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NEW HYBRIDS WITH D-GENOME WHEAT-RELATIVES

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*Contribution from the Missouri Agricultural Experiment Station.
Journal Series Number 9358

ABSTRACT

The cytology of nine new D-genome hybrids involving **Triticum syriacum, T. ventricosum, T. cylindricum, T. juvenale, T. crassum, T. tauschii** and **T. aestivum** is described. The calculation of numerical values of the relative affinity and the patterns of chromosome pairing indicate that the D genome in **T. syriacum** and **T. juvenale** may have been substantially modified and that of **T. crassum** somewhat modified from that of the diploid progenitor, **T. tauschii.**

Triticum aestivum (2n=6x=42), the bread wheat of commerce, is a cultivated species of paramount importance. It is perhaps the most widely and intensively cultivated of all domesticated plant species, a feature which has, paradoxically, led to a considerable erosion of its genetic variability. The probability of a disease epidemic causing widespread losses is therefore greatly enhanced, and this has given rise to an increased interest in locating and introducing new and alien sources of resistance. However, in order to be able to make the cytogenetical manipulations for the introduction of alien variation more logical, it is essential to understand the genomic relationships of the wild species to the cultivated forms (Kimber, 1983).

Genomic analysis was originally based on observations of chromosome pairing in hybrids between diploid analyzers and their polyploid relatives (see review by Lilienfeld, 1951). If the chromosomes paired in multiples of the basic number of the genus, it was assumed that there were genomes in common. Some difficulties were introduced when the number of chromosome pairs were not close to exact multiples of the basic number, and in these cases it was assumed that some genomic differentiation had taken place. Other problems were associated with hybrids in which both parents were polyploid, particularly so when the hybrid was pentaploid or higher. Also it is invalid, in most cases, to infer genomic relationships from chromosome pairing in diploid hybrids, for residual homoeology may lead to synapsis that may be interpreted as homology. Diploid hybrids can, however, be employed to show that two diploid species differ in genomic constitution. Even though the method was subjective, it worked well and provided an essentially correct picture of the evolution in the wheat group (and in other species also).

Recently (Kimber, Alonso and Sallee, 1981; Alonso and Kimber, 1981; Kimber and Alonso, 1981; Espinasse and Kimber, 1981) numerical techniques have been developed that allow measures to be obtained of genomic relationships. This allows some objectivity to be applied to genomic analysis. This technique has been used to confirm the A- and D-genome donors to **T. aestivum** and to show that there is no obvious candidate for either the B genome of **T. turgidum** and **T. aestivum** or the G genome of **T. timopheevii**. Kimber and Abu Bakar (1981) have

suggested that the C^u designation for the genome of **T. umbellulatum** be changed to U since this genome does not show any preferential pairing with the C genome of **T. dichasians.** Similarly, Kimber, Pignone and Sallee (1983) proposed that the M^u genome of **T. uniaristatum** be redesignated Un.

One of the major features of the evolutionary pattern of the wheat group is the clustering of species in which there is a common (pivotal) genome and one, or more, differential genomes (Zohary and Feldman, 1962). One cluster involves the U genome of **T. umbellulatum** and another the D genome of **T. tauschii.** While both groups are of evolutionary significance, the D-genome group has special interest, since any desirable genes in the pivotal genome may be introduced into the bread wheats simply by recombination. Consequently hybrids have been made which will allow an investigation of these genomic relationships. These hybrids appear not to have been recorded previously.

MATERIALS AND METHODS

The species used to produce these hybrids are maintained at the University of Missouri-Columbia, and the original seed was derived from several sources. The sources of the seed of the species used to make the hybrids are listed in Table 1.

Nine hybrids were produced: **T. tauschii** x **T. aestivum**, **T. syriacum** x **T. tauschii**, **T. cylindricum** x **T. crassum**, **T. syriacum** x **T. cylindricum**, **T. ventricosum** x **T. syriacum**,

T. ventricosum x **T. aestivum**, **T. ventricosum** x **T. cylindricum**, **T. juvenale** x **T. ventricosum** and **T. cylindricum** x **T. juvenale**. Meiotic analyses were made on PMC's stained by the Feulgen technique, and optimum values of x were calculated (Alonso and Kimber, 1981). These values, together with the observed value of c, were used to provide the basis for the interpretation of the meiotic data.

The mean arm-pairing frequency (c) varies from 0.0, when there are no chiasmata formed at all, to 1.0, when the maximum number of arms pair in every cell. In triploids it is calculated as the mean number of pairs of chromosome arms, with at least one chiasma, per cell divided by two times the basic chromosome number (Alonso and Kimber, 1981). In tetraploids and pentaploids it is calculated as the mean number of pairs of chromosome arms, with at least one chiasma, divided by four times the basic chromosome number (Kimber and Alonso, 1981).

In order to produce expressions that can be used to predict chromosome pairing in hybrids several assumptions must be made (Alonso and Kimber, 1981). First, the chromosomes are assumed to have a single, possibly terminal, pairing initiation site in each arm. Second, the initiation of pairing occurs independently on each side of the centromere. Third, there is no exchange of pairing partners once synapsis is initiated between two arms in a cell. And fourth, two or more genomes are assumed to be more closely related to each other and they are equally and more distantly related to the remaining genome or genomes. On these assumptions all possible meiotic configurations are drawn

and probabilities assigned to each individual meiotic figure. The probabilities are further divided according to whether the type of association involves the most closely or most distantly related chromosomes. The final expressions are obtained by summing all occurrences of each type of meiotic figure (Alonso and Kimber, 1981). If x is used to represent the relative affinity of the most closely related chromosomes and y the relative affinity of the most distantly related chromosomes then the final expressions are written in terms of x, y and c. Since it is not possible to obtain absolute measures of affinity a relationship between x and y is chosen arbitrarily (x+y=1). This choice allows limits to be placed on the values of x. Values of x equal or close to 0.5 indicate that the chromosomes of a homoeologous group are pairing with each other with similar facility. Values of x close or equal to 1.0 are taken to show a very high relative affinity and, when associated with values of c close to the maximum value expected for the best-fit model (Kimber and Alonso, 1981), to demonstrate homology of two or more genomes. The optimum value of x is calculated by minimum sums of squares of differences between the observed and calculated meiotic figures.

So that the expected pairing patterns may not be confused with alphabetic genomic symbolism the possible patterns are designated by an appropriate series of numbers (Alonso and Kimber, 1981). At the triploid level two possible pairing patterns are expected. First, when all three genomes are equally related, as in a hybrid with three different genomes or in an

autotriploid, and second when two genomes are more closely related to each other than they are to the third. These patterns are designated 3:0 and 2:1. At the tetraploid level there are four possible pairing patterns: 4:0, 2:2, 2:1:1 and 3:1 (Kimber and Alonso, 1981), and at the pentaploid level there are six: 5:0, 2:2:1, 2:1:1:1, 3:2, 3:1:1 and 4:1 (Espinasse and Kimber, 1981). The best-fit model is chosen as that model which gives the smallest sum of squares of differences between the observed and calculated meiotic figures. This model is usually accepted as the model describing the pattern of pairing, and the optimum value of x for this model is indicative of the relationships of the chromosomes present.

RESULTS AND DISCUSSION

At least two mature hybrid plants were obtained in eight of the nine combinations. The optimization analysis of the data collected from the PMC's of each plant was made separately, and only when it was observed that all sibs had a similar pairing pattern were the results amalgamated to provide the data presented in Table 2.

The three hybrids of **T. tauschii** x **T. aestivum** (ABDD) all fit the 2:1:1 model best with very high values of x. The fit between the observed and calculated meiotic figures in all of these hybrids was very good, as may be expected if the genome in common was unmodified. The six hybrid plants of **T. syriacum** x **T. tauschii** (DMSD) all fit the 2:1:1 model best but with a value

of x less than would be expected if the D genome of **T. syriacum** was not modified. The actual value of x may be slightly higher than calculated, for there is evidence of a translocation in this hybrid. Alonso and Kimber (1981) and Kimber (unpublished) have derived the equations for triploid hybrids with various possible translocation situations. From their calculations it would appear that the value of x obtained when there is a translocation is equal to or slightly lower than when a translocation is not present. It is assumed (Kimber and Alonso, 1981) that a similar situation would occur in tetraploid hybrids. The value of c (0.314) is lower than would be expected in a 2:1:1 hybrid with two homologous genomes (Kimber and Alonso, 1981).

The hybrid of **T. syriacum** x **T. cylindricum** (DMSCD) support these observations. Hybrids between **T. aestivum** and either **T. tauschii** or **T. cylindricum** fitted the 2:1:1 or 2:1:1:1 models very well (Kimber and Alonso, 1981; Espinasse and Kimber, 1981), demonstrating that the D genome of **T. cylindricum** is essentially unmodified from the D genome of **T. tauschii.** Consequently the fit to the 3:2 model and the low value of x in the hybrids of **T. syriacum** x **T. cylindricum** must show differentiation of the D genome of **T. syriacum.** The fit to the 3:2 model, rather than the 2:1:1:1, is interesting in this respect also. One cell in one hybrid actually contained seven trivalents, and this unusual event may have biased the observed data towards the 3:2 model. The next-best-fit model was in fact the 2:1:1:1. The optimum value of x calculated for the 3:2 model is the mean of the values of the relative affinity of the group

of three genomes and the group of two, and thus the actual affinity between the D-genome chromosomes may be higher than observed in this case.

The hybrids between **T. ventricosum** and **T. syriacum** demonstrated random, or close to random, association of the homoeologous chromosomes. The strong affinity of the D genome of **T. ventricosum** for the D genomes of **T. aestivum** and **T. cylindricum** is well demonstrated by the values of x and c in the hybrids between these species (Table 2). Further, homology with the D genome of **T. tauschii** with the D genome of **T. ventricosum** is well established (Kimber and Zhao, 1983). Consequently the reduced affinity of the genomes of **T. ventricosum** and **T. syriacum** must indicate modification or reduced homology of the genomes in **T. syriacum.** One cell with a sexivalent was observed in this hybrid, and this probably indicates translocation heterozygosity. The reciprocal of the hybrid **T. ventricosum** x **T. syriacum** has been recorded by Kimber, Pignone and Sallee (1983), and it also showed random association of the homoeologous chromosomes.

The hybrids between **T. juvenale** and **T. ventricosum** also showed random association of homoeologous chromosomes. The best fit of the individual hybrids was with the 3:1:1 (one case) and 4:1 (two cases) models, and in all cases the next-best fits were with models in which only one group of closely related chromosomes was expected (2:1:1:1, 3:1:1 or 4:1). The fits to the 2:2:1 and 3:2 models were always the poorest and with values of x equal or very close to 0.5. Radial multivalents were

observed, and this pattern of pairing is excluded by the assumptions made to derive the equations for the calculation of expected pairing (Alonso and Kimber, 1981). The low value of c also indicates the absence of homologous chromosomes, and the fit to various models is most probably a consequence of the random observation of occasional multivalents or not. Since some meiotic figures increase in some models with increasing values of x and decrease in other models, and since the optimum value of x is calculated as the minimum sum of squares of differences, then at low values of both x and c this pattern of results in sibs in a cross may be expected. Consequently, it is considered that the five genomes present in this hybrid are equally related to each other.

Espinasse and Kimber (1981) obtained best fits to both the 2:2:1 and 3:2 models for the data of McGinnis and Melnyk (1962) for the hybrid **T. juvenale** x **T. cylindricum**. Kimber and Alonso (1981) also from the data of McGinnis and Melnyk (1962) showed that the hybrid **T. juvenale** x **T. tauschii** fitted the 2:1:1 model best. It would appear from these hybrids and the hybrid **T. juvenale** x **T. ventricosum** that there is evidence that a D genome is present in **T. juvenale** but that substantial modification of that genome (and probably of the others also) has taken place.

The five hybrids of **T. cylindricum** x **T. crassum** all fit the 2:1:1 model best but with reduced values of x, indicating that the D genome of **T. crassum** is more remote from the D genome of **T. tauschii** than is the D genome of **T. aestivum** or

T. cylindricum. The hybrid of hexaploid **T. crassum** x **T. cylindricum** (Melnyk and McGinnis, 1962) fitted the 2:1:1:1 model best (Espinasse and Kimber, 1981) and it was assumed that the two D genomes of the hexaploid were pairing preferentially. The triploid hybrid **T. crassum** x **T. tauschii** has been recorded by Kihara (1949) and Kimber and Zhao (1983), but only the latter is amenable to optimization analysis. The value of x ranged between 0.87 and 0.92 in the seven hybrids observed and again is indicative of the differentiation of the D genome of **T. crassum.**

In the evolution of the polyploid complex associated with the D genome of the **Triticeae** it would seem that substantial modifications have taken place in some cases. The D genomes of both **T. juvenale** and **T. syriacum** have evolved, probably by the accumulation of genetic and structural changes, so that they are now demonstrably different from, but still clearly related to, the D genome of **T. tauschii**; while the D genome, or genomes, of **T. crassum** has not undergone such significant change. The D genomes of **T. aestivum, T. cylindricum** and **T. ventricosum** have apparently changed little during their evolution in the polyploid species. The level of the changes in all of these species may not be sufficient to indicate that new genomic symbols are required; however, the changes in both **T. juvenale** and **T. syriacum** are perhaps worthy of the addition of superscripts indicative of the degree of modification.

TABLE 1

The species, ploidy, genomes and source of the parental plants crossed to make the hybrids described in this paper.

Species	Ploidy	Genomes		Source[1]	UMC[2]
T. tauschii	2x	D	K	RL 5288	TD21
T. cylindricum	4x	CD	S	P68-36-2	TX01
T. ventricosum	4x	DL	S	P60-37-1	TH01
T. crassum	4x	DM	P	Line A	TI02
T. juvenale	6x	DMU	T	23-1	TJ01
T. syriacum	6x	DMS	P	**Ae. vavilovi**	TR02
T. aestivum	6x	ABD	S	Chinese Spring	TA11

[1] K = Kerber, University of Manitoba, Canada.
S = Sears, University of Missouri-Columbia.
T = Tanaka, Plant Germplasm Institute, Kyoto, Japan.
P = Plant Breeding Institute, Cambridge, England.;

[2] University of Missouri-Columbia accession number.

TABLE 2

Observed meiotic pairing (mean and range), x, c, best-fit model and genomic formulae in 41 plants in nine D-genome hybrid combinations.

Hybrid	Plants	Cells	I[1]	II	IIc	III	IV	IVc	V	VI	C	X	Model	Genomes
tauschii x aestivum	3	135	14.66 12-20	1.94 0-6	4.44 1-7	0.12 0-1	0.05 0-1	0.02 0-1			0.403	0.999	2:1:1	ABDD
syriacum x tauschii	6	84	13.89 7-21	3.89 0-9	1.14 0-3	0.75 0-3	0.37 0-2	0.00 0-0	0.05 0-1	0.00 0-0	0.314	0.873	2:1;1	DMS[1]D
cylindricum x crassum	5	154	8.51 2-16	3.32 0-7	2.68 0-5	1.84 0-5	0.39 0-3	0.01 0-1	0.04 0-1	0.00 0-0	0.485	0.887	2:1:1	DMCD
syriacum x cylindricum	2	26	12.15 5-23	6.07 2-10	1.27 0-3	2.08 0-7	0.39 0-2	0.00 0-0	0.08 0-1	0.00 0-0	0.509	0.890	3:2	DMS[1]CD
ventricosum x syriacum	3	120	18.39 9-33	4.99 1-11	0.46 0-2	1.50 0-5	0.21 0-2	0.00 0-0	0.06 0-1	0.01 0.1	0.358	0.688	3:1:1	DUnDMS[1]
ventricosum x aestivum	15	514	25.99 19-35	3.35 0-7	0.92 0-6	0.19 0-2	0.00 0-0	0.00 0-1	0.00 0-0	0.00 0-0	0.199	0.986	2:1:1:1	DUnABD
ventricosum x cylindricum	2	40	8.08 3-13	3.10 0-6	3.98 1-6	1.33 0-4	0.35 0-2	0.00 0-0	0.05 0-1	0.03 0-1	0.527	0.944	2:1:1	DUnCD
juvenale x ventricosum	4	96	20.39 12-31	4.41 1-9	0.45 0-3	1.25 0-4	0.25 0-2	0.00 0-0	0.04 0-1	0.00 0-0	0.348	0.500	5:0	DMCDUn
cylindricum x juvenale	1	11	14.36 10-21	5.18 2-7	1.09 0-3	2.45 0-4	0.18 0-1	0.00 0-0	0.00 0-0	0.00 0-0	0.475	0.500	5:0	CDDMU

I = univalents, II = rod bivalents, IIc = ring bivalents, III = trivalents, IV = open quadrivalents, IVc = closed quadrivalents, V = quinqevalents, VI = open sexivalents, C = mean arm pairing frequency, x = relative affinity of most closely related genomes.

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