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C. Möllers · U. Frei · G. Wenzel

Field evaluation of tetraploid somatic potato hybrids

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Abstract Tetraploid somatic hybrids, derived from ten different fusion combinations, were grown in the field to study the inheritance of quantitatively-controlled agronomic traits. The data from two seasons showed that the heterosis in yield varied between 70% and 230% compared to the mid-parent value of the dihaploid fusion clones. The relative yield of the hybrids compared to two standard varieties (= 100%) ranged from 47% to 106%. Despite large differences in yield between the single hybrids of the same fusion combination, no significant differences could be detected between the hybrids. The analysis of the yield components revealed that the heterosis was mainly due to a highly increased tuber weight, whereas the tuber number was in general intermediate to that of the parent clones. In three combinations, the starch content of the hybrids was 15–20% higher than the mid-parent value and in the others it was intermediate. Fusion of clones with long oval tubers and with round or round-oval ones showed the round/round-oval form was dominant. Fusion combinations of tuber flesh colour indicated that in most cases yellow was dominant to white, while red skin colour was dominant over yellow.

Key words *Solanum tuberosum* · Somatic hybrids · Field evaluation

Introduction

Integration of protoplast fusion technology in the potato breeding scheme allows for the incorporation of novel germplasm from sexually-incompatible wild species into actual breeding material, and the asexual combination of selected interdihaploid *S. tuberosum* clones to produce superior tetraploid hybrids. Based on the analytical breeding scheme of Chase (1963), Wenzel et al. (1979) developed a model which proposes breeding at the dihaploid level combined with the protoplast fusion of selected interdihaploids. By this means, agronomically-important traits are combined and tetraploidy, as well as a high level of heterozygosity, is also obtained.

During the last few years, there has been an increasing interest in this area of applied research and the production of tetraploid somatic hybrids from dihaploid *S. tuberosum* clones was reported by several groups (Austin et al. 1985a; Deimling et al. 1988; Waara et al. 1989; Chaput et al. 1990; Möllers and Wenzel 1992; Thach et al. 1993). Frequently, *S. phureja* (Puite et al. 1986) and *S. brevidens* (Austin et al. 1985b; Fish et al. 1988) were also included as one of the fusion partners. However, despite these encouraging results, few reports are available on the field performance of interspecific hybrids (Fish et al. 1988; Mattheij and Puite 1992) and so far there has been only one report on intraspecific hybrids (Munzert et al. 1992).

However, for the analysis of somatic genetics and the evalution of the usefulness of the analytical-synthetical breeding scheme, the genetics of these hybrids and their field performance are important criteria. Studies on the inheritance of dominant traits in somatic hybrids are rare. Helgeson et al. (1986) showed that a major *Phytophthora infestans* gene from *S. tuberosum* is expressed in tetraploid *S. tuberosum* (+) *S. brevidens* hybrids. Möllers and Wenzel (1992) demonstrated that the dominant resistance gene Rol against the nematode *Globodera restochiensis*, and a dominantly inherited tolerance towards the herbicide metribuzin, were both expressed in tetraploid somatic *S. tuberosum* hybrids. Furthermore, it was shown that major genes for PVX and PVY resistance are also expressed in somatic hybrids (Thach et al. 1993).

The present study was conducted to evalute the field potential of intraspecific hybrids and to determine yield

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C. Möllers[1] · U. Frei · G. Wenzel (✉)
Federal Centre for Breeding Research on Cultivated Plants, Institute for Resistance Genetics, D-8059 Grünbach, Germany

Present address:
[1] Institute for Agronomy and Plant Breeding, University of Göttingen, von Siebold Strasse 8, 3400 Göttingen, Germany

components and quality characters. To this end tetraploid somatic hybrid plants derived from different fusion combinations (Deimling et al. 1988; Möllers and Wenzel 1992) were grown over two seasons together with their dihaploid parents and standard cultivars.

Materials and methods

Plant material, protoplast isolation, fusion and the regeneration of hybrids

Twelve different dihaploid potato clones were used in various combinations in PEG (Deimling et al. 1988), or in electrofusion, experiments (Möllers and Wenzel 1992). The respective fusion and regeneration protocols are described in these two papers and in Möllers et al. (1992). Ploidy levels of the hybrids were determined by counting the chloroplast numbers in the stomata and chromosome numbers in the root tips, as well as by scoring the phenotype. Agronomic traits were assessed in ten different fusion combinations (see Table 1).

Field experiments

During the summer of 1990, 1991 and 1992, field experiments with tetraploid somatic hybrids, their dihaploid parents, and different cultivars were performed in the field plots of the Institute of Research Genetics in Grünbach. Hybrids of the fusion combination Hy 9 were grown in 1989, hybrids from Hy 1–10 were grown in 1990, and hybrids of the combinations Hy 12 and 13 were grown in 1991 for the first time in the experimental field. All the experiments were repeated in 1992.

The hybrids were grown in rows of five plants each with the dihaploid parents in every ninth and tenth row. The cultivars were grown as standards either within the same block or in another block but in the same field. In 1992, all the hybrids were grown in rows of ten plants. The tuber characteristics of each hybrid were calculated from five and ten plants (mean value) in 1991 and 1992 respectively and of the parent clones from 2–7 rows, depending on the number of hybrids tested. For statistical analysis, the mean values of the hybrids of each fusion combination (1991/92) were compared with the parent clones; significant differences were determined using Scheffé's test (Scheffé 1953) to compare the hybrids with the mid-parent value.

General performance, flowering, and pollen vitality were scored during the growing period. Only one early treatment with a systemic fungicide was carried out. Fertilizer (N) was applied at a low level (120 kg/ha). Tubers were harvested during the first half of September; tuber weight, tuber yield and tuber number per plant were determined as well as the underwater weight of a tuber sample (about 500g), as a measure of starch content.

Results

Significant phenotypic differences between the diploid parent clones and the tetraploid somatic hybrids were observed in the field. The heterozygous tetraploid hybrids were much more vigorous than the dihaploid parent clones. They were also much more vigorous compared to homozygous tetraploid parent clones which, in some cases, were also grown in the field. In general, the hybrids resembled the cultivars in their habit. Although certain morphological differences between single hybrids of the same fusion combination were observed, no clear classification into two or more distinct groups was possible. Flowering was normal and pollen fertility of 60–90% was also observed. Berries were frequently formed without artificial pollination.

Tuber yield

In the first year, the yield of the hybrids (Fig. 1) was much lower than that of the standard varieties. This might be because minitubers of different maturity were transplanted in the field. The yield improved in the second year, but the mean yield of the hybrids was still very low. However, the tuber yield of the standard varieties was also very low (see Table 2). In the third year, the yield was almost five-fold higher than in the previous year (Fig. 1). Therefore, only yield values of the second and third years were considered (except Hy 12 and Hy 13, where field data from the 1st and 2nd year were combined).

From each fusion combination 4–36 hybrids were grown in the field. Their mean yield varied between 468g and 875g per plant (Table 1). Large differences in yield were also observed between the different hybrids of the same fusion combination (see min/max values in Table 1). The extent of yield variation between single hybrids from Hy 2 is shown in Fig. 2. The yield of the single clones of the hybrids varied between 373 g/plant and 1013 g/plant. However, due to large differences between the 2 years a statistical analysis of the field data did not reveal significant differences between the yeild of the single clones of hybrids for the different fusion combinations. Likewise, for Hy 9 the field data for 3 years did not show a significant difference (data not shown).

The mean yield increase of the hybrids compared to the mid-parent value (MPV) was 73–232% (Table 1) for the ten fusion combinations. Compared to the best parent value (BPV), the increase was still 50–127%. The yield of the hybrids was correlated with the yield of the best parent ($r^2 = 0.78$, data not shown) and also corre-

Fig. 1 Mean yield of tetraploid somatic hybrids in the 1st, 2nd and 3rd field year

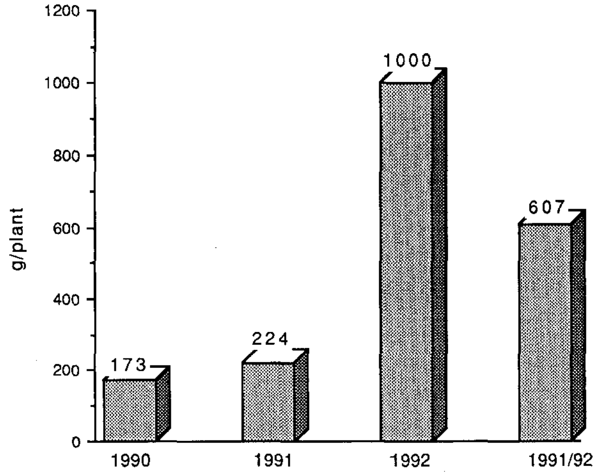

Table 1 Mean yield of tetraploid somatic hybrids and their dihaploid parents (1991/1992)

Hybrid			Parents yield (g)	Mid-parent value (g)	No. of hybrids	Hybrids yield (g)			Yield increase in % of[b]	
						x	Min	Max	MPV	BPV
Hy 1	BP32	(+)	193	234	19	566	387	825	142	105
	H77.417/9		276							
Hy 2	Bp32	(+)	193	291	24	672	373	1013	131	72
	H77.409/13		390							
Hy 3	HH236	(+)	257	266	9	468	128	915	76	70
	H77.417/9		276							
Hy 4	H77.417/9	(+)	276	332	12	576	324	865	73	48
	H77.409/13		390							
Hy 5	BP32	(+)	193	289	14	578	382	812	100	50
	H81.1007/65		385							
Hy 6	H27.33	(+)	304	348	10	722	370	1018	108	85
	H80.1528/6		391							
Hy 9	H75.1207/7	(+)	228	156	10	518	170	964	232	127
	AH78.5111		83							
Hy 10	JM90	(+)	588	487	36	875	543	1186	80	49
	H81.1007/65		385							
Hy 12	AH86.4568/6	(+)[a]	156	274	4	698	821	759	177	94
	H80.1528/6		391							
Hy 13	H1.11	(+)[a]	371	381	4	725	878	834	119	113
	H80.1528/6		391							

[a] Data of first and second field year

[b] MPV = mid-parent value; BPV = best-parent value

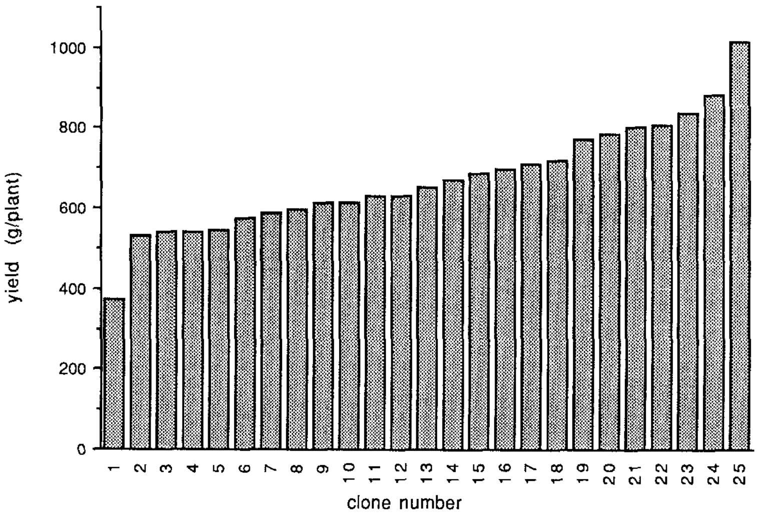

Fig. 2 Mean yield of different clones from Hy 2 (1991/1992)

lated with the MPV ($r^2 = 0.68$, Fig. 3). However, the yield heterosis of the hybrids was not correlated to either the MPV or BPV ($r^2 = 0.2$ and 0.3 respectively).

In Table 2 the fusion combinations with the best, the average, and the lowest yield are compared to the yield of two standard varieties (cvs 'Margit' and 'Ilse'). The best fusion combination, Hy 10, had a relative yield of 87% and 106%, and the others correspondingly less.

Tuber weight and tuber number

The yield increase of the hybrids was mostly due to a much higher tuber weight compared to their dihaploid parents (Table 3). The increase in tuber weight of the hybrids over the MPV varies from 37% to 126%. Of the ten fusion combinations nine showed a significantly higher increase over their respective MPV. The fusion combination Hy2 was not significantly different; in this fusion combination, one parent clone had an extremely low tuber weight and the other a relatively high one.

In contrast to tuber weight, the tuber number per hybrid plant was similar to the mid-parent value (about ± 15%, Table 3). Two fusion combinations showed clearly deviating values. Hy 9 had 63% more tubers than the MPV (13 versus 8) while Hy 5 had 20% significantly less tubers (7.7 versus 9.6). However, the

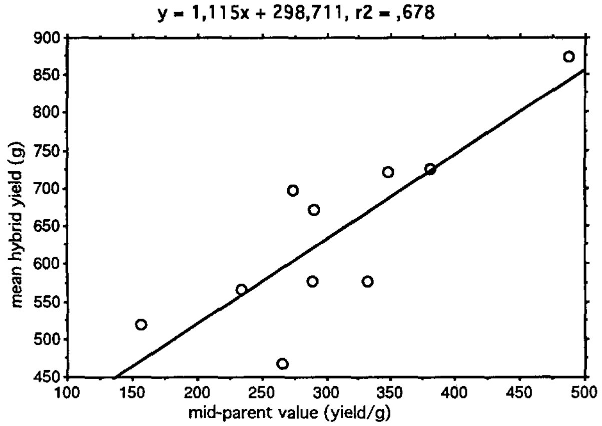

Fig. 3 Correlation between mean hybrid yield and MPV

Table 2 Somatic hybrids with the best, the average, and lowest yielding hybrids compared to the standard cultivars

Cultivar/ hybrid	Yield 1991	Yield 1992	1991/1992	Relative yield in % to	
				cv Margit	cv Ilse
Margit	425	1 230	828	= 100	
Ilse	472	1 540	1 006		= 100
Hy 10	317	1 433	875	106	87
Hy 2	275	1 077	672	81	67
Hy 3	244	662	468	57	47

single data of Hy 9 showed that the non-significant increase in tuber number was due to three different hybrids having average tuber numbers of 18.3–23.3; the remaining hybrids had average tuber numbers of 9, similar to the MPV.

Table 4 Mean starch content of tetraploid somatic hybrids and their dihaploid parents (1991/1992)

Hybrid	Parents starch (%)	MPV[a] (%)	Hybrids starch (%)			Difference in % to MPV[a]
			x	Min	Max	
Hy 1	8.5	12.6	12.6	10.7	13.8	0
	16.7					
Hy 2	8.5	10.4	10.6	8.9	15.1	2
	12.3					
Hy 3	15.7	16.2	14.4	10.9	17.5	−11
	16.7					
Hy 4	16.7	14.5	14.3	10.3	16.3	−1
	12.3					
Hy 5	8.5	11.1	11.6	9.5	14.5	5
	13.7					
Hy 6	14	10.5	12.1	9.5	15.0	15
	7.1					
Hy 9	12.7	12.9	12.0	10.2	14.8	−7
	13					
Hy 10	10.3	12	11.7	10.3	13.4	−3
	13.7					
Hy 12	12.4	9.8	11.9	10.9	13.4	21*
	7.1					
Hy 13	11.3	9.2	11.1	10.1	11.9	21*
	7.1					

[a] MPV, mid-parent value
* Significant difference (Scheffé 0.05)

Starch content

The starch content of the tubers was very low under the growing conditions in the nursery in the 2 years. The dihaploid fusion parents had starch contents varying from 7.1% to 16.7% (Table 4) while the standard varieties also had only 11.6% (cvs 'Margit' and 'Ilse'). The

Table 3 Tuber weight and number of somatic hybrids and their dihaploid parents (1991/1992) (T.w. tuber weight (g), t.n. tuber number, MPV mid-parent value)

Hybrid	Parents		MPV T.w. T.n.	Hybrids Tuber weight (g) Tuber number			% Increase to MPV
	T.w.	T.n.		x	Min	Max	
Hy 1	34	5.2	27	61	35	96	126*
	20	12	8.4	8.9	5.4	15.7	6
Hy 2	34	5.2	36	76	47	112	111*
	37	10	7.7	9.0	5.1	15.4	17
Hy 3	16	11	18	37	21	60	105*
	20	12	11.4	12.4	4.9	17.2	9
Hy 4	20	12	29	55	36	85	90*
	37	10	10.9	10.5	6.2	18.8	−3
Hy 5	34	5.2	29	61	46	91	110*
	25	14	9.6	7.7	5.5	15.2	−20*
Hy 6	53	5.6	64	102	47	156	59*
	75	5	5.3	6.0	3.2	9.5	13
Hy 9	24	8.4	18	35	19	52	94*
	12	7.5	8.0	13.0	7.4	23.3	63
Hy 10	50	13	37	51	35	65	38*
	25	14	13.7	15.3	11.3	21.7	12
Hy 12	8	18	41	56	41	62	37
	75	5	11.5	11.3	9.5	13.5	−1
Hy 13	33	11	54	89	80	103	65*
	75	5	8.0	9.0	7.0	10.0	13

* significant difference (Scheffé 0.05)

Table 5 Tuber characteristics of tetraploid somatic hybrids and their dihaploid parents (*P* parents, *Hy* hybrids *Loval* long oval, *Roval* round oval, *Lyellow* light yellow)

Hybrid	G.i.[a] of tubers after harvest		Tuber form		Tuber skin		Tuber flesh colour[b]	
	P	Hy	P	Hy	P	Hy	P	Hy
Hy 1	8	5	Loval	Roval	Lyellow	Lyellow	5	5
	5		Roval		Lyellow		4	
Hy 4	5	5	Roval	Round	Lyellow	Lyellow	4	6
	3		Round		Lyellow		7	
Hy 5	8	7	Loval	Oval	Lyellow	Lyellow	4	6
	8		Oval		Lyellow		7	
Hy 6	9	8	Oval	Oval	Lyellow	Red	1	3
	8		Loval		Red		3	
Hy 12	9	7	Loval	Loval	Lyellow	Red	7	7
	7		Loval		Red		3	
Hy 13	8	7	Oval	Loval	Lyellow	Red	5	5
	7		Loval		Red		3	

[a] G.i., general impression: 1, excellent; 9, bad

[b] 1, grey; 3, white; 5, light yellow; 7, yellow; 9, dark yellow

starch content of 7 out of the 10 different fusion combinations tested were similar to the mid-parent value (± 11%). In three fusion combinations, Hy 6, 12, 13, in which H80.1528/6 was one of the partners, the hybrids had a clearly higher starch content than the mid-parent value. Depending on the fusion combination, the increase was between 15 and 21%. Differences between the hybrids of the same fusion combination were high. However, even in the best hybrids the starch content was not higher than that of the best parent clone (see max values in Table 4).

Tuber characteristics

The general impression of the tubers after harvest showed that the hybrid tubers had average scores in the range of the better parent (Table 5). In some combinations the tuber form 'round' or 'round oval' was inherited intermediate to 'long oval' while in the others it appeared to be inherited in a dominant manner. Fusion combinations in which one of the parent clones had a red tuber skin showed dominance over light yellow. However, the red tuber skin colour of the hybrids was not so intense as that of the parent clones.

In all fusion combinations, the tuber flesh colour of the hybrids was similar to the parent with the more yellowish flesh colour.

Discussion

The results show that tetraploid somatic hybrids can be obtained by protoplast fusion of dihaploid potatoes which are phenotypically similar to standard potato cultivars. As the dihaploid material used in the present study was produced some years ago (the interdihaploid clone HH 236 was produced by Baerecke in 1965), it is not surprising that the yield of these dihaploid clones is relatively low. Therefore, it is clear that the yield of the somatic hybrids is also relatively low compared to the standard cultivars. However, some hybrid combinations produced yields comparable to the standard cultivars. Thus, the yield of the hybrids was highly correlated with the yield of the parent clones (BPV and MPV), including the importance of the yield level of the dihaploid fusion clones for the yield level of the hybrids. Munzert et al. (1992) reported that tetraploid somatic potato hybrids had yields similar to, or even higher than, that of standard varieties.

In five out of six interspecific fusion combinations between diploid potato clones and *S. phureja*, Mattheij and Puite (1992) found that the yield was similar to the standard variety Bintje. However, despite the tetraploidy and the increased heterozygosity in these combinations the hybrid yield was not higher than the mid-parent value. Only one hybrid clone showed a threefold higher tuber yield than Bintje and the mid-parent value; this heterosis was mainly due to the increase in tuber weight. In our experiments, the yield increase was also mainly due to an increased tuber weight, whereas the number of tubers per plant were similar in most of the combinations to the mid-parent value. In their experiments Mattheij and Puite (1992) observed that the tuber numbers of the hybrids in two fusion combinations were equal to, and in two other combinations much lower than, the mid-parent value.

Although in our experiments the differences in yield between single hybrids of the same fusion combination were high, due to the large variation between years, these were not significant. Further field trials under more controlled conditions, especially involving regular treatment with a fungicide, are necessary to show if differences between the hybrids are significant and if they can be ascribed to differences in the plasmon, to mitotic crossing-over events (Evans et al. 1984), to aneuploidy or to somaclonal variation. Analysis of the plasmon of the hybrids is already in progress (Lössl et al., in preparation). In the present study, the ploidy of the hybrids was determined by routine counting of metaphase chromosoms in the root tip, the chloroplast number in the stomata, and by scoring the phenotype. Therefore, aneuploids at the tetraploid level could still be present among the hybrids tested. Waara et al. (1992) found that some of their euploid tetraploid somatic *S. tuberosum* hybrids differed extensively from other tetraploids. However, they also reported that one of the hybrids, having 49 chromosomes, gave the highest yield. Hexaploid hybrids are also sometimes difficult to distinguish from normal looking tetraploid plants and can give similar yields (Waara et al. 1992). Thach et al. (1993) observed lower than expected virus resistance in some of their hybrids and discuss further explanations for the deviating results.

The starch content of the hybrids was intermediate to that of the parents. However, in three combinations the starch content was 15–21% higher than the mid-parent value, and in two of these combinations it was similar to the best parent value. This heterosis was probably due to the low starch content of the parent clone H80.1528/6 which was used in all these combinations as one fusion partner. The absolute starch content of the parent clones and the hybrids was very low. Some of the hybrids were planted in the nursery of the private plant breeding company; their starch content data were in general much higher, showing that the low starch content in our experiments was due to our specific conditions. For Hy 1, the data also support the intermediate inheritance of starch content.

Scoring of the tuber characteristics demonstrated that red skin colour and yellow tuber flesh colour were inherited dominantly. The yellow flesh colour is supposed to be inherited as a monogenic dominant over 'white', although the existence of modifying genes has also been proposed (Howard 1978). The round/round-oval tuber form dominated in general over long tuber forms. Similar observations were reported by Waara et al. (1992). De Jong and Burns (1993) showed that in cultivated diploid potatoes one major gene controls the round and long tuber shapes and that round is dominant over long.

Intraspecific hybridizations for the production of superior tetraploid potatoes via the protoplast fusion of selected interdihaploid clones require the best available dihaploid material and should therefore carried out in the laboratories of the individual plant breeders. Putative hybrids can usually be identified via isoenzyme analysis and rapid flow-cytometric analysis of the ploidy level can be used beforehand to identify useless, unfused dihaploids, as well as hexaploids and aneuploids at the tetraploid level.

A prerequisite for the successful application of the extended analytical-synthetical breeding scheme, i.e., the immediate production of a superior tetraploid clone which can become a cultivar, is the efficient production of dihaploid potatoes from tetraploid ones. Furthermore, a strong selection for superior dihaploids has to be performed during the prebreeding at the dihaploid level. Compared to the classical breeding scheme of the potato, the selection of agronomically-important traits is much easier at the dihaploid level, while after fusion maximum heterozygosity is obtained at the tetraploid level. For adapted breeding material the maximum heterozygosity hypothesis seems to correlate well with increased tuber yield (Bonierbale et al. 1993). Using molecular markers, genetic distances can be determined between different dihaploid breeding lines to develop gene pools, and dihaploid breeding lines with a large genetic distance can then be selected for protoplast fusion.

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