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Studies on Maternal Inheritance in Polyploid Wheats with Cytoplasmic DNAs as Genetic Markers

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Summary. Restriction fragment patterns of DNA fragments obtained after EcoRI cleavage of chloroplastic (cp) and mitochondrial (mt) DNAs isolated from different wheat species were compared. *T. aestivum, T. timopheevi, Ae. speltoides, Ae. sharonensis* and *T. urartu* gave species specific mt DNA patterns. Consequently, the cytoplasmic genomes of wheat cannot have originated from contemporary *Ae. speltoides, Ae. sharonensis* and *T. urartu* species. It is shown that cp and mt DNAs of *Ae. ventricosa*, a tetraploid used to transfer eyespot resistance into *T. aestivum*, contains cp and mt DNAs differing from DNAs isolated from *T. aestivum* and other wheats. In contrast, the cytoplasmic DNAs of *Ae. ventricosa* and *Ae. squarrosa* reveal an important homology, suggesting that *Ae. squarrosa* was the female parent of *Ae. ventricosa*. Disomic addition lines (*T. aestivum* – *Ae. ventricosa*) in both *Ae. ventricosa* cytoplasm and *T. aestivum* cytoplasm contained cytoplasmic DNAs identical to those of the maternal parent. Restriction patterns of the cp and mt DNAs isolated from eight lines of Triticale differing in their cytoplasm have been compared to those of the maternal parent. A strict maternal inheritance has been observed in each case.

Key words: wheat evolution – Maternal inheritance – Disomic addition lines – Triticale – Chloroplast and mitochondrial DNAs

Introduction

Specific cleavage of the chloroplast and mitochondrial DNAs from higher plants by restriction enzymes (Atchison et al. 1976; Vedel et al. 1976; Quétier and Vedel 1977) has shown that these DNAs can be used as genetic markers to study the phylogeny of different genera (Vedel et al. 1978; Timothy et al. 1979) and to characterize parent and somatic hybrid cytoplasms (Belliard et al. 1978, 1979).

In previous work on wheat phylogeny, we have found that cp DNA restriction patterns of hexaploid *T. aestivum*, tetraploids *T. turgidum* and *T. timopheevi*, and the diploid *Ae. speltoides* were identical, suggesting that *Ae. speltoides* could be the cytoplasmic genome donor to wheats (Vedel et al. 1978). This result was in agreement with ribulose bisphosphate carboxylase analysis (Chen et al. 1975). Nevertheless, it appeared from mt DNA restriction analysis that *Ae. speltoides* mt DNA was distinct from other mt DNAs, indicating that contemporary *Ae. speltoides* could not be the cytoplasmic genome donor to wheats (Vedel et al. 1978).

Recent data on protein electrophoretic patterns, gene frequencies, morphology and cytogenetics have implicated successively *T. urartu* (Johnson 1975; Johnson and Dhaliwal 1978), *Ae. sharonensis* (Paroda 1977), *Ae. longissima* and/or *Ae. searsii* (Feldman 1977, 1979; Feldman and Kislev 1977) as the B nuclear donor to the polyploid wheats. On the other hand, we have shown previously that while *T. aestivum* mt DNA was identical to *T. turgidum* mt DNA, it was different from *T. timopheevi* mt DNA and that normal (cytoplasm *T. aestivum*) and cytoplasmic male sterile (cytoplasm *T. timopheevi*) lines of hexaploid wheat contained mt DNAs identical to *T. aestivum* and *T. timopheevi*, respectively (Quétier and Vedel 1977). These results provided evidence for maternal inheritance of cytoplasmic DNA in wheats.

In this study, we have attempted to identify the contributor of the nuclear B genome by comparing mt DNA restriction patterns from the two diploids *T. urartu* and *Ae. sharonensis* with those from polyploid wheats.

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The non-mendelian transmission of the cytoplasm in wheat and related species has been further investigated by cp and mt DNA restriction analysis of disomic addition lines (wheat × *Aegilops ventricosa*) and of Triticale obtained with various cytoplasms.

Material and Methods

Species Examined

Triticum urartu, (A or B genome), *Aegilops sharonensis* (S^1 genome), *Aegilops speltoides* (S genome), *Triticum timopheevi* (AG genome) and *Triticum aestivum* var. 'Moisson' (ABD genome) were analyzed for their mt DNA in the study on the B genome donor.

Table 1. *T. aestivum – Ae. ventricosa* addition lines used in experiments

Addition lines	Cytoplasms	Reaction to eyespot
v 206	*Ae. ventricosa*	susceptible
16	"	"
52	"	"
204	"	"
109	"	"
318	"	"
191	"	weak susceptibility
193	"	resistant
201	"	"
208	"	"
359	"	"
261	"	"
394	"	"
m 12	*T. aestivum* var. 'Moisson'	susceptible
27	"	"
345	"	"
119	"	"

The cp and mt DNAs of *Ae. squarrosa* (D genome), *Ae. ventricosa* (D^VM^V), *T. aestivum* var. 'Moisson' and the *T. aestivum – Ae. ventricosa* addition lines were compared. Disomic addition lines with one M^V chromosome of *Ae. ventricosa* into the wheat genotype were obtained:

– in *Aegilops* cytoplasm from the cross (*Ae. ventricosa* n°11 × *T. aethiopicum IA*) × *T. aestivum* var. 'Moisson[3]' followed by five self-pollinated generations (v lines).

– in wheat cytoplasm from the cross (*T. aestivum* var. 'Moisson' × *Ae. ventricosa* n° 11) × *T. aestivum* var. 'Moisson[4]', followed by three self-pollinated generations (m lines). The v lines have been obtained and characterized as previously described (Dosba et al. 1979).

Ae. ventricosa n° 11 shows a high level of resistance to eyespot, a fungus disease caused by *Cercosporella herpotrichoides* Fron. It has been observed from eyespot tests at different stages of growth that some addition lines (essentially the v lines) are very resistant to that disease (Table I). The hypothesis that the *Aegilops* cytoplasm may improve disease resistance in comparison to the *T. aestivum* cytoplasm is being tested.

Eight 6× Triticales (Table 2) obtained from crosses between different wheats and rye with either wheat or rye cytoplasms were also analyzed in order to study cytoplasmic inheritance. The alloplasmic lines with *S. cereale* and *T. timopheevi* cytoplasms arose from three Triticale lines:

– T. 949, a primary hexaploid line originates from a cross between *T. durum* and *S. cereale*;

– T 861 and T 333, two secondary hexaploid lines originate from complex crosses between *S. cereale, T. aestivum* and *T. durum* (Table 2). The "cytoplasmic genitors" are:

– the wheat variety 'Chinese Spring' with *T. timopheevi* cytoplasm obtained by Dr. Maan;

– a secondary Triticale with *S. cereale* cytoplasm obtained by Dr. Sanchez-Monge.

Isolation of cp and mt DNAs

Chloroplast and mitochondrial DNAs were isolated as previously described by using CsCl-ethidium bromide gradients (Herrmann et al. 1975; Kolodner and Tewari 1975) with some modifications (Vedel et al. 1980). Cytoplasmic DNAs were specifically cleaved by EcoRI and Sal I restriction enzymes prepared by us according

Table 2. – Triticale lines used in experiments

Original material			New material		
Genealogy	n°	Cytoplasm	n°	Cytoplasm	Conversion generation
T. durum × *S. cereale*	T 949	*T. durum*	T 758	*T. timopheevi*	BC 6
			T 833	*S. cereale*	BC 6
[*T. aestivum* × (*T. durum* × *S. cereale*)] × [(*T. aestivum* × *S. cereale*) × (*T. durum* × *S. cereale*)]	T 861	*T. aestivum*	T 834	*S. cereale*	BC 5
{*T. aestivum* × *S. cereale*} × {[*T. aestivum* × (*T. durum* × *S. cereale*)] × [(*T. aestivum* × *S. cereale*) × (*T. durum* × *S. cereale*)]}	T 333	*T. aestivum*	T 762	*T. timopheevi*	BC 6
			T 832	*S. cereale*	BC 5

to Gingeras et al. (1978) and Greene et al. (1978). Separation of the restriction fragments by agarose gel electrophoresis, gel staining and ultraviolet fluorescence photography have already been described (Vedel et al. 1976; Quétier and Vedel 1977).

Results

The results presented here have been obtained primarily by the cleavage of cytoplasmic DNAs with EcoRI enzyme in order to (1) facilitate comparisons with mt DNA-EcoRI patterns described in a preliminary study on wheat phylogeny (Vedel et al. 1978), and (2) make use of the numerous restriction fragments generated (more than fifty with a molecular weight greater than 10^6 d). Treatments with this enzyme lead to electrophoretic patterns more useful than those obtained with Sal I, Kpn I and Xho I when differences among cytoplasmic DNAs of cereals are to be studied (Vedel et al. 1980). Also, the seed samples (*T. aestivum* excepted) were available in too small quantities to allow mt DNA analysis by several enzymes.

Restriction Analysis of Ae. sharonensis and T. urartu mt DNAs

Figure 1 shows the electrophoregrams of EcoRI fragments obtained with the mt DNAs isolated from *T. aestivum*, *T. timopheevi*, *Ae. speltoides*, *Ae. sharonensis* and *T. urartu*. Each species appears to be characterized by a specific mt DNA pattern, quite distinct from the four others. These results suggest that *Ae. speltoides*, *Ae. sharonensis* and *T. urartu* have not supplied the mitochondrial genomes to *T. aestivum* or to *T. timopheevi*.

Restriction Analysis of Cytoplasmic DNAs Isolated from T. aestivum – Ae. ventricosa Disomic Addition Lines

The cp and mt DNAs of *Ae. ventricosa* have been compared to those of *T. aestivum* var. 'Moisson' and of some wheat related species analyzed previously. The purpose of such comparisons is to distinguish the two parent cytoplasms before analyzing the disomic addition lines and to find homologies between *Ae. ventricosa* (D^vM^v) and diploid and tetraploid species. The results are summarized on Figure 2. *Ae. ventricosa* and *T. aestivum* cp DNAs present important homologies but can be distinguished at the level of the 2×10^6 d-molecular weight-bands. The eighth band of *T. aestivum* cp DNA is particularly lacking in the *Ae. ventricosa* cp DNA pattern. In contrast, the corresponding mt DNA patterns appear quite different.

Among the species belonging to the polyploid series of wheat analyzed previously, the diploid *Ae. squarrosa* (D nuclear genome) presents the greatest homology with *Ae. ventricosa*. *Ae. squarrosa* cp DNA differs primarily from *Ae. ventricosa* cp DNA at the level of the third band, which appears doubled. The eighth band of *T. aestivum* is also absent from the EcoRI pattern of *Ae.*

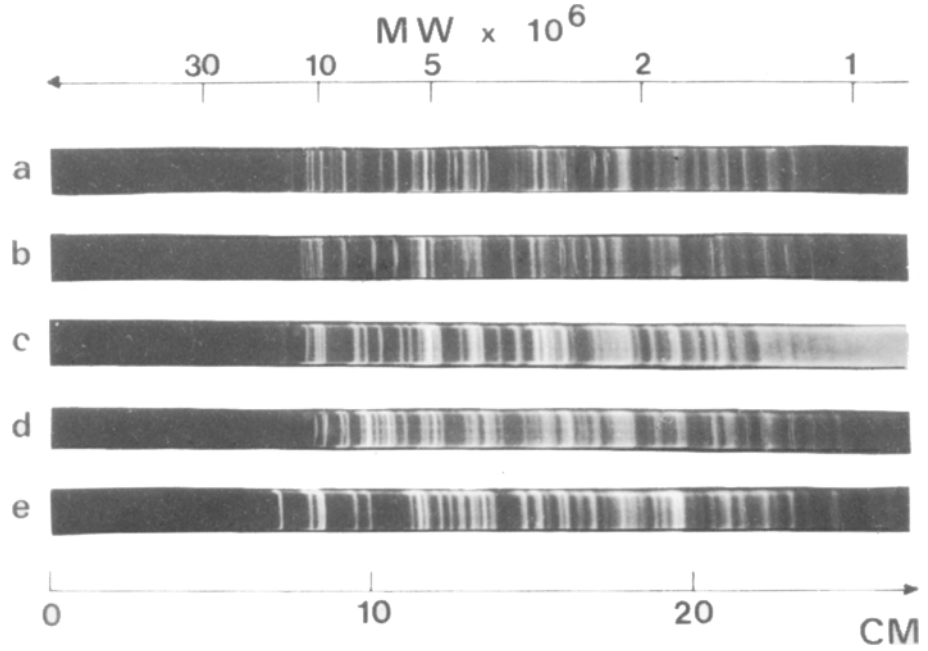

Fig. 1. Agarose slab gel electrophoresis of EcoRI digest of mt DNAs from: *a*, *T. aestivum* var. 'Moisson'; *b*, *T. timopheevi*; *c*, *Ae. speltoides*; *d*, *Ae. sharonensis*; *e*, *T. urartu*

squarrosa cp DNA. The EcoRI patterns of *Ae. squarrosa* and *Ae. ventricosa* (D^vM^v nuclear genome) mt DNAs appear highly homologous suggesting that *Ae. squarrosa* was the female parent of the cross with the diploid species (M^v nuclear genome) related to *Ae. uniaristata* or *Ae. comosa*.

The cytoplasmic DNAs of the v and m addition lines have been cleaved with the EcoRI enzyme. We have found that for each sample the direction of the sexual cross was corroborated both by cp and mt DNAs patterns. All the v addition lines are characterized by cp and mt DNAs identical to *Ae. ventricosa* cp and mt DNAs, and all the

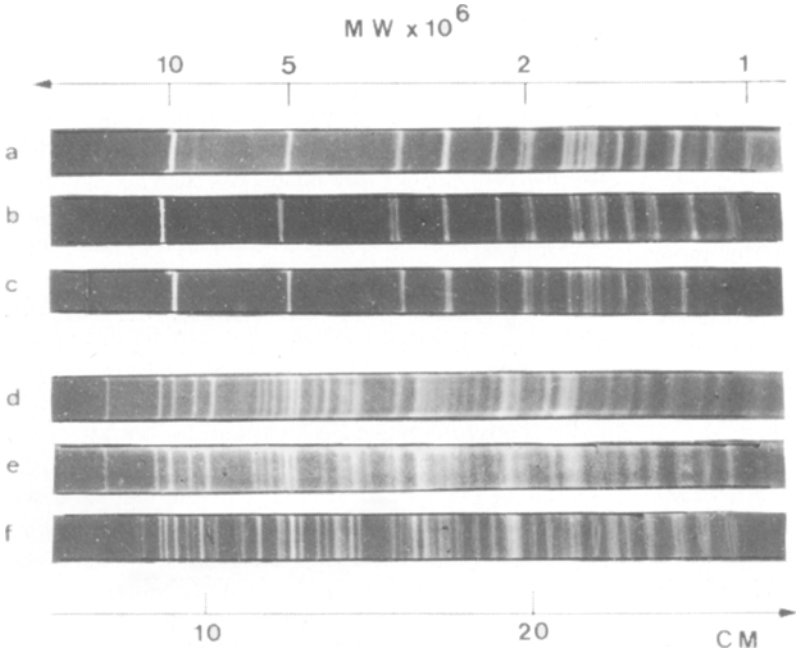

Fig. 2. Agarose slab gel electrophoresis of EcoRI digest of cp DNAs from: *a*, *Ae. ventricosa*; *b*, *Ae. squarrosa*; *c*, *T. aestivum* and of mt DNAs from: *d*, *Ae. ventricosa*; *e*, *Ae. squarrosa*; *f*, *T. aestivum*

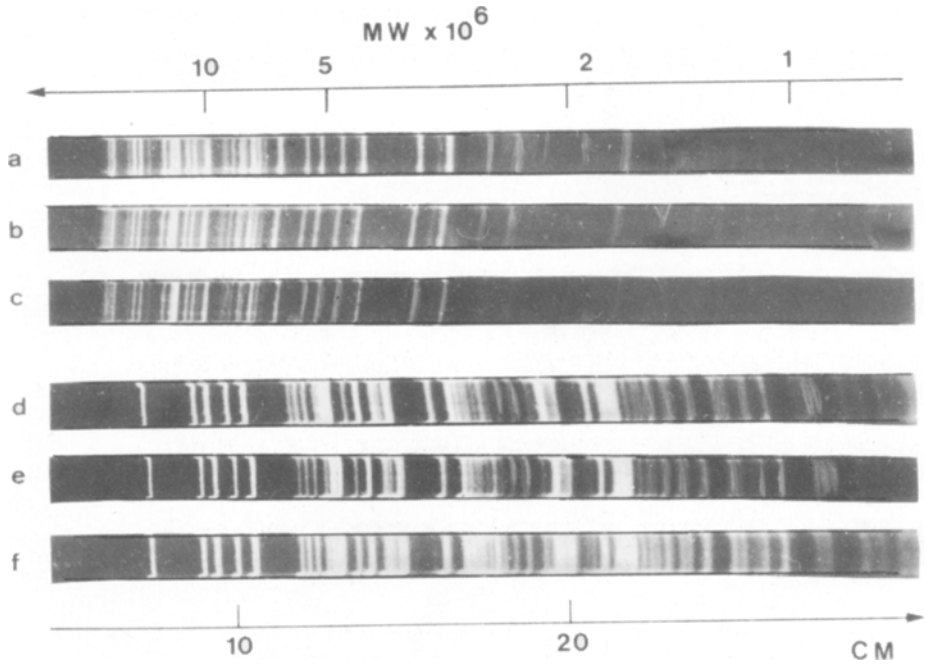

Fig. 3. Agarose slab gel electrophoresis of mt DNA restriction fragments from three addition lines with *Ae. ventricosa* cytoplasm (v lines), after clevage with the Sal I enzyme: *a*, v 52; *b*, v 191; *c*, v 208 and with the EcoRI enzyme: *d*, v 52; *e*, v 191; *f*, v 208. These lines can be distinguished by different responses to the eyespot disease

m addition lines contain cytoplasmic DNAs identical to those of *T. aestivum*. Indeed, cp and mt DNAs analyses by EcoRI and Sal I enzymes (Fig. 3) have shown that it is impossible to distinguish the different addition lines within the v and the m groups respectively. These results indicate that in the sexual crosses leading to the two types of hybrids, the cytoplasms were maternally transmitted. It seems also that the different responses to the eyespot fungus cannot be related, at present, to the gross composition of cp and mt DNAs.

Restriction Analysis of Cytoplasmic DNAs Isolated from Triticales Obtained on Various Cytoplasms

The EcoRI patterns of the cp and mt DNAs of the four female parents used to produce Triticales with different cytoplasms are represented in Figure 4. As previously mentioned, *T. aestivum* and *T. durum* are characterized by identical cp and mt DNAs (diagrams a-b, and e-f). While *T. timopheevi* contains cp DNA identical to *T. aestivum* and *T. durum* cp DNAs (diagram c), its mt DNA is distinct (diagram g). Cp and mt DNAs of *S. cereale* (diagrams d and h) appear distinct from cp and mt DNAs of *T. aestivum, T. durum* and *T. timopheevi*. It is striking that *S. cereale* cp DNA such as that of *Ae. squarrosa* and *Ae. ventricosa* lacks the eighth EcoRI restriction fragment of *T. aestivum* cp DNA.

The EcoRI patterns of the cp and mt DNAs isolated from the three original Triticales, T 949 with *T. durum* cytoplasm, T 333 and T 861 with *T. aestivum* cytoplasm, are identical to the EcoRI patterns presented in Figure 4 a and b and 4 e and f, respectively. Cp and mt DNAs of *T. timopheevi* and *S. cereale* appear identical to cp and mt DNAs isolated from the Triticales with *T. timopheevi* and *S. cereale* cytoplasms respectively. Introduction of a given nucleus into different cytoplasms by repeated backcrosses does not lead to alteration in cp and mt DNAs restriction

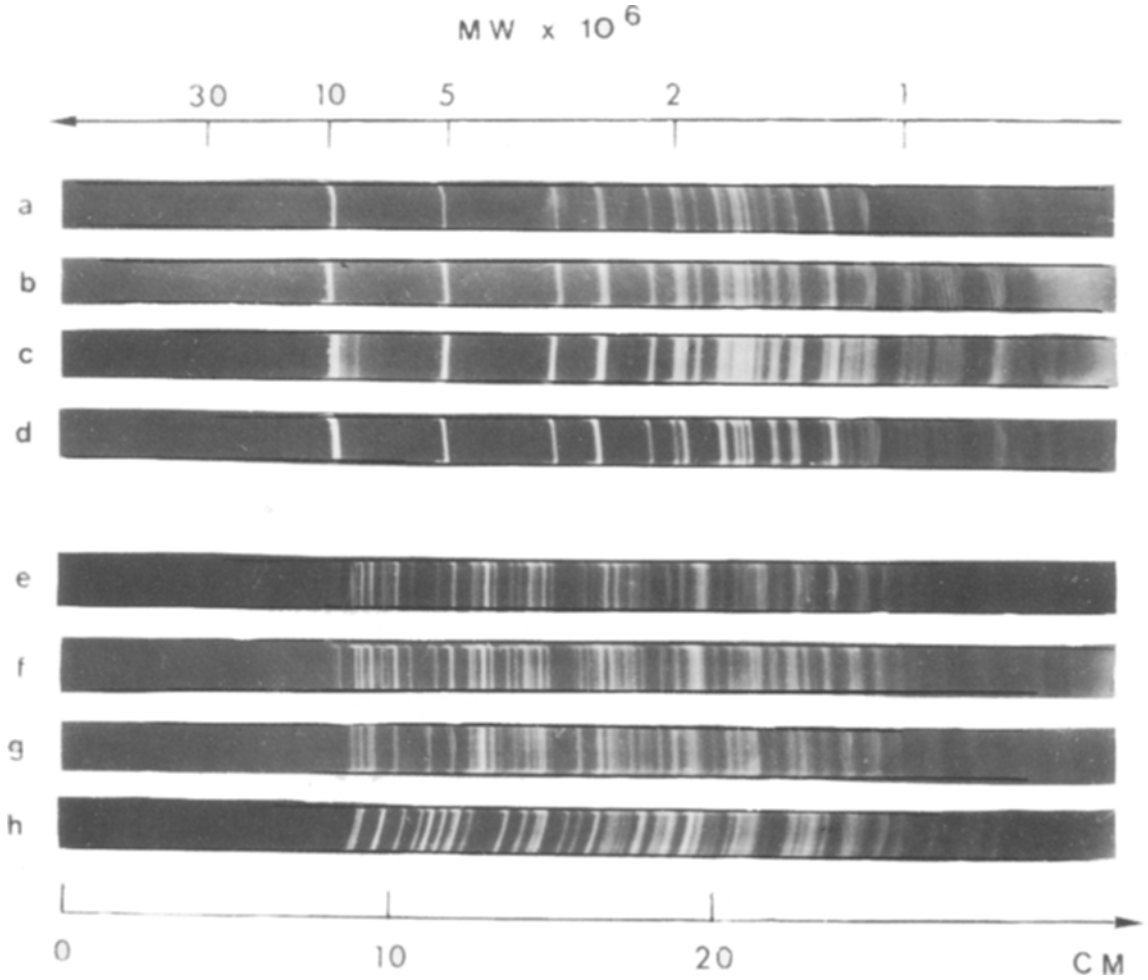

Fig. 4. Agarose slab gel electrophoresis of EcoRI digests of cp DNAs from: *a, T. aestivum; b, T. durum; c, T. timopheevi; d, S. cereale* and of mt DNAs from: *e, T. aestivum; f, T. durum; g, T. timopheevi; h, S. cereale*

patterns. These results show that the cp and mt DNAs in Triticales are maternally inherited.

Discussion

In this work, restriction enzyme patterns of cp and mt DNAs have been used as genetic markers to analyze the cytoplasmic genome donors of wheats and to follow their inheritance in different sexual crosses.

mt DNAs of the two diploids, *T. urartu* and *Ae. sharonensis*, both suspected to be the donor of the nuclear B genome in polyploid wheats, appeared different from *T. aestivum* mt DNA. However, *T. urartu* cytoplasm differs more from *T. aestivum* cytoplasm than from those of *Ae. speltoides* and *Ae. sharonensis*. The *T. urartu* chloroplast genome has been shown previously on the basis of fraction I analysis to differ from that of *T. aestivum* (Chen et al. 1975). *Ae. sharonensis* and *Ae. speltoides* belonging both to the section *Sitopsis* contain cp DNAs identical to *T. aestivum* cp DNA (Vedel et al. 1978). EcoRI cleavage patterns of mt DNAs allow the *T. aestivum, Ae. speltoides, Ae. sharonensis* and *T. urartu* cytoplasms to be distinguished and suggest that none of the diploids we have analyzed are direct donors of the cytoplasmic genome to *T. aestivum*. Consequently, it is also not possible to identify the nuclear B genome donor among these diploid species. Similar difficulties have arisen with most of the approaches to trace the nuclear B genome donor, and several possible reasons for this have been suggested (Konarev et al. 1976; Feldman 1977, 1979; Caldwell and Kasarda 1978; Vedel et al. 1978).

Among the reasons put forward, we consider the following two relevant:

1) The nuclear B genome donor, as well as the cytoplasmic genome donor, may either be extinct or has not been collected and/or analyzed. For instance, it will be important to analyze the mt DNA of other species of the *Sitopsis* section, such as *Ae. longissima* or *T. searsii*, recently discovered and considered by Feldman (1977, 1979), from cytogenic studies, as the most likely donor of the nuclear B genome to polyploid wheats.

2) Cytoplasmic genomes may have changed during the course of evolution and through sexual hybridization. Since evidence for mt DNA recombination has recently been found in parasexual cytoplasmic hybrids of tobacco (Belliard et al. 1979), mt DNA recombination in hybrids of the two diploid parent species characterized by A and B nuclear genomic formulas, cannot be ruled out to explain mt DNA divergence between hexaploid wheats and the nuclear B genome diploid donor (female parent).

Is the cytoplasmic divergence related to the mode of cytoplasmic inheritance? This question was analysed in the second part of this work.

Cytoplasmic DNAs of two kinds of hybrids have been compared to the parent cytoplasmic DNAs. In both the disomic addition lines and in the Triticale lines, a strict maternal inheritance of cp and mt DNAs has been observed. The results suggest further that in the case of the addition lines, *T. aestivum* and *Ae. ventricosa*, cp and mt DNAs are not responsible for the different responses to the eyespot disease among the tested lines. However, a more refined analysis with several other restriction enzymes is required to substantiate this conclusion as the differences may result from point mutations in the cytoplasmic DNA not recognizable by the present analysis. It is noticeable that the cp DNAs of the three following eyespot resistant species, *Ae. squarrosa, Ae. ventricosa* and *S. cereale*, lack the EcoRI restriction fragment number 8 of *T. aestivum* cp DNA. However, a direct relationship between the presence of this cp DNA fragment and eyespot susceptibility remains to be demonstrated.

In sexual hybrids of wheats and related species, as in many genera, the cytoplasms appear to be maternally inherited. Several mechanisms have been suggested for maternal inheritance of chloroplasts and mitochondria in higher plant genetics. The small size of the male generative cell may limit the number of organelles that can be passed on and both the mitochondrion and the chloroplast may be physically altered during microsporogenesis (Vaughn et al. 1980). On the other hand, our knowledge of what happens at the molecular level when the two parent cytoplasms are mixed is scarce. No evidence has been obtained in this study for the presence of both parent cytoplasms or recombination of the cytoplasmic DNAs in the sexual hybrids studied. However, one cannot exclude that such events were not responsible for some cytoplasmic variability during the course of evolution.

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