EXTRACTION OF ANCESTRAL CONSTITUENTS OF NATURAL POLYPLOIDS. I. PRODUCTION OF PENTAPLOIDS (AABBD) FOR EXTRACTING THE TETRAPLOID (AABB) COMPONENT OF THE HEXAPLOID, *TRITICUM AESTIVUM**

K. A. SIDDIQUI

Atomic Energy Agricultural Research Centre, Tandojam.

Abstract

To extract the ancestral tetraploid component, AABB, of the allohexaploid, Triticum aestivum ($2n=6x=42$), *AABBDD, it is necessary to eliminate one genome, that is, one-third of the genotype. An attempt was made to do this by producing pentaploids* ($2n=5x=35$), *AABBD, involving 9 standard varieties of* T. aestivum *(Chinese Spring, Peko, Koga, Svenno, C 591, A.T. 38, April Bearded, C 518 and H-23-42) with 9 tetraploid* ($2n=4x=28$), *AABB, varieties of* Triticum *(Carleton, Mechmudy, Iumillo, Nursi, Samra, Kubankum, T1, 2D 1 and 33D 1) in a number of combinations. Chinese Spring disomic was the most successful hexaploid parent giving a seed set of 22.5% which was more than 3 times than that found with any other hexaploid parent (1 to 7%). Crosses of the tetraploid variety Machmudy with Chinese Spring disomic produced highest (48%) seed set. No seed was obtained by crossing a synthetic tetraploid,* Triticum monococcum x Aegilops speltoides *with varieties (Chinese Spring disomic, Koga and A.T. 38) of* T. aestivum.

Introduction

Interspecific hybridization of tetraploid ($2n=4x=28$, AABB) and hexaploid ($2n=6x=42$, AABBDD) wheats is necessary for producing the pentaploids ($2n=5x=35$, AABBD). These pentaploids are used for the extraction or reconstitution of the ancestral tetraploid (AABB) component of the allohexaploid, *Triticum aestivum* (Kerber 1964, Siddiqui 1964).

Information on the crossability of different monosomic lines (Sears 1959) is important in many cytogenetic studies (Morris *et al.* 1968). Interspecific crosses also offer the possibilities of producing new monosomics and nullisomics (Siddiqui 1964, 1966).

*This is a part of the thesis submitted to the University of Reading, England for the degree of Ph.D.

The present paper deals with the interspecific crosses involving a number of natural and synthetic tetraploids and hexaploids including some monosomic lines.

Materials and Methods

Seed of *Triticum aestivum* (2n=6x=42, AABBDD) var. Chinese Spring (disomic and monosomics) and the synthetic tetraploid *Triticum monococcum* x *Aegilops speltoides* (2n=4x=AABB), was obtained from Dr. E. R. Sears, Missouri, U.S.A.

Four Pakistani varieties (A. T. 38, C 591, C 518 and H-23-42) of *T. aestivum* were obtained from Agricultural Research Institute, Tandojam and seed of *T. sphaerococcum* (2n=6x=42) and *T. durum* var. T1 (2n=4x=28) was supplied by the Agricultural Research Institute, Lyallpur, West Pakistan.

Other material used in the studies viz. varieties Peko, Koga and Svenno of *T. aestivum*; varieties Carleton, Machmudy, Iumillo, Nursi, Samra, Kubankum of *T. durum* and varieties 2D1 and 33D1 of *T. dicoccum* (2n=4x=28) were available in the Department of Agricultural Botany, University of Reading, England.

For the production of F_1 pentaploids, hexaploids were crossed with tetraploids. Florets were pollinated three days after emasculation. Pollen fertility was estimated by squashing anthers in aceto-carmine glycerol jelly (Marks 1954).

Results

The results of all the crosses made with hexaploid varieties are summarised in Table 1. Normal disomic plants of the variety Chinese Spring were the most successful hexaploid parents. The seed set of 22.5% obtained with this variety was 3 times more than that found with any other hexaploid parent (1 to 7%). Monosomic plants of Chinese Spring gave a seed set of about 7%, considerably lower than that of disomic plants of the same variety. Two of the Pakistani varieties C 591 and H-23-42 were the least successful in the crosses, the seed set being 1-2 per cent only.

Ninety one per cent of the seeds from these tetraploid x hexaploid crosses germinated. Some of the hybrid plants died at the seedling stage but 88% grew to maturity. Similar results were recorded by Opeke (1961).

The results of the crosses in which tetraploid varieties were used as pollen parents are summarised in Table 2. The variety Machmudy appeared to be most promising (18% seed set). The differences between the number of seed set with the other varieties of *T. durum* (varying from 7 to 12%) were not very marked although crosses with Samra were relatively unsuccessful (5%). With the other varieties of *T. dicoccum*, 33 D1 seed set was very poor (2-3%).

TABLE 1

Results of the crosses between varieties and species of hexaploid wheats with tetraploid species of* Triticum

	6x parent	No. 4x parents used in crossing	No. florets pollinated	No. seeds obtained	% seed set	No. seeds sown	No. seeds germinated	No. hybrid (5x)** plants	Survived %
	T. aestivum								
1*a*	Chinese Spring (disomic)	8	712	160	22.5	112	100	94	94
1*b*	Chinese Spring (monosomics)	5	698	52	7.5	39	34	31	91
2	Peko	5	472	26	6	21	21	20	95
3	Koga	9	551	27	5	18	18	10	56
4	Svenno	5	295	12	4	11	10	6	60
5	C 518	3	69	5	7	5	5	5	100
6	A.T. 38	5	179	7	4	6	6	4	67
7	April Bearded	2	57	4	7	4	3	3	100
8	C 591	5	152	3	2	—	—	—	—
9	H-23-42	5	149	1	1	—	—	—	—
10	*T. sphaerococcum*	2	53	2	4	2	2	2	100
		Total	3387	299	9	218 (91 %)	199	175	88

* Including monosomics
** Including 34-chromosome plants

TABLE 2

Results of the crosses between varieties and species of tetraploid wheats with hexaploid species of* Triticum

4x parent	No. 6x parents used in crossing	No. florets pollinated	No. seeds obtained	% seed set	No. seeds sown	No. seeds germinated	No. hybrid plants	Survived %
T. durum								
1 Carleton	6	918	89	10	59	53	48	91
2 Machmudy	7	353	62	18	51	47	39	83
3 Iumillo	6	549	60	11	52	52	43	83
4 Nursi	5	190	22	12	8	2	2	25
5 Samra	6	306	15	5	12	10	9	90
6 Kubankum	5	144	13	9	6	6	6	100
7 T 1	8	239	17	7	16	15	15	100
T. dicoccum								
8 33 D 1	4	562	18	3	12	12	11	92
9 2 D 1	4	126	3	2	2	2	2	100
	Total	3387	299	9	218	199 (91%)	175	88

* Including monosomics

The details of seed set for all the hexaploid x tetraploid combinations are presented in Table 3. Three hexaploids, *T. aestivum* (C 518 and April Bearded) and *T. sphaerococcum* were used in a very few combinations only and these are excluded from the total and the mean. Crosses using the normal Chinese Spring disomics and Machmudy, (which were the most successful and 4x varieties respectively) gave the highest seed set (48%). Machmudy was not equally successful with all the 6x varieties and its higher mean success was mainly, but perhaps not entirely, due to the large number of seeds obtained when it was used as pollen parent with normal Chinese Spring disomics. All the tetraploid varieties (except Iumillo) were more successful when they were used to pollinate the Chinese Spring disomics than with any other 6x variety, and therefore the higher mean success of this variety can be attributed to its higher general crossability with tetraploid wheats.

Some details of cross between Chinese Spring disomics and tetraploid varieties are given in Table 4. The variety 33 D1 of *T. dicoccum* gave the lowest seed set (9%) of all the tetraploids when it was used to pollinate the Chinese Spring disomic.

All the hybrid seed germinated well (80 to 100%) except that obtained from the Chinese Spring x Nursi cross (25% germination).

The crossability of different monsomics of Chinese Spring with tetraploid wheat is recorded in Table 5. The results obtained when fewer than 50 florets were pollinated cannot be taken as representative. The crosses with *T. durum* var. Carleton as pollen parent were much more successful on Chinese Spring monosomic (1B) than on monosomic VI (6A) and XV (4 D), the seed set of 27% being similar to that of comparable crosses with Chinese Spring disomics.

The low seed set in many combinations may be due to the low crossability of *T. dicoccum* (33 D1) which also gave poor seed set in crosses with disomic hexaploid varieties.

Varieties of *T. aestivum* were crossed with a synthetic tetraploid 'AABB' (*T. monococcum* x *A. speltoides*). During 1962, Koga was used as pollen parent and 30 florets of the 'AABB' were pollinated but no seed was produced. During 1963, 310 florets of the 'AABB' were pollinated with A.T. 38. None of these crosses was successful. 'AABB' was also used as pollen parent in crosses with Chinese Spring disomic. Ninety florets were pollinated but no seed was obtained. On self-pollination of 156 florets 'AABB' produced 26 seeds in 1962 but self-pollination of 355 florets of the tetraploid did not produce any seed during 1963. Whatever factor was responsible for this seasonal difference may also affect the success of crosses, as most of the crosses with the synthetic tetraploid were made during 1963.

TABLE 3

Seeds obtained in crosses between hexaploid and tetraploid species and varieties of* Triticum.
Number seeds obtained/number florets pollinated (percentage seed set)

Female parents	*Carleton*	*Machmudy*	*Iumillo*	*Nursi*	*Samra*	*Kubankum*	*T1*	33 D 1	2 D 1	*Total or mean*
Chinese Spring (disomic)	46/190 (24)	43/89 (48)	18/152 (12)	22/92 (24)	6/20 (30)	9/46 (20)	9/44 (20)	7/79 (9)	..	160/712 (22.5)
Chinese Spring (monosomics)	27/210 (13)	..	8/20 (40)	..	5/62 (8)	3/18 (17)	..	9/388 (2)	..	52/698 (7.5)
Peko	8/202 (4)	9/52 (17)	9/128 (7)	..	..	..	0/42 (0)	..	0/48 (0)	26/472 (6)
Koga	3/168 (2)	3/53 (6)	18/133 (14)	0/40 (0)	1/24 (4)	1/24 (4)	0/18 (0)	0/80 (0)	1/11 (9)	27/551 (5)
Svenno	5/103 (5)	1/72 (1)	6/54 (11)	..	..	..	0/18 (0)	..	0/48 (0)	12/295 (4)
C 518	..	..	1/37 (3)	..	..	..	2/13 (15)	..	..	5/69 (7)
A. T. 38	..	4/33 (12)	..	0/20 (0)	1/48 (2)	0/20 (0)	2/58 (1)	..	..	7/179 (4)
April Bearded	..	..	0/25 (0)	..	..	..	4/32 (13)	..	..	4/57 (7)
C 591	0/27	2/35 (5)	..	0/18 (0)	1/58 (2)	..	0/14 (0)	..	..	3/152 (2)
H-23-42	0/18 (0)	0/19 (0)	..	0/20 (0)	1/56 (2)	0/36 (0)	..	..	2/19 (11)	1/49 (1)
T. sphaerococcum					0/38 (0)			2/15 (13)		2/53 (4)
Total or Mean (Excluding *T. sphaerococcum* and figures in oblongs and including monosomics)	89/918 (10)	62/353 (18)	59/487 (12)	22/190 (12)	15/268 (6)	13/144 (9)	11/194 (6)	16/547 (3)	3/126 (2)	

*Including monosomics

TABLE 4

Results of crosses between Chinese Spring disomic (female) and varieties of tetraploid wheats

4x *pollen parent*	*No. florets pollinated*	*No. seeds obtained*	*% seed set*	*No. seeds sown*	*No. seeds germinated*	*No. hybrid plants*	*% survived*
Carleton	190	46	24	28	26	26	93
Machmudy	89	43	48	38	34	28	74
Iumillo	152	18	12	18	18	18	100
Nursi	92	22	24	8	2	2	25
Samra	20	6	30	5	5	5	100
Kubankum	46	9	20	3	3	3	100
T1	44	9	20	9	9	9	100
33 D 1	79	7	9	3	3	3	100
Total or mean	712	160	22.5	112	100 (89%)	94	84

Discussion

The first crosses between the hexaploid and tetraploid species and the back-crosses of the pentaploid hybrids to their hexaploid parents all involve two plants with different numbers of chromosomes. Such crosses are usually more successful when the plant with the higher number of chromosomes is used as the female parent than in the reciprocal direction (Thompson 1930, Wakakuwa 1934).

Consistent with the results of these workers the seeds of the F_1 pentaploids produced by using hexaploid varieties as female parents, were of moderate size and 91% of those which were sown germinated (Table 1). Stebbins (1958) discussed the reciprocal crosses in *Triticum* and other genera with reference to the inviability, weakness and sterility of hybrids and suggested that the reciprocal differences are due principally to differences in dosage of genes and genomes in the endosperm and not merely to abnormal chromosome number relationships between embryo, endosperm and maternal tissue.

Three considerations influenced the selection of material for the present extraction studies. First, the great diversity in both *T. aestivum* and *T. durum* wheats prompted the use of a number of varieties of each species. Second, so that the extracted components might be useful in future cytogenetic studies and be comparable with previous aneuploid analysis, the variety Chinese Spring was used. Of all varieties of wheat, Chinese Spring has been used most extensively in monosomic, nullisomic and substitution studies by other workers (Sears 1959, Morris *et al.* 1968). Further, since all the monosomics and nullisomics are already available in Chinese Spring, it is easier to extract all the three tetraploid (AABB, AADD and BBDD) components of Chinese Spring by the 'monosomic and nullisomic' method of extraction. Thus with this variety, and with others in which monosomic lines have been produced more recently, there are possibilities of comparing extractions accomplished by two different methods. Also with Chinese Spring it will be possible to compare and correlate cytogenetic information that is obtained from different methods of analysis. Third, it was hoped that the extracted components might be of value in breeding programmes, and therefore some reputable varieties of bread wheat were selected *e.g.*, Koga, Svenno and C 518. The extracted tetraploids AABB, AADD and BBDD of the varieties might be useful agricultural plants. They might also facilitate the genetic analysis of respective varieties and thereby their future improvement.

Acknowledgment

The author is grateful to Prof. A. H. Bunting and Dr. J. K. Jones of the University of Reading, England for guidance and help.

TABLE 5

Results of crosses between Chinese Spring monosomics (CSM) (females) and varieties of tetraploid wheats

	No. florets pollinated	No. seeds obtained	% seed set	No. seeds sown	No. seeds germinated	No. hybrid plants	% survived
A & B monosomics							
CSM I x Carleton	80	21	27	10	7	6	86
CSM IV x 33 D 1	37	3	8	3	3	3	100
CSM VI x Carleton	50	4	8	4	4	4	100
CSM VI x Iumillo	20	8	40	8	8	8	100
CSM XIII x Kubankum	18	3	17	3	3	3	100
D monosomics							
CSM XV x Carleton	80	2	3	2	2	2	100
CSM XV x 33 D 1	120	1	1	1	1	1	100
CSM XVI x 33 D 1	40	..	..	..	..	..	..
CSM XVII x 33 D 1	40	..	..	..	..	..	..
CSM XVIII x Samra	20	..	..	..	..	..	..
CSM XVIII x 33 D 1	71	1	1	1	1	1	100
CSM XIX x Samra	42	5	12	5	3	2	40
CSM XXI x 33 D 1	80	4	5	2	2	1	50
Total or mean	698	52	7.5	39	34 (87%)	31	79
Summary of crosses							
Chinese Spring monosomics							
x Carleton	210	27	13	16	13	12	92
x Iumillo	20	8	40	8	8	8	100
x Samra	62	5	8	5	3	2	40
x Kubankum	18	3	17	3	3	3	100
x 33 D 1	388	9	2	7	7	6	86
Total or mean	698	52	7.5	39	34 (87%)	31	79

References

Kerber, E. R. 1964. Wheat: Reconstitution of the tetraploid component (AABB) of hexaploids. Science **143:** 253-255.

Marks, G. E. 1954. An aceto-carmine glycerol jelly for use in pollen fertility counts. Stain Tech. **29:** 277.

Morris, R., J. W. Schmidt, P. J. Mattern, and V. A. Johnson, 1968. Quality tests on six substitution lines involving "Cheyenne" wheat chromosomes. Crop Sci. **8:** 121-123.

Opeke, L. K. 1961. Ph.D. Thesis. University of Reading, England.

Sears, E. R. 1959. The aneuploids of common wheat. Proc. First Int. Wheat Genetics Symposium, Winnipeg, 221-229.

Siddiqui, K. A. 1964. Ph.D. Thesis. University of Reading, England.

——— 1966. General Discussion on Aneuploidy in Wheat Genetics. Proc. Second Int. Wheat Genetics Symposium, Lund. Hereditas Suppl. **2:** 392.

Stebbins, G. L. 1958. Hybrid inviability, weakness and sterility. Advances Genet. **9:** 147-215.

Thompson, W. P. 1930. Causes of difference in success of reciprocal interspecific crosses. Amer. Nat. **64:** 407-419.

Wakakuwa, S. 1934. Embryological studies on the different seed development in reciprocal interspecific crosses of wheat. Jap. J. Bot. **7:** 151-187.