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Molecular markers for cytoplasm in potato: male sterility and contribution of different plastid-mitochondrial configurations to starch production

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Summary

Distinct parental cytoplasms were combined in symmetric tetraploid hybrids of potato by somatic cell fusion. This allowed, in the presence of nearly isogenic nuclear genomes, to estimate the contribution of mitochondrial (mt) and chloroplast (cp) genomes to starch content. Analysis of mt-cp configurations in the complete gene pool of german potato cultivars [2n=4x], in a reciprocal dihaploid population [2n=2x], in di-haploid fusion parents [2n=2x] and in their respective hybrids [2n=4x] made visible the effects of different cytoplasmic backgrounds and mitochondrial subgenomic rearrangements. Genotypes identified by markers as cytoplasm Wγ were associated with cytoplasmic male sterility. Evaluation of cytoplasmic types leads to the conclusion, that in starch content the 'wild type' cytoplasms Wα and Wγ have a significant advantage to other cytoplasmic types (Tβ, Wδ, Sϵ). This results from the experiments with a reciprocal population, 180 di-haploids, and from cultivar comparisons. In hybrids an interaction between starch content and different mt-cp combinations could be found. In general the highest field performance, measured in starch content and yield was associated with such cytoplasmic configurations which appeared to a high frequency within a population, when the segregation process was completed. This fact is explained by a selection advantage of clones with optimized organellar segregation already during *in vitro* phase. PCR markers for cytoplasm differentiation are actualized on a website, http://www.flg.tum.de/pbpz/mm/mt/hybrid.html

Introduction

In most cases the influence of the type of cytoplasm is not distinguished from biparental inherited traits. Moreover, cytoplasmic phenotypes only occur in combination with distinct nuclear backgrounds and this hampers their recognition in further breeding work. Unintended counterselection against some organellar types may take place, as long as the agronomic value of different cytoplasms remains unknown. Information about these influences is valuable for selection in sexual breeding but also for production of somatic fusion hybrids.

Following determination of different cytoplasmic types, cytoplasmic, and in part maternal effects can be detected in at least three ways: 1.) Comparison of gene pools of clones (4X cultivars), di-haploids (2X breeding clones), 2.) Comparison of reciprocal populations, and 3.) Comparison of somatic hybrids containing different cp/mt configurations with nearly identical nuclear genomes.

In conventionally bred clones an optimized nuclear-cytoplasmic interaction can be achieved in several traits. This was proven by experiences of conventional plant breeding where reciprocal crosses show differences between cytoplasms: in this respect differences in the photoperiod reaction of different plasms (Sanford & Hannemann, 1982) are relevant as well as maternal effects, reported by Maris (1989) who analyzed a series of diallel crosses between *ssp. tuberosum* and adapted ssp. *andigena* varieties: populations with *tuberosum* cytoplasm were found to be

Table 1. Primer pairs used for identification of cytoplasmic type. The table gives the primer name, the sequence, the primed region and the product size, specific for W/α, T/β and W/γ. ALC primers are used for determination of plastid types, ALM primers for mitochondrial types

Primer name	$5' - 3'$ sequence	Genome region	PCR product specific for		
			W/α	T/β	W/γ
ALC_1	TAG AAT CAG GAG GTC TT	*atp*E	622 bp	381 bp	622 bp
ALC_3	TTA CTC ACG GCA ATC				
ALM_1	CAC AAA TCC ATC TTT GTT TAT GC	*atp6*	1.2 kb	–	1.2 kb
ALM_3	GCG TTG GCT TAC AGC GAA ACT AG				
ALM_4	AAT AAT CTT CCA AGC GGA GAG	*cob, rps10*	2.4 kb	1.6 kb	–
ALM_5	AAG ACT CGT GAT TCA GGC AAT				
ALM_6	ATT TAG GCC CGG CTA GGA ACA	*cob*	–	–	2.4 kb
ALM_7	AAC CCA GTC CCT ATG GTA TCT CCT				

superior in respect to tuber yield whereas the *andigena* cytoplasm provided a higher male fertility. Hilali et al. (1987) observed reciprocal differences in tuber yield, tuber number, vine vigor, average tuber weight, seed germination and pollen vitality. In cytoplasmic substitution backcrosses of potato Amoah et al. (1988) found reciprocal differences between *S. phureja* and *S. tuberosum* backcross progenies.

Nevertheless maternal effects on field performance which are dependent on interorganellar energy metabolism as is starch accumulation and degradation have not been reported so far. This might have been due to the lack of markers capable to differ between cytoplasmic types.

In conventional breeding the differences in sterility, tuber characteristics, seed production and germination are conditioned unlikely by cytoplasmic factors alone. For this reason it is useful to analyze symmetric hybrids, which possess isogenic nuclear genomes and different cytoplasmic backgrounds. By this constellation the effects of various cytoplasms and of plastid-mitochondrial (cp-mt) interaction can be separated from nuclear differences.

In contrast to sexual combinations in somatic fusion the genetic information of the cytoplasms is biparentally inherited. Therefore it is relevant to investigate the influence of the different cp / mt configurations after segregation within a hybrid population, generated in fusion experiments (Schilde-Rentschler et al., 1995; Cardi et al., 1999).

Field trials show variability between hybrid clones from the same parents. This variability can be due to nuclear deviations and different cytoplasmic configurations. The latter were analyzed in detail in an investigation of the fate of organelles after cell fusion by Lössl et al. (1999). The characterization of potato cytoplasms in five main cp/mt types W/α, T/β, W/γ, W/δ and S/ϵ was a precondition for the detection of correlations between cytoplasm and phenotypic data. A set of probes and PCR primers useful for the quick characterization of breeding material was developed and actualized on a website (http://www.flg.tum.de/pbpz/mm/mt/hybrid.html).

Novel configurations of cytoplasmic types occurred in fusion hybrids in shape of partial or complete additions of parental fragments and even novel fragments. In fusion hybrids the strict assortment of cp and mt types was lacking; within regenerants of potato, plastids segregate completely into one of the parental types, whereas mitochondrial genomes are affected from various portions of rearrangements. Cp/mt differentiation serves as a basis for a subsequent evaluation of cytoplasmically influenced traits.

In symmetric hybrids with isogenic nuclear genomes – neglecting protoclonal variation – it could be shown that in general a low degree of chondriome recombination correlated positively with yield potential (Lössl et al., 1994). The question arose, whether mitochondrial performance is influenced by distinct chondriome regions and whether they can interact more efficiently with the new generated genome if they derive from a special parent (Lössl, 1996; Frei et al., 1998). An evaluation of traits being influenced by cytoplasm, is expected to allow predictions about the effects of distinct cytoplasmic configurations for breeding purposes.

Table 2. Frequencies of plastid-mitochondrial types within the German seed board and haploids. T identifies *tuberosum* type, S and W are wild types

Mt Type	Cp Type	Percentage in german assortment
α	W	40%
β	T	47%
γ	W	10%
δ	W	1%
ϵ	S	1%
κ	S	1%

Material and methods

Molecular markers

Restriction fragments specific for the different cytoplasmic types (α, β, γ, δ, ϵ, κ) were cloned and sequenced according to Sanger (1977). The potato mtDNA probes, used in this work can be supplied on request. PCR primers specific for mitochondrial types (α, β, γ) were designed, with the pairs of primers used to detect mt type γ, associated with cytoplasmic male sterility (CMS). They are given in Table 1.

Nearly the complete spectrum of the german cultivar sortiment and 180 dihaploid clones were analyzed on their cytoplasms. Using 4 pairs of PCR primers, and 11 homologous mt-probes they were grouped as mt types α, β, γ, δ, ϵ and κ.

Optimal annealing temperature for mt primers was 57 °C, and for cp primers ALC_1 and ALC_3, to differ between cp type T and wild type plastids (cp type W or S) 44 °C. The markers were used for the estimation of cytoplasm frequencies within the german potato gene pool (Table 2). Plastid type nomenclature was used according to Hosaka & Hannemann (1988).

The fusion hybrids have been checked for their deviations in their nuclear genomes by usage of a set of probes, which covered the 12 chromosomes as performed by Lössl et al. (1994).

Phenotypic evaluation

For the present analysis fusion populations which yielded about 30 hybrid clones, each going back to a single fusion event, were chosen. Fusion hybrids and the dihaploid material derived from the Technical University Munich and from the Institute for Resistance Genetics (Bundesanstalt für Züchtungsforschung) in D-85461 Grünbach. A reciprocal F1 population (Table 3) was provided by Dr Uhrig, MPI Cologne. Fusion populations employed in this work are listed in Table 4.

Field experiments, performed during four years, consisted of blocks in two and three replications with approximately 3,5 m^2 and 16 plants per field plot. Data were evaluated at two locations: in the experimental fields of the Institute for Resistance Genetics, Grünbach and at Roggenstein (Oberbayern) and of the Technical University Munich. Populations (Pop), which were basis for highly significant observations ($p = 0.01$), consisted of more than 30 hybrids. Pop Ia [FAL2 (+) 601] consisted of 35 symmetric hybrids, grown in nine replications, Pop Ib [BP32 (+) 601] of 55 hybrids in two replications, Pop Ic [2006 (+) 626] of 6 hybrids in five replications, Pop IVb [FAL2 (+) M9] of 32 hybrids in two replications, Pop IIc [26–2 (+) 1512_25] of 17 hybrids in five replications, Pop IIIb [2006–10 (+) 576–16] of 10 hybrids in five replications, Pop IVa [BP32 (+) M9] of 58 hybrids in 6 replications, where two plots were lost. They were calculated as an imbalanced trial. Additional field data for the various cultivars were given by the German Seed Board from the 'BundesSortenamt'. Starch and dry matter was determined using a starch weighing machine type MEKU (Pollähne, D-30974 Wennigsen, Germany). For the calculation of the influence of different cytoplasmic compositions on yield components ANOVA and non parametric statistical 'SPSS' programs for imbalanced groups were used.

Test for pollen fertility and vitality was carried out by staining procedures and germinating tests respectively (Stanley & Linskens, 1974).

Results

Differentiation of cytoplasms

Within 4X cultivars predominantly the cp/mt types W/α, T/β and W/γ occurred. Table 2 gives the percentages for this mt type distribution within the German cultivars.

By usage of DNA markers (probes and PCR primers) it is now easy to differ between the cytoplasmic types of potato mt-α, β, γ. Figure 1 shows a comparison of cytoplasmic types W/α, T/β, W/γ with the specific PCR primers. The primers ALM_1, ALM_3, ALM_4, ALM_5, ALM_6 and ALM_7 in combination with ALC_1 and ALC_3 as well as the probes m79, m80, m93, and m112 can be applied as

Table 3. Correlations of effects of different cytoplasms, observed within the spectre of cultivars, in di-haploids and a reciprocal F1 population. Probing was carried out according to Lössl et al. (1999). Replications mean the number of different cultivars and respectively the number of dihaploid clones, which were compared in groups of identical cytoplasm types. Cultivar experiments yielded similar results as published by the official 'BundesSortenamt'

population	cytoplasm combination	primers, probe	correlation [replications]	P	Trait
		probe [*coxI*] primers: ALM_6/7	Wγ > Wα, Tβ [19 : 46 + 79]	$p = 0.01$	male sterility
Cultivars	Wα, Tβ, Wγ	primers: ALM_4 ALM_5	W$\gamma \geq$ Wα > Tβ [19 : 46 + 79]	$p = 0.01$	starch content
		primers: ALM_4 ALM_5	W$\gamma \geq$ Wα > Tβ [19 : 46 + 79]	$p = 0.05$	starch yield
Di-haploid breeding clones	Wα, Tβ, Wγ, Wδ, Sϵ	probe [*coxI*]	Wα, Wγ, Sϵ > Tβ [88: 23 : 18 : 11]	$p = 0.01$	starch content
Reciprocal population	Wα (X) Wδ Wδ (X) Wα	primers: ALM_4 ALM_5	Wα > Wδ [41: 35]	$p = 0.01$	starch content

Table 4. Correlations of different cytoplasmic configurations with starch content, observed within fusion hybrid populations. Probing was carried out according to Lössl et al. (1999)

population	cytoplasm combination	primers, probe	correlation [replications]	significance starch content
I a	Wα (+) Tβ	primers: ALC_1/3	cpT > cpW [270 : 45]	$p = 0.01$
I b	Wα (+) Tβ	primers: ALC_1/3	cpW > cpT [68 : 42]	$p = 0.05$
I c	Wα (+) Tβ	primers: ALC_1/3	cpW = cpT [5 : 30]	not significant
IV b	Wα (+) Sϵ	probe [*atpE*]	cpS > cpW [10 : 54]	not significant
II c	Wα (+) Wγ	probe [*coxII*]	$\alpha > \gamma$ [65 : 20]	$p = 0.05$
III	Wα (+) Wδ	probe [*rps14*]	$\beta > \delta > \alpha$ [35 : 10 : 5]	$p = 0.05$
IVa	Wα (+) Sϵ	probe [*atp6*]	α > Dev.* [317: 29]	$p = 0.01$

* Dev. = Hybrids deviating at *atp6* locus.

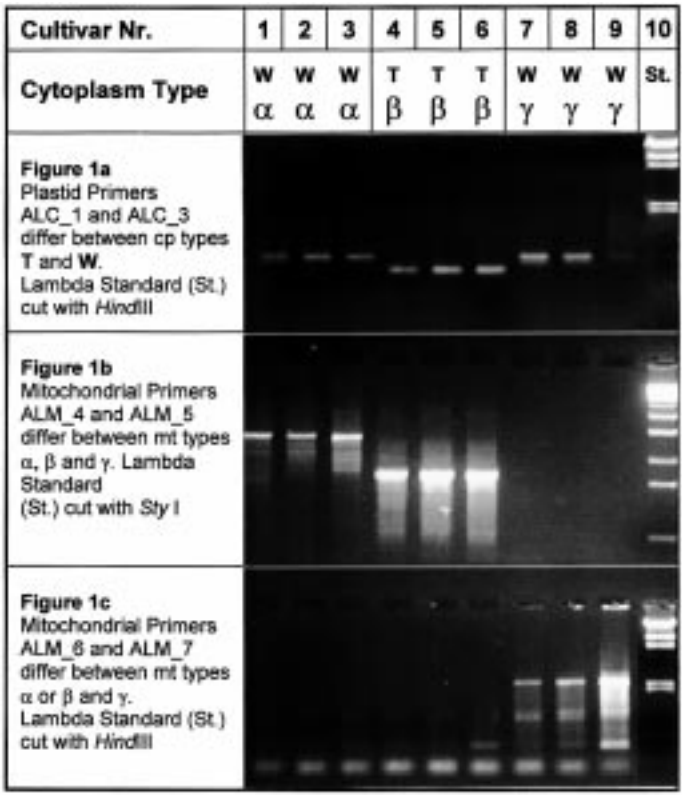

Figure 1. Cytoplasmic types detected by PCR primers. Lanes: 1) Ponto, 2) Adretta, 3) Karlena, 4) Desiree, 5) Sieglinde, 6) Cilena, 7) Assia, 8) Heidrun, 9) Helios, 10). DNA-Standard. DNA product sizes resulting from different PCR primer combinations are given in Table 1.

markers. They detect mt type γ, which is associated with the phenotype of cytoplasmic male sterility. The set of probes for determination of mitochondrial and plastid genome types was tested in non-radioactive hybridization. In this way breeders can perform DNA analysis without isotopic laboratory.

Comparison of field data to cytoplasmic configurations

In order to detect advantageous effects of distinct cytoplasmic types, the di-haploid genotypes, hybrid populations, the reciprocal F1 population (US1) and the whole spectrum of German cultivars, were grouped according to their organellar types and compared in agronomically interesting traits. For this purpose their starch contents, yields and fertility were evaluated (Table 3, Table 4). Cytoplasmic male sterility (CMS) was confirmed by pollen germination tests. Most of the CMS genotypes could be traced back to ancient genotypes as are: I-301, MPI 61-303/34, 56.4129/288, MPI 46.956/68 and Röslau. This maternally inherited sterility was correlated with clones containing mt type γ, as are Aiko, Alwara, Assia, Azur, Bettina, Forelle, Fox, Heidrun, Helios, Petra, Sibu, Uno and Ute.

Cultivars and di-haploid breeding clones

Correlation of field data with cytoplasmic types showed differences between the cytoplasmic groups. Comparison of the cytoplasmic types by Kruskal-Wallis one-way-anova resulted in significantly higher starch contents of varieties with W/α and W/γ type than those with T/β types ($p = 0.01$). The effective difference between culture type (T/β) and wild type (W/α, γ) was 14.7% vs. 16.2%. This clear difference was reduced, but still existent, if starch yield was considered ($p = 0.05$). With slight deviations the field data, measured in this trial, were consistent with the data given by the German Seed Board ('Beschreibende Sortenliste'). In order to exclude a coherence between cytoplasmic type and nuclear genome we performed a cluster analysis by usage of nuclear genomic probes and RAPD (data not shown). Genetic distance of nuclear genomes of 144 varieties did not correlate with their assortment to different cytoplasmic groups.

According to breeders experience and pollen vitality tests nearly all cultivars identified as mt-γ are male sterile. The probes and primers listed in Table 3 can be applied as CMS-markers.

Similarly as found in the cultivar pools, comparison of the cytoplasmic pools within the di-haploid clones confirmed, that the mt-β pool had significantly lower starch contents than those with the 'wilder' plasms mt-α, γ, δ, ϵ. In contrast to the varieties this was associated with a shortened maturity.

Reciprocal population

In starch content the reciprocal crossed F1 population 'US1' revealed a significant positive effect of cytoplasm mt-α to mt-δ. In view of 41 and 35 individuals per cytoplasm pool, it was empirically sure that the different nuclear alleles were represented proportionally within the two groups mt-α and mt-δ. Thus they were comparable. The effective difference was 14.4% (mt-δ) to 15.8% (mt-α).

Fusion populations

For a deeper elucidation of nuclear-cytoplasmic-interactions it was useful to analyze populations of somatic hybrids, with nearly identical nuclear genomes, but containing different plastid-mitochondrial configurations. In hybrids generally the highest field performance was associated with such cytoplasmic configurations which were represented to a high frequency within the concerned fusion population (e.g. Ia, Ib, IIc, IIIb, IVa). Clones, which turned out to have the highest productivity, have already been accumulated naturally during *in vitro* phase. This selection pressure was extremely obvious in population IIIb [W/α (+) W/δ] were 2/3 of the individuals reverted to the configuration of mt type β at *rps14* gene locus.

In contrast to this reconfiguration, hybrids of population IVa revealed a high correlation between homogeneous mt genomes and a high yield potential (Lössl et al., 1994). Continuous evaluation of yield data from 1994 to 1998 detected that an irregular amplification of an additional *atp6* gene copy was correlated with severe depressions in starch yield. In population IVa, these 10% of hybrids, which were affected by a highly replicated additional *atp6* copy (Lössl et al., 1999) had a significantly lower starch content and yield level than those which did exhibit a normal mt genome organization ($p = 0.01$).

Within fusion population Ia plastid segregation ratio was skewed (cpW: cpT = 1: 6). In starch content

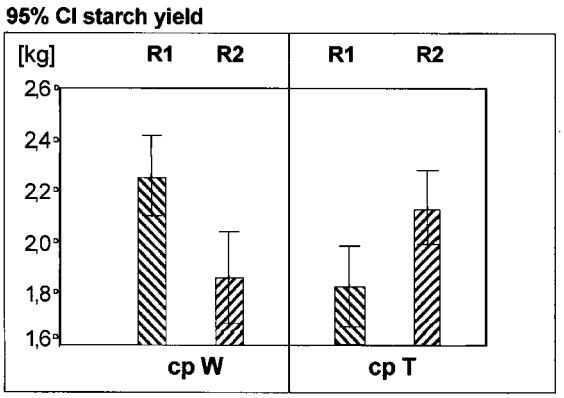

Figure 3. Interdependence between cp- and mt type R1 or R2 of a somatic hybrid population in starch yield (kg/plot). Fusion hybrids of population **Ia** were determined as groups 'R1' and 'R2' and subgrouped according to their plastid types T or W. Given are the 95% confidence intervals for starch yield [kg] of the four groups for four years.

hybrids containing cp type T revealed a clear advantage. But in respect to the novel organization of the hybrids mt genomes the cp pools had to be subgrouped into mt recombination types R1 and R2 (Figure 2). In dependence of the mt type, differences could be detected in starch yield between somatic hybrids. In connection with cpW the mitochondrial recombination type R1 was superior to type R2. This correlation was inverted if mt types R1 and R2 were connected with cp T. The interaction between mt- and cp-types was significant ($p = 0.001$). This interdependence in starch yield is shown in Figure 3.

Discussion

The working hypothesis of a correlation between cytoplasmic genome configuration and field performance measured by starch production could be confirmed in the current investigation.

Evaluation of plasm types lead to the assessment, that in starch content the 'wild type' cytoplasms (W/α and W/γ) are superior in comparison to other cytoplasmic types (T/β, W/δ, S/ϵ). This results from field trials with di-haploids, cultivars and a reciprocal population. W/γ cytoplasm is associated with male sterility and for breeding of a high starch variety W/α and W/γ appears to be the appropriate maternal cytoplasm.

It was generally observed that those cytoplasmic configurations, which appeared to an unproportionally high ratio in somatic fusion hybrids, were also associated with the highest field performance. This

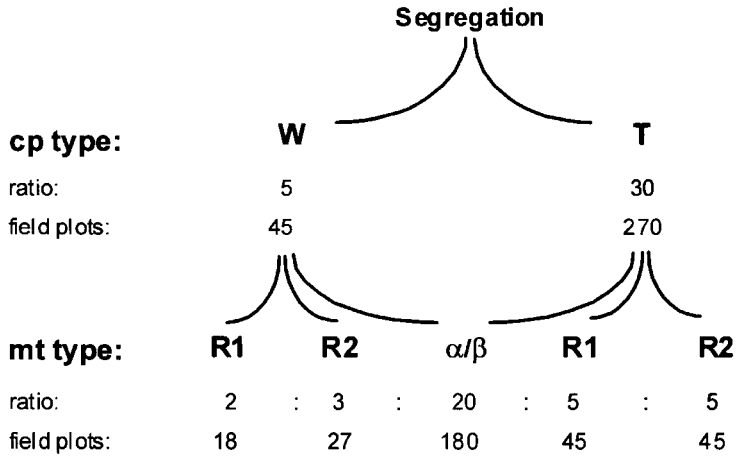

Figure 2. Plastid-mitochondrial segregation in population Ia. The mitochondrial genomes of the fusion population consisted of clones with R1 and R2 and homogeneous mt genomes (α/β). The latter subordinated to the main effect of plastids in population Ia, given in Table 4. Configurations R1 and R2 deviated from this effect and therefore were analyzed on further interactions. The ratio is the absolute number of clones simultaneously.

fact could be explained by a selection advantage of such clones, which works already during *in vitro* segregation and regeneration phase.

Conventionally bred cultivars

Field trials with varieties showed, that the advantage in starch production of cytoplasms W/α and W/γ, representing the wild type cytoplasms was slightly reduced when starch yield was focused. Higher yields seem to compensate the lower starch contents to some extent. The correlation to starch content had a high significance ($p = 0.01$), whereas correlation of the same cytoplasms to starch yield was slightly lower, but still significant ($p = 0.05$). If starch content and yield are considered as complementary breeding aims, the cytoplasmic advantage of W/α and W/γ remains relevant for breeding a high starch cultivar.

It is suggested that cultivars with cytoplasm T/β are optimized for stable yields rather than for high starch contents. Definitely nuclear genes determine starch content and yield, but during starch accumulation and degradation they interact with factors coded by the plastid and mitochondrial genomes. For this reason the complete assortment of cultivars were in parallel investigated in view to their diversity on nuclear genome level. Following an association to cytoplasmic pools W/α, W/γ and T/β the cultivars were tested, whether their cytoplasmic wild type content corresponds with different portions of wild type character in their nuclear genomes. So far there is no indication that varieties with the same cytoplasm could be grouped in the same cluster on nuclear genome level. Nuclear alleles seem to be distributed rather independent from cytoplasmic mating barriers. An exception might be the cytoplasm of W/γ, which is correlated with male sterility.

Mt type γ has been introduced into the German spectrum of cultivars together with PVY-virus resistance. For this purpose the maternal parents from MPI I-301 or its relative MPI-61-303-34 have been employed. These genotypes derive from a mating with a *S. stoloniferum* accession, containing a deviating wild type cytoplasm. Somatic fusions with a parent of mt type γ generate hybrids with different portions of this CMS conferring cytoplasm. Preliminary results with the somatic hybrid populations showed, that this correlation can be broken by the novel cp-mt-configurations. By this constellation it should be possible to limit the responsible CMS. For breeding industry an application of the markers given on the website allows to spare money and time in the crossing work. The identification of male sterile genotypes enables a limitation in the selection of pollen parents. It will further be of advantage, to exchange the cytoplasms of these sterile mt-γ cultivars by the method of Spangenberg et al. (1991) or Rasmussen et al. (2000).

Somatic fusion hybrids

In general the results have to be seen in front of a distinct nuclear genome composition. They are not transferable completely to other fusion populations with similar cytoplasmic combinations.

For confirmation of the effects found, additional fusion populations have to be analyzed; on the other hand it is necessary to check their nuclear genomes for deviations. Within the populations a small portion of somaclonal variation could be proven with a list of probes which covered the whole set of chromosomes. Due to the fact that the main part of mutations have an unfit effect, there is a negative correlation of somaclonal variation with yield parameters. But in none of the euploid hybrids the deviations caused depressions in field performance (Lössl et al., 1994).

From this we can follow, that within the concerned hybrid population the nuclear genomes are not affected from serious aberrations.

Interaction with the novel plastid-mitochondrial factors during accumulation and degradation of polysaccharides constitutes the variable field performance of individual hybrids.

The analysis of cytoplasmic organelle components within somatic hybrid populations revealed high differences between the hybrids cp and mt genomes. The skewed segregation ratio after regeneration phase of 1:6 in cp type W and T was a first indication for an advantage of distinct cytoplasms in fusion population Ia. This deviation from random plastid segregation could be due to different frequencies of replication and organelle division (Glimelius et al., 1981; Donaldson et al., 1994) and is indicative for a higher performance of cp type T during *in-vitro* regeneration phase.

In the analyzed populations I, II, III, IV the segregated plastid genomes did not show any recombinations and were highly conserved, whereas mitochondrial genomes of the hybrids revealed novel DNA organizations R1 and R2. On first view in population Ia somatic hybrids with cp type T had a significant advantage with respect to starch content, but additionally an interaction with the mitochondrial complement was observed by the comparison of recombinant mt genomes. Horvath et al. (1992) suggests that recombinations are processes of adaptation, which improve the nuclear-cytoplasmic interaction. The significant difference in starch yield of recombination type R1 and R2 in dependence of cp type W and T displays the association of distinct chondriome-plastome arrangements with better performance levels.

For an evaluation of this effect mt type was predominantly mentioned for Population Ia. One reason was, that population Ia had the lowest degree of deviations in nuclear genomes. Hereby the risk was reduced, that differences were due to protoclonal variation. Furthermore, in contrast to the other populations only population Ia had a skewed cp type segregation. The skewed ratio was associated with a high difference in starch content between the two groups W and T, whereas the other populations show weak or low significant correlations to starch content. All the populations Ia, Ib, Ic, IIc, IIIb, IVa, IVb are obtained from different fusion parents. Population Ia and Ib contain different nuclear genomes. Therefore it is supposed that the effect, observed in population Ia is dependent on cytoplasmic interaction with the nuclear composition. The other populations listed, show, that there is in general a superiority of these clones, which segregated to a higher number. If this relation seemed to be otherwise, than it turned out, not to be rather significant.

In order to keep an overview, only the significant influences of organellar configurations are stated. Homogeneous mt types had no influence on starch production in population Ia. In order to confine mt regions responsible for interactions with plastid type cpW/cpT, the experiment focused on mt recombination types R1 and R2. No other recombinations in potato mitochondrial genomes have been described so far and mitochondrial types of other populations did not show any significant correlations. Further evaluation of differentially segregated mt types might reveal other interactions, in especially male sterility.

Distinct mt DNA conformations seem to influence mitochondrial performance by their compatibilities with the plastid factors. In this respect it is interesting, that plastid type T lacks five open reading frames which exist in the 5′ region of the *atp*E locus of cp type W (Kawagoe & Kikuta, 1991). In maize Newton et al. (1990) showed that mitochondrial factors may play a role also for chloroplast function when they found a mutant mt gene (*coxII*) which caused an abnormal function of photosystem I. Only an optimized mt-cp configuration allowed an efficient interaction with the new generated cell composition.

It is a question, whether selection pressure works on the level of sugar content in the heterotrophic culture medium. Sugar content in our regeneration media is reduced to one third of the normal concentration in MS medium as a prevention of vitrification effects. This might be a selection pressure, but the bottleneck for cytoplasm performance could also be located in other metabolisms. It is supposed that different compatibility of distinct regions of cp and mt genome is the reason for cytoplasmic interaction: The rare organellar configurations apparently undergo basic problems, reducing vitality during replication of organelles. This

could be any missing recognition of regulatory factors encoded by the nucleus, which affects transcript editing, splicing, processing and translation processes. The deviation concerning the duplicated *atp6* copy in Population IVa is indicative for an abnormal mt genome replication, which contributes to the superiority of homogenous mitochondrial genomes (Lössl et al., 1994). Such differences could be due to 'biological costs', which are associated to nuclear-mitochondrial incompatibilities as reported by McVetty & Pinnisch (1994) for different plasma types in *Brassica*.

In starch content hybrids with an additional *atp6* copy yielded significantly lower than hybrids without this deviation. In this population deviant expression of organellar genomes could be a reason for incompatibilities between cellular compartments. A possible source of variation in this hybrid population is the presence of additional mitochondrial proteins (Lössl et al., 1999). Such deviations in the cytoplasmic expression often are associated with male sterility as a result of mt genome recombinations (Leaver et al., 1988; Köhler et al., 1991; Horn & Friedt, 1999). The reduction in fertility and vitality is in accordance with the observation quoted above, that hybrids affected from extreme recombinations exhibited lower vigor than hybrids with homogeneous chondriomes.

In this respect it will be of relevance to analyze the mt types on their expression level and to find the reasons for the improved nuclear cytoplasmic interaction.

Thus, cytoplasmic analysis is expected to deliver informations for somatic genetics and is involved in polygenic breeding aims, which are dependent on an optimized nuclear cytoplasmic compatibility. Novel mt-cp-configurations generated by cell fusion may be an enrichment for breeding not only in vegetatively grown plants like potato but also in sexually propagated crops.

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