Basic Studies relating to the Transference of Genetic Characters from *Triticum monococcum* L. to Hexaploid Wheat

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Abstract

Two techniques used for genetic transfer of stem rust resistance from einkorn (*T. monococcum*) to hexaploid wheat are described. Success of direct interspecific hybridization between wheat and einkorn was confined to the wild form, and is attributed to differences in survival and development of F_1 hybrids rather than crossability between the two species or germination of hybrid grains. Meiotic pairing between A genome chromosomes in interspecific hybrids was shown to be significantly lower in AABD than in AAB hybrids. These two interspecific hybrids also showed a different distribution pattern of functional female gametes.

Introduction

Each of the three genomes (designated A, B, and D) of common wheat (*Triticum aestivum* L. emend. Thell.) is derived from a diploid progenitor, and two of them are undoubtedly represented in present-day naturally occurring species. These correspond to *T. monococcum* L. (AA) and *T. tauschii* (Coss.) Schmal. (DD). The possible progenitor of the B genome continues to be the subject of much controversy (Johnson 1972; Kimber and Athwal 1972). The A and D progenitors of wheat therefore represent reservoirs of genetic variability which can be explored with a view to possible transference of useful attributes to the economically important hexaploid level. So far, they have been exploited only to a limited extent as sources of genes for resistance to various diseases (Kerber and Dyck 1969, 1973; Gerechter-Amitai *et al.* 1971; The 1973*a*).

Methods for the transference of a gene from *T. monococcum* to *T. turgidum* (durum) have been well documented (Vardi and Zohary 1967). Transference from such tetraploid derivatives to hexaploid wheat presents no technical problems. However, techniques which involve direct hybridization between *T. aestivum* and *T. monococcum* have not been reported. Studies of the early stages of these alternative approaches, together with associated problems and cytological behaviour, are discussed in this paper.

Materials and Methods

For practical purposes einkorn accessions can be divided into two forms, wild and cultivated; the former have brittle spikes which shatter at maturity whereas the spikes of cultivated einkorn disarticulate only upon threshing. However, both forms will be regarded as belonging to the same species, *T. monococcum.* A total of 19 wild and 23 cultivated einkorns, representing a diverse range in origins and types, were used (The 1973b). Most of these accessions were kindly provided by Dr R. Riley,

Plant Breeding Institute, Cambridge, England, and the remainder were received from All Union Institute of Plant Industry, U.S.S.R. Two durum cultivars, Glossy Huguenot W304* and Marruecos W2886 (Williams and Gough 1968), and two hexaploid wheat cultivars, Chinese Spring W1806 and TH3929 W1569, were chosen on account of their susceptibility to *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn. In addition, the hexaploid wheat cultivars were known to cross well with cereal rye. Three additional hexaploid wheat cultivars having relatively poor crossability with rye were also included for comparative studies.

The two methods of gene transfer from einkorn to hexaploid wheat can be summarized as follows:

- (1) Backcrossing the durum \times einkorn F_1 (female) to the durum parent until an AABB tetraploid genotype is derived. This derivative is in turn backcrossed to hexaploid wheat to obtain a stable derivative; or
- (2) Backcrossing the hexaploid wheat \times einkorn F_1 (female) to hexaploid wheat until a stable genotype is isolated.

Crosses were made using the einkorns as male parents. Reciprocal crosses were not attempted since einkorn does not possess durum cytoplasm, and einkorn cytoplasm induces male sterility (Hori and Tsunewaki 1967; Maan and Lucken 1968). The tetraploid or hexaploid wheats were used to pollinate the hybrids in producing the backcrosses. Since the latter parts of the backcrossing programmes are conventional, only observations involving the early generations will be reported.

Cytological observations were performed on materials prepared by the Feulgen technique. Mitotic chromosome counts were determined on root-tip preparations. In order to study chromosome pairing, meiotic observations were conducted at metaphase I in pollen mother cells (PMCs) of anthers individually fixed in acetic acid–alcohol (1:3 v/v).

Results

Crossability of Durum and Hexaploid Wheats with Einkorn

Crosses were made over two seasons under variable field conditions. The number of pollinated florets for each combination varied, and seed sets also fluctuated considerably. The cumulative results are presented in Table 1. Glossy Huguenot and Marruecos showed significant differences in crossability with einkorn. Very low seed sets were occasionally encountered amongst cultivars which normally cross well; this was probably due to unfavourable environmental conditions at the time of pollination. Marruecos was a technically more difficult cultivar to cross due to the presence of awns and the compact nature of the spike. Although this crossability difference could be genetic, technical difficulties and environmental factors probably caused some of the low seed sets observed in crosses involving this cultivar. No difference in crossability with einkorn could be detected between the cultivars Chinese Spring and TH3929. Generally, the wild einkorn produced more seeds than the cultivated einkorn when used in crosses with these hexaploid cultivars. However, there was no difference in crossability when the einkorns were used in crosses with the tetraploid cultivars.

Hexaploid wheats were suspected of having different crossabilities with einkorn, and this aspect was studied in some detail using the cultivated einkorn accession W10. Three additional wheat cultivars Steinwedel W199, Hope W517 and Timstein W2006 were included in this study. The results are presented in Table 2. It is obvious that Steinwedel, Hope and Timstein have very poor crossability with einkorn in comparison with Chinese Spring.

There were considerable differences in the development of the two classes of hybrid grains. Triploid AAB grains of durum \times einkorn crosses were invariably reasonably well filled, but the tetraploid AABD hybrid grains from wheat \times einkorn were very

* W numbers refer to the University of Sydney Wheat Accession Register.

small and shrivelled. Development of the endosperm in tetraploid types appeared normal for approximately 14 days after pollination, after which further development ceased. Hence, the endosperm at maturity was poorly developed, thus accounting for the severe shrivelling (Fig. 1).

Type of cross	No. of crosses	No. of florets pollinated	Seeds set	Contin- gency χ ² (1 d.f.)
Tetraploid \times diploid				
Glossy Huguenot				
× einkorn	20	532	301	
Marruecos			}	27.46***
× einkorn	23	592	ر 210	
Hexaploid $ imes$ diploid				
Chinese Spring				
\times einkorn	29	716	358	·
TH3929				3.43
\times einkorn	39	2020	1130	
Tetraploid $ imes$ diploid				
Durum ×				
wild einkorn	21	612	296)	
Durum \times culti-			}	2.49
vated einkorn	22	512	ر 215	
Hexaploid imes diploid				
Wheat \times				
wild einkorn	27	1497	915	
Wheat \times culti-			}	27.49***
vated einkorn	41	1239	573	

 Table 1. Crossabilities with einkorn types of two cultivars of durum and two cultivars of hexaploid wheat

*** Significantly different (P = 0.001).

 Table 2.
 Seed sets obtained in crosses of einkorn with durum (tetraploid) and bread wheats (hexaploid)

Parent	No. of florets pollinated	Seeds set	Percentage seed set
Glossy Huguenot (tetraploid)	162	97	60
Chinese Spring (hexaploid)	370	285	77
Steinwedel (hexaploid)	177	4	2
Hope (hexaploid)	122	8	7
Timstein (hexaploid)	52	0	0

Germination of F_1 Hybrid Grains

Triploid AAB hybrids displayed normal germination and survival. From a total of 239 hybrid grains, 13 failed to germinate, and these were not confined to any particular cross. On the other hand, low germination and survival rates were found with tetraploid AABD hybrid grains. Grains which developed roots or coleoptiles or both were scored as having germinated (Table 3).

No significant germination difference between hybrids of wild versus cultivated einkorn was evident. On the other hand, the cultivars TH3929 and Chinese Spring were very significantly different in hybrid seed germination rates. Tetraploid hybrids involving TH3929 gave an average germination rate of about 30%, compared with 19% for those of Chinese Spring.

Type of cross	No. of crosses	No. of seeds tested	Germin- ation %	Contin- gency χ^2 (1 d.f.)
TH3929				
× einkorn	70	846	30.1	
Chinese Spring			5	15.15***
× einkorn	34	324	18.9	
Wheat ×				
wild einkorn	48	626	28.9)	
Wheat × culti- vated einkorn	56	548	24.8	2.49

Fable 3.	Germination	of tetraploid	AABD	hybrid	grains	from
	crosses betwee	en hexaploid w	vheat an	d einko	rn	

*** Significantly different (P = 0.001).

Development and Fertility of F_1 Hybrids

Triploid AAB hybrids were vigorous, and generally taller than the durum parent; spikes tended to resemble those of the tetraploid parent. Hybrids were male sterile, and although anthers were formed, these were never observed to dehisce. In most florets the ovules and stigmas were of normal size and shape indicating receptivity. The fact that hybrids were male sterile was exploited in backcrossing, the necessity of emasculation being obviated.

In addition to the germination differences, tetraploid AABD hybrids differed markedly in their subsequent survival and development. A wide range of survival classes was observed from different crosses. Some hybrids died shortly after germination, whilst others developed a few leaves then died before shoot elongation. Others survived until the post-shoot elongation stage, but no reproductive organs were formed. The only useful hybrids were obviously those which developed reproductive organs. Anther development varied from absent to well formed but non-dehiscent (Fig. 2). Of 38 crosses involving TH3929 with 18 wild and 20 cultivated einkorns, only seven hybrids developed reproductive organs, and these crosses were confined to the wild einkorn accessions.

Fig. 1. (a) Left to right, mature grains of durum cultivar Marruecos, the triploid AAB F_1 hybrid, and einkorn accession C68.101. (b) Left to right, mature grains of wheat cultivar TH3929, the tetraploid AABD F_1 hybrid, and einkorn accession C68.113.

Fig. 2. Dissected florets. (a) Normal anthers and ovule of Chinese Spring. (b) Male sterile (nondehiscent) anthers and fertile ovule from a tetraploid AABD hybrid, TH3929 \times diploid accession C69.45. (c) Poorly developed ovule (sterile) from a tetraploid AABD hybrid, TH3929 \times diploid accession C68.120.





Female fertility of AAB and AABD F_1 hybrids is presented in Table 4. In 20 durum crosses involving different diploid accessions, an average seed set of $1\cdot3\%$ was obtained, whilst those of TH3929 were generally more fertile and showed wide variation. Three of the five AABD hybrids involving the diploid accessions C68.123, C68.125 and WS10 gave an average seed set of $2\cdot8\%$. The hybrid of C68.124 showed $7\cdot1\%$ setting, and the most fertile was that of C69.45 with a seed set of $15\cdot4\%$.

Type of cross	No. of crosses	No. of florets pollinated	Seeds set	Percentage seed set
AAB hybrid × durum	20	12806	172	1.3
AABD hybrid \times	3	2772	79	2.8
hexaploid	1	1654	117	7.1
-	1	3924	714	15.4

Table 4.	Fema	ıle ferti	lity o	f AAB	and	AABD	\mathbf{F}_1	hybrid
pollinated	with	durum	and	hexapl	oid	wheats	resp	ectively

Cytological Observations

Whenever possible, chromosome pairing was scored in 100 PMCs per cross, but a minimum of 50 cells was adopted. End-to-end associations were recorded as univalents. Trivalents were included in bivalent classes for computing the average number of bivalents formed; however, for chiasma frequency estimates, trivalents were assumed to have two chiasmata. Results of observations on triploid AAB and tetraploid AABD F_1 hybrid plants are presented in Table 5.

Generally, it can be concluded that there was no significant difference in chromosome pairing in crosses involving the durum cultivars. However, Glossy Huguenot and Marruecos differed slightly in chromosome structural arrangement as shown by the crosses with the diploids. Marruecos consistently formed 0.15 trivalents in all 14 hybrids studied. On the other hand, only 2 out of 18 crosses involving Glossy Huguenot showed a trivalent frequency of a comparable order. The data also show that only 2% of PMCs exhibited the expected pairing of seven bivalents (Fig. 3*a*). An overall average of 5.63 ± 0.18 bivalents (including trivalents) per cell was determined from pooled data.

Meiotic examinations of 11 AABD F_1 plants from four crosses indicated an average chromosome pairing of 3.94 ± 0.47 bivalents per cell. A typical meiotic cell, showing five of the seven possible bivalents, four of which are rods, is shown in Fig. 3b. Meiotic chromosome pairing with regard to bivalent formation in AAB and AABD hybrids differed significantly. In AAB hybrids 1.69 (5.63 - 3.94) more bivalents per cell were formed than in AABD hybrids. Differences in meiotic pairing are accentuated when the types of bivalents are considered. Bivalents in AAB hybrids averaged 1.62 ± 0.07 chiasmata compared with 1.30 ± 0.06 chiasmata for

Fig. 3. Meiotic configurations. (a) Triploid AAB hybrid of Marruecos \times W10, showing five closed bivalents, two rod bivalents and seven univalents. (b) Tetraploid AABD hybrid of TH3929 \times diploid accession C69.45, showing one closed bivalent, four rod bivalents and 18 univalents.

AABD hybrids. These values reflect the greater proportion of rod bivalents found in the tetraploid F_1 plants (compare Figs 3a and 3b).

Table 5.	Percentage frequencies of meiotic chromosome configuration	ns at
	metaphase I	

Data are for triploid AAB F1 hybrids of Glossy Huguenot (column A) and
Marruecos (column B) with einkorn, and for tetraploid AABD F1 hybrids
of TH3929 with four accessions of wild einkorn (column C).
' = univalents; " = bivalents; " = trivalents

Triploid AAB F1 Hybrids			Tetraploid AABD F ₁ Hybrid Meiotic			
configuration	Α	В	configuration	С		
7" + 7'	2.00	2.07	7" + 14'	0.27		
6" + 9'	58.86	57.65	6" + 16'	6.09		
5" + 11'	31.87	20.97	5'' + 18'	25.09		
4" + 13'	6.00	4.00	4" + 20'	28.73		
3" + 15'	0.53	0.14	3'' + 22'	23.36		
2" + 17'	0.07	· · · · ·	2'' + 24'	9.64		
1" + 19'		·	1" + 26'	1.09		
6'' + 6' + 1'''	0.07	0.48	6'' + 13' + 1'''	0.09		
4" + 7' + 2"		0.07	4'' + 14' + 2'''	0.09		
5'' + 8' + 1'''	0.60	10.28	5'' + 15' + 1'''	0.91		
4" + 10' + 1"		3.86	4'' + 17' + 1'''	1.55		
3'' + 12' + 1'''		0.48	3'' + 19' + 1'''	1.73		
2'' + 14' + 1'''		-	2'' + 21' + 1'''	1.09		
1" + 16' + 1"			1'' + 23' + 1'''	0.27		
Total	100.00	100.00		100.00		
Total cells				: .		
observed	1500	1450		1100		
No. of crosses						
studied	18	. 14		4		
Mean bivalent			к.			
frequency	5 · 59	5.68		3.94		
	+0.18	+0.17		+0.47		
Number of chias-	— .	_		1 . .		
mata per cell	8.95	9.36		5.16		
	± 0.58	± 0.62		± 0.78		

In order to determine the breeding behaviour of AAB and AABD F_1 hybrids, chromosome counts were made on backcross seedlings. This information was used to deduce the probable chromosome numbers of the female gametes which produced the backcross seedlings. These results are presented in Table 6.

Approximately 75% of functional female gametes of AAB hybrids had 14 or 15 chromosomes, and subsequent cytological studies confirmed that they were essentially of AB genomic constitution. About 7% of the gametes contained the wholly reconstituted parental chromosome complement of AAB.

Only 5% of the functional gametes of AABD hybrids possessed 21 ± 1 chromosomes. Reconstituted gametes with the approximate parental AABD constitution of 28 ± 1 chromosomes represented 64% of the population. In contrast to AAB hybrids, there was a strong tendency for AABD hybrids to retain the extra A

genome chromosomes in the gametes produced. These observations appear to reflect the greater pairing ability and hence the more normal anaphase and telophase processes in the AAB hybrids which had 5.63 bivalents per cell in contrast to AABD hybrids with 3.94 bivalents. It would appear the process of reconstituting the entire chromosome complement was more successful in producing functional gametes than a random reductional process. This was further supported by the fact that AABD hybrids were more fertile than AAB hybrids.

> Table 6. Chromosome numbers (N) of functional female gametes of triploid AAB and tetraploid AABD F_1 hybrid plants inferred from seedling chromosome counts on backcross progenies

	Values in parenthesis are percentage frequencies								
-	Triploid AAB F_1 hybrids N Frequency			Tetraploid AABD F_1 hybr N Frequency					
	<13	7	(5.60)		< 20	5	(2.86)		
	13	8	(6.40)		20	2	(1 · 14)		
,	14	48	(38.40)		21	4	(2.29)		
	15	47	(37.60)		22	- 3	(1.71)		
	16	3	(2.40)		23	2	(1 · 14)		
1	17				24	6	(3.43)		
	18				25	10	(5.71)		
	19				26	26	(14.86)		
	20	3	$(2 \cdot 40)$		27	43	(24 · 57)		
	21	9	(7.20)		28	52	(29.72)		
	22				29	17	(9.71)		
	> 22				> 29	-5	(2.86)		
	Total	125	(100.00)			175	(100.00)		

Discussion

The success or failure of interspecific hybridization involving einkorn and tetraploid or hexaploid wheats was found to depend on several distinct factors. These included firstly crossability, secondly, germination and subsequent survival of the hybrid, and thirdly, the development of the hybrid plants, i.e. whether reproductive organs were produced. Most of the failures in obtaining hexaploid \times diploid hybrids in the present studies can be attributed to the poor survival and development of the majority of hybrids. However, variability was recognized for the ability to produce some female fertile F₁ hybrids. Types with female fertility in hybrids were confined to the wild forms of *T. monococcum*. However, this degree of difference appears insufficient to merit separate specific classification for cultivated and wild forms.

Triploid AAB and tetraploid AABD hybrids showed different breeding behaviours. The higher fertility of AABD hybrids was unexpected, because they possess more univalents and show less pairing between the A genome homologues than triploid AAB hybrids. Triploid hybrids lose the extra A genome more effectively than AABD hybrids, and thus mainly produce functional gametes of the AB genomic type. Tetraploid AABD hybrids tend to reconstitute the whole chromosome complement, probably due to the large number of univalents, although this process was not fully effective. A similar result was reported by Vardi (1971) in an interspecific hybrid between durum and *T. speltoides*.

Triploid AAB hybrids showed an average meiotic chromosome pairing of 5.63 ± 0.18 bivalents, presumably between the A homologues. This result is consistent with those reported earlier, e.g. 5.22 bivalents by Vardi and Zohary (1967) and 5.49 bivalents by Kerber and Dyck (1973). The slight differences probably can be attributed to the different durum cultivars used as parents, differences in environments, or the sample sizes studied.

Chapman and Riley (1966) noted that mean pairing in tetraploid AABD hybrids ranged from 3.06 to 5.30 bivalents. The mean bivalent score of 3.94 ± 0.47 obtained in this study confirms the relatively low pairing of A genome chromosomes in tetraploid AABD hybrids, especially when this is compared with pairing exhibited in hexaploid wheat \times durum hybrids where 14 or 13 bivalents are regularly observed, indicating very good pairing between the A genome homologues. Riley and Chapman (1960) studied meiotic pairing between the D genome chromosomes in a hexaploid wheat \times T. tauschii hybrid, and found an average of 6.54 bivalents per PMC. This observation supports the contention that the D genome in hexaploid wheat is of more recent origin than the A genome and is consequently less modified. Three tentative explanations can be presented to account for the lower pairing in AABD hybrids: firstly, the extra D genome may in some way affect pairing between the A genome chromosomes; secondly, the presence of the D genome could increase the frequency of rod bivalents to a small extent due to B-D homoeologous pairing; or thirdly, as already suggested, the greater divergence of einkorn from the A genome of hexaploid than from that of tetraploid wheat would be reflected in reduced homology.

Since considerable variation was shown to exist in the survival of tetraploid AABD hybrids, direct hybridization of einkorn and hexaploid wheat is limited in its applicability as a technique of gene transfer. The established technique of gene transfer through the triploid AAB hybrid, however, could be completed more rapidly if the fixation of tetraploid derivative (AABB) is obviated. Since triploid AAB hybrids produce a large proportion of AB functional gametes, such plants can be pollinated with hexaploid wheat to produce AABBD pentaploid populations for use in further backcrossing.

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