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Interaction between cytoplasmic composition and yield parameters in somatic hybrids of *S. tuberosum* L.

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Abstract The nuclear and cytoplasmic composition of five different fusion combinations, consisting of up to 50 hybrid regenerants each, was characterized by RFLP analysis. Simultaneously, the hybrid clones of four fusion combinations were evaluated in field experiments for yield and starch content.

Predominantly complete chloroplast segregation was found with a 1:1 ratio, in all but one fusion combination. Mitochondria, in contrast revealed up to 75% recombination, as proven by the partial addition of parental banding patterns and the altered assignment of the same genotypes with different probes. Newly occuring DNA bands were also indicative of rearrangements in the mitochondrial genome. Correlations between RFLP data and field parameters were calculated. Deviating RFLP patterns of the nuclear genome did not influence yield parameters. Also the assignment of hybrids to different chloroplast genotypes did not affect yield or starch content. However, mitochondrial types could be distinguished with respect to starch content and tuber yield. The more thorough analysis of mitochondrial composition, with different probes homologous to coding regions, revealed a relationship between the homogeneity of the mt genome and the yield level.

Key words Potato · Somatic fusion · Cytoplasm Starch · Variability · Field evaluation

Introduction

Interspecific protoplast fusions within the species *Solanum* were originally developed to introduce novel germplasm from sexually non-compatible *Solanum* species (Austin et al. 1985) in order to broaden the genetic base of *Solanum tuberosum*. Cell fusion is, however, not only a technique to broaden the gene pool of potatoes but also a fast procedure for the combination of qualitative, and especially quantitative, traits within the *S. tuberosum* gene pool. According to the analytical-synthetical breeding scheme (Wenzel et al. 1979) intraspecific protoplast fusion has become a standardized method to combine selected interdihaploids with 2n=2x=24 chromosomes posessing agronomically important traits and to obtain a high degree of heterozygosity at the tetraploid level.

Although several groups have in the last few years reported the production of somatic hybrids (Austin et al. 1985; Deimling et al. 1988; Waara et al. 1989; Chaput et al. 1990) only preliminary reports on the field performance of intraspecific hybrids are available (Möllers and Wenzel 1992; Munzert et al. 1992; Thach et al. 1993; Möllers et al. 1994). Such field evaluations of intraspecific somatic hybrids have revealed new variability within the hybrids of a single fusion combination which theoretically should be uniform (Möllers and Wenzel 1992; Thach et al. 1993). A precise explanation of this unexpected variability has not been given but the differences between hybrids can be ascribed to several reasons such as somaclonal variation, aneuploidy, the composition of the cytoplasm, and its interaction with the nucleus.

Molecular analysis of somatic hybrids, especially of their cytoplasmic composition, has already been performed for the various organelles in the genus *Brassica* (Walters and Earle 1993). Most of the interspecific fusions show an early segregation of chloroplasts into the two parental types, whereas at the mitochondrial level a rather high percentage of recombination events were evident (Morgan and Maliga 1987). Most of the experiments on the analysis of nucleus-organellar interaction refer to studies on cybrids, to avoid any influences originating from the fused nuclear background of hybrids (Perl et al. 1991). In order to establish somatic hybridization in practical breeding programs, emphasis is placed in the present study upon the molecular description of the nucleus and the composition of the cytoplasm in symmetric somatic hybrids from different fusions.

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Materials and methods

Cell culture

For the present analysis, fusions yielding about 30 hybrid clones, each originating in a single fusion event, were selected (Table 1). Isolation, fusion experiments and regeneration of protoplasts follow the procedure published by Möllers and Wenzel (1992). Hybrid regenerants were identified by isoenzyme- and RFLP- analysis and were transferred to the greenhouse for further propagation and tuber production. Chromosome numbers of the hybrids were determined by counting metaphases from squashed root tips.

DNA extraction, cloning, and hybridization

Total genomic DNA was extracted according to Saghai-Maroof et al. (1984). The isolation of mitochondria and chloroplasts was a slightly modified version of the methods of Kemble (1987) and Hosaka and Hannemann (1987) respectively. To distinguish the chloroplast (cp) and mitochondrial (mt) type of fusion parents and hybrid regenerants, total cp- and mt-DNA was digested with restriction enzymes and separated on ethidium bromide-stained agarose gels. Polymorphisms among genotypes were detectable on this basis but, for a more thorough analysis of the composition of cytoplasm, the construction of cp- and mt-DNA-libraries was necessary. These were constructed by shotgun cloning of *Bam*HI-, *Eco*RI-, *Hin*dIII- and *Xba*I- digested organellar DNA into pBSc and pUC vectors (Maniatis et al. 1982). The libraries were first screened with heterologous probes of known cp- and mt-genes (kindly provided by A. Brennicke and W. Schuster, IGF Berlin and R. G. Herrmann, LMU Munich), in order to detect the corresponding homologues of potato and to obtain an orientation within the genomes. Probes found by this method also contain non-coding regions showing a higher potential for the detection of RFLPs. Homologous probes were named according to the reading frame to which they belong. For the analysis of the nuclear DNA, probes were obtained from C. Gebhardt, MPI Cologne.

For all hybridizations total DNA digested with restriction endonucleases (Pharmacia), separated on 0.8% agarose gels and blotted to nylon membranes (Hybond N), was used. For hybridization the probes were random prime labelled with ^{32}P-dCTP. Preliminary experiments demonstrated that, for the organellar probes, Southern blots of total DNA yielded the same banding patterns as blots with the purified mt- or cp-DNA. Probes which revealed polymorphisms between fusion parents were used in the further analysis of the regenerants.

Field experiments

Field experiments were performed during 2 years in two replications. They consisted of incomplete lattices with approx. 4.8 m^2 per plot, each containing 22 plants. Field data were evaluated at two locations: in the experimental fields of the Institute for Resistance Genetics, Grünbach, and in Northern Germany in the experimental fields of Bioplant, Ebstorf. Starch and dry matter were determined using a starch-weighing machine.

Table 1 Fusion combinations analyzed in the present study

Fusion combination				Number of regenerants analyzed
No.	A	(+)	B	
6001	BP 32	(+)	H77.417/9	47
6020	H88.1512/14	(+)	H77.421/2	25
6028	H88.1512/28	(+)	H77.421/2	26
6501	FAL 2	(+)	86.601	35

Results

Analysis of the nuclear DNA

For the RFLP analysis of the regenerants, Southern blots of the genomic DNA, restricted with two different endonucleases (*Eco*RI, *Hin*dIII) were hybridized with two genomic or cDNA probes for each chromosome. Deviating banding patterns, e.g., missing and additional bands, were recorded. In most of the cases missing bands were indicative of aneuploidy. Results of the RFLP analysis of three fusion combinations are summarized in Table 2. The percentage of deviations for each chromosome and the percentage of hybrid genotypes with deviating banding patterns are shown. Nearly all chromosomes were affected by deviations. The percentage of deviating genotypes in different fusions showed rather high variation, depending probably on the duration of the regeneration.

Analysis of the chloroplast genome

The cp-DNA seemed to be highly conserved within the *S. tuberosum* genotypes used in the fusion experiments. Thus only a few polymorphic probes per fusion combination could be selected and used in further analysis.

Relative to their chloroplast genotype somatic hybrids segregated into the two parental types; no mixtures of the banding pattern nor new bands indicating a recombination event could be observed (Fig. 1). In the greater majority of fusion combinations a segregation ratio of 1:1 was found

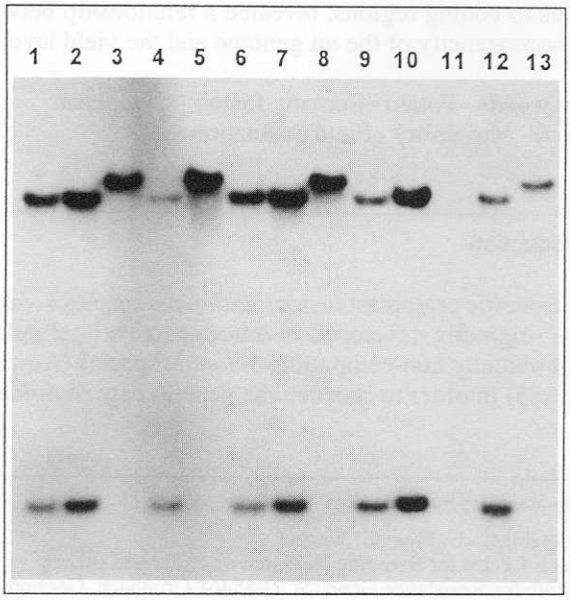

Fig. 1 Autoradiograph of a Southern blot with *Eco*RI-cut DNA, hybridized with cp probe "psbB". *Lanes 1–10,* regenerants of fusion combination 6001; *lane 11*, molecular-weight standard; *lanes 12 and 13*, fusion parents

Table 2 Percentage of hybrid regenerants with deviating RFLP banding pattern. Data for single chromosomes and the whole fusion combination are given. Most of the genotypes deviated on more than one chromosome

Fusion combination	Percentage of regenerants with deviating RFLP banding pattern on chromosome												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
6001	9.3			7.0		2.3	9.3				4.6	11.6	27.9
6020	4.3	8.6		12.9	8.6	4.3	17.2			4.3	17.2		52.0
6028			3.8								7.6		11.0
6501	2.8			2.8				2.8				2.8	8.6

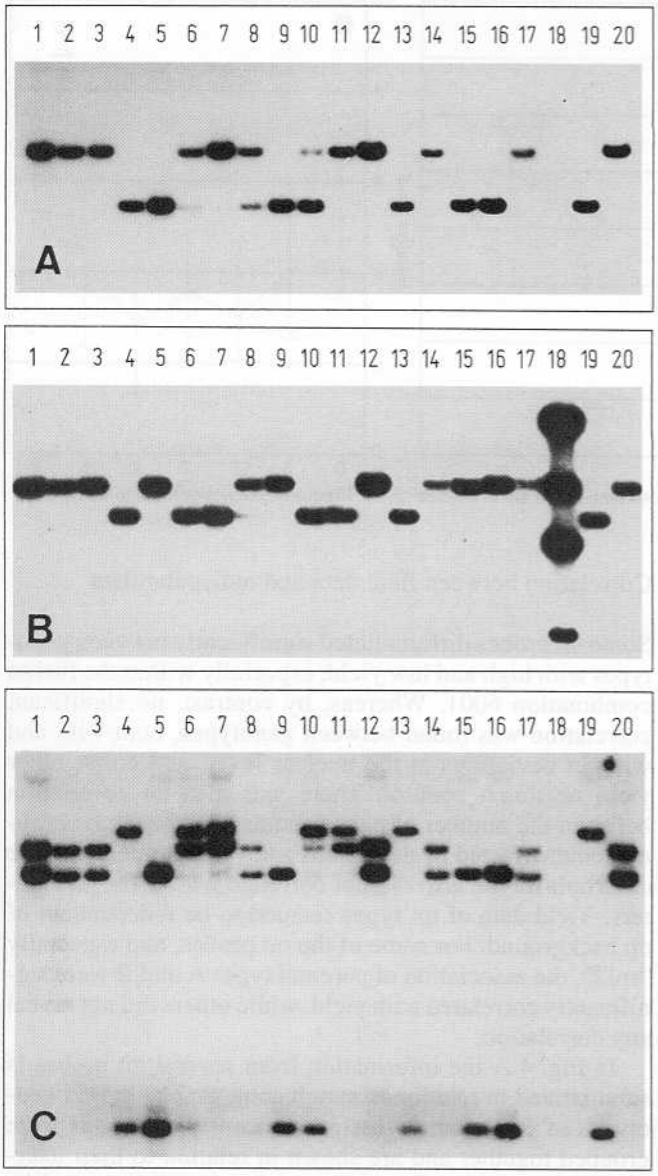

Fig. 2A–C Autoradiograph of Southern blots hybridized with mitochondrial probes. **A** Probe "cob", DNA was cut with *Eco*RI; **B** Probe "rps14", DNA cut with *Hin*dIII; **C** Probe "m100", DNA cut with *Eco*RI. *Lanes 1–17*, regenerants of fusion combination 6001; *lane 18*; molecular-weight standard; *lanes 19 and 20*, fusion parents

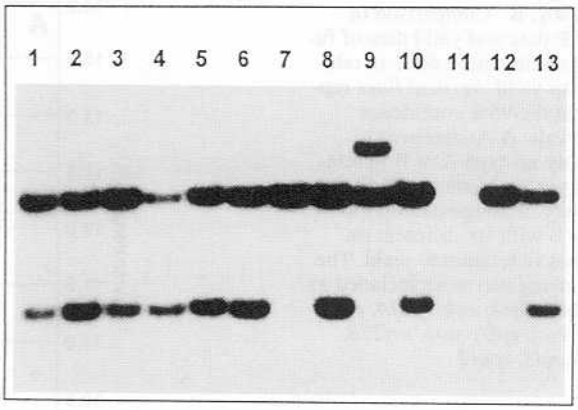

Fig. 3 Autoradiograph of a Southern blot of *Bam*HI-cut DNA hybridized with probe "atp6". *Lanes 1–10*, regenerants of fusion combination 6001; *lane 11*, molecular-weight standard; *lanes 12 and 13*, fusion parents

and in only one case, the fusion combination 6501, did the chloroplasts show a deviating 1:6. segregration.

Analysis of the mitochondrial genome

The mt-DNA composition of somatic hybrids turned out to be more variable. Sufficient polymorphic probes could be detected for all the fusion combinations analyzed. Genomic Southern blots of regenerants and the fusion parents were hybridized with the mitochondrial probe "m100" (Fig. 2C). This probe revealed two different polymorphisms in the genome. Thus, within the regenerants, parental types (lanes 1–4) as well as total and partial additions of banding patterns (lanes 5–7) were observed. Totally new bands were also found (Fig. 3, lane 9),when hybridization was performed with a probe containing the coding region of *atp6*. Altogether, the appearence of totally new restriction fragments was observed in 10% of the hybrid regenerants. The rearrangements, especially those with the "atp6" probe, occurred independently of the respective fusion combination and even in tetraploid non-hybrid fusion regenerants. Partial additions of banding patterns and the occurrence of totally new patterns are both indicative of recombination events. Such recombination is directly visible when the assignment of regenerants to the parental type is shown with different probes. Fig. 2 A–C shows that the association of a single genotype to the dif-

Table 3 Yield and starch content of the different fusion combinations: 6001, 6020, and 6028, field data Grünbach 1993; 6501, field data Bioplant 1992

Fusion combination	Yield			Starch content		
	Average [kg]	Standard deviation	Min/max	Average [%]	Standard deviation	Min/max
6001	4.46	2.6	0.4/13.3	12.05	1.57	8.7/17.7
6020	1.85	1.57	0.1/6.7	11.32	2.82	–/16.0
6028	3.51	2.92	0.2/9.2	11.65	2.27	8.3/15.9
6501	3.09	0.98	0.9/5.9	14.97	1.18	12.7/17.0

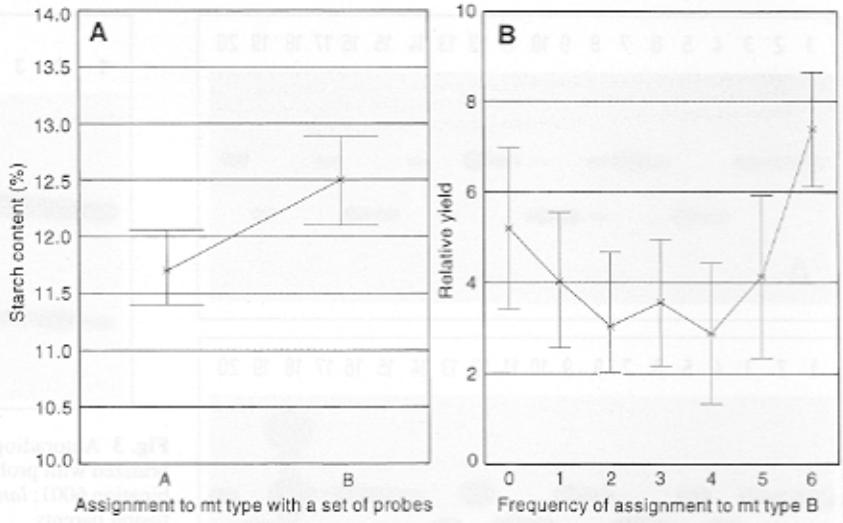

Fig. 4A, B Comparison of RFLP data and yield data of fusion combination 6001 in relation to yield. *Vertical lines* represent the 95% confidence intervals. **A** Assignment to mainly mt-type A or B in relation to starch content. **B** Frequency of assignment to mt-type B with six different mt probes in relation to yield. The following loci were included as probes: *cox1, cob, rps14, rpl5, nd1(b,c), rpl2, atp6, orf228, rpl5, nd3, rps12*

ferent parental mitochondria types depends on the probe used. Apart from the evidence of recombination, the different assignments of genotypes to the parental types reflect the linear arrangement of unknown probes within the mitochondrial genome. Whenever the association to parental type differs between the "cob" (Fig. 2 A) and "rps14" probes (Fig. 2 B), "m100" the probe showed a partial addition of banding patterns (e.g. Fig. 2 C, lanes 5–7). Probe "m100" contains homologous sequences to both *cob* and *rps14* genes. Due to the assignment with these probes, recombination patterns can differ between regenerants (e.g., Fig. 2 B, lanes 5 and 6). Independent of the fusion combination mt-DNA rearrangements were observed in about 75% of the somatic hybrids.

Field data

In order to detect correlations between descriptive molecular data and the corresponding phenotypes of hybrid regenerants, phenotypic data, which were recorded during growing season, and yield parameters were evaluated. A high variability between the regenerants of single fusion combinations was evident with tuber yield and starch content, in particular, differentiating the hybrids (Table 3). Within some fusion combinations (6001, 6028) the starch content showed nearly 100% variation between extreme genotypes. Therefore these parameters were used for the comparisons with the molecular data.

Correlation between field data and molecular data

Some mt probes differentiated significantly between genotypes with high and low yield, especially within the fusion combination 6001. Whereas, by contrast, no significant correlation was found between genotypes, both with and without deviations at the nuclear level, and either tuber yield or starch content. There was also no correlation between the number of chromosomes, or the single chromosome, affected by deviations and yield parameters. The chloroplast type also did not correlate with these parameters. Yield data of mt types seemed to be independent of cp background. For some of the mt probes, and especially "rpl2", the association of parental types A and B were significantly correlated with yield, while others did not reveal any correlation.

In Fig. 4 A the information from several mt probes is summarized in relation to starch content. The hybrid genotypes of 6001 mainly assigned to mt-type A or B were grouped together and are shown in relation to their average starch content. The mt type of parent B seems to be superior in starch content to A. The homogeneity or heterogeneity of the mt genome composition in hybrids of fusion combination 6001 could also be determined with this set of mtprobes. Six of them, homologous to coding regions, were chosen to group together genotypes with same frequency of assignment to parental type A or B and to plot them against their average yield (Fig. 4 B). Genotypes with more homogeneous mt genomes were superior in yield to

the more heterogeneous groups. Also, yield genotypes with a higher proportion of parental type B prevailed over those which were mainly parental type A. The sum of all mt probes show the mt genotype B to be significantly superior, at a level of 5%, to the other genotypes.

Discussion

In order to explain the variability between somatic hybrids of a single fusion combination the nuclear and cytoplasmic DNA composition from hybrid regenerants of different fusion combinations was examined and compared to their field performance. Deviating RFLP banding patterns in somatic hybrids indicate a rather high proportion of genome, chromosome, or point mutations. These probably took place during the in vitro phase of the regenerants. Nevertheless, no influence or direct correlation between the percentage of deviations, the number of chromosomes or the single chromosome affected by deviations, and the phenotypic data obtained from the field could be shown. Therefore, in the present study any influence of nuclear genome deviations for the further analysis of the cytoplasmic composition in relation to the field data were excluded.

Chloroplast segregation has already been reported for different plant species (reviewed by Maliga and Menczel 1986). Chloroplasts seem to segregate at random after a few cell cycles, if the same type of cells, with an equal number, distribution and replication frequency of chloroplasts for both partners, is used (Fluhr et al. 1983; Donaldson et al. 1994). Complete chloroplast segregation was also found in the present study for the greater majority of symmetric hybrids. Equal ratios of both parental chloroplast types indicate that the sorting-out occurs at random. So far no significant similarity was detectable between the type of plastome and the pattern of mtDNA in the hybrid regenerants.

The deviating ratio in one combination (1:6) could not be explained. Different reasons for non-random segregation are discussed in the literature, the most probable is that it may be due to differences in organelle replication rates (Glimelius et al. 1991). Other explanations, such as the tissue source of protoplasts or intergeneric nucleo-cytoplasmic incompatibility (Perl et al. 1991; Sundberg and Glimelius 1991; Bonnema et al. 1992), do not fit these potato fusion experiments.

As expected the mt genome of the somatic hybrids revealed a high percentage of sequence rearrangements. The frequency of about 75% of rearrangements is within the range given by Xu et al. (1993). Kemble and Shepard (1984) showed that mt-DNA rearrangements in *S. tuberosum* also readily occur via intra-molecular recombination and independent of any fusion process. As some of their protoclones showed identical mt genome variation, or else a high degree of similarity in variation, they suggest that one or a few regions of the mt genome are especially susceptible to sequence rearrangements. Intra-molecular mt rearrangements could not be proven, as unfused diploid regenerants were not analyzed. Kemble et al. (1986) proposed that recombinationally active "hot spots" in the interspecific fusion between *S. tuberosum* and *S. brevidens* can reflect the evolutionary changes which separate two species evolved from a common ancestor. Similar results are found in the genus *Brassica*, where Temple et al. (1992) located the rearrangement breakpoints in the mt genome near to the evolutionary breakpoints relating the mt genomes of different *Brassica* species.

Rearrangements, and especially the occurrence of totally new restriction fragments in somatic hybrids, are often discussed in context with the *atpA* genes (Honda and Hirai 1992; Sakai and Imamura 1992). Walters and Earle (1993) report mitochondrial recombination in one-third of their hybrids as revealed by a new fragment near the *atp9* region. In our investigation additional restriction fragments, probably due to a duplication event, could be observed when blots were hybridized with a homologous probe containing the sequence of *atp6* and adjacent regions. The assumption that new reading frames were generated containing a chimeric *atp6* gene is currently being analyzed.

In the present paper a correlation between mitochondrial composition and yield parameters of somatic potato hybrids was evident. Different levels of starch content and tuber yield could be ascribed to different mitochondrial genotypes. Especially for yield, genotypes assigned to mt-type B were significantly superior to those of type A. Also the degree of recombination within the mt genome was foun to influence yield parameters.

Mitochondrial rearrangements may lead to disturbances in nuclear-mitochondrial interaction and thus influence yield parameters. In our experiments, hybrid plants with nearly no recombination in the mitochondria, according to six different probes spread over the mitochondrial genome, gave higher yields than those with more heterogeneous chondriomes. We suggest that viability measured by yield of the various genotypes correlates negatively with the heterogeneity of their mt genome, probably due to disturbed nuclear-mitochondrial interaction. These disturbances might arise from incompatibility between mitochondrial regions derived from different parents or could be due to the doubling, or the deletion, of sequences resulting from unequal recombination events. Most probably, yield parameters are affected, and with different intensity, by more than one locus on the mitochondrial genome. This is reflected in the high correlation of probe "rpl2" with yield data compared to other mitochondrial probes. Only when the results of several mt probes are summarized, can a single genotype with its individual cytoplasmic composition be judged.

At the moment it is impossible to determine, whether homogeneity of the cytoplasm in general, or else the contribution of single regions of the organellar genomes, are decisive for viability. Also, in a practical breeding program it will not be possible to actively influence the composition of the cytoplasm. Therefore, after fusion, selection for regenerants with homogeneous cytoplasmic composition,

or of genotypes which combine regions with positive influence on breeding parameters, is necessary.

It has to be stressed that these experiments have been performed with one pair of parents only; future work will analyze further fusion combinations at the cytoplasmic level and eventually define regions of the mitochondrial genome with a positive or negative influence. Since mitochondria normally are inherited uniparentally there is, however, some logic in the observation that, as a consequence of a selective advantage obtained during evolution, homogeneous cytoplasm is advantageous.

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