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GENETIC DIVERSITY OF *AEGILOPS VARIABILIS* (2n=4x=28; UUSS) FOR WHEAT IMPROVEMENT: MORPHO-CYTOGENETIC CHARACTERIZATION OF SOME DERIVED AMPHIPLOIDS AND THEIR PRACTICAL SIGNIFICANCE

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Abstract

The tertiary gene tetraploid *Aegilops variabilis* (2n = 4x = 28; UUSS) is an alien germplasm resource that provides much needed genetic diversity for resistance to *Cochliobolus sativus* (spot blotch) and *Tilletia indica (*Karnal bunt). This resource has been hybridized with several durum and bread wheat cultivars yielding cytologically normal F_1 hybrids (2n = 4x = 28, ABUS or 2n = 5x = 35, ABDUS) from which fertile amphiploid progenies of 56 (2n=8x=56, AABBUUSS) and 70 chromosomes (2n=10x=70, AABBDDUUSS) were derived. The morphology and cytogenetics of these cross combinations plus their amphiploids, screening data for spot blotch and karnal bunt response under field conditions is reported to elucidate some probable strategies that would permit genetic transfers from *Ae. variabilis* into the recipient durum and bread wheat germplasms.

Introduction

Tertiary gene pool resources, even though complex to utilize are a potent means of enriching wheat germplasm (Mujeeb-Kazi, 2006). *Aegilops variabilis* (2n=4x=28, UUSS) possesses this diversity for providing resistances to at least two important biotic stresses that limit wheat production globally i.e., spot blotch of wheat (*Triticum aestivum* L.) caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur (syn.: *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Helminthosporium sativum* Pammel, C.M. King & Bakke) and karnal bunt caused by *Tilletia indica* (Mitra). Spot blotch is an important pathogen that limits production in many nontraditional hot, humid wheat producing areas of Asia, Africa, and South America. *C. sativus* can attack seedlings, roots, leaves, nodes, spikes, and grains during various stages of plant development (Villareal *et al*., 1995). Yield loss estimates due to spot blotch on wheat vary widely. Losses of 85% were reported from Zambia and 40% from field trials in the Philippines. In addition, yield losses with the highly susceptible cultivar 'Mitacore' in an experiment conducted at Londrina, Brazil ranged from 79 to 87%, and the disease severely affected grain quality. De Milliano & Zadoks (1985) found a 38% yield loss using African wheat cultivars in growth-chamber studies in the Netherlands. Because of the importance of this disease, chemical control is applied in order to obtain crop production stability in many parts of the world. Emphasis is also being given to an integrated pest management approach utilizing resistant cultivars, healthy seed, cultural practices and chemical sprays. Though breeding for resistance is a high priority, it is hampered by scarcity of adequate resistance within *T. aestivum*. Sources of resistance to *C. sativus* in species other than *T. aestivum* (i.e., alien gene pools) are of special interest in breeding programs. We at the International Wheat and Maize Improvement Center (CIMMYT) and NARC (National Agricultural Research Center) have been making some effort to incorporate and exploit

alien resistance genes in a wheat background using diverse sources of genetic variation in order to pyramid genes (Mujeeb-Kazi, 2003). In Pakistan, karnal bunt is emerging as a production concern factor and needs urgent addressing as we advance towards wheat production self-sufficiency envisioning export markets. Since *Ae. variabilis* carries resistance to both these biotic stresses, international and national objectives of *C. sativus* and karnal bunt can be readily addressed by exploiting this species resource via intergeneric hybridization protocols.

The objectives of this presentation are to elucidate the current status of *C. sativus* resistant germplasm by exploiting *Ae. variabilis* (2n = 4x = 28, UUSS) as a resistance donor and elucidate the practical potential of genetic introgression into durum / bread wheats through some gene transfer strategies. Similar strategies can also address the karnal bunt facet for our national goals.

Provided here are the details of F_1 hybridization, derivation of the amphiploid genetic stocks, their cytogenetics and disease screening for *C. sativus* plus karnal bunt. Also presented is the strategy of exploiting the intergeneric tertiary gene pool source *Ae. variabilis* via cytogenetic manipulation to exemplify the use of the amphiploid stocks produced (Mujeeb-Kazi *et al*., 2007).

Materials and Methods

Germplasm: The durum and bread wheat cultivars used in hybridization originated from germplasm banks of CIMMYT, Mexico and NARC, Pakistan. Seed of the cultivars 'Asakasekomugi' and 'Fukohokomugi' were provided by Dr. George Fedak of Agriculture Canada. *Aegilops variabilis* accession (13E) was obtained from Dr. Colin Law (then PBI, Cambridge, UK) and *Triticum aestivum* cv. Chinese Spring, its monosomic 5B plus *ph1b* genetic stocks from late Dr. E.R. Sears (Univ. of Missouri, Columbia, Missouri, USA).

Hybridization, embryo rescue and plantlet regeneration: *Ae. variabilis* seedlings were vernalized for 6 weeks at 8°C with 8h of light. Transplanting was staggered three times every 5 days over 2 weeks. Both durum and bread wheat cultivars were planted over 3 dates at 10 day intervals in order to niche with *Ae. variabilis* pollen availability. The germplasm was maintained in pots under greenhouse conditions of 24/14°C, 14h natural light and approximately 60% RH. Emasculation, pollination, embryo rescue and regeneration procedures were similar to those reported earlier (Mujeeb-Kazi *et al*., 1987).

Cytology of amphiploids and their maintenance: The hybrid plants possessing 2n=4x=28, ABUS or 2n=5x=35, ABDUS chromosomes upon treatment with 0.1% colchicine + 2.0% dimethyl-sulfoxide for 6 hours via aerated root-treatment became the source of the fertile C-o amphiploids (2n=8x=56 or 2n=10x=70). All C-o individual amphiploid combinations were cytologically validated (Mujeeb-Kazi *et al*., 1994), and their seed increased by selfing under glassine bags until adequate seed quantities became available by C-6 for biotic stress screening in hill plots.

***C. sativus* screening:** All durum and bread wheat/*Ae. variabilis* amphiploid combinations, their wheat parents, and two (susceptible 'Ciano 79' and resistant 'Mayoor') bread wheat cultivars (Mujeeb-Kazi *et al*., 1996) were planted in hill plots in Poza Rica, Mexico for *C. sativus* screening. Disease evaluations were based upon foliar

infestation and grain blemish at maturity. A double digit scale measured foliar infestation, where the first digit equated to the height of infection and the second digit with the infection severity. Scale gradations were 1 to 9. For the height of infection a score of 5 was for plants with infection up to the plant center and for a score of 9 infection had spread to the flag leaf. A disease severity score of 1 was for infected leaves exhibiting low disease symptoms, whereas a 9 score reflected total leaf destruction. Grain infection at maturity was scored on a 1 to 5 scale with 1 being low and 5 being a high seed blemish at embryo points.

***T. indica* screening**

a. Inoculum preparation: Teliospores from various locations in the Yaqui Valley, Sonora, Mexico were used to ensure a genetically heterogenous composite of the fungus population. To isolate teliospores, infected kernels were shaken in a water-tween-20 solution for 15 seconds, centrifuged at 3,000 rpm, and sieved using a 60 micron mesh to remove the kernel residue. Thereafter, they were surface-sterilized with 0.5% sodium hypochlorite while centrifuging for about 2 min, rinsed in sterile distilled water, plated on 1.5% water agar and incubated at room temperature. After 5-8 days, germinating teliospores were transferred to potato-dextrose agar (PDA) to which sterile water was added. Nine days later, fungal colonies were scraped and further inoculated onto additional PDA plates. After 8-10 days, the PDA fungus colony was cut into small pieces and placed on the lids of sterile, glass Petri plates. This process enhances the release of many secondary sporidia from the fungal colonies. A small amount of sterile water was added to the bottom of each. Then the allantoid sporidia were counted every 24h using a haemocytometer, and the spore concentration adjusted to 10,000/ml.

b. Inoculation technique and harvest: Ten tillers taken at random from each entry were inoculated during the boot stage, (stages 48-49 according to Zadoks *et al*., 1974), by injecting 1 ml/tiller of the sporidial suspension with a hypodermic syringe. Tillers were tagged with color coding tape to indicate the date of inoculation. There were between 2 to 3 dates of planting during each cycle that got tested. At maturity, 10 spikes were graded for infection and the overall percentage infection calculated for each entry.

Results and Discussion

Hybrid production, morphology and cytology: Both durum and bread wheat/*Ae. variabilis* cross combinations are examples of fairly simple intergeneric crosses as embryo formation frequencies were between 23.8 to 48.4% over all cultivars. All F_1 hybrids were cytologically normal (Fig. 1). Univalency at meiotic metaphase I was dominant. F_1 spike morphology was of intermediate expression and differed from both parents of the combination (Fig. 2). We consider F_1 hybrid phenotype modification a function of alien genetic expression that forms an initial selection sieve for advancing such F_1 combinations for agricultural practicality. In Fig. 3 are several other F_1 spike phenotypes that show this desirable co-dominant phenology trend. Similar phenotypic observations are of common occurrence in intergeneric crosses (Mujeeb-Kazi *et al*., 1987) and specifically reported earlier for wheat/*Ae. variabilis* (Vahidy *et al*., 1991; Zu-jun *et al*., 2001).

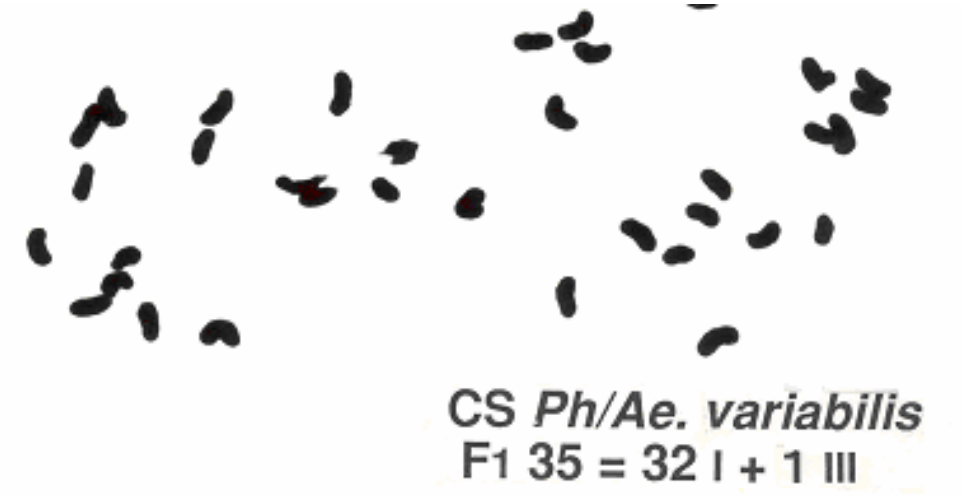

Fig. 1. A meiocyte from the *Triticum aestivum / Aegilops variabilis* F_1 hybrid combination with the *Ph* dominant locus showing negligible chromosomal pairing (2n=5x=35), 32 univalents + 1 trivalent.(Source : Mujeeb-Kazi *et al*., 2001).

Fig. 2. Side and frontal spike views showing from left to right: **a**) *Triticum aestivum*, **b**) *T. aestivum* /*Aegilops variabilis* F_1 hybrid, and **c**) *Ae. variabilis*.

Fig. 3. Spike morphology of bread† and durum wheat* / *Aegilops variabilis* amphiploids showing from left to right, combinations involving the cultivars: Alondra/Pavon†, CS *Ph*†, CS *ph*†, Jauhar†, Lu26†, Pak 81†, Punjab 85†, Altar 84*, Bia*, Laru* with the *Ae. variabilis* accession on the extreme right.

Amphiploid production, maintenance and cytology: F_1 hybrid plants after colchicine treatment produced the C-0 amphiploid progenies which possessed either near 70 or 56 chromosomes respectively. All amphiploids also expressed a codominant phenotype as observed from the spike morphology (Fig. 3). C-0 seed were advanced, cytologically checked and seed quantities accumulated for stress evaluations. Aneuploidy was consistent in the bread wheat based amphiploids with meiosis being varied at each increase generation. Greater stability however, was expressed by the durum based amphiploids where meiocytes with 28 bivalents were abundant. The pedigrees of the amphiploids and their cytological information has been separately reported (Mujeeb-Kazi *et al*., 2007).

***C. sativus* screening:** Under the naturally infested site in Poza Rica, Mexico, disease pressure was very severe and susceptible cultivars were rated 9-7 to 9-9 or 4 to 5 for leaf damage and grain blemish respectively. There were a few modifier combinations but generally an optimum date of planting and normal maturity of the test material provided satisfactory data accumulation. The performance of the various amphiploids is shown in Table 1 where leaf damage scores ranged from 9-2 to 9-5. The bread wheat based amphiploids favored for future exploiting involve the cultivars ‘Jauhar’, ‘Asakasekomugi’, and ‘Fukohokomugi’. For durums the combination with ‘Altar 84’ expressed superior resistance (9-2) and this could be targeted for the subsequent manipulation strategies for the crops improvement.

Table 1. Bread and durum wheat / *Aegilops variabilis* cytological details, plus field screening data for Co*chliobolus sativus* leaf damage and *Tilletia indica* response (% infection).

Amphiploid combination *T. aestivum** cultivar *T. turgidum*§ cultivar	Somatic chromosome range	Leaf a	Damage† b	Grain Blemish	Bunt Grain Infection (%) Cross	 Parent
***T. aestivum* based amphiploids**						
Ald/Pvn* // *Ae. variabilis*	64 to 68	9-4	9-5	2	0.26	37.8
Faisalabad* / *Ae. variabilis*	65 to 68	9-3	9-4	2	0.84	43.7
Chinese Spring* *Ph*/*Ae. variabilis*	66 to 71	9-5	9-5	2	1.48	53.4
CS* *ph* / *Ae. variabilis*	65 to 72	9-5	9-5	2	1.62	52.9
Jauhar* / *Ae. variabilis*	65 to 79	9-3	9-3	2	1.10	48.0
Lu26* / *Ae. variabilis*	67 to 71	9-5	9-5	2	1.94	59.3
Pak 81* / *Ae. variabilis*	66 to 70	9-5	9-5	2	1.55	49.9
Punjab 84* / *Ae. variabilis*	66 to 71	9-5	9-5	2	1.90	60.2
Asakasekomugi* / *Ae. variabilis*	64 to 67	9-3	9-3	2	1.73	38.5
Fukohokomugi* / *Ae. variabilis*	68 to 70	9-3	9-3	2	2.03	41.2
***T. turgidum* based amphiploids**						
Altar 84§ / *Ae. variabilis*	53 to 56	9-2	9-2	2	0	0.3
Bia§ / *Ae. variabilis*	52 to 56	9-4	9-5	2	0	1.1
Laru§ / *Ae. variabilis*	53 to 57	9-3	9-4	2	0	0.8
Control cultivars						
WL-711 (Susceptible)						67.2
Ciano79(Susceptible)		9-6	9-9	5		
Mayoor(Resistant)		9-2	9-9	2		

†- Leaf damage score is based upon double digit scoring where the first digit relates to the height of the infection and the second digit the severity of the infection (9 is the susceptible limit), scoring done at (a) milk stage and (b) dough stage of grain development.

***T. indica* screening:** Under the artificial field inoculation screening all amphiploids expressed a resistance trend to percentage bunt infection. The range across bread wheats was from 0.26 to 2.03. All durum combinations were immune (Table 1). Bread wheat parents ranged in susceptibility from 37.8 to 60.2% with WL-711 the recognized susceptible check cultivar being 67.2%. Comparison of scores between the bread wheat parents and their derived amphiploids gives a clear indication from the present study that the *Ae. variabilis* accession 13E is contributing to the low infection percentage in this diverse germplasm for which greater support comes from the durum screening. Earlier (Warham *et al*., 1986) had reported resistance in the same accession and here when it has been combined with various cultivars that resistance has shown desirable expressivity thus adding strength to its future usage for wheat improvement. The normally field resistant durums did show some infection when the parent types were inoculated and the range was from 0.3 for Altar 84 to 1.1 for Bia. The corresponding amphiploids were immune which added strength to the interpretation that *Ae. variabilis* was a potent source for karnal bunt resistance. Since all the bread wheat / *Ae. variabilis* amphiploids express satisfactory resistance to bunt, each source can be utilized for further breeding targets. However, preference should be given to those stocks that have good agronomic plant types in which case those that are tall, late to mature and have open crowns could be avoided. The above structure is common for Chinese Spring forms. Undesirable also are the combinations with Asakasekomugi and Fukohokomugi. From the remaining cultivars a choice can be further made if the recipient goal needs to access the T1BL.1RS

translocation or not. Two (Ald/Pvn and Pak-81) amphiploids have this translocation and can be selected for usage if so desired.

The practicality focus will be with bread wheat materials since it is doubtful if durum cultivation will expand into regions of the world where there is any constraint for *C. sativus*. Also should karnal bunt become an issue, the levels of field resistance are high enough in durums to preclude any concern warranting use of complex intergeneric strategies for gene transfers from *Ae. variabilis*.

Genetic manipulation strategies for alien introgression: Amphiploids are an ideal means of storing usable genetic diversity and providing a continuous means of generating stress disease diagnostic data. For biotic/abiotic stress resistances with an ill-defined mode of inheritance, use of disomic alien addition lines may be another classical and lengthy methodology to utilize for subsequently effecting alien transfers. However, when the meiotic process does not facilitate recombination based alien transfers, other gene transfer strategies become essential, of which a few have been described (Jauhar, 1993; Kimber, 1993). By using the monosomic 5B and *ph* route for F_1 production, enhanced chromosome pairing is observed (Fig. 4a and 4b). Such associations were earlier reported by Sharma & Gill (1986) for *ph* wheat / *Aegilops* species hybrids, but a constraint was identified in producing their BCI derivatives. We have used the *ph* based F_1 hybrid combination of wheat / *Ae. variabilis* and successfully backcrossed it twice to eventually produce BCI and BCII selfed derivatives (Fig. 5a and 5b). This germplasm offers a means of *C. sativus* screening and further exploitation. Molecular diagnostics in the future on resistant euploid progenies are anticipated to identify alien introgressions resulting from F_1 meiotic associations (Fig. 5b); an aspect that occurs in high frequency as shown for manipulations involving *T. aestivum* and *Thinopyrum bessarabicum* combinations around the *ph* system (Mujeeb-Kazi, 2003).

Other strategies: Considering the strength of molecular diagnostics, we are of the opinion that maximizing alien transfers at the F_1 stage may be advantageous in the quest of producing practical products. If possible, producing new *ph* based F_1 hybrids is one option where back- or top-crossing can build up valuable germplasm rapidly. Alternatively, where *ph* based hybrids may be difficult to produce or advance by backcrossing, direct utilization of the wheat (*Ph*) / alien F_1 combinations produced may be a solution. Such *Ph* F_1 male-sterile hybrids become maternal parents amenable for trigeneric product generation aimed at suppressing the *Ph* locus and promote alien introgression. Further, the existing *Ph* F_1 wheat/alien species hybrids can be crossed with the *ph* wheat, yielding heterozygous *Ph ph* BCI derivatives. Polyhaploids produced from these BCI derivatives (BCI x *Zea mays*, Millet (*Pennisetum* sp.) or *Tripsacum* sp.) will segregate for the dominant *Ph* or *ph* recessive loci, get cytologically identified and the appropriate high pairing *ph* combination advanced further to yield wheat/alien chromosome translocations (Mujeeb-Kazi, 2003). In order to provide additional novel diversity for *C. sativus* resistance, other alien species like *Th. curvifolium, Th. elongatum* and *Th. scirpeum* with diversified genomes are ideal alternate sources. We have either amphiploids or fertile BCI derivatives of these species with *T. aestivum.* These germplasm products have incorporated both the above cytogenetic manipulation strategies in attempts to introgress the *C. sativus* resistances into wheat, thus adding to the UUSS contribution of *Ae. variabilis* and the D genome resistance donation already incorporated from several *Ae. tauschii* accessions (Mujeeb-Kazi *et al*., 2001).

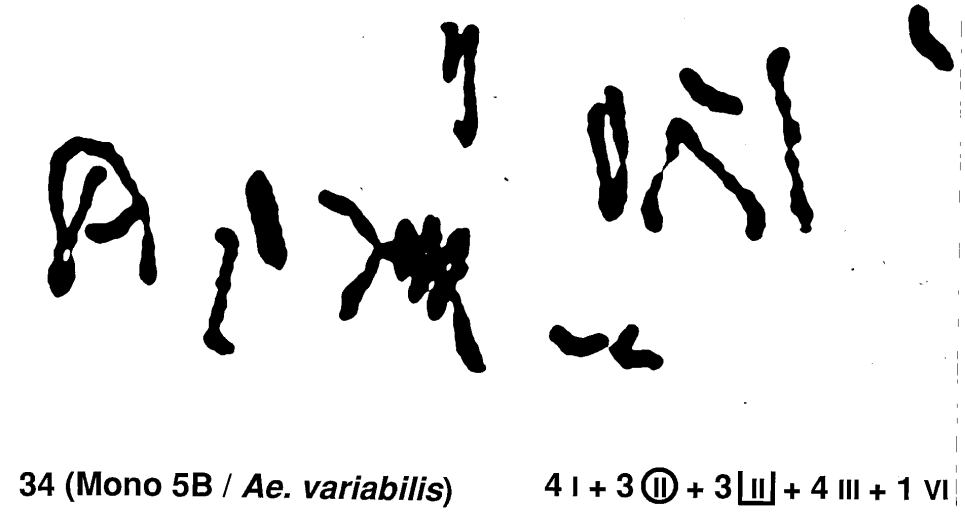

Fig. 4. **a**) Meiotic association in a Chinese Spring monosomic 5B / *Aegilops variabilis* F_1 hybrid (2n=5x=34) showing a relationship of 4 univalents + 3 ring bivalents + 3 rod bivalents+ 4 trivalents + 1 hexavalent (Source: Mujeeb-Kazi *et al*., 2001) **b**) Meiotic association in a CS *ph* / *Ae. variabilis* F_1 hybrid (2n=5x=35) showing a relationship of 9 univalents + 2 ring bivalents + 8 rod bivalents + 2 trivalents.

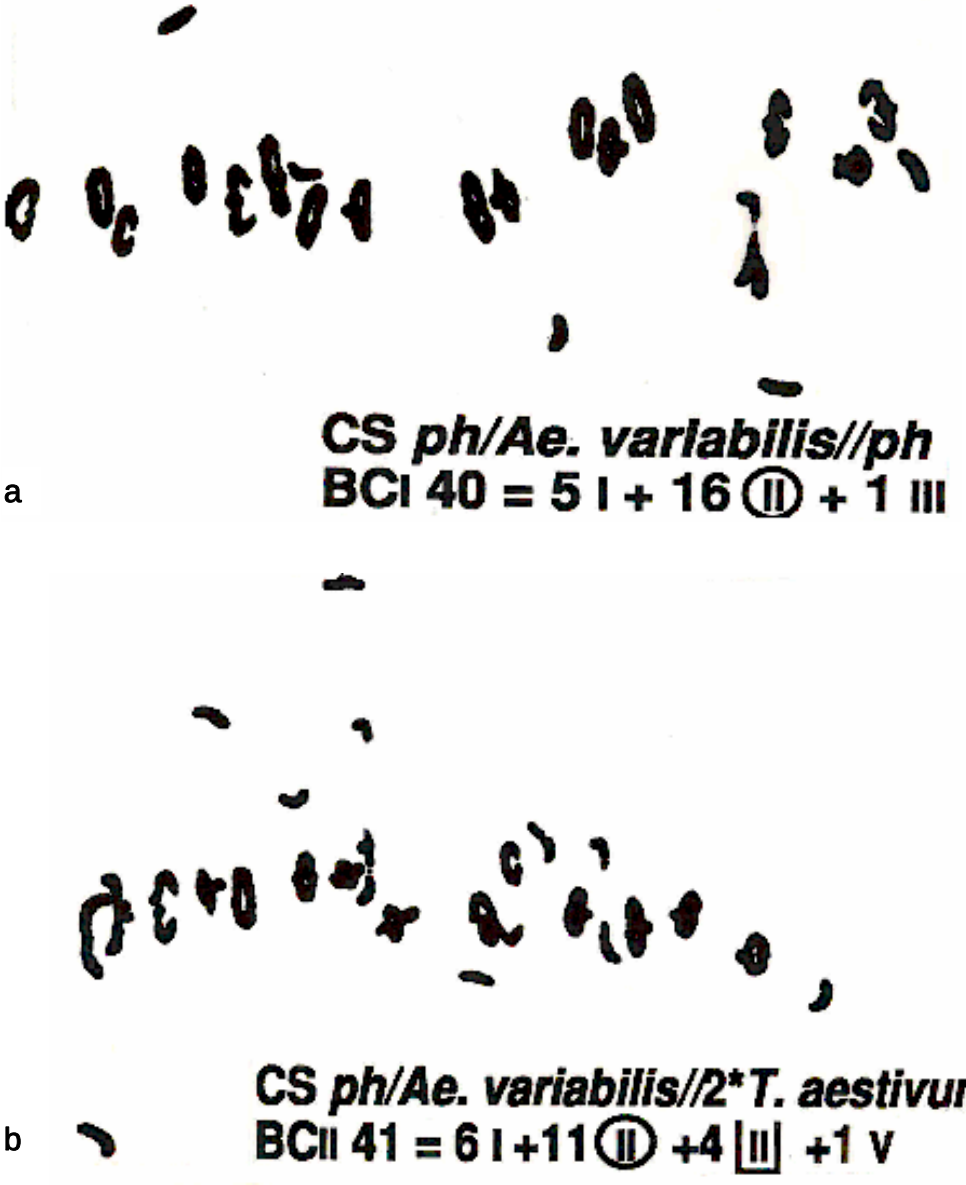

Fig. 5. Meiocytes from BCI and BCII derivatives of the CS *ph / Aegilops variabilis* derivatives showing associations in: **a**) 5 univalents + 16 ring bivalents + 1 trivalent, and **b**) 6 univalents + 11 ring bivalents + 4 rod bivalents + 1 pentavalent

References

De Milliano, W.A.J. and J.C. Zadoks. 1985. The effect of early foliar infection by *Helminthosporium sativum* on some yield components of two African wheats. In: Wheats for More Tropical Environments - A Proc. of the Int. Symp. CIMMYT, Mexico, D.F., pp.154-157.

Jauhar, P.P. 1993. Alien gene transfer and genetic enrichment of bread wheat. In: *Biodiversity and Wheat Improvement*, (Ed.): A.B. Damania. John Wiley & Sons, pp. 103-19.

Kimber, G. 1993. Genomic relationships in *Triticum* and the availability of alien germplasm. In: *Biodiversity and Wheat Improvement*, (Ed.): A.B. Damania. John Wiley & Sons, pp. 9-16.

Mujeeb-Kazi, A. 2003. New genetic stocks for durum and bread wheat improvement. In: *Tenth International Wheat Genetics Symposium*, Paestum, Italy pp. 772-774.

Mujeeb-Kazi, A. 2006 Utilization of Genetic Resources for Bread Wheat Improvement. CRC Series. (Eds.): R.J. Singh and P.P. Jauhar. Publishers: Taylor and Francis Group, Boca Raton, FL, USA pp. 61-97.

Mujeeb-Kazi, A., S. Roldan, D.Y. Suh, L.A. Sitch and S. Farooq. 1987 Production and cytogenetic analysis of hybrids between *Triticum aestivum* and some Caespitose *Agropyron* species. *Genome*, 29: 537-553.

Mujeeb-Kazi, A., Q. Jahan and A.A. Vahidy. 1994. Application of a somatic and meiotic cytological technique to diverse plant genera and species in the Triticeae. *Pak. J. Bot*., 26: 353-366.

Mujeeb-Kazi, A., R.L. Villareal, L.I. Gilchrist and S. Rajaram. 1996. Registration of five wheat germplasm lines resistant to *Helminthosporium* leaf blight. *Crop Sci*., 36: 216-217.

Mujeeb-Kazi, A., S. Cano, V. Rosas, A. Cortes and R. Delgado. 2001. Registration of five synthetic hexaploid wheat and seven bread wheat germplasm lines resistant to wheat spot blotch. *Crop Sci*., 41: 1652-1653.

Mujeeb-Kazi A., A. Gul, M. Farooq, S. Rizwan and J.I. Mirza. 2007. Cytogenetics of some *Triticum aestivum* and *T. turgidum* x *Aegilops variabilis* intergeneric hybrids and their derived amphiploids. *Cereal Res. Commun*., (SUBMITTED).

Sharma, H.C. and B.S. Gill. 1986. The use of *ph1* gene in direct transfer and search for *Ph*-like genes in polyploid *Aegilops* species. *Z. Pflanzenzuchtg*, 96: 1-7.

Vahidy, A.A., F.A. Durrani, Q. Jahan and A. Mujeeb-Kazi. 1991. Production and Cytogenetics of intergeneric hybrids of *Triticum aestivum* with *Aegilops variabilis* and *Ae. vavilovi*. *Pak. J. Bot*., 23: 213-222.

Villareal, R.L., A. Mujeeb-Kazi, L.I. Gilchrist and E.D. Toro. 1995. Yield loss to Spot Blotch in Spring bread wheat in warm nontraditional wheat production areas. *Plant Dis*., 79: 893-897.

Warham, E.J., A. Mujeeb-Kazi and V. Rosas. 1986. Karnal bunt (*Tilletia indica*) resistance of *Aegilops* species and their practical utilization for *Triticum aestivum* improvement. *Can. J. Plant Pathol*., 8:65-70.

Zadoks, J.C., T.T. Chang and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. *Weed Res*., 14: 415-421.

Zu-Jun, Y., Y. Wu-Yun, L. Guangrong and R. Zheng-Long. 2001. Genetic identification of an amphiploid between *Triticum aestivum* and *Aegilops variabilis*. *Wheat Information Service*, 93: 9-13.

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