

WHEAT X MAIZE AND WHEAT X TRIPSACUM HYBRIDIZATION FOR PRODUCTION OF WHEAT HAPLOIDS.

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ABSTRACT

Haploids in wheat (*Triticum aestivum* L., $2n=6x=42$, AABBDD) have been earlier produced by anther culture and crosses of wheat with *Hordeum bulbosum*. Both approaches have constraints associated with genotypic specificity. More recently, wheat x maize or x *Tripsacum* crosses have led to wheat haploid production in acceptable economical frequencies. The procedure relies on a post-pollination 2,4-dichlorophenoxy acetic acid treatment and embryo rescue after 14 to 16 days. Our embryo formation/rescue has a mean success of 29% with 80% plantlet regeneration followed by a colchicine doubling rate of 60 to 70%. The procedure is applicable to all wheat cultivars and is independent of the maize pollen source. We have applied the methodology to wheat breeding, development of cytogenetic stocks and mapping populations for wheat RFLP's.

INTRODUCTION

Haploid production in Triticeae has relied upon anther culture and sexual crosses with *Hordeum bulbosum* L. Both techniques have limitations; aneuploidy, somaclonal variation and genotypic specificity with anther culture and the homoeologous group 5 crossability loci (*Kr1*, *Kr2*) with *H. bulbosum* crosses (Falk and Kasha 1981). In order to avoid aneuploid variation the sexual route to haploid production is seemingly more desirable. Recently, crosses of wheat and other members of the Triticeae with maize have been promising for haploid production (Laurie and Bennett 1986, 1988). Maize is insensitive to the *Kr* crossability alleles of wheat (Laurie and Bennett 1987), that yields polyhaploids across different genotypes (Suenaga and Nakajima 1989; Inagaki and Tahir 1990), that makes it a potentially superior haploid induction system. In addition, gametoclonal variation induced in double haploid lines using the maize system was similar to that found in doubled haploids obtained from wheat x *H. bulbosum* L. crosses (Laurie and Snape 1990).

Use of 2,4-D is critical in promoting seed set and embryo formation (Inagaki and Tahir 1990). High frequency of wheat polyhaploid recovery was reported for crosses between the wheat cultivar Morocco and the maize population "Pool 9A" (Riera-Lizarazu and Mujeeb-Kazi 1990). We have since obtained and herein report polyhaploids of additional *Triticum aestivum* and *T. turgidum* L. cultivars, and a few *T. turgidum* x *Aegilops squarrosa* L. synthetic hexaploids with *Zea mays* crosses using a detached tiller culture method. Cytological features of these polyhaploids and their doubled progeny were characterized. Additionally, the polyhaploid production limits were extended in the Triticeae through crosses with *Tripsacum dactyloides*. Cytogenetic application of the above procedures simplified disomic and multiple disomic alien chromosome addition line production of salt tolerant *Thinopyrum elongatum* to *T. aestivum*.

MATERIALS AND METHODS

Plant material. Field grown plants of *Triticum aestivum* ($2n=6x=42$), *T. turgidum* ($2n=4x=28$), *T. turgidum* x *Aegilops squarrosa* synthetic hexaploids ($2n=6x=42$), *Tripsacum dactyloides* and *Zea mays* grown at El Batan, CIMMYT, Mexico, were used. A bulk pollen sample from several maize cross-pollinating populations and one accession of *T. dactyloides* was used for all crosses. Backcross II plants from *Thinopyrum elongatum*/3¹ *T. aestivum* with 43 to 45 chromosomes were maintained in the greenhouse at El Batan and were crossed with *Z. mays*. The environmental regimes were 15 hours light, 24°C day, 12°night temperature and approximately 60% relative humidity. Crossing procedures were similar to those reported earlier (Riera-Lizarazu and Mujeeb-Kazi 1990; Riera-Lizarazu et al., 1992; Mujeeb-Kazi et al., 1987).

Cytogenetic Analysis

Cytology. Somatic and meiotic cytology of all regenerated plants utilized plants utilized the acetoorcein (Mujeeb-Kazi and Miranda 1985) and a modified alcoholic carmine procedure (Mujeeb-Kazi et al., 1993; Snow 1963). Pairing associations were calculated from 25 meiocytes at metaphase I for some of the bread wheat and synthetic hexaploid polyhaploids. Disomic and doubled disomic additions derived from *Thinopyrum elongatum*/*T. aestivum*/*Z. mays* polyhaploids were also analyzed using standard fluorescent *in situ* genomic hybridization protocols.

Colchicine treatment. Cytologically identified polyhaploid plants were treated with colchicine as described previously (Mujeeb-Kazi et al., 1987). Successful chromosome doubling was inferred from seed setting on the colchicine-treated polyhaploid plants.

RESULTS AND DISCUSSION

We recovered haploids from many different wheat genotypes following pollinations with maize similar to the reports of others (Inagaki and Tahir 1990; Laurie and Reymondie 1991). In addition, we extended polyhaploid recovery to include some durum wheats and synthetic hexaploids. There was a wide range of embryo recovery frequencies. The recovery averages were 15.6% for bread wheats, 16.9 for durum wheats, and 19.8% for synthetic hexaploids respectively. These frequencies were 20.6, 26.8 and 23.5% for crosses with *T. dactyloides*. Mean plant regeneration frequencies were 68.5 for bread wheat, 73.9% for durum wheats and 74.5% for synthetic hexaploids respectively for polyhaploids from maize. These frequencies were 78.5, 66.7 and 75.5% from *T. dactyloides* combinations. Successful chromosome doubling of polyhaploid plants averaged 60.7% for *T. aestivum* cultivars, 69.5% for *T. turgidum* cultivars and 63.6% for the synthetic hexaploids. Polyhaploids obtained from the *T. dactyloides* procedure were not subjected to colchicine induced doubling. The wheat polyhaploid plant production frequencies obtained are adequate to meet economical threshold levels (Comeau et al 1988). In this study as compared with earlier findings (Riera-Lizarazu and Mujeeb-Kazi 1990), the lower frequencies are attributed to problems inherent in use of the detached tiller culture system in an adverse field crossing environment.

Regenerated plants possessed the haploid complement of 21 chromosomes for *T.*

aestivum and 14 chromosomes for *T. turgidum*. Minimum aneuploidy was observed in these polyhaploids. Each wheat parent had the euploid number of $2n=6x=42$ or $2n=4x=28$. Polyhaploid *T. aestivum* cultivars and the synthetic hexaploid showed low allosyndetic pairing. Ring bivalents were rare with chiasmata ranging from 0.44 to 1.96 per meiocyte. The *T. aestivum* polyhaploids of several had a mean metaphase I chromosome association frequency of 18.6 univalents + 0.01 ring bivalents + 1.24 rod bivalents + 0.06 trivalents. Values for the synthetic (*T. turgidum* x *Ae. squarrosa*) polyhaploid were 20.1 univalents + 0.44 bivalents. This degree of chromosome pairing is consistent with earlier data (Riley and Chapman 1958; Kimber and Riley 1963). The low pairing observed indicates that the dominant *Ph* locus remained intact over the haploid induction process. A similar low chromosome pairing trend at metaphase I was also prevalent for the *T. aestivum* and the synthetic hexaploid polyhaploids derived from *T. dactyloides* crosses.

The use of the maize system for haploid production in the Triticeae is encouraging since stringent genotype specificity is not apparent. Like with maize, polyhaploid production in the Triticeae with *Tripsacum* is dependent upon a 2,4-dichlorophenoxy acetic acid post-pollination treatment. Pollinations with *Tripsacum*, together with maize offer an extended crossing cycle of at least two months under our conditions and extends the range of alien species available for polyhaploid production. Reaching homozygosity will accelerate cereal breeding progress and include both spring and winter wheats based upon recent results (Inagaki dan Tahir 1990; Laurie and Reymondie 1991).

Other applications of polyhaploidy

a) Developing RFLP mapping populations. Towards RFLP mapping of wheat F_1 hybrids of three wheat cultivars and a synthetic hexaploid with high levels of polymorphisms were crossed with maize, haploids were produced which were cytologically analyzed and colchicine doubled giving rise to a doubled haploid population of at least 100 plants for each of the three combinations. Ruff "S"/*Ae. squarrosa* was the synthetic hexaploid and the wheat cultivars were Buc, Ciano 79 and Opata. Ruff "S"/*Ae. squarrosa* possesses resistance to Karnal bunt, *Septoria nodorum*, *Helminthosporium sativum* and tolerance to salinity; attributes to which the above wheat cultivars are susceptible upto various degrees.

b) Cytogenetic stocks for salt tolerance. Salt tolerance breeding through intergeneric hybridization has utilized two diploid ($2n=2x=14$) alien species of *Thinopyrum*; *bessarabicum* and *elongatum*; in our program. As one method the development of single disomic additions of these species in wheat is the first step for controlled salt tolerance screening followed by genetic manipulation to induce cryptic introgressions. Three aspects have influenced this addition line development that inclined us to use the polyhaploid system:

1) More than one alien chromosome influencing salt tolerance i.e. 3E, 4E and 7E of *Th. elongatum* as reported previously (Dvorak et. al., 1988). We produced the reciprocal combination to capture any cytoplasmic effects of *Th. elongatum* and incorporated a semi-dwarf commercial wheat Goshawk "S" in the cross.

- 2) In producing single disomic chromosomal additions poor paternal transmission constraints occurred for the *Th. elongatum* chromosomes, and
- 3) Since several chromosomes may influence salinity having disomic additions of the contributing chromosomes appeared plausible. Hence, producing polyploids from BCII plants with 43 to 45 chromosomes (21 bivalents + 1 to 3 univalents) would resolve not only the alien paternal chromosome transmission constraints, but also yield disomic and multiple disomic additions. These additions have to be characterized and tested for salinity. Initial fluorescence *in situ* hybridization analyses enabled identification of the alien *Th. elongatum* chromosomes

We have integrated the sexual route of haploid production with maize or *Tripsacum* routinely into the various facets of crop improvement, genetic analyses and cytogenetic areas. These aspects have been presented in this paper. Other alien sources like pearl millet (Ahmad and Comeau 1990), *Teosinte* (Ushiyama et al., 1991) and Sorghum (Ohkawa et al., 1992) emphatically augment our contention that the above methodology as a process for cereal crop improvement may become routinely adapted.

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