Polyhaploidy in the Triticeae Mediated by Crosses of *Triticum* Species with *Zea Mays* and *Tripsacum Dactyloides*.

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ABSTRACT

Triticum aestivum L. (2n=6x=42, AABBDD) polyhaploid production has relied heavily on anther culture and to a certain extent on wheat crosses with *Hordeum bulbosum*. Both approaches have constraints associated with genotype specificity and dominant *Kr* alleles on homoeologous group 5. More recently, wheat polyhaploids have been obtained through wheat x maize crossing. The cross success was independent of the material wheat or paternal maize genotypes. The crossing and embryo rescue procedures have since been modified in our laboratory yielding substantially improved wheat polyhaploid frequencies. Embryo recoveries from our first experiment ranged from 16 to 41 percent (%); a mean of 29.0%; with a 81.0% germination success and a colchicine doubling rate between 60.0 and 70.0%. The procedure has been successfully applied to *T. turgidum*, to a project on RFLP mapping of wheat aimed at producing inbreds derived from F_1 hybrids of polymorphic *T. aestivum* cultivars x polymorphic *T. turgidum/T. tauschii (Aegilops squarrosa)* synthetic hexaploids and to synthetic hexaploids between durum /*T. tauschii* (2n=6x=42, AABBDD). Application of this procedure has been made to study the development of multiple disomic chromosome additions in advanced backcross derivatives from the *Thinopyrum elongatum* x *T. aestivum* hybrids and haploid production from some Triticeae x *Tripsacum dactyloides* crosses, the latter being a novel means of obtaining haploids.

INTRODUCTION

Haploid production of the Triticeae cereals has mostly relied on anther culture and sexual crossing with *Hordeum bulbosum* L. Limitations to these haploid production strategies include aneuploidy, somaclonal variation and genotypic specificity in the former approach, with the homoeologous group 5 crossability loci (*Kr1, Kr2, Kr3*) being the essential limiting factor in the latter method (Falk and Kasha 1981). In order to avoid tissue culture associated somaclonal variation, a sexual route to haploid production is seemingly more desirable. Recently, crosses of wheat and other members of the Triticeae with maize have been suggested as an alternative sexual route for haploid production (Laurie and Bennett 1986, 1988b). Since maize appears to be insensitive to the *Kr* crossability alleles of wheat (Laurie and Bennett 1987), polyhaploids can be recovered across different genotypes (Suenaga and Nakajima 1989; Inagaki and Tahir 1990), thus making it potentially superior to the *H. bulbosum* system. In addition, gametoclonal variation induced in doubled haploid lines using the maize system was simialr to that found in doubled haploids obtained from wheat x *H. bulbosum* L. crosses. The use of 2,4-D in promoting seed set and embryo formation in wheat x maize crosses is critical (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). In this paper we report successful polyhaploid embryo production of additional *Triticum aestivum* and *T. turgidum* L. cultivars, and of a few *T. turgidum* x *Aegilops squarrosa* L. synthetic hexaploids following crosses with *Zea mays* using a detached tiller culture method. Cytological features of these polyhaploids and their doubled progeny are also characterized. Additionally is reported an extension of polyhaploid production limits of the Triticeae through crossing with *Tripsacum dactyloides*. Application of the above procedures in the development of alien disomic and multiple disomic chromosome additions of the salt tolerant *Thinopyrum elongatum* to *T. aestivum* is demonstrated.

MATERIALS AND METHODS

Plant material: Field grown plants of breadwheat (*Triticum aestivum*), durum wheat *(T. turgidum),*

T. turgidum x Aegilops squarrosa amphiploids and *Tripsacum dactyloides (Zea mays)* grown at El Batan, CIMMYT, Mexico, were used (Table 1). A bulk sample of pollen from several maize cross-pollinating populations and *T. dactyloides* was used for all crosses (Table 1). Backcross II plants from *Thinopyrum elongatum/3* T. aestivum* with 43 to 45 chromosomes were maintained in the greenhouse at El Batan. These plants were crossed with *Z. mays* as the pollen source. The environmental regimes were 15 hours light, 24°C day, 12°C night temperature and approximately 60% relative humidity. The crossing, detached spike culture, embryo rescue, plant regeneration and transplantation procedures were similar to those reported earlier (Mujeeb-Kazi et al. 1987; Riera-Lizarazu et al. 1990; Riera-Lizarazu et al. 1992).

Cytogenetic Analysis

Cytology: Somatic cytology of all regenerated plants utilized the aceto-orcein method (Mujeeb-Kazi and Miranda 1985). Meiotic analyses used a modified alcoholic carmine procedure (Snow 1963; Mujeeb-Kazi et al. 1993) for high contrast, intense staining and reduced stickiness. 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 inferrd from seed setting on the colchicine-treated polyhaploid plants.

RESULTS AND DISCUSSION

Sexual crossing between members of the Triticeae and maize offers a new alternate route to haploid production. This avoids somaclonal variation induced aneuploidy plus genotypic specificity associated with anther culture and the *H. bulbosum* system.

As reported by others (Inagaki and Tahir 1990; Laurie and Reymondie 1991) we also recovered haploid embryos from many different wheat genotypes following pollinations with maize. Our initial results (Riera-Lizarazu et al. 1990) gave mean values of 29.0% for embryo recovery, 81.0% for germination and a colchicine induced doubling frequency range between 60 to 70%. In addition, our results obtained by using detached tillers indicate that the use of the maize system, can be extended to recover polyhaploids in durum wheats and *T. turgidum* x *Ae. squarrosa* amphiploids (Table 1). There was a wide range of embryo recovery frequencies in this experiment among hexaploid wheats, tetraploid wheats and the *T. turgidum* x *Ae. squarrosa* synthetic hexaploids with recovery averages of 15.6, 16.9, and 19.8%, respectively (Table 1). These frequencies were 20.6, 26.8 and 23.5% for the embryos derived from crosses with *T. dactyloides*. Mean plant regeneration frequencies calculated as a percentage of embryos recovered for bread wheats, durum wheats and *T. turgidum* x *Ae. squarrosa* amphiploids were 68.6, 73.9, and 74.6%, respectively for polyhaploids from maize. These frequencies were 78.5, 66.7 and 75.5% from *T. dactyloides* polyhaploid derivation. Successful chromosome doubling of polyhaploid plants treated with colchicine averaged 60.7% for *T. aestivum* cultivars, 69.5% for *T. turgidum* cultivars and 63.6% for the synthetic hexaploids (Table 1). The polyhaploids obtained from the *T. dactyloides* procedure were not subjected to colchicine induced doubling since we felt that this aspect has established a routine trend. Our average doubled haploid plant recovery frequencies for *T. aestivum, T. turgidum* and the synthetic hexaploids ranged from 63.6 to 69.5%, average embryo excision frequencies were 15.6 to 19.8%, and the mean plant regeneration frequency range was 68.6 to 74.6% (Table 1). The wheat polyhaploid plant production frequencies obtained in this study more than adequately meet the economical threshold level reported (Comeau et al. 1988). The low haploid embryo recovery frequencies in this study as compared with earlier findings (Riera-Lizarazu and

Table 1. Embryos produced, recovery percentage, plants regenerated and colchicine doubled of *Triticum aestivum* L., *T. turgidum* L. and *T. turgidum* x *Aegilops squarrosa* L. lines following crosses with maize and *Tripsacum*

	T. aestivum		*T. turgidum*		Synthetics	
	M	T	M	T	M	T
Florets pollinated	2839	654	841	168	595	450
Embryos	442	1 5	142	45	118	106
Percent Produced	15.6	20.6	16.9	26.8	19.8	23.5
Plants Regenerated	303	106	105	30	88	80
Percent Regenerated	68.6	78.5	73.9	66.7	74.6	75.5
Plants Doubled	184	---	73	---	56	---
Percent Doubled	60.7	---	69.5	---	63.6	---

Mujeeb-Kazi 1990), is attributed to problems inherent to the detached tiller culture system.

Cytologically analyzed plants possessed the expected haploid complement of 21 chromosomes for *T. aestivum* and 14 chromosomes for *T. turgidum*, where each wheat parent had the euploid number of 2n=6X=42 or 2n=4X=28. Minimum aneuploidy was observed in the polyhaploid. Polyhaploids of *T. aestivum* cultivars and the synthetic hexaploids showed very low allosyndetic pairing. Ring bivalents were rare with chiasmata ranging from 0.44 to 1.96 per meiocyte (Table 2). The earlier reported (Riley and Chapman 1958) wheat polyhaploid (n=3X=21) meiotic associations were 18.05 univalents + 1.38 bivalents + 0.07 trivalents. Subsequently (Kimber and Riley 1963), was reported for bread wheat a mean frequency of 19.18 univalents + 0.90 bivalents + 0.008 trivalents from analyses of eight euhaploids; values indicating very low allosyndetic pairing. This degree of chromosome pairing is consistent with our data where the *T. aestivum* polyhaploids of several cultivars 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 low pairing indicates that the wheat cultivars and the synthetic hexaploid used had the dominant *Ph* locus that restricts homoeologous pairing and that the 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 based polyhaploids (Table 2) derived from *T. dactyloides* crosses.

The use of the maize system for haploid production in the Triticeae is very encouraging since stringent genotype specificity is not apparent. Like with maize, polyhaploid production in the Triticeas with *Tripsacum* is dependent upon a 2,4-dichlorophenoxy acetic acid post-pollination treatment that promotes embryo development. No strong genotypic specificity is prevalent. Pollination with *Tripsacum*, together with maize offer an extended crossing cycle of at least two months under our conditions, in addition to extending the range of alien species available for producing polyhaploids in the Triticeae. Reaching homozygosity in earlier generations will accelerate cereal breeding progress. Recent results (Inagaki and Tahir 1990; Laurie and Reymindie 1991) corroborate this contention where high frequency haploid production has been reported in spring and winter wheat x maize crosses.

Other applicatios of polyhaploidy

a) Developing RFLP mapping populations. The D genome linkage map is being exhaustively developed in molecular laboratories to which the highly polymorphic synthetic hexaploids (*T. turgidum* x *Ae. squarrosa*); as a consequence of *Ae. squarrosa*; shall positively contribute. So far, three *T. aestivum* cultivars (Buc, Ciano 79, Opata) have been identified as being highly polymorphic (M. Sorrells, personal communication) together with the synthetic Ruff "S"/*Ae. squarrosa* (seed source Dr. Dhaliwal,

India). The F_1 hybrids of these three wheat cultivars with the synthetic hexaploid upon crossing with maize, cytological analyses and colchicine doubling have built the double haploid population indicated in Table 3. 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 variours degrees.

Table 2. Mean chromosome pairing at metaphase I in some polyhaploids of *Triticum aestivum* L. and of *T. turgidum* x *Aegilops squarrosa* synthetic hexaploids derived from their crosses with maize (M) and *Tripsacum* (T)

Polyhaploid combinations	Metaphase I chromosomal associations (25 meiocytes)						
	I	Bivalents		Total II	Trivalents		Total III
		rings	rods		chain	pan	
T. aestivum x M	18.6 (15-21)	0.01 (0-1)	1.24 (0-2)	1.25	0.06 (0-1)	0	0.06
T. aestivum x T	17.7 (15-19)	0	1.63 (1-3)	1.63	0	0	0
Synthetic x M	20.1 (17-21)	0	0.44 (0-2)	0.44 (0-2)	0	0	0
					0	0	0
Synthetic x T	20.9 (19-21)	0	0.05 (0-1)	0.05	0	0	0

Table 3. Polyhaploid embryo production of three F_1 DNA polymorphic crosses between *Triticum aestivum* L. (cvs. Buc, Ciano 79, Opata) and a synthetic hexaploid (*T. turgidum* L. x *Aegilops squarrosa*) using the maize polyhaploid induction system. Also included are values for plants regenerated and doubled, and percentages in parenthesis

Characteristic observed	Buc/ Synthetic	Ciano 79/ Synthetic	Opata/ Synthetic
Number of Embryos	245	207	260
Plants Regenerated	172 (70.2)	154 (74.4)	180 (69.2)
Plants Doubled	107 (62.2)	115 (74.7)	136 (75.6)

b) Cytogenetics stocks for salt tolerance. Breeding for salt tolerance through intergeneric hybridization has utilized two alien species of *Thinopyrum; bessarabicum* and *elongatum*; in our program. Both species are diploids, 2n=2x=14. Development of single disomic additions of these species in wheat is the logical first step for controlled salt tolerance screening followed by genetic manipulation protocols to generate subtle introgressions. Three aspects have influenced this addition line development that have made us use the polyhaploid sexual 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) when *Th. elongatum* disomic addition lines in a *T. aestivum* cv. Chinese Spring background were tested for salinity. We have produced the reciprocal combination to capture any cytoplasmic effects and incorporated a semi-dwarf commercial wheat Goshawk"S" in the cross.

2) In producing single disomic chromosomal additions poor paternal transmission constraints occurred, and.

3) Because several chromosomes may influence salinity response having multiple disomic additions of

the contributing chromosomes seemed plausible. Hence, producing polyhaploids from BCII plants with 43 to 45 chromosomes (21 bivalents + 1 to 3 univalents) resolved the alien paternal chromosome transmission constraints, yielding disomic and multiple disomic additions (Table 4). These additions have to be characterized and tested for salinity. Initial fluorescence *in situ* hybridization analyses enabled identification of the alien *Th. elongatum* chromosomes.

Table 4. Polyhaploids derived by crosses of BCII *Thinopyrum elongatum/Triticum aestivum* cv Goshawk plants, their somatic cytology, doubled mitotic status and seed fertility

Number of Polyhaploid Plants	Somatic Chromosome Frequency	Doubled Somatic Range	Seed Set Range (/Plant)
342	22 (273)	42-44	24-183
	23 (57)	42-46	22- 83
	24 (12)	46	47-151

Integration of the sexual route of haploid production either with maize or *Tripsacum* into the various facets of crop improvement, genetic analyses and cytogenetic areas is fast becoming a routine process. These aspects have been presented in this paper. Other alien sources like pearl millet (Ahmad and Comeau 1990) and *Teosinte* (Ushiyama et al. 1991) augment the above contention.

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