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Chromosomal and molecular rearrangements in somatic hybrids between tetraploid *Medicago sativa* and diploid *Medicago falcata*

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Abstract The aim of this study was to produce somatic hybrids between tetraploid ($2n = 4x = 32$) *M. sativa* and diploid ($2n = 2x = 16$) *M. falcata* and analyse their genomic structure. Protoplasts from genotypes selected for regeneration ability from the cultivar Rangelander of *M. sativa* and Wisfal-1 of *M. falcata* were electrofused. Seven somatic hybrid calli were produced and one of them regenerated plants. The hybrid nature of these plants and their genetic composition were assessed with morphological, cytological, and molecular analyses. The resulting plants were hyperaneuploid ($2n = 33$) and contained one extra long chromosome, indicating that a translocation had taken place. The presence of both types of parental sequences in the RAPDs analysis confirmed the true hybrid nature of the plants. Rearrangements within the parental genomes and the presence of somaclonal variation among hybrid plants were observed through an RFLP analysis of the nucleolar organizing region (NOR). The possible causes for the gross genomic alterations, and the suitability of this method for transferring useful agronomic traits from wild species to cultivated alfalfa, are discussed.

Key words Somatic hybridization · rDNA locus · Chromosome rearrangements · Gene transfer · Forage legumes

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Introduction

As a potentially useful method for genetic exchange between incompatible taxa, somatic hybridization is an alternative to sexual crosses and to genetic transformation, which is appropriate only for traits under the control of single, well-defined and cloned genes.

In the genus *Medicago* attempts to apply somatic hybridization started in the early eighties in order to be able to transfer important agronomic traits, such as resistance to biotic and abiotic stresses and regional adaptation potential (Barnes et al. 1977; Arcioni et al. 1982), from wild *Medicago* species to tetraploid *Medicago sativa* (alfalfa), the most cultivated forage legume in the world.

In many early experiments hybrid calli were obtained, but did not regenerate plants (Gilmour et al. 1987; Damiani et al. 1988; Deak et al. 1988; Walton and Brown, 1988; Pupilli et al. 1991). The first hybrid plants were obtained from the interfertile tetraploid *M. sativa* + *M. falcata* by (Téoulé (1983) and later repeated by Mendis et al. (1991). Somatic hybrid plants were obtained from *M. sativa* + *M. arborea*, both of which are tetraploid but genetically very distant (Nenz et al. 1996).

Medicago species at different ploidy levels have been fused through asymmetric hybridization (Kuchuk et al. 1990). The only case of plant regeneration from symmetric hybrid calli between *Medicago* species at a different ploidy level was reported by Pupilli et al. (1992), who fused *M. sativa* ($2n = 4x = 32$) with *M. coerulea* ($2n = 2x = 16$). These two species are genetically very similar (Quiros and Bauchan 1988) but, because of their different ploidy levels, can be sexually crossed through unreduced gametes (McCoy and Bingham 1988). As *M. coerulea*, *M. falcata* belongs to the "sativa-falcata-coerulea" *Medicago* complex, it is interfertile with *M. sativa* when at the same ploidy level (Mariani and Veronesi 1979); and is cultivated in the northernmost

area of cultivation of alfalfa because of its resistance to cold environments (Michaud et al. 1988).

In the present paper we describe the fusion of protoplasts from *M. sativa* (2n = 4*x* = 32) with those from *M. falcata* (2n = 2*x* = 16), the regeneration of hybrid plants and an analysis of their genetic make up. Previous experiments indicated that hybrids of *M. sativa* with different partners showed modification in chromosome number and the loss of species-specific sequences (Arcioni et al. 1994).

The work described in the present paper was carried out not for transferring agronomical traits from one species to a closely related one but rather to add information about the influence of genetic distance, different ploidy levels, and cell-cycle rates between parents, on the production and genomic rearrangements of *Medicago* somatic hybrids. These data should be of value for the planning of somatic hybridization experiments between alfalfa and diploid wild *Medicago* species which contain genetic resources for resistance to insects and pathogens, and for tolerance to salinity, drought, and other stress conditions (Arcioni et al. 1994).

Materials and methods

Plant material

The tetraploid *M. sativa* genotype R15, which was selected from cv Rangelander for high regeneration ability through somatic embryogenesis from leaves and protoplast-derived calli, was kindly supplied by Dr. P. J. Larkin (CSIRO, Plant Industry, Canberra).

The diploid *M. falcata* genotype P3, which was selected for regeneration ability from callus within the WISFAL-1 population, was kindly supplied by Prof. E.T. Bingham, University of Madison, Wisconsin. Plants were propagated through cuttings and maintained in the greenhouse.

Protoplast isolation

M. sativa protoplasts were isolated from 30 heart-shaped, hair-free, fully expanded leaflets following the protocol of Pupilli et al. (1991) and using the enzyme mixture described by Nenz et al. (1996) diluted 2:1 with the protoplast culture medium KMP8 (Kao and Michayluk 1975). *M. falcata* protoplasts were isolated from 3-month-old leaf-derived calli which were cultured on UM medium (Uchimiya and Murashige 1974) supplemented with 2 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid) and subcultured every 2 weeks; 3 g of creamy callus were digested for 7 h, at the same conditions used for R15, in 30 ml of the enzyme mixture used by Nenz et al. (1996) in order to isolate protoplasts from calli of *M. arborea*. Protoplast isolation was performed as described by Pupilli et al. (1991) using CPW salts (Frearson et al. 1973) supplemented with 25% (w/v) sucrose as a floating solution.

Protoplast electrofusion

Protoplasts from both species were re-suspended in the electrofusion solution (0.6 M mannitol, 0.6 mM Histidine-HCl, 0.2 mM $CaCl_2$, pH 6.5) at 3×10^5 prot/ml. Equal volumes of mesophyll and callus protoplasts were mixed and fused in 0.5-ml aliquots using the electrofusion apparatus described by Damiani et al. (1988); the fusion chamber was a polysulphonate pipette with two stainless-steel coaxial electrodes at a distance of 1 mm from one another. The fusion conditions included an alternating current field strength of 170 V/cm with three interspersed pulses of direct current for 35-μs duration and a voltage of 1700 V/cm. The fused protoplasts were placed in a Petri dish (2 cm diameter) with 2 ml of KMP8 and a drop of *M. sativa* mesophyll protoplasts as a "nurse culture". Just before adding the untreated protoplasts the percentage of binary heterokaryons was determined through microscope observation, both in terms of the different colours of the two parental protoplasts and the different size and shape of the binary fusions with respect to the multi-protoplast ones.

Protoplast culture

One-day after fusion, the protoplasts from four Petri dishes were combined, centrifuged (60 g, 10 min), re-suspended in 1 ml of unfused mesophyll protoplasts (4×10^4 prot/ml) and embedded on a semi-solid medium as described by Pupilli et al. (1991). The heterokaryons were identified by virtue of the double colouring in fluorescent light: red from mesophyll protoplasts and green from callus protoplasts labelled with the fluorescein-isothiocianate solution (Nenz et al. 1996). The positions of hybrid cells were recorded and followed during the culture period (28 ± 1°C in the dark).

Regeneration of hybrid plants

When microcalli reached the dimension of 70–80 cells, they were picked up from the agarose with a 1-ml-syringe needle and transferred to a Petri dish (6-cm diameter) containing 0.5% agarose-solidified KM8 (Kao and Michayluk 1975) and surrounded by small highly dividing calli.

After 3 weeks of culture in the dark at 28 ± 1°C, with subculturing every 4–5 days, the calli were transferred to the growth chamber ($27\ \mu E\ m^{-2}\ s^{-1}$, 23 ± 1°C, 12-h photoperiod). After a few days, calli were transferred to 0.8% agar-solidified UM medium supplemented with 2 mg/l of 2,4-D and kept in the same environmental conditions. A 1-cm-diameter portion of putative hybrid calli was transferred to five different regeneration media all based on MS medium (Murashige and Skoog 1962) and supplemented with: (1) 0.1 mg/l of indol-3-acetic acid and 1 mg/l of 2iP (N^6-Δ^2-isopentenyl-adenine) (CDM1); (2) 0.5 mg/l of BOA (α-butoxyacetanilide) and 1 mg/l of 2iP (CDM1-BOA); (3) 10 mg/l of 2,4-D (MS10); (4) no growth regulators (MS0); and (5) 1 mg/l of α-napthalenacetic acid and 1 mg/l of 6-benzylaminopurine (MSN1). As soon as "embryo-like" structures appeared they were transferred to CDM1 medium to induce secondary embryos. When these embryos developed into shoots they were moved to Magenta vessels (6 × 6 × 10 cm), containing RL medium (Phillips and Collins 1979) without growth regulators or MS0, for root formation.

Plantlets of 8–10-cm height were placed in pots, covered with plastic bags to prevent dessication, kept in a controlled environment cabinet ($216\ \mu E\ sec^{-1}\ m^{-2}$, 20 ± 1°C, 12-h photoperiod 80% relative humidity) and after 3–4 weeks transferred to the greenhouse.

Morphological analyses of hybrid plants

Ten *sativa* + *falcata* (*S* + *F*) hybrid plants regenerated from the same callus and ten cuttings of each parent were evaluated for the following nine morphological characters: (1) growth habit, by scoring from 1 (prostrate) to 5 (erect), (2) flower colour, by visual observation, (3) leaf shape, represented by the ratio leaf length/leaf

width), (4) number of teeth per leaf, (5) tooth depth (μm), (6) number of stomata per mm^2, (7) stomata length (μm), (8) stomata width (μm), and (6) number of leaf hairs per mm^2. Traits 3–9 were recorded from the three leaflets of the trifoliate leaves positioned on the median internode of the longest stem. Data were submitted to ANOVA using the GLM procedure of the SAS program and a Duncan test was performed.

Cytological analysis

Chromosome analysis was carried out on mitotic cells of root tips excised from cuttings of $S + F$ hybrid plants. Root tips were pretreated in ice-water followed by 3 h in alpha-bromonaphthalene at 4°C and fixed in ethanol-glacial acetic acid (3 : 1) for at least 24 h. Staining was performed according to the Feulgen method, followed by squashing in 1.5% acetic orcein. At least 20 well-spread metaphases per plant were considered. Silver staining was also performed, as described by Calderini et al. (1996), in order to detect transcriptionally active rDNA loci.

Southern analysis of rDNA intergenic spacer (IGS) variants

DNA, isolated from individual plants as described by Cluster et al. 1996, was restriction-digested (1 μg each) with seven enzymes (*Ase*I, *Bcl*I, *Dra*I, *Eco*RI, *Nco*I, *Nsi*I and *Ssp*I; New England Biolabs), electrophoresed in 1% agarose gels, blotted onto Hybond N+ membranes (Amersham), and hybridized according to manufacturer's instructions with ^{32}P-dCTP-labelled probes (Ready To Go kit; Pharmacia) at 65°C. Two heterologous conserved-coding sequence rDNA probes for the 25 gene were employed (Fig. 1). The probes 25*S*-B (800 bp) and 25*S*-C (850 bp) were *Eco*RI/*Bgl*II double-digest fragments of the flax 25*S* gene from pBG35 (Goldsbrough and Cullis 1981). They were isolated as bands cut from Sea Plaque low-melting agarose (LMA; FMC Bio Products) and labelled directly in LMA. 25*S*-B contained 400 bp of 25*S* sequences and 400 bp of extraneous pAT153 cloning-vector sequences. All 850 bp of 25*S*-C were 25*S* gene sequences.

RAPD analysis

Twenty two random primers (RP1–RP22), identical to those of Kangfu and Pauls (1993), were utilised. PCR conditions for each 50-μl reaction were: 30 ng of total DNA, 27 pmol of each primer used one at a time, 5 μl of 10 × PCR buffer minus $MgCl_2$ (Gibco BRL), 3 mM of $MgCl_2$, 200 μM of each dNTP and 2.5 U of *Taq* DNA polymerase (Gibco BRL). Amplification was carried out on an MJ Research Programmable Thermal Controller with the following thermal profile: (1) a hot start at 96°C for 90 s, (2) melting at 96°C for 35 s, annealing at 40°C for 35 s and extension at 72°C for 90 s for 35 cycles, and (3) a final extension at 72°C for 10 min. Ten microliters of completed reactions were electrophoresed in 1.5% agarose gels containing ethidium bromide and photographed.

Gels containing the RAPD amplification products (10 μl) were Southern blotted and probed as described above. Blots of RAPDs produced with RP7 were probed with a 530-bp *M. falcata*-specific RP7 fragment (called FAL-7). RAPDs from RP10 were probed with a 1.2-kb *M. falcata*-specific RP10 fragment (FAL-10). Both probes were isolated by excising the appropriate fragments from RAPDs electrophoresed in LMA.

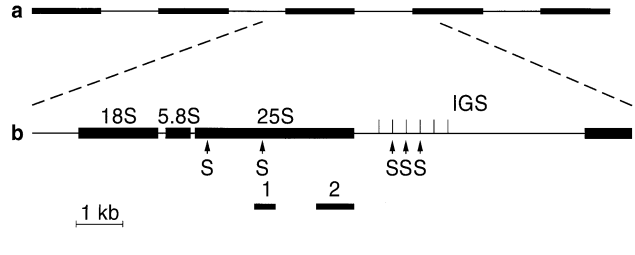

Fig. 1a, b rDNA diagram. **a** The tandem organization of rDNA genes. *Thick lines* represent coding sequences and *thin lines* noncoding IGS regions. **b** Expanded map of one complete gene unit. *SspI* sites (*S*) are from Cluster et al. (1996). Regions encoding the 18*S*, 5.8*S* and 25*S* ribosomal RNA products are indicated. Probes used in Southern analysis of rDNA IGS: *1* (25*S*-B) and *2* (25*S*-C). IGS subrepeats are indicated by *vertical lines*

Results

Protoplast isolation

Fifteen Rangelander leaves released about 5×10^6 protoplasts per gram of fresh weight; protoplast size was uniform (20–30 μm diameter) and chloroplasts were well distributed. About 75% of the protoplasts started dividing after 7 days.

When *M. falcata* callus was dispersed in liquid medium, it resulted in many single cells (100–110 per ml) plus a few colonies of 5–20 cells (4–8 per ml), as well as and a few tightly clumped colonies of 20–50 cells (two per ml). The use of CPW 25*S* (see Materials and methods) allowed the selection of 5×10^4 prot/g to be derived from the small aggregates. The *M. falcata* protoplasts were highly cytoplasmatic, vacuolized, uniform, and similar in size to the R15 protoplasts. They regenerated cell walls 4–5 days after culture, the first mitotic divisions occurred within 14 days and plating efficiency was 7% after 3 weeks.

Protoplast fusion, culture and plant regeneration

The percentage of heterokaryon production was 5–10% of the protoplasts submitted to the electrofusion process; among these, 60% were binary fusion products, 30% derived from the fusion of 3–4 protoplasts and 10% resulted from the fusion of more than four protoplasts. After 3 days the positions of binary heterokaryons, which appeared highly cytoplasmatic with chloroplasts well distributed and placed not too close to other protoplasts, were recorded. In total, 80 heterokaryons were selected; 30 of these (37.5%) underwent the first mitotic division 6 days after planting and 19 (23.7%) underwent a second division 2 days later; after 10 more days, seven heterokaryons (8.75%) had developed into microcalli constituted by 10–20 cells.

Three hybrid calli survived the transfer to UM medium, only one of which regenerated plants when cultured on MS10 for 10 days followed by CDM1 for 3 months. Unlike the result with another *Medicago*

somatic hybrid (Nenz et al., 1996), the addition of the synthetic auxin BOA to the regeneration medium was ineffective.

Analyses of hybrid plants

The *M. sativa* parent differed from *M. falcata* in most of the morphological characters evaluated (Table 1). Among the characters measured, *M. sativa* and *M. falcata* were indistinguishable only for stomatal dimensions. $S + F$ plants were intermediate for tooth depth and showed the lowest number of leaf hairs but did not differ from *M. sativa* for any of the remaining traits.

The chromosome number of all the ten hybrids analysed was $2n = 33$ (Fig. 2 a); therefore 15 parental chromosomes were lost in the hybrids. Because chromosomes of the parental species are indistinguishable with the Feulgen-staining technique, it was not possible to determine the relative contribution of the two parents to the hybrid chromosome sets. In all metaphases, two chromosomes of unexpected length were observed which could be the result of a translocation. One was extra long (Fig. 2 a, double arrow) and one extra short (Fig. 2 a, single arrow). The long chromosome had two secondary constrictions which resembled the ribosomal secondary constrictions of satellited chromosomes. However, only one of these two constrictions contained ribosomal genes and only five of six parental NORs were retained in the hybrids (Fig. 2 b).

The rDNA patterns analysed with seven restriction enzymes and two rDNA probes were remarkably similar for both parents. A few minor (low-copy number) variants were found, however, which differentiated *M. sativa* from *M. falcata*. With *Eco*RI, a *M. sativa*-specific variant and a *M. falcata*-specific variant were observed and both were present in hybrid $S + F$ plants (Fig. 3 a, arrows). Surprisingly, the copy numbers of several variants were dramatically amplified in hybrid plants. Figure 3 a also shows that several new rDNA variants, not present in the parents (all greater than 10 kb in *Eco*RI digests), appeared in hybrid plants. New variants were observed in all nine $S + F$ plants examined. The creation of new rDNA variants occurred differentially among hybrids (Fig. 3 b). Considering that the copy numbers drastically increased and that new variants were found, this analysis does not unequivocally demonstrate that both parents contributed to the hybrid genome; in fact it was possible that the *falcata*-like variants could have been derived from *M. sativa* as a consequence of rearrangements occurring in tissue culture.

For these reasons, further investigations utilizing RAPDs were undertaken to determine if *M. falcata* had contributed to the $S + F$ genome. In the RAPD analysis, the amplification products of 22 RPs were screened with DNA samples from *M. sativa*, *M. falcata* and $S + F$ lines. From among the 22 RPs screened, nine produced at least one *M. falcata*-specific band that also

Table 1 Evaluation of hybrid $S + F$ and parent plants (*M. sativa* and *M. falcata*) for leaf characters and flower colour. Values followed by the same letter do not differ significantly for $P \leq 0.05$

Character	*M. sativa*	*M. falcata*	$S + F$
Growth habit	4.2 ± 0.3a	2.0 ± 0.4b	4.8 ± 0.6a
Leaf shape	1.4 ± 0.2b	2.3 ± 0.1a	1.5 ± 0.2b
Flower colour	Purple	Yellow	Purple
Number of teeth	15.8 ± 1.3a	10.6 ± 1.1b	18.4 ± 2.9a
Tooth depth	35.1 ± 3.2a	19.1 ± 2.7c	24.6 ± 1.9b
Stomata number/mm^2	68.2 ± 4.6a	45.9 ± 3.8b	66.4 ± 5.1a
Hair number/mm^2	28.9 ± 2.2a	13.2 ± 1.5b	5.3 ± 1.1c

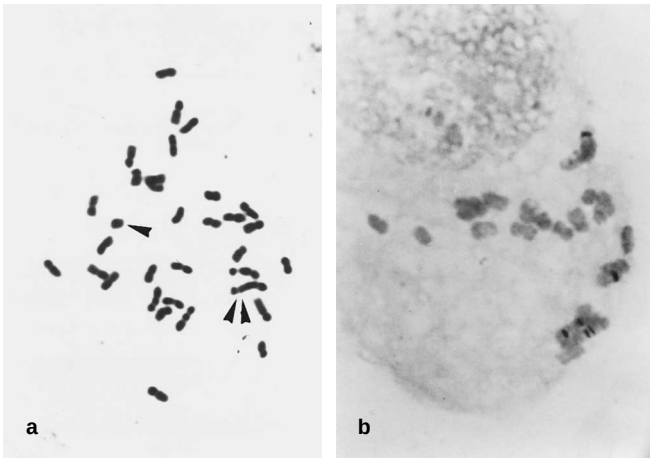

Fig. 2 **a** Metaphase spread of a hybrid $S + F$ plant. The two *arrows* indicate two chromosomes of unexpected length, probably a consequence of a translocation phenomenon. **b** A metaphase stained with silver nitrate

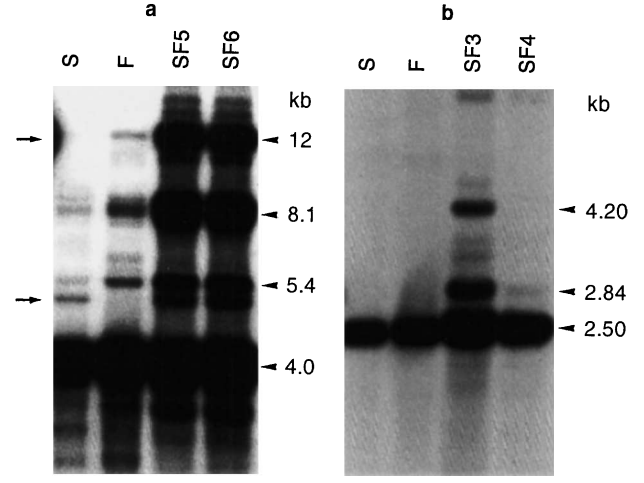

Fig. 3 **a, b** Southern rDNA patterns. **a** EcoRI fragments probed with 25*S*-C; *sativa*- and *falcata*-specific variants are indicated by *arrows* on the left. *Lane S*: *sativa*, *F*: *falcata*, *SF*: $S + F$ somaclones. **b** *Ssp*I fragments probed with 25*S*-B

appeared in $S + F$ plants. Among the RAPDs produced, an abundance of *M. sativa* and *M. falcata*-specific bands, as well as bands in common to both parents, were found (Figs. 4 a and 5 a). The $S + F$ RAPD patterns generally reflected the combination of the two parents, though some parental RAPDs were lost.

The *M. falcata*-specific FAL-7 and FAL-10 probes were hybridized, respectively, to blots of RP7 and RP10 RAPDs from parental and hybrid lines (Figs. 4 b and 5 b). Both probes gave similar results. The FAL-7 probe hybridized with two *M. falcata*-specific RAPDs, among which one was also found in $S + F$ RAPDs and none in *M. sativa*. The FAL-10 probe hybridized with two *M. falcata* RAPDs which were also present in the hybrids, but not in *M. sativa*. It was thus confirmed that *M. falcata* gene sequences were in fact integrated in the $S + F$ genome. No variation between different $S + F$ or parental lines was detected in the RAPD analysis.

Discussion

Regeneration is the most critical step in the production of somatic hybrids. In alfalfa, it is under complex genetic control and different genotypes require different culture conditions (Crea et al., 1995); in somatic hybrids, the combination of the two genomes complicates the process. It has been reported (Arcioni et al., 1994) that regeneration from hybrid calli can be achieved either when the two parental protoplasts are both regenerable or when one parent releases morphogenetic protoplasts and the other is characterized by a low division and low regeneration ability. Although the present hybrids were obtained with two morphogenetic genotypes, they required a long time for regeneration and only one hybrid callus out of three was able to regenerate plants.

From previous experiments of somatic hybridization with *Medicago* species carried out in our laboratory it was supposed that the considerable genetic distance between the parents played a major role in preventing successful somatic hybridization and in inducing large chromosome loss and rearrangements. As a matter of fact, fully symmetric hybrids were obtained fusing *M. sativa* with genetically close species, such as tetraploid *M. falcata* (Téoulé 1983) and diploid *M. coerulea* (Pupilli et al., 1992), while chromosome loss was reported in the somatic hybrid *M. sativa* + *M. arborea* (Nenz et al., 1996), which are the two most distant species of the genus *Medicago*.

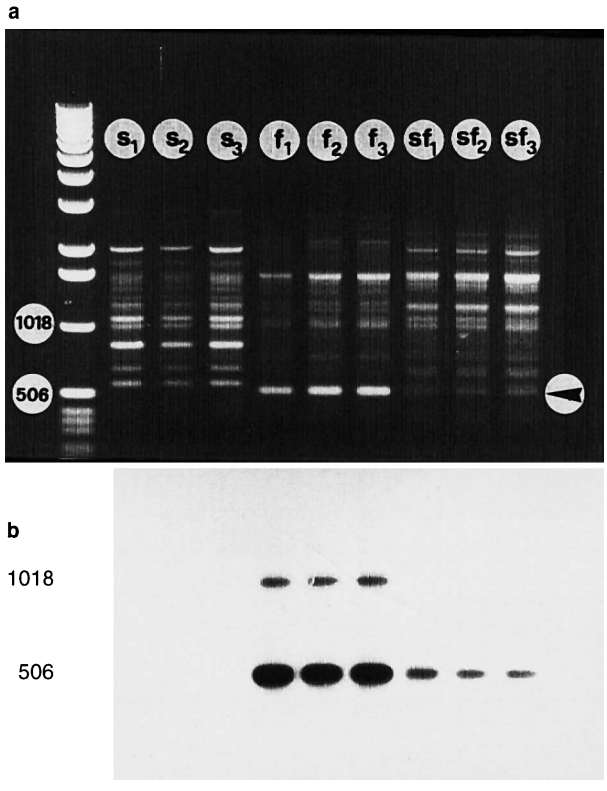

Fig. 4 **a** RAPD analysis obtained with RP7. **b** Southern analysis of the RAPD from RP7 (probe: 530-bp *falcata*-specific RP7 fragment, FAL-7). Somaclones of *M. falcata* (*s1–s3*) and *M. sativa* (*f1–f3*) parental plants were analysed

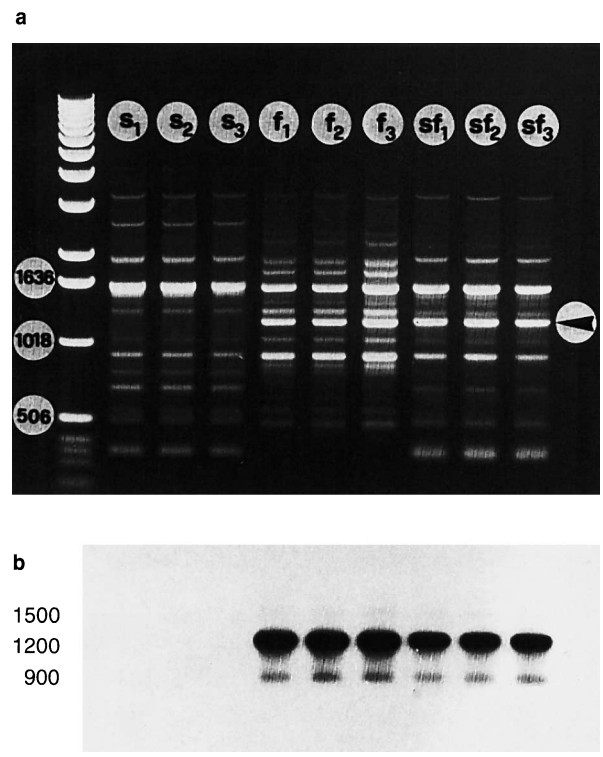

Fig. 5 **a** RAPD analysis obtained with RP10. **b** Southern analysis of the RAPD from RP10 (probe: 1.2-kb *falcata*-specific RP10 fragment, FAL-10). Somaclones of *M. falcata* (s1–s3) and *M. sativa* (f1–f3) parental plants were analysed

Since *M. coerulea*, *M. falcata* and *M. sativa* are genetically very close (Quiros and Bauchan 1988), it was expected that the diploid *M. falcata* genome would combine with that of *M. sativa* in somatic hybrids, as did *M. coerulea*. On the contrary, however, $S + F$ hybrids retained only 33 chromosomes rather than 48 (the sum of chromosomes from both parents). Consistent chromosome loss in $S + F$ hybrids has also been reported when tetraploid *M. falcata* was utilized as a parent (Coulaud et al., 1990; Mendis et al., 1991). One possible reason for chromosome elimination in the hybrids is the significant difference between parental nuclei in their cell-cycle rates as witnessed by differences in the division frequency and rate of the parental protoplasts, mesophyll protoplasts of *M. sativa* being much more active than those of *M. falcata*.

Previous reports have shown that chromosome elimination is correlated with differential parental cell-cycle rate in hybrid plants. All such studies indicated that the parent with a slow cell cycle had contributed less to the hybrid genome (Stutz 1962; Gupta 1969). More recent studies have provided evidence that the phenomenon of premature chromosome condensation (PCC) occurred in the presence of heterophasic fusion products. (Szabados and Dudits 1980). Mitotic nuclei induce a premature condensation of fused interphasic nuclei that leads to the fragmentation of single-stranded, incompletely condensed, chromosomes that might be integrated into the genome during the subsequent division (Dudits 1982). Therefore, chromosome elimination should occur preferentially from the parent with the longest cell-cycle time (i.e. *M. falcata*). $S + F$ hybrids may thus contain nearly the entire chromosome complement of *M. sativa* plus one or a few chromosomes from *M. falcata*.

The fact that regenerants closely resembled *M. sativa* for most traits, including flower colour which is intermediate in tetraploid sexual hybrids, suggests a large genetic contribution of *M. sativa* to the hybrid plants. However, the values for tooth depth and hair number indicate the presence of some genes specific to *M. falcata* in the hybrids. Also chromosomes counts, where $2n = 33$ was far in excess of the 16 chromosomes of *M. falcata*, led to the conclusion that *M. sativa* contributed significantly to the genome of the $S + F$ plants.

Notwithstanding the long callus phase of the hybrid prior to regeneration, no somaclonal variation among hybrid plants was observed for chromosome number or RAPD patterns. The variability observed for the rDNA RFLP patterns was probably due to the combined effects of in vitro culture and somatic hybridization. In fact, the many mitotic cycles which occurred in vitro increased the probability of unequal recombination in somatic lineages among partially homologous sequences such as the parental rDNA, resulting in the production of new variants. The potential application of tissue culture to induce rearrangements between alien chromosomes has already been demonstrated (Larkin and Banks 1994); in vitro culture is in fact permissive for the production of gross nuclear changes. As examples, somatic-hybrid calli produced cells with 120–250 chromosomes (Arcioni et al., 1988) and allowed the elimination of non-viable mutations through the sieve of somatic embryogenesis.

In conclusion, our results indicate that the use of somatic hybridization to improve the gene pool of *M. sativa* is affected by the syncrony of protoplast division of parents more than by genetic distance and ploidy levels *per se*. The differences between cell-cycle rates or cell-cycle phases appear to be related to chromosome loss in the hybrid plants, while cell culture also induces chromosome rearrangement and recombination between parental genomes. In the light of these results, somatic hybridization could also be considered as a method of genetic transformation suitable for transferring traits of agronomic interest controlled by "multigenic systems".

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