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Unusual inheritance of the mitochondrial genome organization in the progeny of reciprocal crosses between alloplasmic hexaploid wheat regenerants

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Abstract The transmission of a structurally-hypervariable fraction of the mitochondrial genome has been studied in 42 F_1 progenies obtained from reciprocal crosses between self-pollinated alloplasmic wheat plants regenerated after long-term somatic embryogenesis. This fraction of the genome is maternally and stoichiometrically inherited. In contrast, some additional restriction fragments specific to regenerated plants display a more complex mode of sexual transmission: one of the additional fragments was stoichiometrically and systematically inherited whereas two others were detected only in certain F_1 hybrids. Assuming that the detection, by Southern analysis, of such a fragment in regenerated plants is due to the amplification of a pre-existing substoichiometric molecule generated by the activation of a rare recombination event, our results suggest that the probability of detecting a novel fragment in the F_1 hybrids could be determined by the length of the repeated sequence at which recombination occurs.

Key words Wheat · Somatic tissue culture · Regeneration · Reciprocal crosses · Mitochondrial DNA variability

Introduction

It is now well documented that the mitochondrial genome of higher plants is large and heterogeneous (Newton 1988). Most of the mitochondrial information is likely to exist as a collection of circular minichromosomes, or subgenomic molecules, derived, by recombination events involving direct repeats (Lonsdale et al. 1984; Palmer and Shields 1984), from a master circular molecule encompassing the entire sequence complexity. Plant cell and tissue culture has demonstrated that the plant mitochondrial genome can undergo various and numerous alterations in its structure (McNay et al. 1984; Grayburn and Bendich 1987; Hartmann et al. 1987; Rode et al. 1987; Brears et al. 1989; Dörfel et al. 1989; Saleh et al. 1990; Shirzadegan et al. 1989, 1991). As in-vitro cultured plant tissues can regenerate into fertile plants, it is theoretically possible to follow not only the pattern of reorganization of the mitochondrial genome through an entire cycle of in-vitro culture (explant–cultured tissue – regenerated plant) but also the pattern of transmission of the reorganized structures in sexual crosses.

In a previous report we showed, by using wheat mitochondrial RFLP markers, that the selfed progeny of plants regenerated after long-term culture of immature embryos of the wheat variety Chinese Spring had novel and various organizations of their mitochondrial genome (Hartmann et al. 1989). Plants possessing the Chinese Spring nuclear background and various cytoplasms can be regarded as alloplasmic lines. A first source of alloplasmy was due to the fact that two defined, and differently-sized, fractions of the mitochondrial genome could disappear through in-vitro culture and were not detected in regenerated plants. Furthermore, novel and various subgenomic configurations could be specifically detected in some of the regenerated plants, thus creating a second source of alloplasmy.

Until now, the inheritance of the mitochondrial genome of plants regenerated after somatic embryogenesis has not been extensively studied. Only preliminary results dealing with maize resistance to pathotoxin have been obtained (Gengenbach et al. 1977). What we know to date is that maternal inheritance of mtDNA is the general rule in animals (Hutchison et al. 1974; Giles et al. 1980) and plants (Vedel et al. 1981; Schmitz 1988) although some exceptions have been reported in plants (Fairbanks et al. 1988; Neale et al. 1989; Erickson and Kemble 1990). In the same way, no data is available that

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the subgenomic configurations revealed by the passage of tissue culture onto regeneration medium are effectively transmitted through sexual crosses.

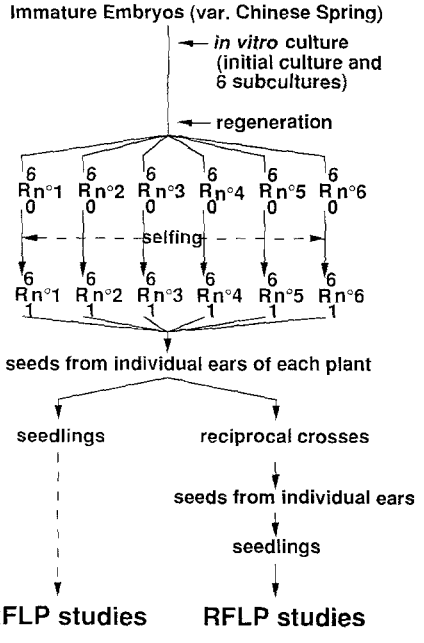
To address this question, we have developed a biological system in which both the selfed progeny of alloplasmic regenerated plants and the starting variety Chinese Spring were used as parents in a table of reciprocal crosses. DNA of the F_1 hybrids was then probed with selected mitochondrial RFLP markers. The results are consistent with the fact that mtDNA variability detected in regenerated plants is not stoichiometrically transmitted through sexual reproduction.

Materials and methods

Plant material

The wheat variety Chinese Spring (CS) and six plants regenerated after CS long-term somatic embryogenesis (Fig. 1), referred to as R_0^6n°I–R_0^6n°6 according to the nomenclature of Rode et al. (1988), were used. The regenerated plants were grown in a growth chamber (16h light, 200μE. $m^{-2}.s^{-1}$ at 21°C and 8h darkness at 17°C). The ears were bagged before flowering to ensure self-pollination. Six progenies (referred to as R_1^6n°1-R_1^6n°6) were obtained. Seeds from individual ears where harvested. To perform crosses, plants were grown in the field in spring conditions (sowing in February). At the time of ear emergence, flowers were emasculated after removing central florets of the spikelets and apical and basal spikelets. The prepared spikes were enclosed in plastic bags for 3–4 days, pollinated with freshly-collected pollen, and bagged again. The hybrid seeds from reciprocal crosses were harvested at maturity.

Fig. 1 Flow chart showing how the regenerated plants and their progenies were obtained. The nomenclature assigned to regenerated plants takes into account the number of subcultures before the regeneration process (*superscript suffix*) and the number of selfings after regeneration (*subscript suffix*)



Isolation of total DNA

Total DNA from individual plantlets obtained from seeds of parental variety (Chinese Spring), of plants R_1^6n°1–R_1^6n°6 and of the 42 reciprocal F_1 hybrids, was prepared has described by Dellaporta et al. (1983) with minor modifications (Rode et al. 1987).

RFLP studies

DNA restriction, electrophoresis, blotting and hybridization were performed as previously described (Rode et al. 1987). The *Sal*I-cloned wheat mtDNA fragments K_3 and K′, according to the nomenclature of Quétier et al. (1985), were used as labelled probes. As previously shown (Aubry 1990) fragments K′ and K_3 have homology to ctDNA fragments (respectively S5b and S4, according to the nomenclature of Bowman et al. (1981)).

Results

Structural features of the mitochondrial DNA reorganization in self-pollinated regenerated plants

Total DNA of single seedings of each R_1^6 progeny was probed with two mitochondrial RFLP markers already known to reveal genomic variability induced by in-vitro culture, i.e., the *Sal*I-cloned wheat mitochondrial DNA fragments K′ and K_3 (Hartmann et al. 1987; Rode et al. 1987). Both fragments, located in a so-called "hypervariable region" of the genome, contain an internal recombinogenic repeated sequence. In the starting variety, Chinese Spring, each of them thus hybridizes to four genomic arrangements corresponding, on the one hand, to *Sal*I fragments J′, K_1, K′ and M (Fig. 2) and, on the other hand, to *Sal*I fragments E_1, G_3, K_3 and N_3 (Fig. 3).

Figure 2 shows that only the R_1^6n°1 plant had retained the four *Sal*I fragments J′, K_1, K′ and M. The other five plants (R_1^6n°2 to n°6) had lost fragments J′ and K′. On the other hand, all the six R_1^6 plants has lost fragments E_1 and K_3 whereas only the R_1^6n°1 plant had retained fragment N_3 (Fig. 3). Thus these rearrangements, which correspond to a first type of alloplasmy, involve the disappearance (or, at least, the marked decrease) of molecular configurations present in the parental variety and give rise to two novel genome organizations.

The same probes also hybridized to additional fragments representative of one (or several) molecular configuration(s). Such an additional hybridizing fragment (6.2 kb) was found in plant R_1^6n°2 after probing with fragment K′ (Fig. 2). In the same way, additional hybridizing fragments were detected in plants R_1^6n°1 (19.5 kb) and R_1^6n°4 (11 kb) after probing with fragment K_3 (Fig. 3). The presence of these three fragments generates a second type of alloplasmy. Together, both types of alloplasmy define four different organizations of the mitochondrial genome (R_1^6n°1 plant; R_1^6n°2 plant; R_1^6n°4 plant; R_1^6n°3, 5 and 6 plants).

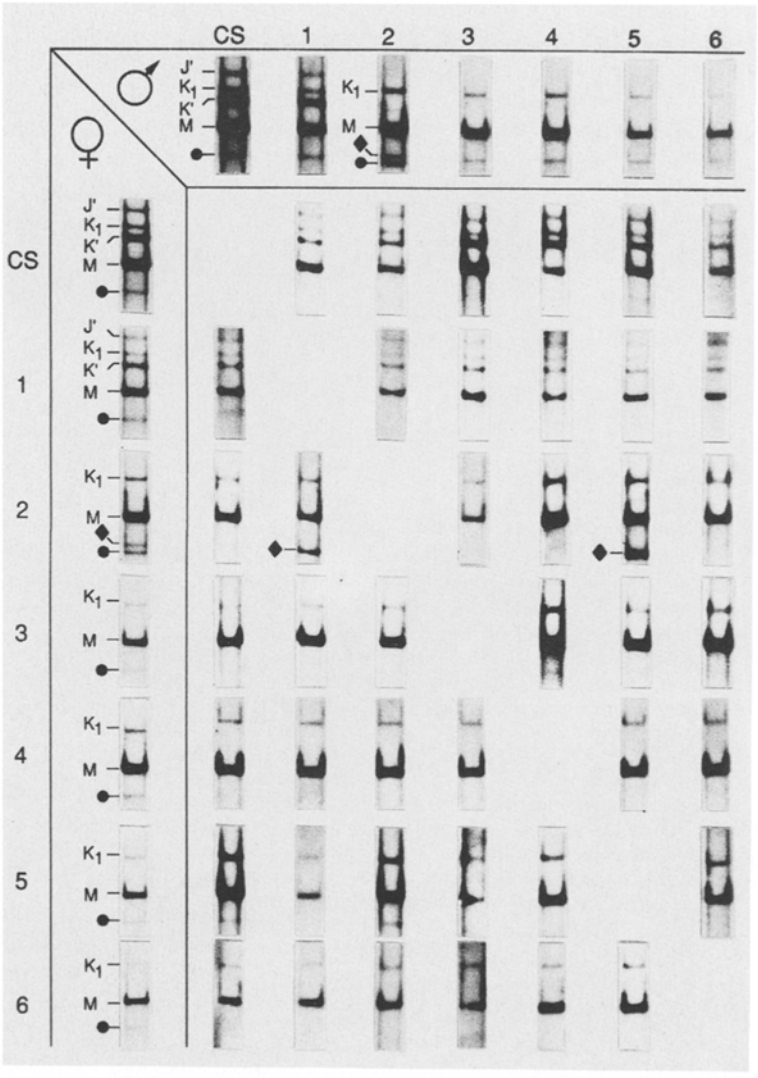


Fig. 2 Southern-blot analysis of DNA prepared from the reciprocal F_1 hybrids. Total cellular DNA was prepared from the starting variety Chinese Spring (*CS*), from R_1^6n°1-n°6 plants (1–6) obtained from seeds of self-pollinated regenerated plants and from the corresponding 42 reciprocal F_1 hybrids. *Sal*I-restricted DNA was probed with fragment K′. Top, male parents and corresponding hybridization patterns. Left, female parents and corresponding hybridization patterns. J′, K_1, K′, M: *Sal*I fragments hybridizing to the probe. ◆: an additional hybridizing fragment (6.2 kb) specifically detected in the R_1^6n°2 plant. ●: a cp DNA *Sal*I fragment [S5b, according to the nomenclature of Bowman et al. (1981)] with homology to the probe (Aubry 1990)

Structural features of the mitochondrial DNA reorganization in the reciprocal F_1 hybrids

The banding patterns obtained with fragment K′ as a probe (Fig. 2) showed (1) that the fragments common to both the parental variety and the reciprocal F_1 hybrids were strictly maternally inherited, (2) that the molecular configuration containing the 6.2-kb hybridizing fragment, when present, was also maternally inherited but detected in only two F_1 hybrids (F_1 crosses R_1^6n°2 × R_1^6n°1 and R_1^6n°2 × R_1^6 × R_1^6n°5).

Figure 3 shows the hybridization patterns of probe K_3 with DNA of the F_1 hybrids corresponding to the reciprocal crosses of plants R_1^6n°1 and R_1^6n°4 with the selfed progeny of both the regenerated plants and the parental variety Chinese Spring. Once more, a strict maternal heredity of fragments common to both the parental variety and the reciprocal F_1 hybrids was found. The situation was not as easy to understand as far as the additional hybridizing fragments were concerned. Indeed, the 19.5-kb hybridizing fragment specifically detected in plant R_1^6n°1 was stoichiometrically inherited in all six F_1 hybrids having plant R_1^6n°1 as a female parent, whereas the 11-kb hybridizing fragment, specific to plant R_1^6n°4, was maternally inherited but detected in only three F_1 hybrids (F_1 crosses R_1^6n°4 × R_1^6n°2, R_1^6n°4 × R_1^6n°3 and R_1^6n°4 × R_1^6n°5).

Discussion

Some selected mitochondrial RFLP markers define several alloplasmic wheat lines in regenerants derived from long-term somatic embryogenesis of the variety Chinese Spring. The reciprocal crosses involving both the parental variety Chinese Spring and the various alloplasmic lines have allowed us to obtain a collection of F_1 plants suitable for the study of RFLP inheritance.

Our results show that the organization of that part of the hypervariable region of the mitochondrial genome common to the parental variety and the self-pollinated progenies of regenerated plants is strictly maternally

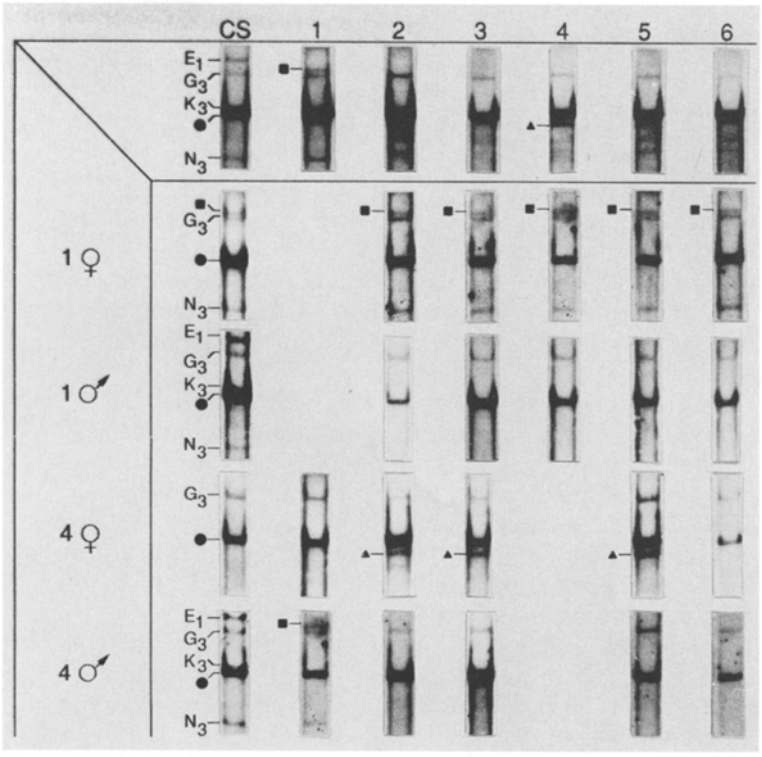


Fig. 3 Southern-blot analysis of the inheritance of the 19.5-kb (■) and 11-kb (▲) additional fragments in the reciprocal F_1 hybrids obtained by crossing the starting variety Chinese Spring (*CS*) and the R_1^6n°1–n°6 plants (1–6) with R_1^6n°1 and R_1^6n°4 plants. Total cellular DNA samples were *Sal*I-restricted and probed with fragment K_3.

E_1, G_3, K_3, N_3: *Sal*I fragments hybridizing to the probe. ●: a cp DNA *Sal*I fragment [S4, according to the nomenclature of Bowman et al. (1981)] with homology to the probe (Aubry 1990)

transmitted to the hybrids. In the same way, the subgenomic configuration containing the 19.5-kb additional *Sal*I fragment is stoichiometrically transmitted to all the hybrids having plant R_1^6n°1 as a female parent. Materal inheritance has already been shown, from the study of restriction patterns, in the progeny of various *Triticeae* (Vedel et al. 1981). In that case, all the mitchondrial subgenomic structures of the female parent were stoichiometrically inherited. Nevertheless, the present work shows for the first time that some fragments specifically detected in the mitochondrial genome of the selfed progenies of certain regenerated plants are not found in all the F_1 hybrids for which the female parent contains such a fragment.

Both the nuclear and the mitochondrial genome of plants can undergo rearrangements in culture (for a review see Brown 1991; Karp 1992). Various events can trigger changes in the nuclear genome: polyploidy, aneuploidy, chromosomal deletions, inversions and translocations (Lee and Phillips 1988), activation of transposable elements (Peschke et al. 1987), and single-base changes (Dennis et al. 1987). In contrast, a body of information now suggests that mitochondrial genomic variability could only be the consequence of variations in the relative amounts of certain subgenomic molecules. As these changes in structure have been shown to be nuclearly encoded (Hartmann et al. 1992), it may be that they are strictly governed by changes affecting nuclear genes involved in the building of the mitochondrial genome architecture. However, mitochondrial variability specifically detected in the progenies of sexual crosses involving regenerated plants cannot be directly ascribed to changes in the nuclear genome induced by the in-vitro process. In this case, the regenerated plants and their selfed progenies could segregate for nuclear changes and thus have a high potential for nuclear instability following the in-vitro period preceding the regeneration process. Thus, the plant-to-plant mitochondrial genomic variability detected in some F_1 lines would reflect this nuclear instability. However, it is striking that these changes in the mitochondrial DNA organization affect some subgenomic structures found only in regenerated plants. This particular feature suggests that nuclear variability could be confined to particular regions containing genes governing either the replication rate or the recombinational frequency of these subgenomic structures.

By using the PCR, we have recently shown that the 19.5-kb hybridizing fragment, present in all the F_1 lines, was in fact present in the parent variety in sub-stoichiometric amounts (Hartmann et al. 1994). Thus, the appearance of an additional fragment in the mitochondrial genome of a plant regenerated from tissue culture corresponds to the dramatic amplification of a pre-existing structure present in the parental plant and the tissue culture in copy numbers too low to be detected by conventional Southern-blot analysis. Furthermore, the 19.5-kb fragment was shown to arise from a reciprocal recombination event at a short (242 bp) repeated DNA sequence. The pattern of appearance of the 19.5-kb fragment in a single regenerated plant is thus consistent with the fact that a recombination event involving a short repeat is thought to occur at a low frequency and not to be easily reversible as indicated by the presence of

this fragment in all the F_1 hybrids derived from plant R_1^6n°1 as a female parent (for a review see André et al. 1992). In contrast, the appearance of both the 11-kb and 6.2-kb hybridizing fragments could have arisen from a marked increase in recombinational activity at larger repeated sequences. In this case, the frequency of reversibility of the recombination event would be higher and would explain why some F_1 hybrids have retained these fragments whereas others have not. Unfortunately, it was not possible to determine the lengths of the repeated sequences leading to the generation of the 6.2-kb and 11-kb additional fragments as we did not succeed in cloning them.

In a general way, the appearance of mitochondrial variability could depend on two, not mutually exclusive, parameters: first, the triggering of a modulation in the expression of nuclear genes which, in turn, would trigger either the amplification or the marked decrease of certain subgenomic structures, and second, the lengths of the recombinogenic repeated sequences through which these subgenomic molecules are generated.

At first sight, the possibility of obtaining fertile alloplasmic lines from in-vitro culture could represent an useful tool in studying organelle heredity. However, our results show that the problem is more intricate than expected as in-vitro mediated mtDNA variability is not systematically transmitted. Therefore, further work is required to check for the coding properties of the unstable regions of the mitochondrial genome.

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