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Somatic hybrids and cybrids of *Senecio fuchsii* Gmel. (x) *jacobaea* L.

G. R. Wang, H. Binding

Botanical Institute, Christian-Albrechts-University, Olshausenstrasse 40, D-24098 Kiel, FRG

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Abstract. Twenty fusants of *S. jacobaea* L. (chlorophyll deficient) (x) *fuchsii* Gmel. (wild-type) were investigated by studying chromosome number, isozymes, and restriction fragment length polymorphism (RFLP). Sublines were established from primary shoots, and clones were grown from isolated protoplasts. Out of the 12 hybrid lines 9 had the amphidiploid chromosome number of 80 (2x = 40) as concluded from each one subline. Three hybrid lines showed reduced chromosome numbers between 60 and 76, indicating karyotypic instability. Additive isozyme complements with peroxidase, esterase, malate dehydrogenase, and glutamate-oxalacetate transaminase activities were found. In the RFLP analyses, three ptDNA probes were able to demonstrate the origin of plastomes from either one or both parents, but singular chondriomes from either parent were traced with a mtDNA probe. The results of detailed studies with sublines and clones of two hybrid lines revealed that the chlorophyll-deficiency mutation of one parental *S. jacobaea* clone is located in the genome and that of the other one is located in the plastome. Eight fusant lines were putative cybrids. Mutant plastids were not transmitted into the regenerated putative cybrid shoots. The mtDNA of *S. jacobaea* alone was detected with a mtDNA probe in seven putative cybrid lines; in one line mtDNA fragments of both the parents were traced. In three lines, the nucleus and the plastids only showed *S. fuchsii* characters, but the mtDNA was of *S. jacobaea*.

Key words: *Senecio* – Somatic hybrid – Cybrid – RFLP – Isozyme

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Correspondence to: G. R. Wang

Abbreviations: χ, Chondriome; mtDNA, mitochondrial DNA; π, plastome; ptDNA, plastid DNA, *S.f* or *f*, *Senecio fuchsii*; *S.j* or *j*, *Senecio jacobaea*

Introduction

Somatic hybridization in higher plants is an effective way to create novel combinations of nuclear and extranuclear genetic materials, some of which cannot be achieved by sexual crosses due to sexual incompatibility, maternal inheritance, and meiosis preceding gametogenesis. In Asteraceae, which contains several species of economic interest, a relatively low number of somatic hybridization experiments have been performed (Al-Atabe et al. 1990; Rambaud et al. 1990; Matsumoto 1991; Wang and Binding 1993), even though efficient regeneration from protoplasts has been possible since 1980–1981 (for *Senecio:* Binding and Nehls 1980; for *Cichorium* and *Gaillardia:* Binding et al. 1981). The genus *Senecio* is considered to be an appropriate model system for this family because of the particularly fast regeneration displayed by several of its species (Binding and Nehls 1980; Binding et al. 1981). In the present article, detailed investigations with somatic hybrids and cybrids of *S. fuchsii* (x) *jacobaea* (Wang and Binding 1993) are described.

Materials and methods

Plant material

The provenance of the species *Senecio fuchsii* Gmel. and *S. jacobaea* L., the production of the cholorophyll-deficient mutants *S.j*-Wa1 and *S.j*-Wa2 (*S.j* π-Wa2, as concluded from the

present studies), as well as the establishment, visual characterization, and preliminary identification of 25 somatic hybrid lines and eight putative cybrid lines of *S.f* (x) *j* have been discribed in an earlier publication (Wang and Binding 1993a). Sublines which were established from primary shoots of 12 hybrid lines and the eight cybrid lines were maintained on MS medium (Murashige and Skoog 1962) containing 0.8% agar and 2.5 μ*M* 6-benzylaminopurine. Protoplast-derived clones were grown applying the agarose gel lens technique (Binding et al. 1988a). Hormone-free MS agar medium was used for growing the material to be harvested for isozyme and restriction fragment length polymorphism (RFLP) analysis.

DNA probes specific for plastids and mitochondria

Four ptDNA clones (from Dr. K. Krupinska, Hamburg) and two mtDNA clones (from Prof. E. Pratje, Hamburg) were used to trace the organelle types of the fusants. The plasmid constructs (Table 1) were propagated in *Escherichia coli*, strain DH5α.

Chromosome counting

Protoplasts of the shoot tips of invitro cultures were isolated and cultured in streaky agarose gel lenses of about 10 μl (Binding et al. 1988a). After 3–8 days, the lenses were transferred into 95% ethanol and acetic acid (3:1 v/v) and, after 2–24 h, into acetocarmine. Squashing for the analysis of mitotic figures was obtained simply by heat-melting the agarose lens on a slide under a coverglass (Binding et al. 1992a).

Isozyme analysis

Soluble proteins were isolated from shoots of 30-day-old subcultures. The extraction and purification of the proteins, poly-acrylamide gel electrophoresis, and peroxidase, esterase, malate dehydrogenase, and glutamate-oxalacetate transaminase staining (according to Allen et al. 1984; Brewer 1970; Gabriel 1971) were performed as described by Wang and Binding (1993 submitted).

RFLP analysis

Total DNA from fresh plant material of fusants and their parental species grown in vitro was prepared using a CTAB protocol (Rogers and Bendich 1988) modified by Mettler (1987). The procedure is described in detail by Wang and Binding (1993 submitted).

Plasmids with inserts of ptDNA and mtDNA were isolated from the *E. coli* strain DH5α and purified on Qiagen columns (Qiagen, Düsseldorf, FRG) according to the manufacturer's instruction. Insert DNA was restricted according to the particular restriction sites, separated in 0.8–1.5% agarose gel by electrophoresis, and collected with NA45 DEAE membranes (Schleicher and Schuell, Dassel, FRG). The insert DNA was labelled with [^{32}P]-ATP (NEN-DuPont, Bad Homburg, FRG) using random hexanucleotides (Boehringer Mannheim) as primers (Feinberg and Vogelstein 1983).

The isolated plant DNA (10 μg) was digested with the restriction endonucleases *Eco*RI, *Bam*HI, and *Hin*dIII, both singularly and in various combinations, following the manufacturer's instruction (Boehringer Mannheim). Separation by agarose gel electrophoresis, Southern blotting of the DNA fragments, and probing of the blots were as published in Wang and Binding (1993 submitted). The investigations were carried out with each of two independent digests in order to avoid any misinterpretation arising from incomplete cleavage.

Results

Lines and sublines with phenotypic characters indicative of a fusant nature were established from primary shoots of 33 lines. Differently pigmented shoots commonly grew from hybrid calluses of *S.f* (x) *j*-Wa2. Protoplast-derived clones from variegated shoots were either green or albinotic. On the other hand, *S.f* (x) *j*-Wa1 lines produced green shoots exclusively, and only rarely were small chlorophyll-deficient spots observed in the leaves of a few shoots. The hybrid lines additionally produced primary shoots that showed purely parental habitus. The shoot populations of the putative cybrid lines were uniform in their phenotypes. The constitutions of the fusants as concluded from the present studies which were performed roughly a year after the fusion treatment, are indicated in Table 2. Cell graft chimeral shoots were never found in the fusant lines.

Genetic constitutions of the nuclei of the fusants

The nuclear constitutions of the hybrids were investigated in 12 sublines, each of which was established from a separate line. Nine hybrid sublines had the expected amphidiploid chromosome number of 80

Table 1. The DNA inserts for RFLP probing. The ptDNA probes derived from barley were cloned in the pUC plasmid, and the mtDNA probes derived from maize were cloned in the pBR322 plasmid

Donor site	Insert fragment	Reference
ptDNA probes		
ndh E + G	1205 bp *RSa*I segment (192-2) of pHvC 192	Krupinska 1992
*psb*A	800 bp *Eco*RI/*Hin*dIII segment of pHvC 147	Falk et al. 1993
*rbc*L	200 bp *Pst*I/*Eco*RI segment of pHvC 209	Falk et al. 1993
psb F + E	350 bp *Eco*RI segment of pHvC 186	Krupinska and Berry-Lowe 1988
mtDNA probes		
Cob	2900 bp *Hin*dIII segment	
CoxII	2300 bp *Pst*I/*Eco*RI segment	

Table 2. Genetic constitutions of somatic hybrids and putative cybrids of *S. fuchsii*(x) *jacobaea* as concluded from present investigations

Fusant line	Composition of the fusant line			Chlorophyll content	Chromosomes
	Genome	Plastome	Chondriome		
Somatic hybrids					
***S.f*(x)*j*-Wa2**					
H1	***S.f*(x)*j***	$\pi f+j$	χj	**ch1$^+$/ch1$^-$**	80
H2	*S.f*(x)*j*	πj	χj	**ch1$^-$**	60–70
H3	*S.f*(x)*j*	$\pi f+j$	χj	**ch1$^+$/ ch1$^-$**	68–75
H4, 5	*S.f*(x)*j*	$\pi f+j$	χf	**ch1$^+$/ch1$^-$**	80
***S.f*(x)*j*-Wa1**					
H6	*S.f*(x)*j*	πj	χf	**ch1$^+$**	80
H7	***S.f*(x)*j***	$\pi f+j$	χj	**ch1$^+$**/(**ch1$^-$**)	80
H8	*S.f*(x)*j*	$\pi f+j$	χj	**ch1$^+$**	80
H9	*S.f*(x)*j*	πj	χf	**ch1$^+$**	80
H10	*S.f*(x)*j*	πj	χj	**ch1$^+$**	80
H11	*S.f*(x)*j*	πf	χj	**ch1$^+$**	80
H12	*S.f*(x)*j*	πj	χf	**ch1$^+$**	65–76
Putative cybrids					
***S.f*(x)*j*-Wa1**					
C1, 2	**(x)*S.f***	πj	χj	**ch1$^+$**	**40**
C3, 4, 5	**(x)*S.f***	πf	χj	**ch1$^+$**	**40**
***S.f*(x)*j*-Wa2**					
C6	**(x)*S.j***	πf	$\chi f+j$	**ch1$^+$**	**40**
C7, 8	**(x)*S.j***	πf	χj	**ch1$^+$**	**40**

Bold, constituents of the line; normal case, constituent of a single subline; within brackets, occasionally in small sectors

(2n = 40 from each parent); in the other 3 hybrid sublines chromosome numbers between 60 and 76 indicated karyotypic instability (Table 2). Isozyme investigations revealed biparental patterns in all of the 12 hybrid sublines. Figure 1a depicts the patterns of the peroxidase isozyme. One peculiarity of these peroxidase patterns is that there is one band specific for *S.f* that is obviously less intense in hybrid lines 1–3, 5–8, 10, and 12 than in the parental sample.

The putative cybrid lines had the expected 2n = 40 chromosome number (Table 2). Correspondingly, only uniparental isozyme patterns were obtained indicating the presence of the *S.f* genome in five lines and the *S.j* genome in three lines. As an example, the patterns of peroxidase activity are shown in Fig. 1b.

The plastid complements of the fusant lines

Species-specific RFLP bands were visualized with all of the ptDNA probes (Table 1) except for the *psb*A probe. The same hybrid sublines used for nucleus analysis were screened. Six sublines displayed the specific bands of *S.j* plastids, 4 sublines, those of *S.f* and 2 sublines, those of both parents. As an example, the RFLP pattern after hybridization with the *ndh* E + G probe is demonstrated in Fig. 2a. Further, 11 sublines of each of 2 selected hybrid lines containing chlorophyll-deficient mutants *S.j*-Wa2 and *S.j*-Wa1, respectively, were investigated. The RFLP obtained with the line *S.f*(x)*j*-W2-H1 shows that chorophyll-deficiency was strictly correlated to the ptDNA pattern of *S.j*, whereas in green sublines only the ptDNA of *S.f* was detected (Fig. 3a). RFLP of the sublines of the green line *S.f*(x)*j*-Wa1-H7 showed *S.j* ptDNA bands in 5 sublines, *S.j* ptDNA bands in 2 sublines, and those *S.j* both parents in 4 sublines (Fig. 3b).

The results from RFLP analysis with the putative cybrid lines suggest that plastid segregation had been completed at the time of the investigation (about 12 months after the fusion experiment) and that only tissue with either one or the other plastid type was left in the investigated material. All three lines with *S.j*-W2 (C6-C8) had the constitution (x)*S.j*πf. The lines C1 and C2 with *S.j*-W1 were (x)*S.f*πj, and the lines 3–5 appeared to be (x)*S.j*πf (Fig. 2b).

The mitochondrial complements of the fusant lines

Only one of the two mtDNA probes (Table 1), namely *CoxII*, was suited for tracing species-specific mtDNA.

Of the 12 representative hybrid sublines 7 showed the RFLP pattern of *S.j*; in the other 5 hybrid sublines the mtDNA of *S.f* was indicated (Fig. 4a).

Out of eight putative cybrid lines, seven lines had only *S.j* mtDNA fragments. Line C6 appeared to be (x)*S.j* $\pi f \chi f + j$, with a prominent band of *S.f* and a little one of *S.j* (Fig. 4b). Lines 3–5, in which purely *S.f* nuclei and plastids were traced, were identified as fusants of the (x)*S.f* $\pi f \chi j$ nature containing heterologous mtDNA.

Discussion

The fusant nature of the 20 lines studied was confirmed, thereby indicating that the morphological characters such as types of hair, leaf shape, leaf margin, leaf point, and pigmentation (Wang and Binding 1993) had been appropriate selection criteria. Clearcut proof of the fusant nature was provided by the combination of nuclear characters with RFLP patterns of the plastids and/or mitochondria (Table 2).

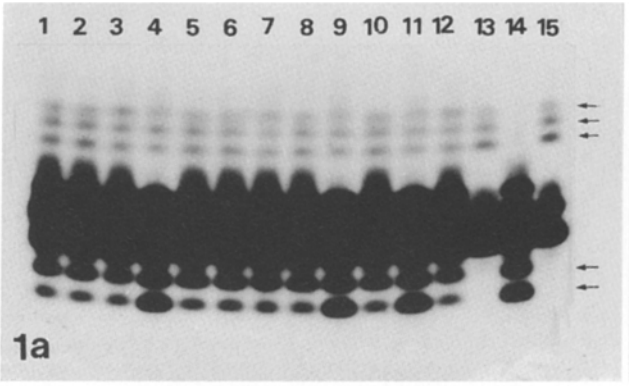
Some of the callus lines from which the fusant sublines were grown were composed of more than one protoplast-derived clone since both uniparental primary shoots as well as hybrid shoots formed. However, fusant(+)parent cell graft chimeral primary shoots which would have resulted in uniparental segregants within sublines and protoplast-derived clones were never observed. This suggests that the heterogeneity between sublines of hybrid lines was caused by the segregation of organelles.

While the chromosome numbers and isozyme patterns of the hybrids fitted the expectation, if the phenotypes and organelle DNA had not been additionally considered misinterpretation could not have been avoided. Confusion with autotetraploids would have been possible because no species-specific karyotypic characters were available; the isozyme patterns were purely additive, lacking hybrid bands and, hence, would not have been able to distinguish the respective patterns of cell graft chimeras most frequently observed in Solanaceae (Binding et al. 1992b).

Some hybrid lines showed karyotypic instability. This was indicated by reduced chromosome numbers in 3 of the 12 lines as well as by the occasional occurrence of albinotic spots in line H7 with the nuclear constitution *S.f*(x)*j*-Wa1. With respect to the albinotic spots, apparently pairs of chromosomes or chromosome fragments which complemented the mutation were lost. Furthermore, less pronounced *S.j* bands of peroxydase in some of the hybrids may be taken as an indication of the loss of a single chromosome or chromosomal fragment carrying the corresponding gene. However, the expected frequency of such an event would probably be much lower than suggested by its occurrence in 9 of the 12 lines.

The fusant nature of the putative cybrid lines was provided by specific combinations of the investigated nuclear and organellar traits. The formation of putative cybrids might have occurred (1) by the loss of a complete chromosome set of a hybrid, (2) by the loss of one nucleus type of a heterokaryon, or (3) by fusion of a subprotoplast to a protoplast (Binding et al. 1986). The cybrids are still classified as "putative": a probable derivation from a hybrid might have resulted in an extremely asymmetric hybrid rather than pure cybrids, the definition demanding a pure uniparental nucleus (Gleba and Sytnik 1984). Such an asymmetric hybridity, which has already been found by Power et al. (1975), Dudits et al. (1980), Gupta et al. (1984), and others, must be particularly taken into consideration for the putative cybrids (x)*S.f* $\pi f \chi j$; they were originally selected by malformations which might have been caused by mitochondrial, but which also could have been caused by nuclear factors. When considered from another point of view, the loss of chromosomes is unlikely due to the homogeneity of the populations of

Figs. 1–4. **1** Peroxidase isozyme activity patterns of the fusants. **a** The hybrids *S. fuchsii* (x) *jacobaea*-H1–H12 (Table 2) and the parents. *Lanes 1–12* correspond to the hybrids, *lane 13 S. jacobaea*-W a1, *lane 14 S. fuchsii, lane 15 S. jacobaea*-Wa2. **b** The putative cybrids *S. fuchsii* (x) *jacobaea*-C1–C8 and the parents. *Lanes 1–8* correspond to the putative cybrids (Table 2), *lane 9 S. fuchsii, lane 10 S. jacobaea*-W a1, *lane 11 S. jacobaea*-Wa2. **2** RFLPs of ptDNA of the somatic fusants and the parents hybridized with a barley plastid probe of the *ndh* E + G genes. Total DNA was isolated from each a sublines of the hybrids or each line of the cybrids and digested with *Eco*RI + *Bam*HI. The DNA fragments were seperated by agarose gel electrophoreses and Southern blotted. The kb values were concluded from the positions of λDNA fragments digested by *Hin*dIII. **a** The somatic hybrids *S. fuchsii* (x) *jacobaea*-H1–H12 and the parents. *Lanes 1–12* correspond to the hybrids, *lane 13 S. jacobaea*-W a1, *lane 14 S. fuchsii.* **b** The putative cybrids *S. fuchsii* (x) *jacobaea*-C1–C8 and the parents. *Lanes 1–8* correspond to the putative cybrids (Table 2), *lane 9 S. jacobaea*-Wa1, *lane 10 S. fuchsii.* **3** RFLPs of ptDNA of each of 11 sublines of 2 hybrids. DNA was prepared and probed as described for Fig. 2. **a** *Lanes 1–11* Sublines from *S. fuchsii* (x) *jacobaea*-Wa2-H1; sublines *1–7* were chlorophyll deficient and sublines *8–11* were green. *Lane 12 S. jacobaea*-Wa2 *lane 13 S. fuchsii.* **b** *Lanes 1–11* Somatic hybrid sublines from *S. fuchsii* (x) *jacobaea*-Wa1-H7 (Table 2); all sublines were green. *Lane 12 S. jacobaea*-Wa1, *lane 13 S. fuchsii.* kb values as for Fig. 2. **4** RFLPs of mtDNA of the somatic fusants and the parents hybridized with a maize mtDNA probe of the *CoxII* gene. Total DNA was isolated from each a subline of the somatic hybrids or each line of the putative cybrids and digested with *Eco*RI. The DNA fragments were separated by agarose gel electrophoreses and Southern blotted (kb values as for Fig. 2). **a** The somatic hybrids *S. fuchsii* (x) *jacobaea*-H1–12 and the parents. The lanes are ordered as in Fig. 2a. **b** The putative cybrids of *S. fuchsii* (x) *jacobaea* and the parents. The lanes are ordered as in Fig. 2b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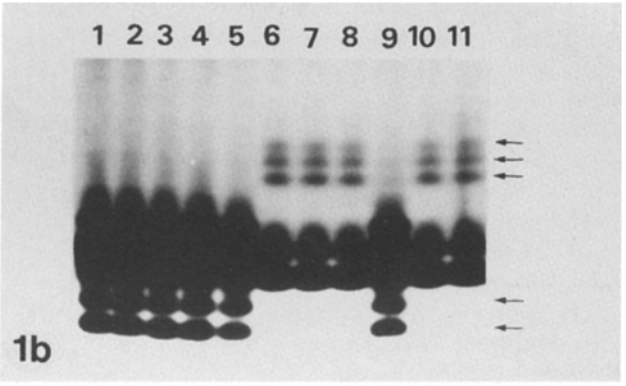
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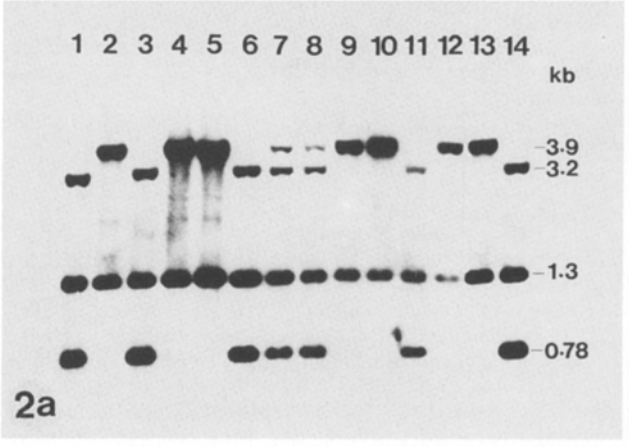
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kb
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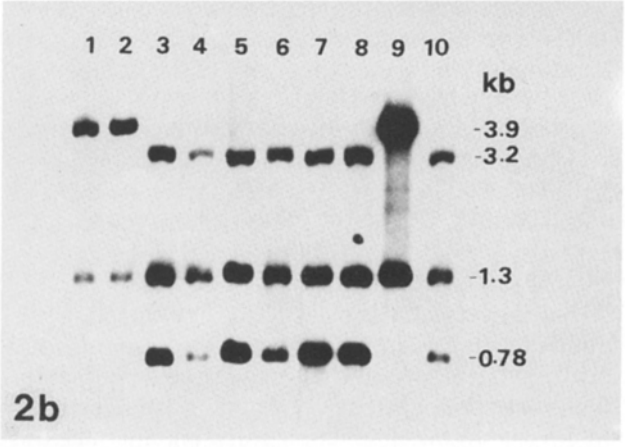
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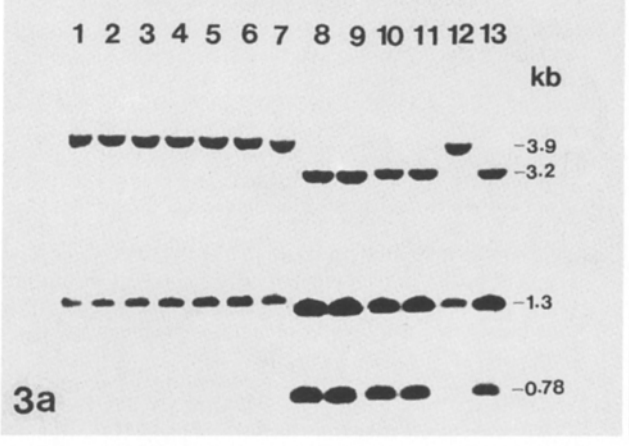
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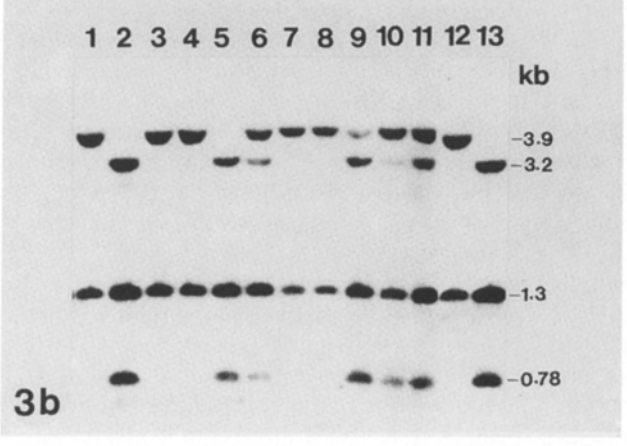
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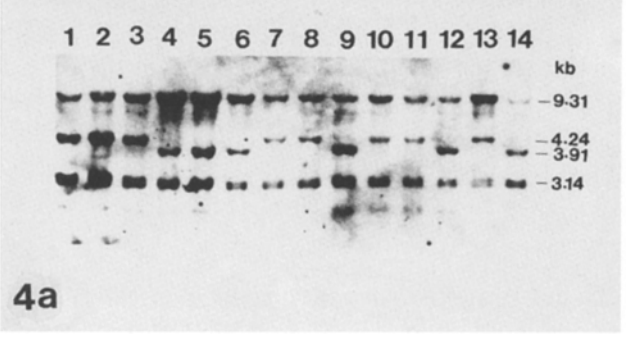
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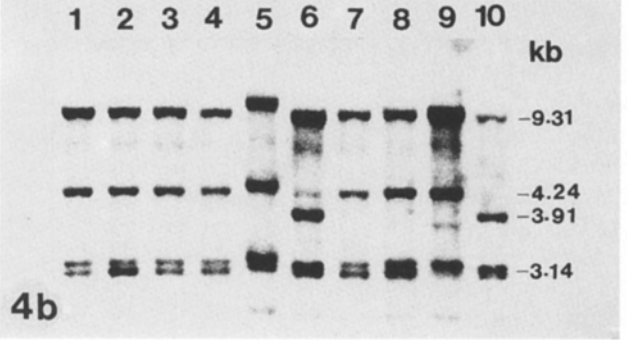
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primary shoots in any cybrid line. Another explanation, incomplete rounding up of the fusion body caused by the strength of the agarose gel, might have limited the migration of nuclei and organelles in the heterokaryon. The relatively high numbers of cybrids – ranging around 25% of the fusant – found also with *Solanum* (Binding et al. 1988b) favour this suspicion of a correlation between embedding in agarose and cybridization. Subprotoplast fusion also cannot be excluded. However a constitution as (x)*S.j* π *f* which completely lacks *S.j*-Wa2 plastids is not sufficiently explained by either of these processes.

The chlorophyll deficiency of *S.j*-Wa2 which appeared to be located in the plastome was a convenient and sensitive means for the investigation of plastid segregation during the development of the fusants. In most of the hybrid sublines and all of the putative cybrids only uniparental plastids were detected, suggesting that the segregation of plastids was soon completed. This is particularly indicated by the absence of variegated clones in protoplast regenerants. Rapid segregation of plastids has also been found in other fusion combinations (e.g. Morgan and Maliga 1987).

The drawing of a conclusion on the direction of organelle segregation may be precarious due to the limited numbers of fusant lines, fusant sublines, and DNA probes investigated. A unilateral plastid segregation directed to the elimination of the mutant plastids was indicated in the putative cybrids with a (x)*S.j* π *f* nature. However, there is no determinable reason for the elimination of the *S.j* plastids in the presence of the *S.j* nucleus. Furthermore, the domination of the mtDNA of of *S.j* particularly in the cybrids cannot be reasonably explained. Cybrid lines or sublines with the *S.j*-Wa1 nucleus have most probably not been recognized because of their similarity to uniparental *S.j*-Wa1 lines, with both having the same morphology and both leaking chlorophyll by the nuclear mutation.

The DNA probes from barley and maize were appropriate for the detection of species-specific DNA fragments of the plastids and mitochondria, respectively. The high degrees of homology between monocot and dicot ptDNA were also utilized in *Solanum* (x) *Potentilla* cybrids (Wang and Binding 1993 submitted). However, the number of probes investigated here was not sufficient to recognize potential organelle DNA recombinants that have been frequently found in mitochondria (first by Belliard et al. 1979) and rarely plastids (Medgyesy et al. 1985; Thanh and Medgyesy 1989; Wang and Binding 1993 submitted).

The fusants were useful in allocating the mutation of *S.j*-Wa1 into the nuclear genome and the *S.j*-Wa2 mutation into the plastome. The plastid-specific RFLP patterns and the pigmentation were totally congruent in the sublines of hybrids and putative cybrids of *S.f*(x)*j*-Wa2. In contrast, *S.f*(x)*j*-Wa1 hybrids only formed green shoots, whereas some of these lines contained plastids of *S.j* or both parents. The localization of genes into different compartments of the cell by somatic hybridization was first investigated in *Nicotiana* by the group of Gleba (see Gleba and Sytnik 1984) and by Aviv and Galun (1985), and has also been successful in *Solanum* (Binding et al. 1989).

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References

Al-Atabee JS, Mulligan BJ, Power JB (1990) Interspecific somatic hybrids of *Rudbeckia hirta* and *R. laciniata* (Compositae). Plant Cell Rep 8:517–520

Allen RC, Saravis CA, Maurer HR (1984) Gel electrophoresis and isoelectric focusing of proteins, selected techniques. Walter de Gruyter, Berlin, New York

Aviv D, Galun E (1985) An in vitro procedure to assign pigment mutations in *Nicotiana* to either the chloroplast or the nucleus. Hered 76:135–136

Belliard G, Vedel F, Pelletier G (1979) Mitochondrial recombination in cytoplasmic hybrids of *Nicotiana tabacum* by protoplast fusion. Nature 281:401–403

Binding H, Nehls R (1980) Protoplast regeneration to plants in *Senecio vulgaris* L. Z Pflanzenphysiol 99:183–185

Binding H, Nehls R, Kock R, Finger J, Mordhorst G (1981) Comparative studies on protoplast regeneration in herbaceous species of the Dicotyledoneae class. Z Pflanzenphysiol 101:119–130

Binding H, Krumbiegel-Schroeren G, Nehls R (1986) Protoplast fusion and early development of fusants. In: Reinert J, Binding H (eds) Results and problems in cell differentiation 12-differentiation of protoplasts and transformed cells, Springer, Berlin Heidelberg New York, pp 37–66

Binding H, Görschen E, Jörgensen J, Krumbiegel-Schroeren G, Ling HQ, Rudnick J, Sauer A, Zuba M, Mordhorst G (1988a) Protoplast culture in agarose media with particular emphasis to streaky culture lenses. Bot Acta 101:233–239

Binding H, Zuba M, Rudnick J, Mordhorst G (1988b) Protoplast gel fusion. J Plant Physiol 133:409–413

Binding H, Bünning D, Görschen E, Hansen U-P, Ling HQ, Röhl S, Zuba M. (1989) Protoplast plating and fusion in agarose lenses applied to potato. Vortr Pflanzenzuecht 15:18–10

Binding H, Görschen E, Hassanein AM, Ling HQ, Mordhorst G, Puck G, Rudnick J, Wang GR, Truberg B (1992a) Plant development from protoplasts of members of Bryophyta, Pteridophyta and Spermatophyta under identical conditions. Physiol Plant 85:295–300

Binding H, Lee DW, Ling HQ, Buch G, Tank G, Rudnick J, Wang GR (1992b) Interspecific interactions in the regeneration from protoplasts. In: Oono K, Hirabayashi T, Kikuch S, Handa H, Kajiwara K (eds) Plant tissue culture and gene

manipulation for breeding and formation of phytochemicals. NIAR, Japan, pp 27–34

Brewer GJ (1970) An introduction to isozyme techniques. Academic Press, New York, San Francisco, London

Dudits D, Fejer O, Hadlaczky G, Koncz L, Lázár GB, Horwarth G (1980) Intergeneric gene transfer mediated by plant protoplast fusion. Mol Gen Genet 179:283–288

Falk J, Schmidt A, Krupinska K (1993) Characterization of plastid DNA transcription in ribosome deficient plastids of heat-bleached barley leaves. J Plant Physiol 141:176–181

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Analytical Biochemistry 132:6–13

Gabriel O (1971) Locating enzymes on gels. In: Jakoby WB (ed) Enzyme purification and related techniques. (Methods in enzymology, vol 22.) Academic Press, New York, London, pp 578–604

Gleba YY, Sytnik KM (1984) Protoplast fusion. Springer, Berlin Heidelberg New York Tokyo

Gupta PP, Schieder O, Gupta M (1984) Intergeneric nuclear gene transfer between somatically and sexually incompatible plants through asymmetric protoplast fusion. Mol Gen Genet 197:30–35

Krupinska K (1992) Transcriptional control of plastid gene expression during development of primary foliage leaves of barley grown under a daily light-dark regime. Planta 186:294–303

Krupinska K, Berry-Lowe S (1988) Characterization and in vitro expression of the cytochrome b-559 genes of barley. I. Localization and sequence of the genes. Carlsberg Res Commun 53:43–55

Matsumoto E (1991) Interspecific somatic hybridization between lettuce (*Lactuca sativa*) and wild species *L. virosa*. Plant Cell Rep 9:531–534

Medgyesy P, Fejes E, Maliga P (1985) Interspecific chloroplast recombination in a *Nicotiana* somatic hybrid. Proc Natl Acad Sci USA 82:6960–6964

Mettler IJ (1987) A simple and rapid method for minipreparation of DNA from tissue-cultured plant cells. Plant Mol Biol Rep 51:346–349

Morgan A, Maliga P (1987) Rapid chloroplast segregation and recombination of mitochondrial DNA in *Brassica* cybrids. Mol Gen Genet 209:240–246

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tissue cultures. Physiol Plant 15:473–497

Power JB, Frearson EM, Hayward C, Cocking EC (1975) Some consequences of the fusion and selective culture of *Petunia* and *Parthenocissus* protoplasts. Plant Sci Lett 5: 197–207

Rambaud C, Dubois J, Vasseur J, Lejeune B, Quetier F (1990) Obtention de chicorées males-stériles (*Cichorium intybus* L. var 'Magdebourg') par fusion de protoplastes. Colloq INRA Paris 51:315–316

Rogers SO, Bendich AJ (1988) Extraction of DNA from plant tissue. In: Gelvin SB, Schilperoort RA, Verma DPS (eds) Plant molecular biology manual. Kluwer Academic Publ, Dordrecht Boston London, A6:1–10

Thanh ND, Medgyesy P (1989) Limited chloroplast gene transfer via recombination overcomes plastome-genome incompatibility between *Nicotiana tabacum* and *Solanum tuberosum*. Plant Mol Biol 12:87–93

Wang GR, Binding H (1993) Somatic hybridization between *Senecio fuchsii* Gmel. and *S. jacobaea* L. Acta Hortic 336:315–320