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The first and second backcross progeny of the intergeneric fusion hybrids of potato and tomato after crossing with potato

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Abstract Somatic fusion hybrids between the diploid potato and tomato were backcrossed to several genotypes of potato. Two ploidy levels of fusion hybrids, 4x and 6x, were used as female parents in backcrosses with five clones of 4x-potato. An estimate of the berry set and "seed set" in immature berries harvested 14–21 days after pollination indicated that crosses between certain combinations of 6x-fusion hybrids and male parents were more successful than others. The culture of over 4000 young seeds from berries harvested 2–2.5 weeks after pollination gave rise to a single seedling, 93.6701, from the cross between the 6x-fusion hybrid C 31-17-1 and the 4x-potato AM 66.42. This seedling was found to possess a pentaploid chromosome number, which was expected of a 6x × 4x cross. Isozyme analysis and DNA hybridisation studies confirmed that the seedling 93.6701 was indeed a backcross (BC_1) progeny. Morphologically, this BC_1 plant resembled potato with respect to plant habit, leaf shape, stolons and tuber characteristics, while some of the characters, such as floral morphology and the fragrance of the crushed leaves (typical of tomato), were intermediate. It was male sterile but could be successfully hybridized with 4x-potato through in vitro culture of yound seeds; thus, BC_2 plants were obtained. The possibilities of backcrossing and the potential use of BC_1 and BC_2 plants in genetics and breeding are discussed.

Key words Protoplast fusion · Potato · Tomato · Post-fertilisation barriers · Introgression

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Introduction

Somatic hybridisation is a biotechnological tool for combining genomes of distantly related plant species. This additional possibility for creating genetic variation is intensively used in several crops to introduce desired traits like cytoplasmic male sterility and herbicide resistance through the introduction of mitochondrial and chloroplast DNA, respectively, or resistance to biotic factors and tolerance to abiotic factors, which are inherited chromosomally. For the first two classes of traits, cybridisation is sufficient but for chromosomally encoded traits, species' hybridisation followed by a backcross programme and meiotic recombination between homoeologous chromosomes are necessary. In potato, the introduction of *Erwinia* resistance has been reported using *Solanum brevidens* as a fusion parent, where backcrosses with *S. tuberosum* could be made (Austin et al. 1988).

Fusion hybrids between potato and tomato were reported more than 15 years ago (Melchers et al. 1978). Since then, many such hybrids have been produced due to their potential for transferring traits from potato to tomato or vice versa. Most of the attention has been paid to the possibility of transferring interesting traits like chilling resistance from potato to tomato (Smillie 1979). Despite the large genetic homology between the genomes of both genera found by restriction fragment length polymorphism (RFLP) analysis (Gebhardt et al. 1991), no successful backcross has ever been reported.

Recently, we recommenced research on somatic hybridisation and are now investigating more systematically factors like using different fusion parents and backcross parents to overcome the various problems. Several fusion combinations have been made (Jacobsen et al. 1992) and used as female parents, and several potato male parents have been tested for berry and seed set. Some of those were known to possess a good combining ability for seed set in potato breeding.

Material and methods

Plant material

The somatic fusion hybrids which were used as female parents included both tetra- and hexaploids. These hybrids were derived from the fusions of the following genotypes of the diploid potato (*Solanum tuberosum* 2n = 2x = 24) and tomato (*Lycopersicon esculentum* 2n = 2x – 24): (1) the amylose-free potato clone, 87.1029/31 (*amfamf*) + ALRC (albino red cherry tomato), coded as S1; (2) the *amfamf* potato clone, 87.1030/5 + ATW 4015 tomato, coded as S2 and (3) the *Amfamf* potato clone, 87.1017/5 + C31 tomato, a nitrate reductase-deficient mutant that is resistant to tomato mosaic virus (Schoenmakers et al. 1991), coded as the C31-17 series. The details of the S1 and S2 series of hybrids have been described earlier (Jacobsen et al. 1992) and the C31-17 series was made by H C. H. Schoenmakers (Department of Genetics, Agricultural University Wageningen).

With respect to the ploidy levels of the fusion hybrids, all of the genotypes of the S1 and S2 series, with the exception of S1-60, were tetraploid (2n = 4x = 48); among the C31-17 series, with only one exception, the 4x-C31-17-50, all of the others, C31-17-9, -17, -24, -42 and -51, were hexaploid (2n = 6x = 72) (see Jacobsen et al. 1992; unpublished data). The potato backcross parents were (1) two nulliplex genotypes for amylose-free starch (*amf*) (Jacobsen et al. 1989), 91.6016-11 and 91.6020-22; (2) three cultivars, 'Desiree', 'Escort' and 'Mansour' and (3) two breeding clones, AM 66.42 and MPI 19268.

All plants were grown in a temperature-controlled glasshouse during the growth season April–September. The fusion hybrids were emasculated before the opening of the flower buds and pollinated with fresh pollen when the stigmas were receptive.

In-vitro culture

The ovule culture technique according to Jacobsen et al. (1993) was used to rescue the developing embryos. Plants were grown on basal MS medium (Murashige and Skoog 1962) for in vitro multiplication and rooting. Rooted plants were transferred to sterilized soil and grown in 100% humidity in the glasshouse for about 1–2 weeks.

Cytological observations

Chromosomes were counted in the dividing cells of root tips, and meiosis was investigated after collecting young anthers as described earlier by Jacobsen et al. (1992).

Isozyme analysis

For biochemical analysis of offspring plants were investigated for the isozymes, GOT (glutamate oxaloacetate transaminase) and SDH (shikimic acid dehydrogenase), according to Suurs et al. (1989). Electrophoresis was carried out using the Phast System at 80 °C following the methodology of applying these enzymes described by Suurs et al. (unpublished).

Dot-blot analysis using species-specific repetitive DNA probes

In order to determine the ratio between the potato and tomato DNA content in somatic potato-tomato hybrids dot-blot analyses were performed as described by Wolters et al. (1991). The tomato-specific, repetitive DNA probe pTHG2 (Zabel et al. 1985) was kindly provided by Dr. P. Zabel, the potato-specific, repetitive DNA probe P5L (Visser et al. 1988) by Dr. R. G. F. Visser and the ribosomal probe pJL4324 (Landsman and Uhrig 1985) by Dr. L. Landsman.

Results

In all, 31 somatic hybrids belonging to three different parental combinations were used as female parents to investigate cross compatibility and combining ability for berry and seed set. The berries were harvested 2–3 weeks after pollinations, and developing seeds were isolated and cultured on solid ovule culture and embryo rescue media. Of the female parents 16 were hexaploids (2n = 6x = 72) and 15 tetraploids (2n = 4x448),

Backcrosses between somatic hybrids and tetraploid potato

In the crosses with tetraploids, 533 pollinations gave rise to only 11 berries containing 91 developing seeds (Table 1). At least 1 or more seeds were present in each berry. There were no notable differences between the female parents with regard to seed and berry set. Genotypes like S2-36 and S2-29 produced, respectively, after 107 and 125 pollinations, no or only 1 berry, the latter containing 2 developing seeds. The other extreme was C31-17-50 with 5 berries containing 24 developing seeds after 50 pollinations. The average berry set and seed set per pollination were 0.01 and 0.17, and those of the best performing female, C31-17-50, -10 and -48, respectively. The best performing male parents were cv 'Mansour' and *amf* mutant 91.6022.

In the combination with hexaploid hybrids, 403 pollinations were made, resulting in 102 berries containing 3498 developing seeds (Table 1). In comparison with the tetraploid females, a much larger variation in berry set and seed set was observed. Most of the seriously tested females gave both berry set and seed set, indicating the much higher compatibility in this ploidy combination. The best performing genotypes were C31-17-5, -24, -42 and S1-60 with a relatively high berry set and seed set of up to 0.76 and 29.94 per pollination, respectively. Analysis of the data according to the male parents indicated that *amf* mutant 91.6011 and the wild-types AM66.42 and MPI 19268 were the best performing genotypes with a berry and seed set per pollination of 0.42 and 13.42, 0.33 and 10.03, and 0.30 and 13.82, respectively. It is clear from these observations that hexaploid hybrids were much more successful in crosses with tetraploid potato than tetraploids and that genotypic selection even within a fusion combination is fruitful.

From the 4147 ovules that were cultured for the germination of embryos only 1 seedling was obtained. The early development of this seedling was very slow, and it took more then 3 months before it could be multiplied in vitro. This seedling (Fig. 1), designated 93.6701, originated from the hexaploid somatic hybrid C13-17-1 as female and the tetraploid potato AM66-42 as male.

Table 1 Berry and 'seed set'[a] in tetraploid (A) and hexaploid (B) fusion hybrids of diploid potato and tomato crossed with 4x-potato clones as male parents

Male/ Female parent	AM 66.42			Escort			Mansour			Desiree			MPI 19268			91-60-11			91-60-20			Σ			≠ of plants	
Weeks after pollination	1[b]	2[c]	3[d]	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
A																										
4x																										
S1-54	1	1	35	4	0	0	3	0	0	4	0	0	2	0	0	1	0	0	–	–	–	15	1		0	0
S2-29	32	0	0	21	0	0	8	0	0	8	0	0	2	0	0	29	1	2	8	0	0	107	1		2	0
S2-36	80	0	0	6	0	0	6	0	0	4	0	0	14	0	0	13	0	0	2	0	0	125	0		0	0
S2-(12x)[e]	110	1	9	5	0	0	11	1	13	27	0	0	63	0	0	31	0	0	13	2	0	236	4		65	0
C31-17-50	13	1	0	5	0	0	14	2	20	9	1	1	1	0	0	6	1	3	2	0	0	50	5		24	0
Total	236	3	44	41	0	0	42	3	33	52	1	1	82	0	0	80	2	5	25	2	0	533	11		91	0
B																										
6x																										
C31-17-1	9	1	6	6	1	7	1	0	9	4	0	0	4	0	0	2	0	0	6	0	0	34	2		13	1
C31-17-5	16	14	495	4	4	109	2	1	0	5	3	96	19	12	623	2	2	89	1	1	55	49	37		1467	0
C31-17-24	12	5	89	3	2	0	–	–	–	1	1	35	8	5	284	8	6	271	1	0	0	33	19		679	0
C31-17-42	9	4	90	4	1	14	6	0	0	5	1	15	1	0	0	1	1	10	1	0	0	26	7		129	0
S1-60	30	7	302	12	0	0	12	4	245	11	0	0	31	8	291	2	0	0	8	0	0	106	19		838	0
C31-17(10x)[e]	44	10	227	25	1	7	8	2	8	21	0	8	34	3	87	16	4	43	3	0	0	155	18		372	0
Total	120	41	1209	54	9	137	29	7	262	47	5	154	97	28	1285	31	13	413	20	1	55	403	102	3498	1	

[a] Developing seeds in berries collected 14–21 days after pollination
[b] Number of pollinations
[c] Number of berries
[d] Number of seeds
[e] Twelve somatic hybrids of series S2, 10 somatic hybrids of series C31-17

Analysis of the germinated seedling

Theoretically, seedling 93.6701 can be the result of a normal fertilization or from other processes like parthenogenesis or androgenesis of reduced or unreduced gametophytic cells. The expected ploidy level after normal fertilization is a pentaploid because of the hexaploid and tetraploid chromosome number of the female and male parents involved (Table 1). Extensive chromosome countings in the dividing root-tip cells clearly showed a pentaploid ploidy level. This was a very important first indication that 93.6701 resulted from a normal fertilization in which both parents were involved. In biochemical studies in which GOT and SDH were used additional evidence was found for this hypothesis. The combination of zymograms of both GOT and SDH clearly indicated that tomato as well as the male parent AM66.42 were involved in the backcross plant (Fig 2).

Observations on 93.6701 (Fig. 1) showed clearly a morpological similarity to potato with respect to plant shape, stolon/tuber development, leaf arrangement on stems, leaf shape and leaf size. The leaves were dark green and the petals were white, the same colours as those of both potato parents were used during somatic fusion and backcrosses. The shape of the corolla was more comparable with that of the somatic hybrid and the tomato fusion parent than with that of potato. The flower size was as small as that of the diploid potato fusion parent 87.1017/5. The anthers of 93.6701 were as in the fusion hybrid, not united into a column at the tips but as separated as in potato. After being wounded the leaves of the BC_1 plant gave the typical tomato fragrance. Tuber formation in both male parent AM 66.42 and BC_1 93.6701 was localised in subapical parts of the stolon, whereas in the female, fusion hybrid C31-17-1, tuber development was not localised on the stolons. Tuber shape was round and regular in the BC_1 plant and highly irregular in the somatic hybrid. It is clear from all of these observations that 93.6701 is potato-like for most of the characters, intermediate for some of them and tomato-like for a few. All of these observations indicate that 93.6701 is the result of a normal back-cross between C31-17-1 and AM 66.42.

Genomic constitution of BC_1 93.6701

An important question to answer is just what is the genomic constituation of this BC_1 plant. The answer is entirely dependent on the genomic constitution of the hexaploid female parent C31-17-1. This somatic hybrid is the result of a fusion between the diploid tomato line C31 (TT) and the diploid potato clone 87.1017/5 (PP) and consists, theoretically, of PPPPTT or PPTTTT. Molecular studies by dot-blot analysis showed a 2 (potato):1 (tomato) ratio between the potato and tomato

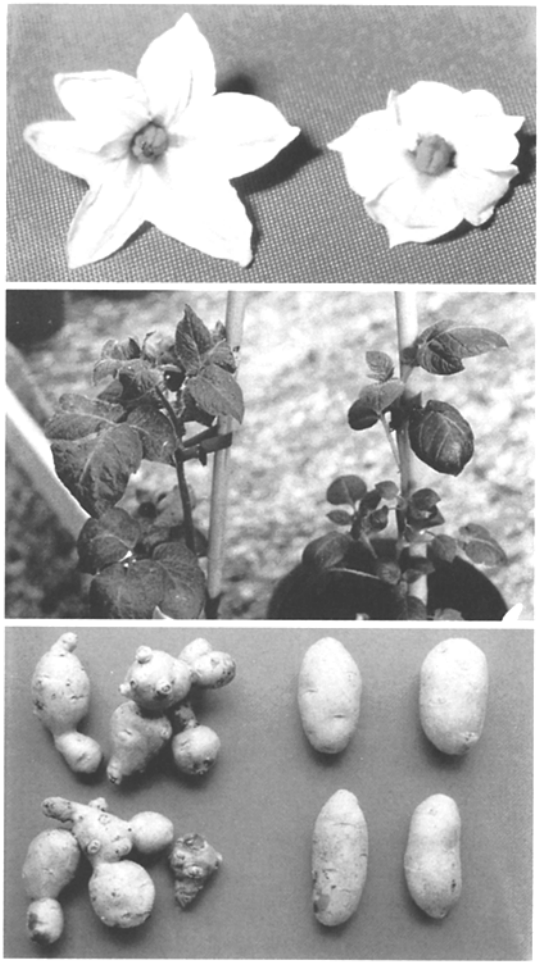

Fig. 1 Comparison of fusion hybrid C31-17-1 (*left*) and BC_1 93.6701 (*right*)

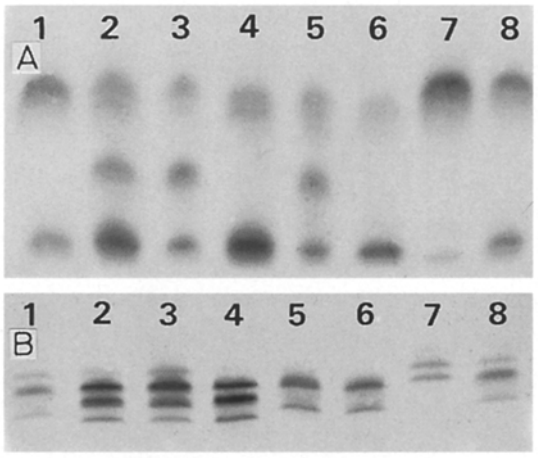

Fig. 2 A, B Isozyme analysis of fusion parents, fusion hybrid C31-17-1, backcross male parent AM 66.42 and 93.6701 using **A** Got (glutamate oxaloacetate transaminase) and **B** SDH (shikimic acid dehydrogenase). *Left-right*: C31 + 87.1017-5 (mixture), AM66.42 + 93.6701 (mixture), 93.6701, AM66.42, C31.1017-1, 87.1017-5, C31, C31 + 87.1017-5 (mixture)

DNA content of the fusion hybrid (data not shown). The tomato-specific probe THG2 and the potato-specific probe P5L were used to estimate the quantity of tomato and potato DNA, respectively. The ribosomal probe pJL 4324 was used as a reference for the total amount of DNA. These quantitative data confirmed the results of the isozyme analyses. This means that the genomic constitution of the pentaploid BC_1 plant 93.6701 unless this expected balance of the genomes was not transmitted by the C31-17-1 parent through homoeologous recombination between the potato and tomato chromosomes.

Fertility of BC_1 93.6701

Pollen stainability studies and pollinations showed that the pentaploid BC_1 plant 93.6701 was male sterile but female fertile. The female fertility of 93.6701 was tested during the winter using potato pollen. Fifteen pollinations with the 4x *amf* male parents 90.6020/22 and 90.6016/11, gave 6 berries varying in seed number between 26 and 91. The seeds from 5 berries were harvested 4 weeks after pollination and tested for germination in vitro. They showed normal size and development and contained an embryo. This was a clear indication that the female fertility of BC_1 93.6701 is normal. Seed germination in vitro was poor. Improvement could be made by removing the seed coat in order to produce more BC_2 seedlings. Pollinations with 2x potato were without any berry or seed set.

Discussion

The circumvention of crossing barriers between distantly related species by protoplast fusion is an interesting tool. This possibility has successfully been used in the combination between potato and the non-tuberous species *Solanum brevidens* (Austin et al. 1988). Crossing experiments between hexaploid and tetraploid fusion hybrids of this combination and tetraploid potato have been successful in giving rise to BC_1 offspring. Embryo rescue in vitro, starting 3–4 weeks after pollination, did increase the rate of success of BC_1 seedling development. It became clear from these observations that BC_1 seedlings could be obtained easily from 6x × 4x crosses and less frequently from the 4x × 4x combination (Jacobsen et al. 1993). The fertility of the resulting tetraploid or pentaploid BC_1 plants was improved or almost normal. In this way, several resistances from *S. brevidens* have become available for normal potato breeding. The fact that 6x × 4x crosses can also be successful in interspecific hybridisation has been shown in the past in breeding for resistance to *Phytophthora infestans* using sexual hybrids between hexaploid *S. demissum* and tetraploid *S. tuberosum* (Wastie 1991). It was also found

that in this combination the resulting pentaploid hybrid could be used easily as a parent in backcrosses with tetraploid potato. In the combination between allotetraploid or allohexaploid somatic hybrids of potato-tomato the same phenomenon has been found. Berry set with developing ovules is much more frequently observed in the 6x × 4x backcross than in the 4x × 4x combination. Pilot experiments with the pentaploid BC_1 plant showed the same phenomenon of almost normal berry and seed set as described above for other 5x BC_1 plants. It is clear from this experience that fusions between tetraploid potato and diploid tomato resulting in an hexaploid are recommended in order to optimize the chance for BC_1 offspring plants in ovule cultures. A second important factor is the selection of good combining female somatic hybrids and pollinators (Table 1). This factor was also of importance in circumventing crossing-barriers by bridging species (Hermsen and Taylor 1979).

Our observations (Table 1) show that sexual offspring can be obtained at a low frequency from the somatic potato-tomato hybrid. Histological studies (data not shown) at different time intervals after pollination on developing fruits and ovules showed that in most of the ovules abnormal developments are visible within 5–7 days, frequently leading to callus development during in vitro culture. Obviously, the frequency of ovules with normally developing embryos is decreasing rapidly. In our case this led to only 1 seedling out of over 4000 ovules when cultured 2–2.5 weeks after pollination. This type of research has been started not only in backcrosses using this species combination but also in the somatic combination between diploid potato and hexaploid *S. nigrum* (unpublished data). Other examples of successful early ovule rescues have been described in tobacco, 5–7 days after pollination (Redd and Collins, 1978), and in cotton, 2–4 days after pollination (Stewart and Hsu 1978), for the isolation of interspecific hybrids.

The pentaploid BC_1 plant appeared to be female fertile and male sterile. The male sterility could be the result of an exchange of mitochondrial DNA causing cytoplasmic male sterility (CMS) in potato. Indications have been found that cybrids in the reciprocal combination delivered cms in tomato (Melchers et al. 1992). That the first backcross is the most difficult step in starting a successful backcross programme has also been ascertained in the combinations between potato and *S. brevidens* (Jacobsen et al. 1993) and between *Lycopersicon esculentum* and *Solanum lycopersicodies* (Gradziel and Robinson 1989).

The BC_1 and BC_2 plants are basic material for introgression research by chromatid recombination on the one hand and for the creation of potato genotypes with one or two additional chromosomes of tomato on the other. The availability of genotypes with different chromosome additions enables the localisation of all kinds of morphological and biochemical traits of tomato, such as storage proteins, which are expressed in potato and unknown in the existing genetic variation within tomato. The investigation of introgressions enables localisation of these traits within particular chromosomes and integration with molecular markers (Gebhardt et al. 1991). The successful application of the technique of chromosome painting, based on heterologous hybridisation of genomic DNA, is an efficient tool for a clear detection and localisation of both introgressions and chromosome additions (Anamthawat-Jónsson et al. 1993).

The results shown indicate that tomato-encoded characters like chilling resistance and all kinds of disease resistances are available for potato breeding. The transfer of these important characters is clearly dependent on the measure of introgression, which is based on the frequency of recombination between homoeologous chromosomes, enabling the separation of desired and undesired traits by crossing-over.

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