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CYTOGENETICS OF SOME *TRITICUM AESTIVUM* AND *T. TURGIDUM* × *AEGILOPS VARIABILIS* INTERGENERIC HYBRIDS AND THEIR DERIVED AMPHIPLOIDS

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Abstract

Amongst alien germplasms that provide much needed genetic diversity for resistance to leaf-blotch (caused by *Cochliobolus sativus*) and karnal bunt (caused by *Tilletia indica*) is the tertiary gene pool tetraploid *Aegilops variabilis* (2n=4x=28; UUSS) resource. In order to exploit this species potential, it is crucial that intergeneric hybrids are first produced from which stable genetic stocks are developed for subsequent agricultural utilization. The hybridization of several durum and bread wheat cultivars with *Ae. variabilis* is reported herein which have yielded cytologically characterized normal F_1 hybrids (2n=4x=28, ABUS or 2n=5x=35, ABDUS). These upon colchicine treatment have generated fertile octoploid and decaploid amphiploids. The amphiploids in all cases possessed aneuploidy that was more pronounced with bread wheat cultivars (2n=10x=70, AABBDDUUSS). Durum amphiploid combinations had more normal 56 chromosome plant types with perfect bivalent meiosis (2n=8x=56, AABBUUSS and 28 bivalents across variable ring and rod associations). Reported here is the cytogenetics of these intergeneric hybrid cross combinations that have enabled the production of a novel genetic resource in the form of fertile amphiploids that hold the potential to address future work on improving wheat cultivars for resistance to *C. sativus* and *T. indica*.

Introduction

Genetic resources are paramount for providing diversity that supports crop improvement for various biotic and abiotic stress production constraints. In the Triticeae this much needed diversity is distributed within three gene pools (Jiang *et al*., 1995); primary, secondary, tertiary; and the exploitation of these alleles is a function of the resources genetic distance from wheat genomes (A, B, D). Though primary gene pool genera and accessions are a top priority for wheat improvement (Mujeeb-Kazi, 2003), tertiary gene pool resources, even though complex to utilize are also a potent means to enrich the wheat germplasm (Mujeeb-Kazi, 2006). The focus here is on *Aegilops variabilis* (2n=4x=28, UUSS) for providing resistance to two important biotic stress wheat production constraints; leaf blotch and karnal bunt that are globally distributed and are of significant importance. Sources of resistance to *C. sativus* and *T. indica* in species other than *T. aestivum* (i.e., alien gene pools) are of special interest in breeding programs. The International Maize and Wheat 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). These programs encompassed two distinct methodologies for introgressing alien genetic diversity (Mujeeb-Kazi & Hettel, 1995): a) Intergeneric hybridization route for incorporating distant gene pools, and b) Interspecific hybridization aimed at utilizing closely related genetic diversity. The objectives of this presentation are to elucidate the current status of usable genetic diversity generated around accession 13E (Source: Former PBI, Cambridge, England) of *Ae. variabilis* (2n=4x=28, UUSS) recognized as a resistance biotic stress donor. Elucidated are details of the F_1 hybrid production, cytological validation, and amphiploid generation coupled with phenology aspects.

Materials and Methods

Germplasm: The *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). The durum and bread wheat cultivars used in hybridization originated from CIMMYT's germplasm bank and NARC, Pakistan, except for 'Asakasekomugi' and 'Fukohokomugi' that were provided by Dr. George Fedak (Agriculture Canada).

Hybridization, embryo rescue and plantlet regeneration: All durum and bread wheat cultivars were planted over 3 dates at 10 day intervals in order to niche with *Ae. variabilis* pollen availability. *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. 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 hybrids, colchicine doubling and cytology of amphiploids: At least 15 differentiated potential hybrid plants/combination were grown in the greenhouse. From each potted plantlet, root-tips were collected and somatically analyzed (Mujeeb-Kazi *et al*., 1994) to validate hybridity. The hybrid plants possessing 2n=4x=28, ABUS or 2n=5x=35, ABDUS chromosomes were treated with 0.1% colchicine + 2.0% dimethyl-sulfoxide for 6 hours *via* aerated root-treatment for doubling the chromosome number in order to obtain fertile amphiploids (2n=8x=56 or 2n=10x=70) of which seed setting was the initial identifier. All amphiploid seed produced were germinated, somatically analyzed to validate the amphiploid status and for obtaining a seed increase. Meiotic analyses were conducted on F_1 plants of each combination and on their amphiploids.

Results and Discussion

Hybrid production, morphology and cytology: There are distinct hybridization barriers amongst the Triticeae species for which genetic control involving *Kr1, Kr2, Kr3 and Kr4* genes has been unequivocally demonstrated (Falk & Kasha 1981; Luo *et al*., 1992; Riley & Chapman 1967). Nevertheless, for most intergeneric hybrids, cross manipulation procedures abound that circumvent hybrid production constraints of complex combinations and further enhance frequencies for those that cross readily. The present wheat / *Ae. variabilis* cross combination is under our situation an example of a fairly simple cross where embryo formation frequencies range between 23.8 to 48.4% over all durum and bread wheat cultivars. The higher percentage was with Chinese Spring (*Ph*) and F_1 seed had well formed endosperm. Embryos, however, were still rescued to speed up germplasm development and the project pace. We do contend however, that where well-formed F_1 wheat cultivar / alien species hybrid seed is produced in a high frequency (as with CS *Ph*) and embryo rescue is not essential, such combinations be preferred since penalties of not having the recessive *Kr* crossability genes show a nominal influence on hybrid production. Also when the commercial cultivar / Species crossability levels are high, use of such wheat cultivars instead of Chinese Spring that possesses a poor agronomic type would be advantageous for wheat breeding targets that require good plant types swiftly as end-products. All F_1 hybrids were somatically normal with 2n=4x=28 (*T. turgidum* based) or 2n=5x=35 (*T. aestivum* based) chromosomes. Their genomic combinations are represented as ABUS and ABDUS respectively. The mean meiotic associations indicated a range of chromosomal relationships at metaphase I that characterized low pairing predominantly as a consequence of the *Ph* locus. Univalency at meiotic metaphase I was dominant. F_1 spike morphology was of intermediate expression and differed from both parents of the combination. 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. All other F_1 spike phenotypes showed this desirable co-dominant phenology trend consistent with similar prior phenotypic observations within intergeneric crosses (Mujeeb-Kazi *et al*., 1987) and specifically reported earlier for wheat / *Ae. variabilis* (Mujeeb-Kazi *et al*., 2007; Vahidy *et al*., 1991; Zu-jun *et al*., 2001).

Amphiploid production, maintenance and cytology: F_1 hybrid plants after colchicine treatment produced spikes that were glassine bagged. Several spikes set seed that represented the C-0 amphiploid generation. There were 10 bread wheat and three durum wheat based amphiploids that possessed either near 70 or 56 chromosomes respectively (Figs. 1a, c). All amphiploids also expressed a co-dominant phenotype as observed from the spike morphology. C-0 seed were advanced, cytologically checked and seed quantities accumulated for future stress evaluations. Aneuploidy was consistent in the bread wheat based amphiploids with meiosis being varied at each increase generation (Fig. 1b). Greater meiotic stability however, was expressed by the durum based amphiploids (Fig. 1d). The pedigrees of the amphiploids and their cytological information is elucidated in Table 1.

Some perceptions: The hybrids of durum wheat and bread wheat with *Ae. variabilis* were somatically normal (2n=4x=28; 2n=5x=35). All hybrids expressed meiotic associations that do not facilitate recombination exchange products. Amphiploids that have been induced, are a potent resource to screen for *C. sativus* and *T. indica* resistances both for the *T. aestivum* and *T. turgidum* combinations. Ideally *ph* based F_1 hybrids (eg. with cv. Chinese Spring) facilitate recombinational events from which hard to produce advanced derivatives (BC's selfed) can be developed. However, in the absence of such hybrids and in order to harness other globally available amphiploid germplasm we propose that *Ph* based transfers be mediated by the p*h* genetic manipulation protocol of Mujeeb-Kazi (2003). This exploits all existing *Ph* based hybrids or their amphiploids by crossing onto them *Ph* suppressor species, but more preferably the *ph* stock, as viable options around this chromosome 5B locus for affecting genetic wheat / alien chromosome exchanges. The current stock of amphiploids are a valuable arsenal of usable diversity for addressing wheat production constraints around biotic and abiotic stresses.

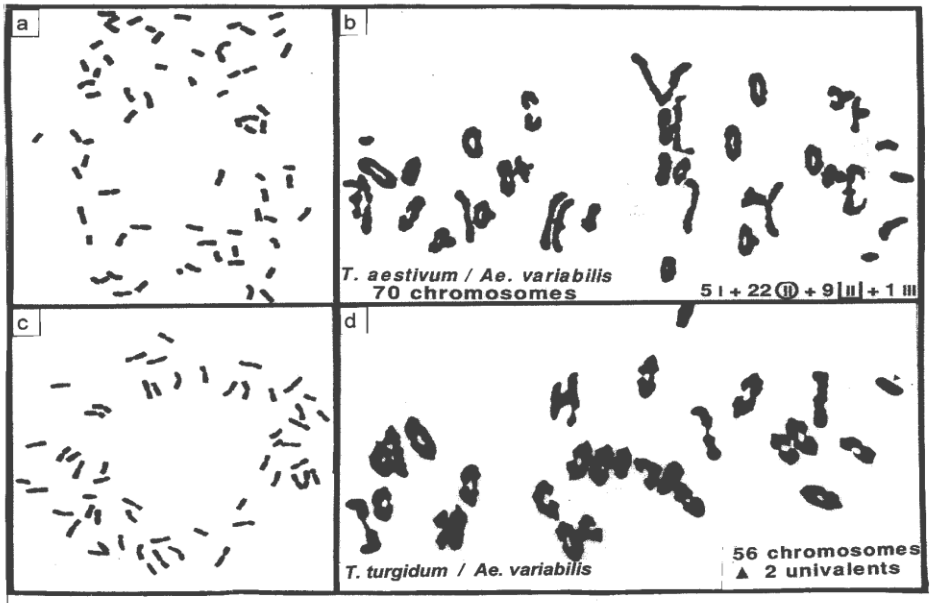

Fig. 1. Mitotic and meiotic microphotographs of bread and durum wheat / *Aegilops variabilis* amphiploids showing in: a) *Triticum aestivum / Ae. variabilis* mitotic cell with 68 chromosomes including 2 telocentrics .b) A meiocyte of a *T. aestivum / Ae. variabilis* amphiploid with chromosomal associations of 5 univalents + 22 ring bivalents + 9 rod bivalents + 1 trivalent c) *T. turgidum / Ae. variabilis* mitotic cell with 55 chromosomes, including 1 telocentric d) A meiocyte of a *T. turgidum / Ae. variabilis* amphiploid with 56 chromosomes of which 2 are univalents.

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