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Phylogenetic relationships in some diploid species of *Triticineae*: cytogenetic analysis of interspecific hybrids

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Summary. Ten diploid species from genera *Triticum, Aegilops, Haynaldia* and *Secale* were included in a diallel crossing program. Forty-one different interspecific hybrids were obtained. The number of associations between chromosome arms at metaphase I of meiosis in pollen mother cells from the hybrids was taken as an indication of the degree of homology between parental genomes. Genome relationships were defined and indicated a possible pattern of differentiation from a common ancestor. Breeding strategies based on this information are proposed.

Key words: Phylogenetic relationships – *Triticineae* – Meiotic behavior – Homology

Introduction

Wild relatives of cultivated wheat are an important gene reservoir. Characters of particular interest for improvement of wheat are: high level and quality of grain proteins, disease and insect resistance, climatic adaptation, salt tolerance, and early ripening. Some cultivated wheat varieties already derive from interspecific crosses (Sharma and Gill 1983). Although some authors have successfully transformed cereals by genetic engineering techniques (Potrikus et al. 1985; Lörz et al. 1985; de la Pena et al. 1987), it seems that interspecific hybridizations could still be useful in the immediate future.

An attempt has been made here to more precisely define relationships between diploid species of the subtribe *Triticineae*, in order to suggest breeding strategies, based on the transfer of alien genetic material to wheat. This paper presents results of a cytogenetic study of interspecific hybrids.

Materials and methods

Diploid species (2n = 2x = 14) used in this study were all obtained from the collection of the I.N.R.A. station (Le Rheu – Rennes, France) except *Ae. longissima* TL01, kindly provided by Dr. M. Feldman (Rehovot, Israel). Accessions were designated by the following numbers: *Triticum urartu* Tuman. 7891 (*Au*), *T. boeoticum* Boiss. 165 and 166 (*A*), *Aegilops longissima* Schweinf. & Muschl. TL01 (S^1), *Ae. squarrosa* L. 38 (*D*), *Ae. umbellulata* Zhuk. 1 (*U*), *Ae. uniaristata* Vis. 2 (*Un*), *Ae. comosa* Sibth & Sm. 1 (*M*), *Ae. caudata* L. 2 (*C*), *Haynaldia villosa* L. 107 (*V*) and *Secale cereale* L. 601 (*R*) (letters in brackets are species genomic symbols, Kimber and Sears 1983).

Plants were grown in a greenhouse at a night temperature above 10 °C and a day temperature not exceeding 28 °C. Accessions were subjected to diallel crossing. The embryo rescue technique was applied to all hybrid grains obtained. Embryos were excised 10 to 14 days after pollination in a drop of Monnier liquid medium (1976) containing 50 g/l saccharose. They were plated on the same medium containing 7 g/l agarose in Petri dishes, and covered with a droplet of a modified Monnier medium containing 4 g/l saccharose, 200 mg/l glutamin, and 2 g/l agarose (J. Tempé, personal communication). Embryos were cultured at 22 °C with 16 h illumination (20,000 Lux) per day. After germination, seedlings were transferred onto Orchid agar medium (Difco), and then to soil in a humidity chamber for 48 h. After 8 weeks in a vernalisation chamber, plants were grown in the greenhouse. Anthers containing pollen mother cells (PMCs) at first metaphase of meiosis (MI) were extracted and fixed in 3:1 absolute alcohol:glacial acetic acid, then stored at 4 °C. They were prepared using the acetocarmine squash method for meiotic studies. Meiotic behavior for different hybrid combinations was compared statistically using Student's *t*-test.

Results

In order to study the degree of homology between the genomes of the eleven accessions used, 95 of the 110 possible hybrid combinations of F1 were attempted.

Table 1. Mean number of associations between chromosome arms in the PMCs of F1 hybrids. (Authors will be pleased to send detailed data on request)

♀ \ ♂	*T. urartu* (Au)	*T. boeoticum* 165 (A)	*T. boeoticum* 166 (A)	*Ae. longissima* (S1)	*Ae. squarrosa* (D)	*Ae. umbellulata* (U)	*Ae. uniaristata* (Un)	*Ae. comosa* (M)	*Ae. caudata* (C)	*H. villosa* (V)	*S. cereale* (R)
T. urartu					6.92					1.70	
T. boeoticum 165	13.19				3.14						
T. boeoticum 166	13.43			1.29	4.32	4.12	2.35		2.88	2.47	
Ae. squarrosa	7.23			7.38		7.01	3.53	5.78	5.82	1.24	0.62
Ae. umbellulata	4.58	4.56	4.12	2.38	6.51		3.64	3.26		1.31	1.33
Ae. uniaristata			2.89	3.13				5.37		0.71	0.69
Ae. comosa	5.27		2.63		5.54	4.08			4.49		
Ae. caudata			2.43			5.96				1.18	

Forty-one different interspecific hybrids were obtained and provided anthers whose PMCs were observed at MI. Because it was sometimes difficult to determine whether the chromosomes were linked at MI as a result of crossing-over, the word association between chromosome arms was preferred to the word chiasma. The mean number of associations between chromosome arms in the PMCs was taken as an indication of the degree of homology between genomes of the parental species. This was negatively correlated ($r = -0.94$) with the mean number of univalents which indicated lack of homology between genomes. An attempt was made to observe at least 100 PMCs for each hybrid from one or several anthers of a same or different plants. The mean number of associations for the different hybrids is given in Table 1.

Among the 41 hybrids analyzed, seven pairs of reciprocal hybrids were obtained. The mean number of associations was compared statistically to assess whether there was a reciprocal effect. If PMCs were observed in more than one anther, between-anther variance was eliminated. Results are shown in Table 2. Four of seven combinations showed statistically significant differences between the two reciprocal hybrids, but they did not exceed 0.82 associations.

Three hybrid combinations were obtained with both *T. boeoticum* 165 and *T. boeoticum* 166 as parents. Meiotic behavior of hybrids was compared for the two accessions to assess an eventual accession effect. There was no significant difference between the mean number of associations of *T. boeoticum* 165 × *T. urartu* 7891 (13.19 associations) and that of *T. boeoticum* 166 × *T. urartu* 7891 (13.43 associations). Mean numbers of associations in PMCs of *T. boeoticum* × *Ae. squarrosa* 38 and *Ae. umbellulata* 1 × *T. boeoticum* differed significantly whether accession 165 or 166 was used ($P<0.01$). The highest value was not always obtained for the same accession.

Meiotic behavior of all F1 hybrids was analysed to determine the genome relationships between the parental species. Results of diploid accessions involved in the origin of hexaploid wheat are presented first.

Table 2. Comparison of the reciprocal hybrids

Cross	X_H[a]	X_{RH}[b]	$X_H - X_{RH}$[c]
T. urartu 7891 × *Ae. squarrosa* 38	6.92	7.23	NS
T. boeoticum 166 × *Ae. umbellulata* 1	4.12	4.12	NS
T. boeoticum 166 × *Ae. uniaristata* 2	2.35	2.89	**
T. boeoticum 166 × *Ae. caudata* 2	2.88	2.43	*
Ae. squarrosa 38 × *Ae. umbellulata* 1	7.01	6.51	**
Ae. squarrosa 38 × *Ae. comosa* 1	5.78	5.54	NS
Ae. umbellulata 1 × *Ae. comosa* 1	3.26	4.08	**

[a] Mean number of associations in the PMCs of hybrids
[b] Mean number of associations in the PMCs of reciprocal hybrids
[c] Statistical analysis of the difference between X_H and X_{RH}
* Significant difference ($P<0.05$); ** Highly significant difference ($P<0.01$)
NS: non significant

Meiotic behavior of hybrids between the presumptive genome donors to hexaploid wheat

The mean number of associations between chromosome arms in PMCs of hybrids between *T. boeoticum* and *T. urartu* 7891 nearly attained the maximum expected for a fully fertile diploid line (13.19 and 13.43).

The hybrid between *T. boeoticum* 166 and *Ae. longissima* TL01 had the following meiotic behavior: $11.51' + 1.21'' + 0.03'''$. Therefore very few chromosomes were paired at MI.

A number of associations approaching seven and univalents approaching three were found in PMCs of the hybrid between *T. urartu* 7891 and *Ae. squarrosa* 38. However, *T. boeoticum* × *Ae. squarrosa* hybrids had fewer associations (3 to 4) and more univalents (7 to 8).

The *Ae. squarrosa* 38 × *Ae. longissima* TL01 hybrid had an average of 7 associations and 2.5 univalents in its PMCs.

Meiotic behavior of other diploid hybrids

Ae. squarrosa 38, when crossed with *Ae. umbellulata* 1, *Ae. uniaristata* 2, *Ae. comosa* 1, and *Ae. caudata* 2 showed more associations with their chromosomes than the presumptive donors of *A* and *B* wheat genomes.

In contrast, *H. villosa* 107 seemed to be more homologous with *T. boeoticum* 166 and *T. urartu* 7891 genomes than with the *D* genome of *Ae. squarrosa.*

Hybrids between *Ae. umbellulata* 1, *Ae. uniaristata* 2, *Ae. comosa* 1, and *Ae. caudata* 2 had a mean number of associations ranging from three to six. Diploid hybrids containing one genome from *S. cereale* 601 or *H. villosa* 107 had a very low number of paired chromosomes in their PMCs.

Discussion

Significant differences in pairing frequency between anthers from a given plant were observed in some of the hybrids studied (Lucas 1986), which could be ascribed to temperature differences at meiosis for different spikes or florets (Bennett et al. 1972). The observed differences between the reciprocal hybrids and between hybrids involving the two accessions of *T. boeoticum* may be due to the same cause. Even though the differences observed were statistically significant, they did not exceed 0.82 associations for the reciprocal hybrids (*Ae. umbellulata* 1 × *Ae. comosa* 1) and 1.18 in the case of *T. boeoticum* × *Ae. squarrosa* 38.

Several authors have suggested that cytoplasm can influence meiotic behavior, e.g. Larter and Hsam (1973) and Roupakias and Kaltsikes (1977) for alloplasmic lines of *Triticale* in either a 6X or a 4X wheat cytoplasm. Some proteins involved in the recombination process could conceivably be specific to the nucleus/cytoplasm complex. The non-equivalence of cytoplasm and nucleus in interspecific hybrids might lead, in some instances, to differences in meiotic behavior of reciprocal crosses.

Genetic systems increasing or decreasing the number of associations between chromosomes have been reported in several diploid species of *Triticineae* (Mello-Sampayo 1971; Avivi 1976; Lelley 1976; Attia et al. 1979; Miller and Reader 1980; Blanco et al. 1983). The presence of such genetic systems has been indicated by variability of meiotic behavior in hybrids between different accessions of those species and 6X wheat. Despite the fact that *Ae. longissima* TL01 is a low-pairing line (Avivi 1976), the mean number of associations in *Ae. squarrosa* 38 × *Ae. longissima* TL01 is fairly high (7.38). Therefore it is likely that if genetic systems controlling pairing at MI were present in the accessions used, they are not expressed in diploid hybrids. Meiotic analysis is based on the assumption that genetic systems controlling pairing does not alter the mean number of associations in diploid interspecific hybrids. Nevertheless, this possibility must be assessed in further studies.

Relationships between the presumptive genome donors of hexaploid wheat

Given the number of associations in the PMCs of the hybrids between *T. boeoticum* and *T. urartu* 7891, the *A* and *Au* genomes appear to be appreciably homologous. The *Ae. longissima* TL01 S^1 genome shows little homology with the *T. boeoticum A* genome, since the mean number of associations was as low as 1.29 in the PMCs of the hybrids. The mean number of associations between chromosome arms in the hybrid *Ae. squarrosa* 38 × *Ae. longissima* TL01 is high (7.38), indicating that their genomes are partially homologous. The results suggest that the genomes of *T. urartu* (*Au*), *T. boeoticum* (*A*), *Ae. longissima* (S^1), and *Ae. squarrosa* (*D*) genomes are linked as follows: *A* and *Au* closer to *D* than to S^1 (in accordance with the results of Dvořák (1976) and Hutchinson et al. (1983); S^1 closer to *D* than to *A* or *Au*; and, *D* closer to S^1 than to *A*, but as close to *Au* as to S^1.

Relationships between the genomes of the presumptive ancestors of wheat and of the other diploid species

It is interesting to know how closely related the wild species genome is to the A, B and D genomes when transferring genetic information into cultivated wheat. The chromosomes of *Ae. umbellulata* 1, *Ae. comosa* 1, and *Ae. caudata* 2 showed a high degree of association with those of *Ae. squarrosa* 38 (5.5 to 7 associations). The number of associations between chromosome arms in *Ae. squarrosa* 38 × *Ae. uniaristata* 2 was lower (3.53). However, these four genomes are more homologous to the *D* genome than to the *A*, *Au*, or S^1 genomes. These results lead to the assumption that they could have been derived from *Ae. squarrosa* or from a genotype closely related to it. On the contrary, the *V* genome of *H. villosa* seems to be closer to the *A* than to the *D* genome; however, the number of associations is very low. No hybrid between rye and the presumptive *A* and *B* wheat genome donors was obtained. Meiotic behavior of *T. monococcum* × *S. cereale* reported by Sodkiewicz (1982) indicates that the affinity between *A* and *R* genomes could be as low as that found here between *D* and *R* (0.62 associations).

Relationships between the genomes of Aegilops species not involved in the origin of 6X wheat

Two of the diploid hybrids obtained from crosses between species not implicated in the origin of hexa-

ploid wheat showed a high number of associations between chromosome arms at MI of meiosis: *Ae. caudata* 2 × *Ae. umbellulata* 1 (5.96), and *Ae. uniaristata* 2 × *Ae. comosa* 1 (5.37). This result indicates that *Ae. caudata* and *Ae. umbellulata* on the one hand, and *Ae. uniaristata* and *Ae. comosa* on the other, could have a common ancestor or could have been derived one from the other.

From the results of several authors (Kihara 1954; Kimber and Abubakeer 1981; Miller 1981) and our own data, it is evident that *Ae. umbellulata* and *Ae. caudata* are distinct species, though quite close. We believe that one differentiated from the other or from a common ancestor. The fact that *Ae. umbellulata* seems to be closer to *Ae. squarrosa* than to *Ae. caudata* suggests that the latter could have been derived from *Ae. umbellulata.*

Designation of the *Ae. comosa* genome by *M* and that of *Ae. uniaristata* by M^u was proposed to indicate the closeness of the two species (Kihara 1937). Kimber et al. (1983) proposed change of the genomic symbol to *Un* for *Ae. uniaristata* because they found no preferential pairing between chromosomes of the *M* and M^u genomes. Our results agree more with those of Tanaka (1985) who concluded, from a morphological and cytogenetical analysis, that *Ae. comosa* and *Ae. uniaristata* derived from a common ancestor. Nevertheless, it seems to us that the divergence is sufficient to designate them by different letters. However, the *Un* symbol proposed by Kimber et al. (1983) for *Ae. uniaristata* is rather confusing, because it seems to indicate a relationship between it and *Ae. umbellulata* (*U* genome). We prefer use of the letter *N* as proposed by Chennaveeraiah (1960) and more recently by Teoh et al. (1983).

A possible pattern of species differentiation

In view of our results, a possible pattern of differentiation from a common ancestor for the diploid species used can be proposed (Fig. 1). This scheme does not fit Kihara's classification (1954), which placed *Ae. squarrosa* closer to *Ae. uniaristata* and *Ae. comosa* (*M* group) than to *Ae. caudata* and *Ae. umbellulata* (*C* group). Our data indicates that the *D* genome of *Ae. squarrosa* is closer to the *C* and *U* genomes.

Because *H. villosa* 107 and *S. cereale* 601 chromosomes rarely showed associations with those of other species, it is likely that the differentiation of *H. villosa* and rye from the genera *Aegilops* and *Triticum* occurred a long time ago. No hybrid between *H. villosa* and *S. cereale* was obtained in this study, but Nakajima (1950) observed an average of one bivalent in its PMCs. For that reason, we propose differentiation between *H. villosa* and *S. cereale* quite early in the evolutionary pattern.

In agreement with MacKey's (1968) classification, *T. urartu* and *T. boeoticum* clearly stand apart from *Aegilops* species contradictory to Morris and Sears' classification (1967), which regroups all these species in the genus *Triticum.*

The differentiation pattern from a common ancestor proposed accounts for all the genome relationships established from the meiotic behavior of the interspecific hybrids we obtained. The choice of a diallel cross enabled numerous hybrid combinations, and partially compensates for the fact that we did not use more than one accession per species, except for *T. boeoticum.* In order to compare genomes from species which did not give any hybrid when crossed, crosses between amphiploids have also been undertaken in our laboratory.

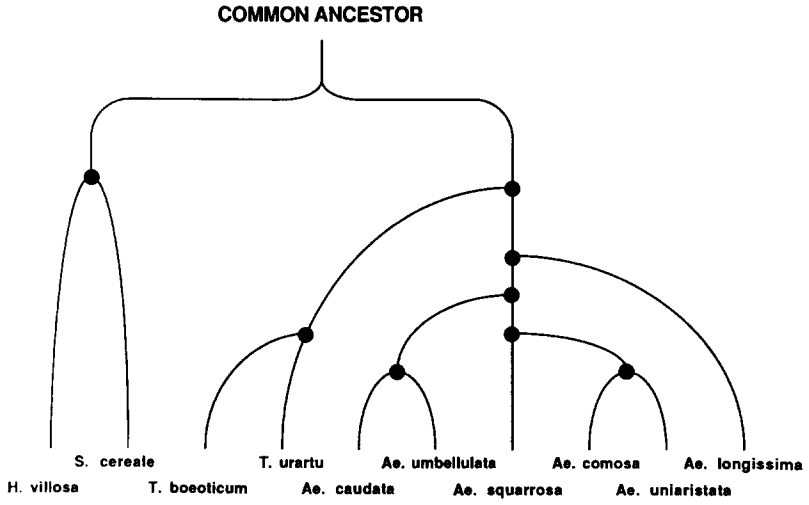

Fig. 1. Differentiation scheme for some diploid species of Triticineae from a common ancestor. The number of branchings (●) to pass from one species to another accounts for the number of associations in the hybrid PMCs, e.g. *Ae. umbellulata* is closer to *Ae. squarrosa* (2 ●) than to *Ae. longissima* (3 ●)

Conclusion

The cytogenetical study of interspecific hybrids has allowed us to define the relationships between the genomes of some diploid *Triticineae* more precisely, and to propose a pattern of species differentiation. In addition to phylogenetic understanding, the results obtained can be used as a basis for breeding strategies involving transfer of alien genetic material to cultivated wheat.

As *Ae. umbellulata, Ae. uniaristata, Ae. comosa,* and *Ae. caudata* are closer to *Ae. squarrosa* than to the presumptive *A* and *B* wheat genomes donors, a transfer of genetic material from the former species to the *D* genome of hexaploid wheat should be attempted. The use of *AABBXX* amphiploid structures (*X* being the alien species genome) in crosses with 6X wheat lacking the Ph gene should increase the likelihood of success. In other respects, it seems that the transfer of characters from *H. villosa* or *S. cereale* could be improved by targeting to the *A* genome of wheat. However, this might be difficult given the lack of wild *BBDD* genomic structures.

We also believe that the expression of alien genes in wheat could be enhanced by their transfer into the wheat genome closest to that of wild species.

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