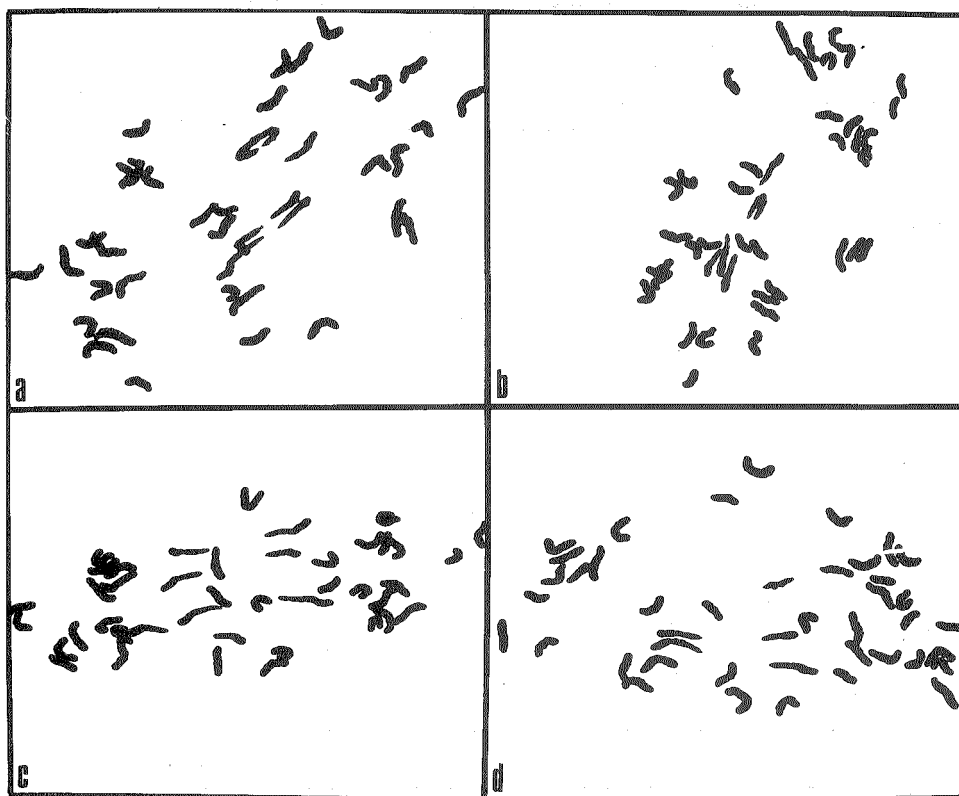


Fig.3. Meiotic metaphase-I chromosome pairing associations in an F_1 hybrid of *Triticum aestivum* L. cv. Chinese Spring (CS) x *Aegilops vavilovi* with 42 chromosomes.

(a) 33 univalents + 1 ring bivalent + 2 rods bivalents + 1 stretched trivalent. (b) 37 univalents + 1 rod bivalent + 1 trivalent. (c) 34 univalents + 4 rod bivalents. (d) 38 univalents + 2 rod bivalents.

Intergeneric hybrids of *T. aestivum* cv. Chinese Spring, Buc"S", Sind 81 and ZA-77 with *Ae. vavilovi* were produced in frequencies of 31.3, 20.4, 1.7 and 5.0% respectively (Table 1). The hybrids possessed 42 chromosomes (Figs. 1c & 1d) that were meiotically associated according to the genetic control characteristics of the *Ph* locus i.e., low pairing; 33.31 univalents + 0.19 ring bivalents + 2.94 rod bivalents + 0.81 trivalents with a 4.938 mean chiasmata frequency (Figs. 3a to d & Table 3).

The meiotic relationships for *T. aestivum* x *Ae. variabilis* (Table 2) and *T. aestivum* x *Ae. vavilovi* (Table 3) indicate ample choice of breeding methodologies for incorporating procedures pertaining to introgression of alien genes for wheat improvement. The low pairing hybrids would facilitate germplasm procedures for addition line development, though induced pairing between homoeologous genomes will be the natural cytogenetic means of genetic transfers (Vahidy *et al.*, 1991) via recombination. The *ph1b* mediated enhanced pairing or use of the 5B based genetic stocks is an ideal means of effecting alien transfers and such progenies also have the potential of being utilized in molecular diagnostic processes of which restriction fragment

Table 3. Mean metaphase-I meiotic chromosomal associations in an F₁ hybrid of *Triticum aestivum* L. cv. Chinese Spring x *Aegilops vavilovi* (2n=6x=42, ABDDMS).

Number of Cells	Metaphase-I Chromosomal Associations				% Cells	Chiasmata/Cell
	Rings		Rods			
	I	II	II	III		
1	28	0	4	2	3.13	
1	30	0	3	2	3.13	
2	30	0	6	0	6.25	
4	31	1	3	1	12.50	
3	31	0	4	1	9.38	
1	32	0	2	2	3.13	
1	33	1	2	1	3.13	
3	33	0	3	1	9.38	
1	34	1	3	0	3.13	
3	34	0	4	0	9.38	
6	35	0	2	1	18.75	
2	36	0	3	0	6.25	
3	37	0	1	1	9.38	
1	38	0	2	0	3.13	
Total:	32	1066	6	94	26	100
Mean		33.31	0.19	2.94	0.81	4.938
Range		28-38	0-1	1-6	0-2	

length polymorphisms (RFLP) may be one powerful example. This approach could be applied swiftly to suit breeding programmes in not too distant a future.

In intergeneric hybrids a constraint is related with alien genetic expressivity. For a hybrid to be of agricultural value an initial selection sieve would be its morphology where a modified F₁ phenotype as compared to the phenotype of the female *T. aestivum* parent is indication of alien genetic epistasis and considered practically desirable (Mujeeb-Kazi & Asiedu, 1990). Spikes of *T. aestivum* x *Ae. variabilis* (Fig.4) possessed a modified co-dominant phenotype supporting the concept of alien genetic expression in a *T. aestivum* background. Similar phenotypic expression also existed for the *T. aestivum*/*Ae. vavilovi* F₁ hybrid. Advancing such hybrids may consequently have merit for agricultural practicality; an aspect that is to be pursued further when the F₁ hybrids are advanced by back- or topcrossing to *T. aestivum* cultivar/s.



Fig.4. Spike morphology (left to right) of *Triticum aestivum* cv. Chinese Spring (CS), CS x *Aegilops variabilis*, F₁ hybrid and *Ae. variabilis*.

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