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Alien Germplasm for Wheat (*Triticum Aestivum* L.) Improvement Facilitated By Cytogenetic Manipulation and Use of Novel Techniques

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Introduction

There are approximately 250 perennials amongst the 325 Triticeae species (Dewey, 1984), and relatively few of these have been hybridized with wheat. Those that have been are predominantly included in the *Thinopyrum* group. Over the last decade and a half, phenomenal success has been achieved in the production of complex hybrids amongst the Triticeae, leading to a potential stock of valuable alien genetic material introduced from wide hybrids. Wide hybrids provide cytological data, evolutionary or phylogenetic information about the parental species, and the practical motivation associated with the introduction of significant characteristics from the alien species for wheat improvement. These alien transfers can diversify variability for both biotic and abiotic situations, two aspects that are of considerable functional difference. In one case, the pathogenic system is vulnerable to mutational events leading to breakdown of resistance, whereas the other aspect deals with physiological traits devoid of mutational changes, being thus categorized as dynamic and static systems (Mujeeb-Kazi and Kimber, 1985). Recognizing the above and also the fact that variation can be incorporated into wheat via intervarietal crosses, alien genetic variability is additive to plant breeding efforts due to its unique origin. This is based upon diverse alien genome categorizations, a variability source that would otherwise be inaccessible to breeders adopting strict conventional plant improvement procedures.

Successes of usable alien genetic incorporation are not numerous, but they have certainly made their impact (Sharma and Gill, 1983a; Mujeeb-Kazi and Kimber, 1985) despite the fact that these achievements have primarily been for simply inherited genetic traits. Hence, objectives associated with selection within populations that permit genetic expressivity of simplistically heritable attributes rank high in exploitation of intergeneric alien gene transfers. The vulnerability of simple genes is quite obvious even when these are pyramided with other genes. Other attributes with ill-defined genetic information, circumstantially inferred to be controlled by polygenic recessive systems, have complex heritability

and may have more success via interspecific en-bloc gene transfers rather than staggered intergeneric exchanges. Some of these complex attributes are associated with resistances or tolerances to *Fusarium graminearum, Helminthosporium sativum, Neovossia indica,* salt, drought, and mineral deficiencies or toxicities.

Several complexities exist in effecting successful alien introgressions, and there are different methodologies of attaining wheat x alien species combinations. The alien species could be screened for specific resistances or tolerances and then be hybridized with wheat. Alternatively, the species could be first hybridized and lead to advanced derivative screening. Irrespective of the procedure adopted, hybrid production (intergeneric or interspecific) is the critical initial base from which additional manipulations may promote effective genetic transfers.

Germplasm

The alien species (annual or perennial) utilized in wide hybridization are of *Aegilops, Agropyron, Elymus, Haynaldia villosa, Hordeum vulgare, Heteranthelium, Henrardia, Secale cereale, aeniantherum, Triticum monococcum, T. boeticum, T. urartu, T. araraticum,* and *T. tauschii.* The species are characterized by genomic diversity or substantial similarity and are either monotypic or comprised of several hundred accessions.

Hybrid Production

Barriers to production of wide crosses occur at various stages in the ontogeny of the hybrid comprising of: (a) parental choice, (b) emasculation procedure, (c) prepollination treatment, (d) pollination process, (e) post-pollination treatments, and (f) embryo excision plus culture (Mujeeb-Kazi and Kimber, 1985). The most simple hybrids, it appears, were produced by the conventional wheat crossing procedures. Variations originated when distant crosses were attempted, leading to the adoption of an array of manipulative techniques. These enabled the (a) to (f) characterization of the salient hybrid production stages that are more a reflection of constraints for the intergeneric hybridization category rather than the interspecific combinations used in the program. The crossability barriers are less critical in the latter case, but where prevalent can be readily eliminated through incorporation of bud pollination and embryo rescue techniques. Plantlet processing after embryo differentiation in Murashige and Skoog (1962) or Taira and Larter (1978) media has been elaborated in reports of Mujeeb-Kazi and Rodriguez (1983a, 1983b). Hybrid identification procedures are morphological, cytological, or biochemical (Mujeeb-Kazi et al., 1987;

Asiedu and Mujeeb-Kazi, 1987). Their practical utilization is determined through genomic analysis made possible by the development of numerical methods of assessing genomic affinity (Kimber and Hulse, 1978; Kimber et al., 1981; Alonso and Kimber, 1981; Kimber and Alonso 1981; Espinasse and Kimber, 1981). Interpretations from the above methods enable application of pertinent techniques relative to alien transfers (Kimber, 1984). When the mean arm-pairing frequency and the relative affinity have values approaching one, the transfer can be made by recombination. At intermediate values of mean arm-pairing frequency, increased homologous pairing can be induced by changes in the systems regulating chromosome pairing. At very low values of the mean arm-pairing frequency, irrespective of the value of the relative affinity, irradiation or centric break-and-fusion in derived aneuploids is the optimum method (Kimber, 1984).

Alien Transfer Constraints and Some Solutions

In alien genetic transfers so far, only rather simply inherited traits have been introgressed (Dewey, 1984; Knott and Dvorak, 1976). Transferring polygenically controlled characters that are presumably recessive in nature appears too difficult to achieve via intergeneric hybridization if short-term projections are made. Where polygenes are located on more than one alien chromosome, each gene could be introduced into a separate wheat background prior to pyramiding these independently transferred genes into a single line or variety. We must not, however, preclude the possibility of major gene influences in a polygenic system that would bestow major resistant/tolerant influences in a simple genetic manner and yield variable segregating patterns.

Despite the complexity of polygenic systems, solutions do exist that could facilitate gene transfer methodology. These are associated with:

(a) Chromosome 5B mechanism. There is considerable merit in attempting to enhance recombinations in F_1 hybrids by incorporating the *Ph* system (Darvey, 1984; Forster and Miller 1985; Mujeeb-Kazi et al., 1984, 1987; Sharma and Gill, 1983a, 1983b, 1983c). The procedures involve the use as maternal parents of (i) nulli-5B tetra 5A or 5D wheat stocks, (ii) mono- 5B, or (iii) *PhPh* mutant, etc., in the production of F_1 hybrids. Such F_1 hybrids would exhibit a high meiotic chromosome pairing frequency, thereby promoting incorporation of alien transfers in ideal locations within the wheat genomes.

(b) Partial or complete synthetic genomes. In the alien species, several are autotetraploids (*A.* or *P. stipaefolium*), segmental allotetraploids, or partial autopolyploids (*A.* or *T. curvifolium*, *A.* or *T. junceiforme*, *A.* or *T. scirpeum*), segmental allohexaploids (*A.* or *T. junceum*), or segmental autoallohexaploids (*A.* or *T. podperae*, *A.* or *T. varnense*) where there are two closely related genomes and the third genome is distinctly different from the other two.

In segmental allotetraploids or partial autopolyploids, repeated selfings of the backcross I self-fertile derivatives could potentially lead to "complete" synthetic genome formation (Mujeeb-Kazi and Miranda, 1984) (Figure 1). In segmental autoallohexaploids, there is the likelihood of the unrelated third genome to be eliminated and of fertile backcross I derivatives leading to formation of stabilized 'partial' synthetic genomes (Figure 2). The selfing is expected to promote reorganization of complex polygenes as a consequence of recombination.

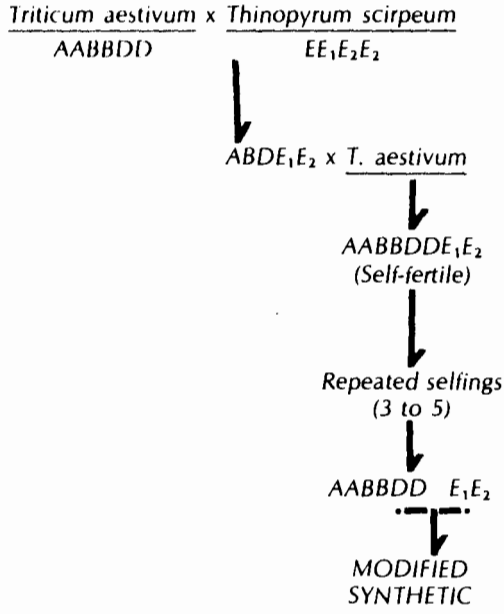

Figure 1. Development of a complete synthetic genome from hybridization of Triticum aestivum x Thinopyrum scirpeum *via Backcross I production and its repeated selfings to enhance E_1E_2 genomic reorganization.*

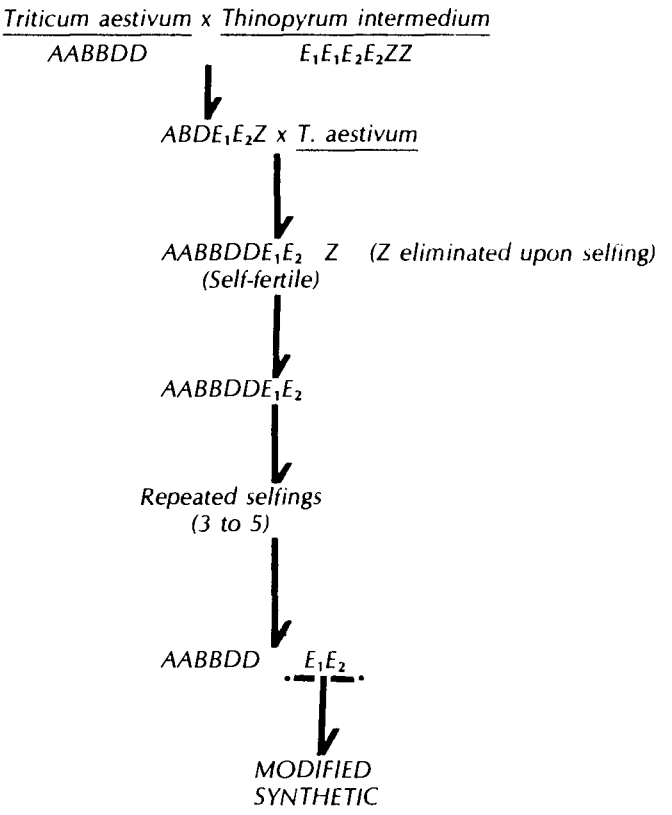

Figure 2. Development of a partial synthetic genome from hybridization of Triticum aestivum x Thinopyrum intermedium *via backcross I production and its repeated selfings to enhance E_1E_2 genomic reorganization with elimination of the unrelated Z genome.*

(c) Translocations have contributed significantly to disease resistance transfers (see review by Sharma and Gill, 1983a). Presumably, the major impact so far has been from the 1A/1R and 1B/1R translocations, greater for the 1B/1R (Rajaram et al., 1983). Other translocations of practical interest are the 5A/5R for copper efficiency and the probable utilization of 6RL rye arm for cereal cyst nematode resistance. In intergeneric hybridization, the advance of the F_1 hybrid to BCI via unreduced egg formation is a potent manipulative source of wheat-wheat, wheat-alien, or alien-alien exchanges (Mujeeb-Kazi et al.,1987). Numerous translocations have been reported in triticale-wheat crosses (Lukaszweski and Gustafson, 1982),

and this additionally supports the significance of translocations for practical applications, particularly wheat x rye (Asiedu and Mujeeb-Kazi, 1987). Ter Kuile et al. (1987) have proposed a scheme for inducing general or specific wheat D genome x alien chromosome-induced translocations. The general translocation induction process involves crossing of the *T. aestivum* x alien F_1 hybrid with *T. turgidum* that produces univalency for the entire wheat D and the alien genome chromosomes at meiosis (Figure 3). Specific alien translocations involve crossing a specifically desired disomic alien addition line that is *T. aestivum* based by *T. turgidum*. This would promote univalency at meiosis for the single alien addition chromosome and the entire *T. aestivum* D genome chromosomes, facilitating controlled centric-break-fusion between the entire D genome and the alien univalent (Figure 4).

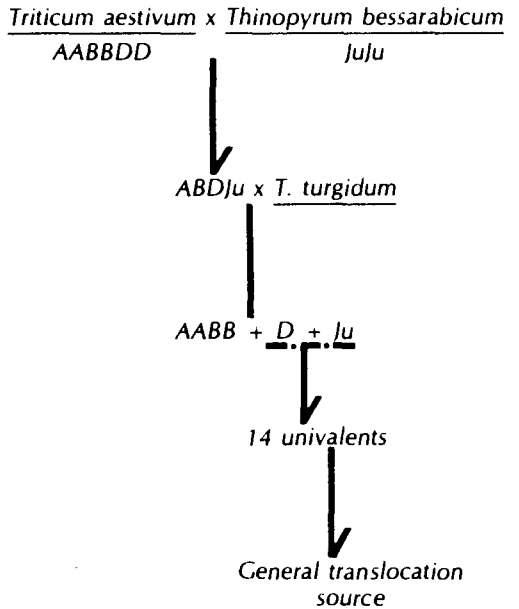

Figure 3. The general translocation induction process that utilizes 14 univalents (7 each) of the wheat D and T. bessarabicum *Ju genomes as a consequence of centric-break-fusion event/s.*

(d) Interspecific hybridization. Though rather simplistic as compared to intergeneric hybridization, it has the theoretical methodology advantage of yielding rapid practical returns over relatively short-term durations. The above concept is based on the following salient aspects: (i) ease of hybridization, (ii) field

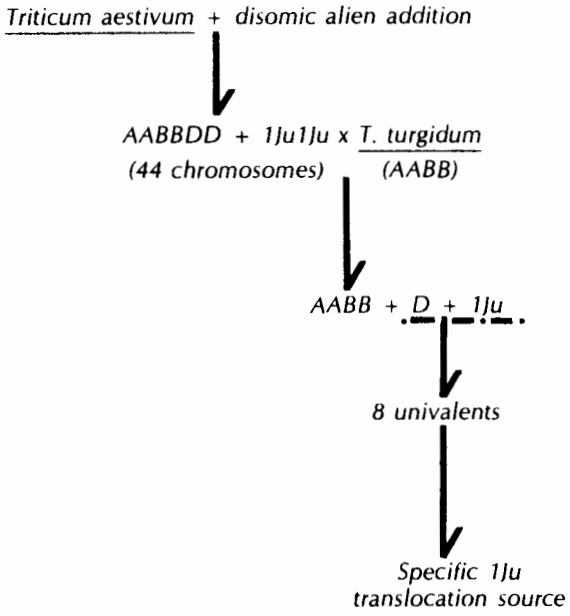

Figure 4. Specific translocation of a desired disomic alien addition and D genome of wheat involving 8 univalents (7 of D genome + 1 of the alien species).

oriented research, (iii) genomic similarity of the species with durum and bread wheat genomes, and (iv) as a consequence of high recombination the potential of en-bloc recessive polygenic transfers. There is tremendous flexibility of genomic manipulation approaches, and those with *T. tauschii* (2n = 2x = 14, DD) are represented in Figure 5 for developing *T. turgidum* x *T. tauschii* synthetics, in Figure 6 for developing AABBDD synthetics by crossing extracted AABB from elite *T. aestivum* cultivars with *T. tauschii,* and in Figure 7 for improving elite *T. aestivum* cultivars by direct crossing with *T. tauschii* accessions. There are constraints pertaining to lack of expression of D genome characteristics but the range of variability in various species is so diverse that the advantages definitely warrant a thorough investigation. The above schematics of genome manipulation and utilization can be extended to the other genome sets.

Supporting Systems

In essence, the brief consideration of the gene transfer methodology presented under the above categories provides reasonable insight

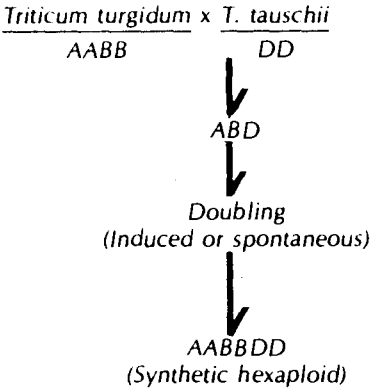

Figure 5. Derivation of synthetic hexaploids (AABBDD) from Triticum turgidum x T. tauschii *crosses.*

into the manipulative flexibility of the wheat cytogenetic system for facilitating alien genetic transfers. There are additionally several unique supporting systems that can extend the research potential of *Triticum*. These are all complementary facets for a coordinated approach at effecting alien transfers and a few are mentioned:

(a) Embryo and callus culture. Despite phenomenal successes in embryo culture, there is still ample need to manipulate embryo culture media and thus enable more distant hybrid embryos to differentiate. Embryo culture- based callus induction and plant regeneration has been studied with keen interest worldwide. More recently (TCCP, 1987), several *T. aestivum* and *T. turgidum* cultivars have been demonstrated to possess superb long-term callusing and regeneration capabilities. Cytological variation is a correlated aspect with regenerated plant development. The observed variation in advanced generations integrates with segregation of simply or polygenically inherited traits, and appropriate selections can be made. We do not feel, however, that callus-induced variation would be any different in nature as to that obtained in mutation breeding programs during M_2 to M_4 macro- and micromutation observational stages. In vitro screening has a decided advantage for the Triticeae that may include salt, aluminum, *Helminthosphorium* sp., fusaric acid, etc. The callus culture methodology further provides a means of promoting changes and/or alien genetic transfers (Lapitan et al., 1984, 1986, 1988). This approach is being applied to other wheat x alien F_1 hybrids that normally are poor recombinations; *T. aestivum* x *Aegilops variabilis* (Ter Kuile et al.,

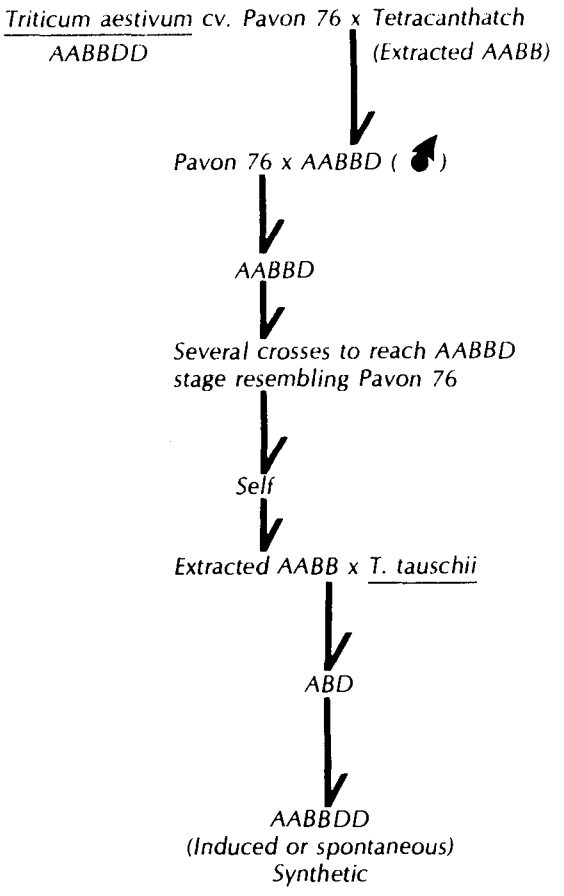

Figure 6. Extraction of AABB genomes from Triticum aestivum *L. cv. Pavon 76; hybrid with* T. tauschii *(DD) to produce the synthetic AABBDD.*

1987). More remarkable is the recent observation of chromosome number doubling in *T. aestivum* and *T. turgidum* x *Ae. variabilis* F_1 hybrids that have eluded conventional doubling attempts for approximately three decades. The doubling phenomenon needs further evaluation and warrants application towards other hard to double intergeneric combinations. The cytogenetic implications of callus-induced doubling are highly significant for controlled genetic transfers. A stable amphiploid is anticipated to eliminate aneuploidy that otherwise is rampant in backcross I derivatives (Jewell and Mujeeb-Kazi, 1982), thus eluding complete genetic evaluation.

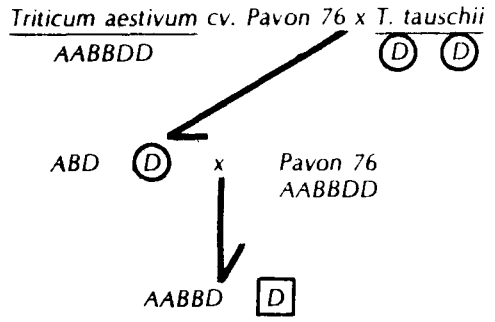

Figure 7. A mechanism for incorporation of DD genome variability into an elite Triticum aestivum *cultivar. Note that after 1 backcross only, 5/6 of the Pavon 76 genotype is restored.*

(b) Polyhaploid production. Presumably, the genetic system of polyhaploid generation in wheat based upon *T. aestivum* L. cv. Chinese Spring x *H. bulbosum* hybridization (Barclay, 1975) is superior both in terms of polyhaploid generation frequency and euploid formation upon doubling. The procedure also was extended to recover alien disomic additions (Islam and Shepherd, 1981; Islam et al., 1978) in wheat x barley crosses. Polyhaploids of wheat have also been reported from *T. aestivum* L. cv. Chinese Spring x *Zea mays* crosses in reasonably high frequencies (Laurie and Bennett, 1986). The anther culture-based wheat polyhaploid generation results are below the frequencies obtained in the above-mentioned examples, and when compared with this sexual cross study in a joint experiment (Inagaki et al., 1987), limitations of *T. aestivum* anther culture are obvious. Varietal responses abound in both approaches but apparently the *kr* genes on 5A, 5B and 5D are a major source of the high success of Chinese Spring in the sexual combinations.

Anther cultures or sexual processes of polyhaploid production in polyploid aliens have significant merit and warrant greater attention in order to better resolve the prevalent genomic relationships. This information will undoubtedly have tremendous impact on alien genetic transfer methodology.

(c) *Diagnostic markers and their significance.* A number of heritable characteristics have been associated with specific genomes, chromosomes, chromosome segments or genes, and these serve as markers for the identification of alien chromatin in wheat. Some of the markers are also applicable during the initial screen of alien populations for maximum genetic

variability and avoidance of unnecessary duplication. They provide the information required to make the right choice of materials, methods, and population size necessary for efficient and precise transfer of alien genetic material into wheat and the characterization of the transferred segments. Specific genes of agronomic importance could be tagged more quickly and easily in a population once they have been linked to some of these markers. Other applications include aneuploid identification, genetic analysis, chromosome assays, hybrid confirmation, and establishment of alien-wheat chromosome homologies. Four major groups of markers have applications in wheat wide crosses—morphological, genetic, cytological, and biochemical. They all have their advantages and limitations so that combinations of two or more classes of markers for specific projects are not uncommon (Landry et al., 1987).

Generally, however, the most useful markers are those that show high levels of polymorphism, are rapid, can be applied to seeds or during seedling stages, have no deleterious pleiotropic effects, and are inherited in a codominant fashion.

Morphological markers

The loss of chromatin from wheat or its gain from an alien source often results in modifications in plant morphology. Some of these changes have been associated with specific homologous groups (Miller and Reader, 1987) and thus serve as markers for those groups. Hairy peduncles of wheat-rye derivatives have long been associated with the presence of rye chromosome 5R (Chang, 1975).

Genetic Markers

Genes for resistance to a number of diseases and pests and tolerance to such environmental stresses like micronutrient toxicity or deficiency have been located on specific chromosomes of wheat and/or its relatives. For instance, resistance to leaf, stem, and yellow rusts serve as useful markers for rye chromosome 1RS (Koebner and Shepherd, 1986).

Cytological Markers

Direct identification of plant chromosomes using such features as chromosome size, arm ratio, possession of satellites, and/or secondary constrictions have been used either alone or in conjunction with other techniques (Endo and Gill, 1984; Fujigaki and Tsuchiya, 1985; Hsiao et al., 1986). Numerous stains have been used for this purpose including feulgen, aceto-carmine, and carbol fuchsin (Evans and Reed, 1981).

C-banding, which reflects constitutive heterochromatin, has been very useful in the identification of chromosomes or their segments in plants and animals since its discovery by Pardue and Gall in 1970. The C-banding patterns of wheat cultivars have since been reported (Gill and Kimber, 1984; Iordansky et al., 1978a, 1978b; Seal, 1982; Seal and Bennett, 1982) as have those of the relatives of wheat (Vosa, 1984; Teoh and Hutchinson, 1983; Sybenga, 1983; Friebe et al., 1987). Chromosomes of wheat and its relatives have also been identified on the basis of their N-banding patterns (Gerlach, 1977; Jewell, 1979; Schlegel and Gill, 1984; Endo and Gill, 1984). The value of chromosome banding in identification of wheat and alien chromosomes involved in translocations has been demonstrated (Gill and Kimber, 1977; Jewell, 1979; Lukaszewski and Gustafson, 1983). While Giemsa has been most extensively used in banding, other dyes like Leishman's, Wright's (Seal, 1982), Hoechst 33258, and DAPI (Sarma and Natarajan, 1973; Schlegel and Gill, 1984) have been applied in differential staining of chromosomes of the *Triticeae*. These staining techniques enable more detailed meiotic analysis through positive identification of chromosome segments involved in synapsis (Naranjo and Lacadena, 1979; Giraldez and Orellana, 1979; Singh and Shepherd, 1984).

The introduction of DNA sequences as cytological markers through *in situ* hybridization (Gall and Pardue, 1969, John et al., 1969) has further expanded the capacity for chromosome identification (Rayburn and Gill, 1987a). While some of the chromosome staining patterns revealed by the technique are similar to those produced by conventional Giemsa C-banding (Hutchinson and Lonsdale, 1982; Teoh et al., 1983; Hutchinson, 1983) there are many reports of situations where *in situ* hybridization has proven either clearly advantageous or a valuable complement (Hutchinson et al., 1980; Miller et al., 1982; Hutchinson et al., 1982; Teoh et al., 1983; Lapitan et al., 1986; Rayburn and Gill, 1986, 1987b). Many early workers used probes labeled with radioactive nucleotides like ^{3}H or ^{125}I and detected hybridization by autoradiography (Gerlach and Peacock, 1980; Hutchinson and Lonsdale, 1982; Hutchinson et al., 1982) but the use of biotin-labeled probes and detection by immunological and staining techniques is now in vogue (Manuelidis et al., 1982; Rayburn and Gill, 1985, 1986, 1987a, 1987b; Lapitan et al., 1986; Gillam, 1987). The nonradioactive probes have the comparative advantage of safety, stability, and speed.

Biochemical Markers

Biochemical markers may be subdivided as protein and DNA markers. The former has been more extensively used in work on the *Triticeae* and is comprised both of isozyme and storage protein

markers. Hart and Gale (1987) have presented a recent summary of chromosomal locations of biochemical/molecular loci reported in hexaploid wheat cultivar Chinese Spring which shows the short arm of chromosome 2 as the only arm not thus marked. They (Hart and Gale, 1987) also tabulated chromosomal locations of orthologous loci in other species in the tribe *Triticeae*. More of such loci are being established for many other species of the tribe (Asiedu and Mujeeb-Kazi, 1987). The many advantages of these markers are the speed and applicability to seeds and early seedling stages of plant growth. Using the Glucose Phosphate Isomerase enzyme locus on wheat chromosome arm 1BS as a marker, the 15th, 18th, and 21st International Bread Wheat Screening Nurseries of CIMMYT were demonstrated to carry 41, 43, and 52 percent, respectively, of the 1BL/1RS translocation (unpublished). This involved the assaying of about 250 lines per day (at 2 to 4 seeds per line) by one person which is only a fraction of the time required using cytological methods.

DNA markers in the form of restriction fragment length polymorphisms (RFLPs) are relatively new within the *Triticeae*, but genetic maps of crops like maize, tomato, and lettuce have been constructed or are being updated with RFLPs as markers (Helentjaris et al., 1986; Landry et al., 1987). Conceptually, RFLPs have the potential to saturate the genetic maps of most crops with markers as they have the capacity to detect many differences or changes in the DNA sequence which may not result in detectable gene products or changes in morphology. A number of laboratories are developing probes suitable for or specific to genomes in the *Triticeae* (Appels, 1986). As more of these become available, there would be a great improvement in our capacity to identify smaller and smaller fragments of alien chromatin transferred into wheat.

Conclusions

Since the pioneering work of the late Anton Kruse (Kruse, 1969, 1973), advances in intergeneric hybridization in the Triticeae have been extensive (Sharma and Gill, 1983a; Mujeeb-Kazi and Kimber, 1985). Hybrid production technology is simplified, hybrid identification procedures are well-developed, and hybrid advance methodology is clarified, with gene transfer constraints adequately identified and partially overcome. The need for novel complementary areas and superb resolution of various diagnostic techniques appear to be well- documented. This consequently sets in appropriate balance for the Triticeae crops a wide cross alien gene transfer program that encompasses sophisticated current approaches without any retraction from the important rapid field application practical goals. Research objectives are carefully weighed and are

principally dependent upon practical returns. Collaborative research arrangements follow cautious evaluation as to their crop production duration significance, that should not be too basic a research function.

There seems to be no major impediment in wheat wide crosses to restrict genetic advances at the plant level. The germplasm that emanates as a consequence of cytogenetic manipulation forms the backbone for diagnostic technology applications to thrive upon and simultaneously enables the breeding component to forge ahead without crucial time lapses between the various developmental phases of the program. Collaborative research in areas of novel system applications and diagnostic procedures is, and will further become, a major incentive towards effective alien genetic transfer research understanding, productivity, and end-product dissemination at budget-efficient standards. The program perspectives of short-term (interspecific hybridization) and long-term (intergeneric hybridization) stretched over 7 to 12 years are anticipated to provide quality returns for the applied mandate of crop improvement via alien genetic variability incorporation.

The program structure has precise facets of further linking the plant level manipulation phase with cellular and molecular approaches, two aspects that are essential contributors to program functionality and effectiveness. A number of very desirable approaches are anticipated to subsequently emerge for the monocotyledonous plants, notably the Triticeae. When these research breakthroughs are functionally refined and applicable they undoubtedly will either find complementary usage in wheat improvement, or may possess the potential to totally replace several conventional stages of genetic manipulation. We are receptive to and cognizant of these futuristic changes and have not delved into any detail at this stage due to lack of adequately reported progress within the tribe Triticeae.

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