

Aspects of fusion combining ability of dihaploid *Solanum tuberosum* L.: influence of the cytoplasm

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Summary

Creation from 4x hybrid clones from protoplast fusion of 2x clones of potato was evaluated. Besides combined nuclear genomes, composition of the cytoplasm significantly influenced the phenotypic traits of hybrid clones. To ascertain the influence of parental cytoplasm on the success of protoplast fusion and regeneration of hybrid plants, data from 74 fusion combinations of 50 dihaploid clones were analyzed. The majority of dihaploid breeding clones belonged to the cytoplasm types W α , T β and W γ .

When the closely related mt types α , β and γ were used, fusion combinations had a better combining ability compared with more distantly related cytoplasm types δ and ϵ . Fusions containing the same mitochondrial type (homofusions) were not superior to closely related mitochondrial types. However, homofusions of cytoplasm type W α yielded significantly more hybrids than homofusions of type T β . In general, parental cytoplasm types had little impact on the fusion combining behaviour. Thus the cytoplasm type of the fusion parents is not a suitable marker for predicting the combining ability in protoplast fusion experiments.

Introduction

Somatic hybridization of dihaploid *S. tuberosum* L. clones has been successfully applied to combine desirable characters in different dihaploids and to resynthesize the tetraploid state in one breeding step. Heterozygous gene combinations become fixed without further meiotic rearrangements and are stably inherited in the tetraploid fusion product (Möllers & Wenzel, 1992; Waara et al., 1992; Thach et al., 1993). The use of somatic fusion in dihaploid breeding programmes therefore offers an effective means of combining several traits, such as disease resistance and yield components for the production of new cultivars (Hofferbert, 1996).

There are still some practical problems that limit the widespread use of somatic fusion technology. For example, no genotype independent regeneration systems exists (Millam et al., 1995), and hybrid efficiency depends strongly on the genotype of parental clones. Furthermore, the breeder is interested not only in the production of hybrid populations but also in predicting how genotypes and their specific traits will harmonize in the fusion product. Thus, means of predicting hybrid efficiency and the final performance of the tetraploid fusion product would be highly desirable.

First field evaluations of somatic hybrids revealed an unexpected variability between the hybrids of a single fusion combination (Munzert et al., 1992; Möllers et

al., 1994), possibly due in part to somaclonal variation (Fish et al., 1988; Ramulu et al., 1989). Theoretically, the fused nuclear genomes should result in one hybrid genome, and therefore a fascinating factor contributing to hybrid variability is the new constitution of the cytoplasm after fusion. Biparental inheritance of cytoplasm is a characteristic of somatic hybridisation and contrasts with the uniparental plasmone inheritance of sexual fertilization.

Molecular analysis of the cytoplasm composition of fusion products showed that organelles are typically involved in processes such as chloroplast segregation and recombination or rearrangements of mitochondrial (mt) DNA (Lössl et al., 1994). Somatic hybrids predominantly possess chloroplasts (cp) from only one fusion parent but in most fusion combinations equal numbers of the two parental cp types can be found. Nevertheless, combinations could be detected showing a strong selection for one of them (Lössl, 1996). By contrast, the mtDNA organized in a main circle and different subcircles revealed a more complex fusion behaviour. There is strong evidence that parts of the mtDNA (subcircles) are exchanged at an early stage of protoplast regeneration after fusion (Miyake et al., 1996). The different division activity of these new formed mitochondria thus results in new predominating mt types. In this way, protoplast fusion does not only generate hybrids with added nuclear information, but also combines it deliberately with the parental cp types and, to some extent, recombined mt types (Lössl, 1996).

Field experiments with somatic hybrids showed that there is a correlation between the composition of the cytoplasm and the phenotype (Lössl et al., 1994). Statmmann (1996) correlated morphological and fertility differences of somatic hybrids between medicinal *Solanum* wild species with their recombined cytoplasm composition. Thus generation of completely new cytoplasm compositions via protoplast fusion, as yet found in nature, may complement the classical breeding strategy with regard to improvement of cytoplasmically inherited traits.

These observations concerning the influence of the de novo arranged cytoplasm on somatic hybrid performance led us to the following questions: (1) Regarding the cytoplasm types of the fusion parents, what is the role of the cytoplasm types in protoplast fusion experiments? (2) Do they have an impact on the somatic combining ability of the clones? And (3) do certain cytoplasm types show a superiority in protoplast fusion that may result in an enhancement of hybrid efficiency? Therefore, the fusion data of 74 different fusion combinations was analyzed. The fusions were performed with respect to the cytoplasm type of the fusion parents at the Institute of Resistance Genetics, BAZ Grünbach and by the practical breeding company Bioplant GmbH, Ebstorf.

Material and methods

In total 74 different fusion combinations, yielding more than 10 regenerants, were selected from 102 combinations. There were 50 different dihaploid genotypes involved in these fusion experiments. The influence of the cytoplasm type on the fusion combining ability of the dihaploid potato genotypes was analyzed.

The origin of the parental 2x clones is given in Table 1 and represent a cross-section from the diploid breeding material of several German and Dutch breeding companies. Fusion combinations were done in 1994 and in 1995 at the Institute for Resistance Genetics, BAZ Grünbach (28 fusions), and as part of the routine breeding programme at Bioplant GmbH, Ebstorf (46 fusions). At both sites isolation of protoplasts, fusion experiments and regeneration procedures were identical to those originally described by Möllers & Wenzel (1992). Only one shoot per regenerating callus was collected, thus representing a single fusion event. Hybrid regenerants were analyzed with either RFLP and/or RAPD technique. The percentage of tetraploid

Table 1. Dihaploid genotypes, their cytoplasm composition, number of fusion combinations and average hybrid yield.

Cytoplasm type	Genotype ^a	No of FC ^b	Hyb% ^c	Cytoplasm type	Genotype	No of FC	Hyb %
Tβ	NH08	4	2.0	Wα	FH02	11	11.4
Tβ	NH13	1	0.0	Wα	FH09	2	10.5
Tβ	NH24	1	38.0	Wα	GB13	5	29.2
Tβ	NH38	2	15.5	Wα	GB17	3	8.3
Tβ	NH43	3	39.7	Wα	GB19	2	29.0
Tβ	NH45	5	5.2	Wα	GB20	1	15.0
Tβ	NH48	1	0.0	Wα	GB22	4	19.5
Tβ	NH55	4	3.8	Wα	MH01	4	14.5
Tβ	NH61	3	0.0	Wα	MH02	3	30.3
Tβ	NH64	1	0.0	Wα	MH19	4	3.0
Tβ	NH66	1	0.0	Wα	MH39	1	72.0
Tβ	NH68	2	41.5	Wα	NH12	5	23.8
Tβ	NH69	3	5.0	Wα	NH23	2	0.0
Tβ	NH76	2	7.5	Wα	NH32	3	15.0
Tβ	NH81	4	26.3	Wα	NH47	1	0.0
Tβ	RH15	8	10.9	Wα	NH56	1	0.0
Tβ	RH16	2	0.0	Wα	NH57	1	0.0
Tβ	RH20	2	38.5	Wα	NH62	2	0.0
Tβ	RH37	2	0.0	Wα	RH71	8	36.4
Tβ	RH38	1	0.0				
Tβ	RH42	3	10.7	Wγ	GB11	2	12.0
Tβ	RH88	4	0.5	Wγ	NH58	4	26.5
Tβ	RH89	2	3.0	Wγ	NH72	3	42.0
Tβ	RH91	1	72.0	Wγ	RH97	1	0.0
Tβ	RH96	1	77.0				
Wδ	MH03	4	5.0	Se	GB16	2	3.5

^a Origin of genotypes: NH: Nordkartoffel GmbH, Ebstorf, Germany;

RH: Agrico Research, Emmeloord, Netherlands;

MH: MPI, Cologne, Germany;

FH: FAL, Braunschweig-Völkerode, Germany;

GB: BAZ, Grünbach, Germany;

^b No of FC: Number of fusion combinations with the respective genotype;

^c Hyb%: mean hybrid recovery.

hybrid clones among the regenerated clones was calculated in order to define the fusion combining ability of two genotypes.

DNA of the 50 diploid potato genotypes involved in the analyzed fusion combinations were hybridized with a set of mtDNA probes in order to determine their cytoplasm type (Lössl, 1996). The nomenclature of the cp types follows that proposed by Hosaka & Hannemann (1988).

As the percentage of hybrids recovered from the fusion combinations was not normally distributed, non parametric methods were chosen for statistical analysis (Lienert, 1986): H-test (Kruskall, Wallis), U-test (Mann-Whitney) and Dunn-test.

Results

Cytoplasm composition of the fusion parents. The analysis of the cytoplasm of the 50 dihaploid potato clones used as parents for the somatic fusions is summarized in Fig. 1. Two chloroplast types were detected: 48% show the W type and 50% the T type. Only one genotype showed the rare S type (2%). Analogous to the chloroplasts, two mitochondrial types (α and β) prevailed with 38% and 50% respectively. Three further mt types (γ , δ , and ϵ) could also be found at low frequencies. Analysis of the plasmons also showed that cp types and mt types of the parental clones had a clear correlative pattern; for example T type chloroplasts only occurred in combination with β type mitochondria.

Cytoplasm of the parents selected for analysis reflected a representative cross-section of European cultivars. Since some cytoplasm types are very rare, not all possible cytoplasm combinations were carried out. Moreover, only a small number of fusions could be evaluated from some cytoplasm combinations.

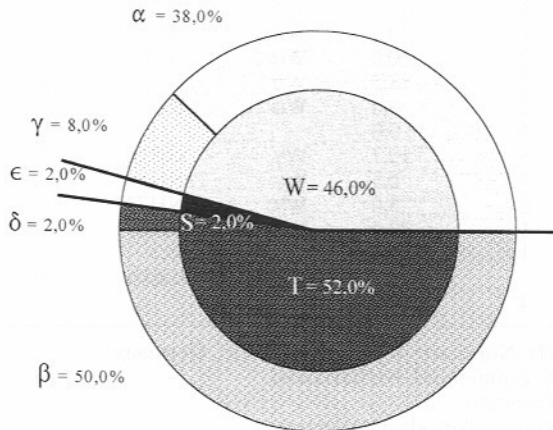


Fig. 1. Distribution of the cytoplasm composition of dihaploid potato clones. Inner circle: cp types, outer circle: corresponding mt types.

Cytoplasm types and hybrid recovery. Approximately 3500 regenerants from 74 fusion combinations were analyzed and 15.8% were found to be tetraploid hybrids. The hybrid recovery varied strongly between 0 and 83%, with a high percentage of fusion combinations (37.8%) yielding no hybrids at all. Up to 60% of the fusion combinations had 10% or less hybrid regenerants. Only 5% showed a hybrid recovery higher than 70%.

Average percentages of hybrid recoveries of the different cytoplasm combinations are given in Fig. 2. Combinations with the mt types δ and ϵ were under represented and therefore not included in statistical analysis. There were statistically significant differences between the hybrid recoveries of the different cytoplasm combinations with the cytoplasm types $W\alpha$, $T\beta$ and $W\gamma$. The pairwise comparison of fusion combinations with parents of the same cytoplasm type (homofusions) revealed that homofusions of the cytoplasm type $W\alpha$ (22.0%, 16 fusion combinations) yielded significantly more hybrids than combinations of the $T\beta$ type (14.1%, 15 fusion combinations). The superiority of the cytoplasm combination $W\alpha$ with $W\gamma$ (38.3%, 4 fusion combinations) could not be proved. This may have been due to the small number of fusion combinations. Fusion combinations with the participation of mt types δ and ϵ yielded 5.5% and 3.5% hybrids respectively, a relatively low hybrid efficiency compared with the average of all combinations. These findings could not be evaluated statistically due to the small number of fusion combinations resulting from these rare cytoplasm.

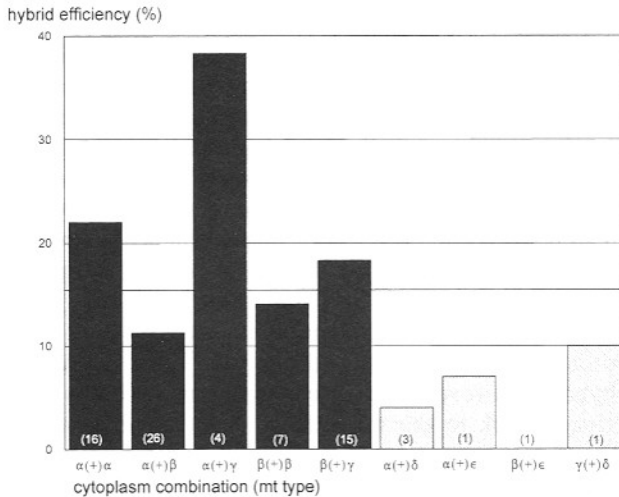


Fig. 2. Mean hybrid efficiency of different cytoplasm combinations. Dark columns: fusion combinations with the main cytoplasm types $W\alpha$, $T\beta$ and $W\gamma$, bright columns: fusion combinations with the rare cytoplasm types $W\delta$ and Se , not included in further statistical analysis. The number of fusion combinations analyzed are given in brackets.

Discussion

Intraspecific somatic hybrids of a single fusion combination show rather a high variability of phenotype in field evaluations (Matteij & Puite, 1992). This variability is ascribed to somaclonal variation, aneuploidy and to the new cytoplasm constitution of the hybrid genotypes (Gleba & Sytnik, 1984; Austin et al., 1985; Lössl et al., 1994). The present paper shows that, despite the influence of cytoplasmic factors on the phenotype of somatic hybrids, the cytoplasm of fusion parents has only a small effect on the combining ability in protoplast fusion experiments.

The occurrence of five different mt types in the dihaploid gene pool is in agreement with the findings of Lössl (unpublished), who observed a similar distribution among German potato cultivars: 32% were of the α type, 55% of the β type, and 13% of the γ type. Differences in the distribution of mt types within tetraploid cultivars and diploid breeding clones can eventually be traced back to the intensive selection for high male and female fertility of dihaploid clones. The strict order of cp and mt types of the fusion clones (Fig. 1) is also found in tetraploid cultivars and breeding clones, provided they are the products of sexual processes. In tetraploid fusion products, this order can be disrupted and completely new assortments of mt and cp types established (Lössl et al., 1994).

An explanation for the predominance of the two cytoplasm types T β and W α has to be seen in the context of the history of the European potatoes. Originally introduced potatoes probably possessed the Se type cytoplasm (*S. andigena*), but they were nearly all decimated by the late blight epidemics in the last century, and replaced by T β type accessions (*S. tuberosum*) from Chile (Grun, 1990; Hawkes, 1994). Breeding for disease resistance at the beginning of this century led to the introduction of W α type accessions (e.g. *S. demissum*, *S. gourlayi*) (Ross, 1986). Thus the intensive use of wild species in potato breeding resulted in the combination of all cytoplasm types of species *tuberosum* with the actual material of the subspecies *tuberosum*. Phylogenetically, the cytoplasm types of the cultivated potato are more distant than their nuclear background that has been restricted by selection for agronomically important traits. Similarly, the comparative analysis of the phylogenetic distances within the German potato cultivars revealed no congruence between the clustering of genotypes based on nuclear probes on the one hand and cytoplasmic data on the other (Lössl, pers. com.).

In a cluster analysis of *Solanum* wild types the mt types δ and ϵ were grouped apart from the more closely related α , β and γ types (Lössl, unpublished). Thus the relatively low hybrid recovery of fusion combinations with this rare cytoplasm can eventually be traced back to the relatedness of mt types influencing the general combining ability of the cytoplasm. In fusion experiments with medicinal *Solanum* wild species, Statmann (1996) also found better fusion combining ability for genotype combinations with closer related cytoplasm compositions than for distantly related species. Consequently, a better hybrid efficiency for homofusions should be expected compared with fusion combinations with different cytoplasm compositions (heterofusions). In the present analysis only homofusions of two cytoplasm types

were used. Their hybrid recovery did not significantly exceed the recovers of heterofusions. Therefore the relatedness of cytoplasm within the *S. tuberosum* breeding material cannot explain differences in hybrid recoveries of the cytoplasm compositions. A significant difference between cytoplasm combinations could be found between homofusions of the $W\alpha$ and $T\beta$ types. As $W\alpha$ also seems to yield higher hybrid numbers in combinations with $W\gamma$ and Se in comparison to $T\beta$ (+) $W\gamma$ and Se , there is an indication for a better general combining ability of this cytoplasm compared with $T\beta$. The good hybrid efficiency of the fusion combinations $W\alpha$ (+) $W\gamma$ might indicate a high specific combining ability of the two cytoplasm types. Although the general influence of cytoplasmic factors on the combining ability of dihaploid potatoes seems to be low, there are indications that specific cytoplasm combinations in fusion may be superior to others. In order to validate the present results it would be necessary to add to the further fusion combinations and especially more homofusions and heterofusions of the rare cytoplasm types.

The main criteria of plant breeders for the selection of fusion parents are the agronomical traits of clones and the knowledge of how they will combine in the fusion product. Initially, fusions are mostly done by trial and error and only later on basis of the already known fusion data of single clones. It is thus essential for the breeder to know his material well. This is evident from Table 1 where the average hybrid efficiency for each genotype of our study is listed. Because of limitations of donor and recovered plant material, no clear statistical information about the influence of the cytoplasm type on the hybrid recovery efficiency could be provided. There are nevertheless interesting genotypes e.g. RH71 ($W\alpha$), NH12 ($W\alpha$), FH02 ($W\alpha$) or RH15 ($T\beta$), that show, in a series of different fusion combinations, high hybrid recoveries. A thorough analysis of the nuclear background of their fusion combining ability is therefore called for. Until more information is available, the cytoplasm type of the fusion parents should be seen only as an additional information, which to keep in mind when creating new valuable genotypes.

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