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Allotriploid somatic hybrids of diploid tomato (*Lycopersicon esculentum* Mill.) and monoploid potato (*Solanum tuberosum* L.)

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Abstract. Allotriploid somatic hybrids were obtained from fusions between protoplasts of diploid tomato and monohaploid potato. The selection of fusion products was carried out in two different ways: (1) The fusion of nitrate reductase-deficient tomato with potato gave rise only to hybrid calli if selection was performed on media lacking ammonium. Parental microcalli were rarely obtained and did not regenerate. (2) The fusion of cytoplasmic albino tomato with potato gave rise to albino and green hybrid calli and plants. Allotriploids were identified from the two somatic hybrid populations by counting chloroplast numbers in leaf guard cells and by flow cytometry of leaf tissue. Although some pollen fertility of allotriploids and pollen-tube growth of tomato, potato and *Lycopersicon pennellii* into the allotriploid style were observed, no progeny could be obtained. The relevance of allotriploid somatic hybrids in facilitating limited gene transfer from potato to tomato is discussed.

Key words: *Lycopersicon esculentum* – *Solanum tuberosum* – Allotriploid somatic hybrids – Nitrate reductase deficiency

Introduction

Asymmetric somatic hybrid plants, which combine the total genome of a recipient species with part of the genome of a donor species, can be constructed in various ways. Many investigators have treated donor cells or protoplasts with ionizing irradiation prior to their fusion with recipient protoplasts. The irradiation causes changes in or the loss of bases, different types of crosslinking and DNA fragmentation, and consequently the elimination of damaged nuclear DNA of the donor in fusion products. However, the extent of elimination of donor DNA is often unsatisfactory, and the irradiation treatment also reduces the efficiency of recovering somatic hybrids. Furthermore, endoreduplication during cell culture increases the ploidy level of asymmetric somatic hybrids, the genomic balance is often disturbed and, in general, fertility of the hybrids is low or absent, which prevents further elimination of donor DNA during meiosis by possible recombination between recipient and donor genomes.

Techniques such as the fusion of donor microprotoplasts which contain micronuclei with one or a few chromosomes (Verhoeven et al. 1991) with recipient protoplasts and the micro-injection of isolated chromosomes (de Laat et al. 1989) or micronuclei theoretically result in highly asymmetric hybrids. However, these procedures are technically complicated and require cell cultures of the donor species in which the cell cycle can be synchronized efficiently.

A more established approach to reduce the contribution of the donor genome to that of the hybrid is to use haploid unirradiated genotypes for fusion instead of the diploid irradiated donor protoplasts that are normally used. Haploid protoplasts can be either of gametophytic or of sporophytic origin. In theory, one complete set of chromosomes (one genome) is transferred by such a fusion. For phylogenetically related combinations this means that the genomic balance of the somatic hybrids is less disturbed than in asymmetric somatic hybrids that are constructed with the aid of ionizing radiation.

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Experimental data suggest that both the efficiency of recovering triploid somatic hybrids of related solanaceous species by this procedure and the fertility of these allotriploid somatic hybrids are higher than those of asymmetric somatic hybrids constructed with ionizing radiation (Lee and Power 1988; Pental et al. 1988; Pirrie and Power 1986).

Partial fertility has also been described for sexual allotriploid hybrids between solanaceous species. Soost (1958) described the construction of fertile allotriploid (sesquidiploid, 2n = 3x = 36) hybrids derived from the interspecific cross *Lycopersicon esculentum* (tomato) (2n = 4x = 48) × *L. peruvianum* (2n = 2x = 24). Progenies from open pollination, assumed to be allotriploid × *L. peruvianum* (2n = 2x = 24) crosses, showed reduced chromosome numbers that ranged from 26 to 34. Rick et al. (1986, 1988) performed intergeneric crosses between *L. esculentum* (2n = 2x = 24) and *Solanum lycopersicoides* (2n = 2x = 24), doubled the ploidy level of the F_1 hybrid and subsequently were able to make the backcross with *L. esculentum* (2n = 2x = 24). The resulting allotriploids (2n = 3x = 36) could be crossed with *L. pennellii* (2n = 2x = 24). Progenies from these crosses showed the introgression of *S. lycopersicoides* traits into diploid lines.

In this report we describe the isolation and analysis of allotriploid somatic hybrids between diploid tomato and monohaploid potato (*Solanum tuberosum*). Tomato and potato are related, both being solanaceous species with the same basic chromosome number (x = 12); however, they cannot be hybridized sexually. Somatic hybrid plants between tomato and potato have been described by Melchers et al. (1978) (hyperallotetraploids), Shepard et al. (1983) (allohexaploids) and Jacobsen et al. (1992) (allotetraploids and allohexaploids).

Materials and methods

Plant material

Two different tomato (*Lycopersicon esculentum*) genotypes were used in fusion experiments. The nitrate reductase-deficient apoenzyme (*nia*) mutant C31, described by Schoenmakers et al. (1991), was backcrossed with its wildtype, cv 'GT'. 'GT' is a tomato mosaic virus-resistant line and was kindly provided by DeRuiterseeds, Bleiswijk, the Netherlands. The F_3 line C31-244 (derived from 'GT' × C31 crosses) was used for fusion; it was homozygous for chlorate resistance and did not show any other visually detectable phenotypic abnormalities that were not associated with the *nia* mutation. The tomato with a cytoplasmic inherited albino mutation was obtained as follows: In vitro-grown shoots of a cytoplasmic albino mutant of tomato cv 'Large Red Cherry' ['ALRC'; originating from Dr. M. R. Hanson, Section of Genetics and Development, Cornell University, Ithaca, USA; for description see Hosticka and Hanson (1984)] were grafted in vitro onto tomato cv 'Moneymaker' root-stock. Grafted albino shoots were transferred to the greenhouse and crossed with tomato genotype MsK8, which exhibits good regeneration capacity (Koornneef et al. 1987), as a staminate parent. From the totally albino F_1 population, genotype ALRC × M8-7 was selected because of its good regeneration capacity from root and hypocotyl explants (Koornneef et al. 1993). The potato (*Solanum tuberosum*) 7322 monoploid originated from Dr. G. Wenzel, Germany. For detailed description see de Vries et al. (1987). Plants were grown in vitro in 380-ml plastic containers on 50 ml Murashige and Skoog (1962) medium without hormones supplemented with 58.4 m*M* (for C31-244 and 7322) or 175 m*M* (for 'ALRC' × M8-7) sucrose, pH 5.8 before autoclaving, and solidified with 8 g/l agar (these media are designated MS20 and MS60, respectively), at a light intensity of 10 W/m^2 under a 16 h/8 h light/dark regime at 25 °C.

For the pollinations various species were used. Homozygous recessive morphological markerlines of tomato were used as pistillate parents. Various diploid tomato lines, including cv 'Moneymaker' and MsK9, the F_1 hybrid (*L. esculentum* cv 'Solentos' × *L. peruvianum* LA 2157) and *L. pennellii* LA 716 were used as staminate parents, as was the dihaploid (2n = 2x = 24) potato RH87-343-25, kindly provided by R. Eijlander, Department of Plant Breeding, Wageningen Agricultural University, the Netherlands.

Isolation, fusion and culture of protoplasts; regeneration and culture of plants

The isolation, fusion and culture of protoplasts was carried out as described by Wolters et al. (1991). Minicalli were subcultured monthly on solidified TMcμZ greening medium (Wolters et al. 1991). Calli with a diameter of approximately 5 mm were transferred to 1Z medium [modified 2Z medium (Thomas and Pratt 1981) with 1 instead of 2 mg/l zeatin] to induce shoot regeneration. Regenerated green shoots were rooted on MS20 medium; albino shoots were rooted on MS60 medium. Vegetative propagation of somatic hybrid plants was carried out in vitro. Aseptic shoots were rooted in Jiffy-7 peat pellets (Jiffy Products, Norway) and transferred to the greenhouse. Cuttings of somatic hybrid plants were grafted onto tomato root-stock for better growth and flowering.

Flow cytometric analysis

For the flow cytometric analysis of leaf tissue approximately 0.1 g of tissue was placed in a 6-cm petri dish, and 0.5 ml of a nuclei buffer, consisting of 10 m*M* sperminetetrahydrochloride, 200 m*M* hexylene glycol, 10 m*M* NaCl, 10 m*M* TRIS-HCl, 0.025% (v/v) triton-X100 and 2.5 μg/ml 4,6-D-diamidino-2-phenylindole (DAPI), pH 7.0, was added. The tissue was chopped into small pieces with a very sharp razor blade, and the suspension was filtered through a 85-μm pore nylon filter. The filtrate was used directly for flowcytometry. For the flowcytometric analysis of protoplasts, the protoplasts were suspended in nuclei buffer (10^4 protoplasts/ml) and disrupted mechanically by passing them twice through a 26-gauge needle prior to the flowcytometric assay.

Cytological analyses

The number of chloroplasts per guard cell pair was determined in the lower epidermis from leaves of in vitro-grown plants or in lower epidermis strips from leaves of greenhouse-grown plants as described by Koornneef et al. (1989). Chromosome counts and karyotype analyses were performed on unbanded chromosome preparations of spread root-tip meristems prepared as follows [Modified after Pijnacker and Ferweda (1984)]. Root tips were collected from in vitro-grown hybrids and from the parental species grown in the greenhouse. They were incubated in 2 ml

2 m*M* 8-hydroxyquinoline for 4 h at 17 °C and afterwards fixed in cold Carnoy's solution (ethanol–glacial acetic acid, 3:1) for 18 h or more at 4 °C. The fixed root tips were rinsed in demineralized water (demiwater) and subsequently incubated in 1–2 ml 10 m*M* citric acid–sodium citrate buffer, pH 4.2–4.5, containing 0.1% each of cellulase RS, pectolyase Y23 and cytohelicase enzymes for 1 h at 37 °C. Then the enzyme solution was carefully removed and demiwater was added. With a Pasteur pipette root meristems were transferred to a clean slide (cleaned in 70% ethanol). Excess water was removed, and the cells were loosened with the aid of fine needles under a binocular. A small drop of 60% acetic acid was quickly added with a Pasteur pipette, and the cells were suspended in this drop. Then, with a pipet tip the suspension was surrounded with freshly prepared, ice-cold Carnoy's solution, after which one drop of Carnoy's solution was put on top of the suspension. The slide was air dried for ± 1 h. For staining the slides were first incubated for 15 min in 2 × SSC at 60 °C and subsequently rinsed for 1–2 min in demiwater. Then the chromosomes were stained in 2% Giemsa in 10 m*M* Sörensen's phosphate buffer pH 6.8 for 30 min at room temperature, rinsed in Sörensen's buffer and demiwater, air dried and mounted in Entellan with a large cover slip.

Isoenzyme analysis

Polyacrylamide gel electrophoresis (PAGE) of glutamate-oxaloacetate transaminase (GOT; EC. 2.6.1.1) and 6-phosphogluconate dehydrogenase (6-PGdH; EC. 1.1.1.49) was performed according to Schoenmakers et al. (1992).

Morphological analysis and fertility

In vitro- and greenhouse-grown plants and scions were analyzed for their leaf and flower morphology. Pollen viability was determined by: (1) staining with a solution consisting of 6 m*M* fluorescein diacetate (FDA) and 0.2 *M* sucrose in a 1:1 (v/v) mix of dimethylsulfoxide (DMSO) and water (viable pollen stained yellow under UV; non-viable pollen did not stain), and (2) pollen germination on a solidified medium consisting of 0.3 *M* sucrose, 2.2 m*M* $Ca(NO_3)_2 \cdot 4H_2O$, 1.6 m*M* H_3BO_3, 2.0 m*M* citric acid, 0.6% agar, pH 5.9. Reciprocal crosses were made between allotriploid somatic hybrids and diploid tomato, *Lycopersicon pennellii*, *L. peruvianum* and potato. Pollen-tube growth in the pistils was assessed 24 h or 48 h after pollination according to Ramanna and Mutsaerts (1971) with aniline blue. Observations were made with a Nikon microscope equiped with high quality fluor objectives and filter set DM400.

Results

Fusion and culture of protoplasts; regeneration of hybrid plants

From the fusion combination C31-244 (+) 7322, subsequently designated C7, 170 hybrid calli (each from a single fusion event) yielded somatic hybrid plants. Parental microcalli were rarely obtained in control and

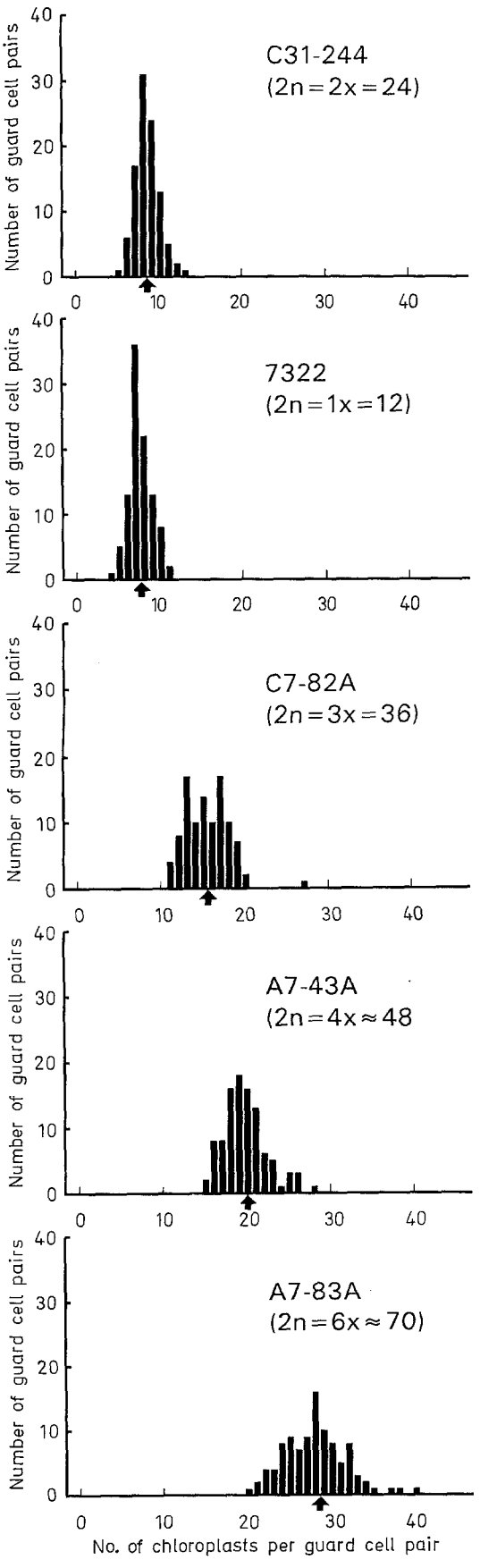

Fig. 1. Number of chloroplasts per guard-cell pair of in vitro-grown tomato C31-244, potato 7322, allotriploid C7-82A, allotetraploid A7-43A and allohexaploid A7-83A. The average number of chloroplasts per guard cell pair is indicated with an *arrow*. Number of guard-cell pairs counted = 100

fusion plates; they turned brown at a very early stage. From the combination 'ALRC' × M8-7 (+) 7322, subsequently designated A7, few viable, albino tomato calli could be obtained in control and fusion plates. Here, also neither tomato nor potato regenerants were found. From the A7 combination 275 hybrid calli gave green somatic hybrid plants, while 5 hybrid calli gave albino plants. The hybridity of calli and plants was determined with GOT and 6-PGdH isoenzyme analysis (zymograms not shown). The somatic hybrid vigor of fusion products of tomato and potato has also been observed in earlier experiments (Schoenmakers et al. 1992). Up to four independent, adventitious shoots (designated A, B, C and D) were regenerated per fusion callus. These shoots were rooted on MS20 (green plants) or MS60 medium (albino plants).

Chloroplast number in leaf guard-cell pairs

For tomato (Koornneef et al. 1989) and potato (Frandsen 1968; Karp et al. 1984) the number of chloroplasts per guard-cell pair is positively correlated with the ploidy level. This correlation was also found for the somatic hybrids of tomato and potato (Fig. 1). However, because in vitro-rooted shoots formed significantly more chloroplasts than non-rooted shoots and because of the small differences between allotriploid and allotetraploid somatic hybrids, it was difficult to identify allotriploids from the somatic hybrid populations on the basis of the average number of chloroplasts per guard-cell pair only. Within well-rooted allotriploid, allotetraploid and allohexaploid populations no significant differences were found between the average numbers of chloroplasts per guard-cell pair.

Flow cytometric analysis

Parental protoplast populations were analyzed for their relative DNA content, expressed as C-values, by means of flow cytometry (Table 1). The C-values of diploid tomato (2C) and diploid potato (2C) are almost equal. The presence of higher C-values (especially 4C in diploid tomato and 2C and 4C in monoploid potato) can be explained either by the presence of cells in the G2 phase or because of polyploidization, as is described by Nootebos et al. (1989) for tomato and by Uijtewaal (1987) for potato.

With this procedure, it was possible to detect 1 triploid leaf sample in a mixture of 20 leaf samples of equal weight, each derived from a different plant. For each flow cytometric analysis 10-mg leaf pieces of 10 somatic hybrids were pooled. Subsequently, plants from pools containing triploids were analyzed individually (Table 1, Fig. 2). From the somatic hybrid population C7 one albino and 9 green allotriploids were selected; from population A7 12 green allotriploids were selected. The discrepancy between the numbers of isolated allotripoids and the numbers as estimated from summed flow cytometric histograms from 10 plants each (Table 1) is probably related to differences in (1) the degree of polyploidization per plant, (2) the fraction of cells in G2, (3) the cell weight, and (4) to equipment imprecision.

Chromosome numbers

In addition to flow cytometric selection of allotriploids, the triploid character was confirmed by counting chromosomes in root-tip cells as is shown in Table 2 and Fig. 3. Although the karyotypes of the tomato and potato genotypes used are very similar, the satellite chromosomes (the numbers 2 in both parental karyotypes) can easily be distinguished in mitotic metaphase plates by the strikingly larger size of the tomato satellites, relative to the potato satellites. In most allotriploids both tomato satellite chromosomes were present, but in 3 hybrids containing 35 chromosomes, one chromosome 2 of tomato was missing. It appeared that less than half of the hybrids contained the euploid triploid number (36) of chromosomes.

Table 1. Relative C-value distribution (in percentages) of parental protoplast populations (ppp) and somatic hybrid populations (shp) C7 and A7. For shp the distribution was estimated from summed flow cytometric histograms from pools of 10 plants each

Population	Number of C equivalents									n^a
	1C	2C	3C	4C	5C	6C	7C	8C	>8C	
ppp C31-244		86		14				0		841
ppp ALRC × M8-7		82		18				0		704
ppp 7322	66	30		4						989
shp C7			5	60	11	12	0	12	0	170
shp A7			8	56	12	12	0	11	1	280

[a] n = number of protoplasts or somatic hybrids analyzed

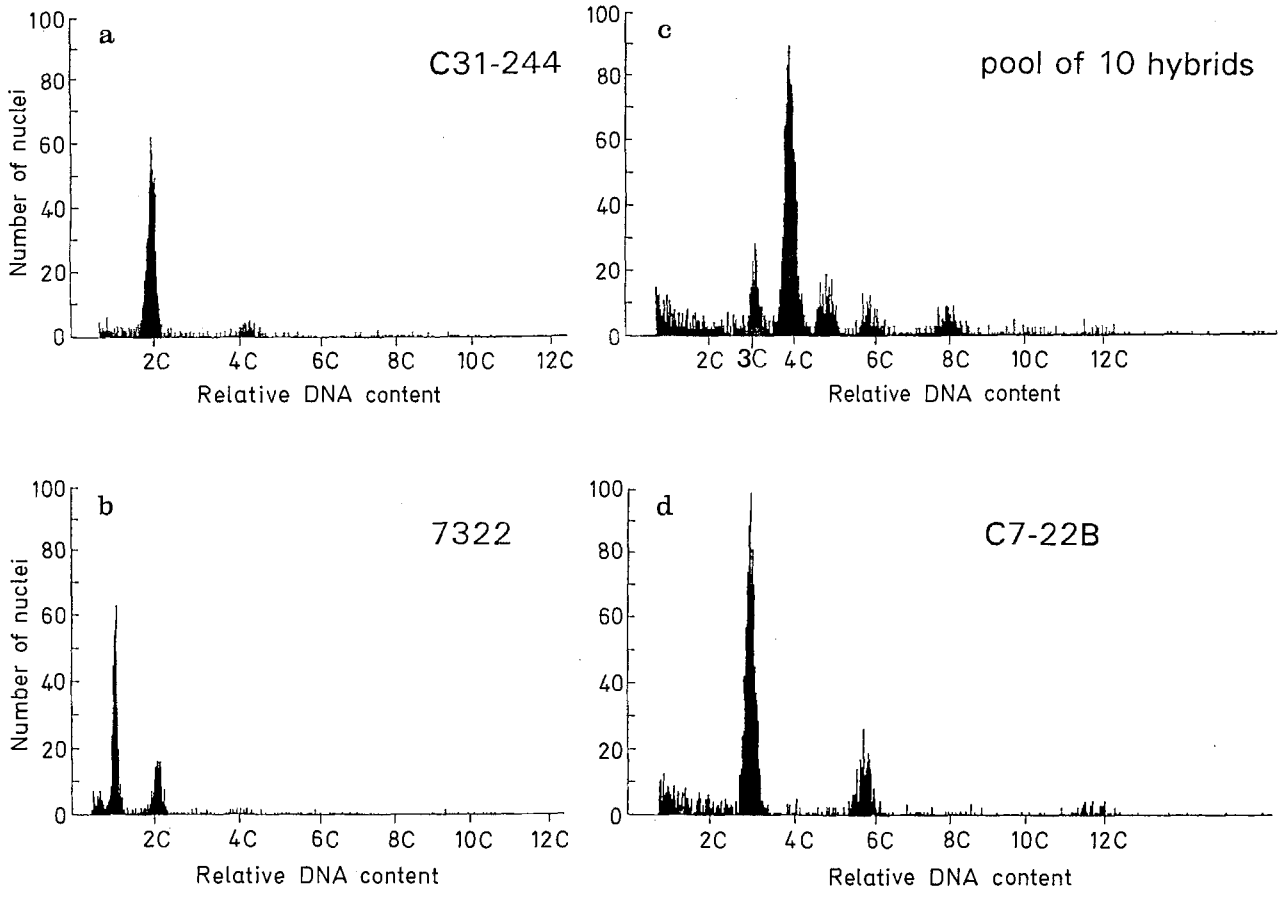

Fig. 2a–d. Flow cytometric histograms of protoplasts of fusion parents tomato C31-244 (2n = x = 24) (**a**) and potato 7322 (2n = 1x = 12) (**b**), leaf samples from a pool of 10 somatic hybrid plants (**c**), and allotriploid fusion product C7-22B (2n = 3x = 36) (**d**)

Morphology

Although growth of the allotriploids in vitro was vigorous, greenhouse-grown plants displayed a retarded growth and developed local necrosis. Upon grafting both growth and flowering improved, but poorly growing plants still produced no flowers. The leaf morphology of both allotriploids and allotetraploids was intermediate between the two fusion parents and was highly variable between allotriploids. Allotriploids were more tomato-like than allotetraploids. The color of the young flowers of allotriploids was intermediate (light-yellow) between tomato (yellow) and potato (white); This light-yellow color faded to white in older flowers. The flower morphology was variable between allotriploids (Fig. 4a, b). Allotetraploid flowers (Fig. 4c) were white. The anther morphology of allotriploids was tomato-like (Fig. 4d).

Surprisingly, there was no obvious link between the morphology of the plant and flower and the number of chromosomes or the absence of satellite chromosomes of these plants. Vigorously growing plants, which had

Table 2. Number of allotriploid somatic hybrids of diploid tomato and monoploid potato containing 34, 35, 36 or 37 chromosomes. The number of hybrids with 35 chromosomes possessing one or two tomato satellite chromosomes ("sat") are indicated, all other hybrids possessed both satellite chromosomes from tomato

Fusion combination	Number of triploid hybrids with a chromosome number of					
	34	35 (1 sat)	35 (2 sat)	36	37	Total
C31-244 (+) 7322	1	2	3	5	–	11
ALRC × M8-7 (+) 7322	2	1	4	5[a]	2	14

[a] One hybrid formed roots with cells containing 36 chromosomes and roots with cells containing 37 chromosomes

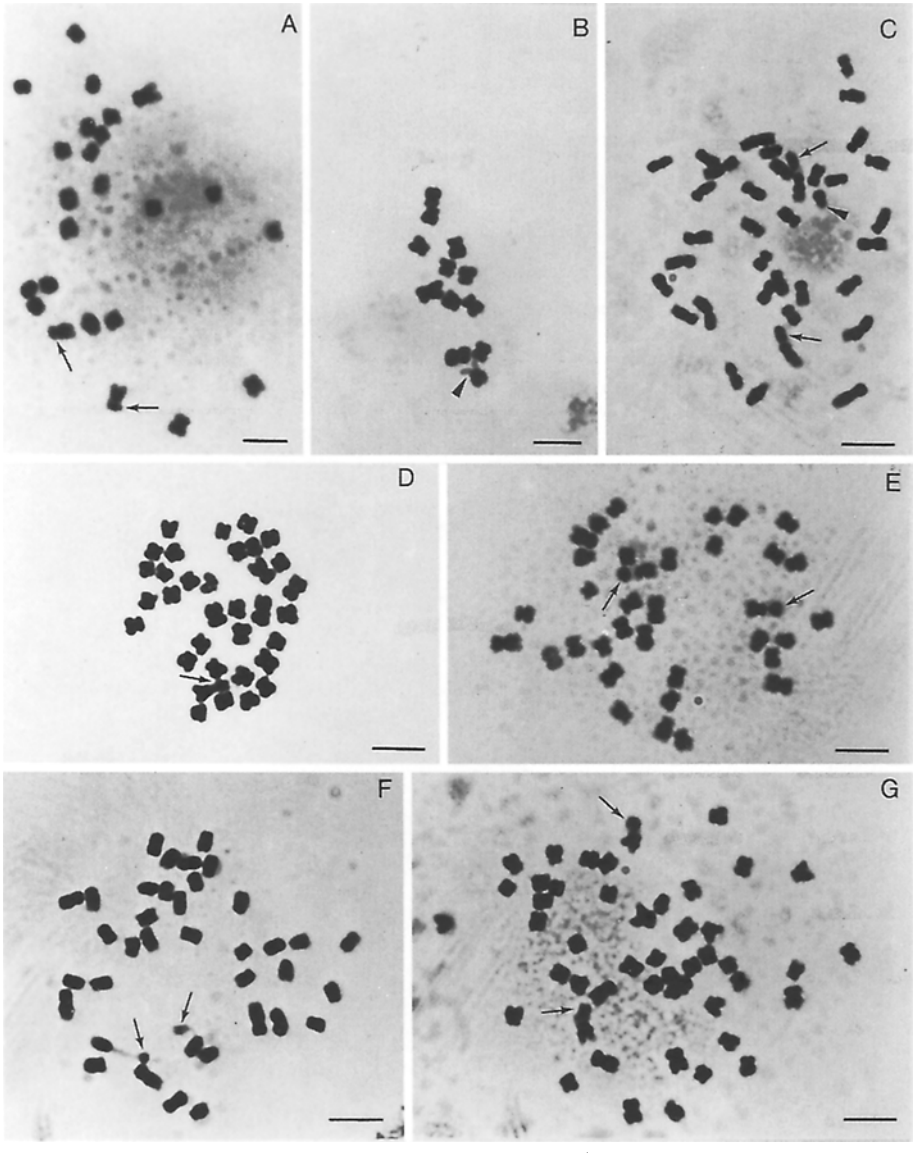

Fig. 3A–G. Metaphase plates of root-tip cells from **A** tomato C31-224 (2n = 2x = 24), **B** potato 7322 (2n = x = 12), **C** allotriploid somatic hybrid C7-133A (2n = 3x = 36), **D** allotriploid C7-149A (2n = 3x = 35, with one tomato satellite chromosome), **E** allotriploid C7-22A (2n = 3x = 35, with two tomato satellite chromosomes), **F** allotriploid A7-74B (2n = 3x = 37), **G** allotetraploid C7-23A (2n = 4x = 48). *Arrows* indicate tomato satellites; *arrowheads* indicate potato satellites. *Bars*: 5 μm

normal appearing leaves and produced many flowers, were found in both euploid and aneuploid populations. On the other hand, several euploid plants remained stunted in their development.

Fertility

Only 6 of the 22 allotriploid somatic hybrids (C7-22A, C7-167A, A7-58A, A7-74B, A7-82A, B, C, D A7-146A, B, C, D) produced full-grown flowers with calyx (sepals), corona (petals), pistils and stamen. The pollen viability of these hybrids, as tested with FDA staining, was low (approximately 5%) for A7-82A (2n = 36), A7-82B (2n = 35) and A7-146D (2n = 34) and less than 1% for C7-22A (2n = 35), C7-167A (2n = 35), A7-58A (2n = 36) and A7-74B (2n = 36/37). Pollen germination on artificial medium was rarely observed (less than 0.1%; control tomato 45%) as was pollen-tube growth in the styles of tomato and allotriploid somatic hybrids. Therefore, allotriploids were mainly used as the staminate parent in crosses with tomato, *Lycopersicon pennellii* and potato. These crosses did not yield progeny. Tomato and potato pollen-tube growth was inhibited in the style (Fig. 5a). While *L. pennellii* pollen

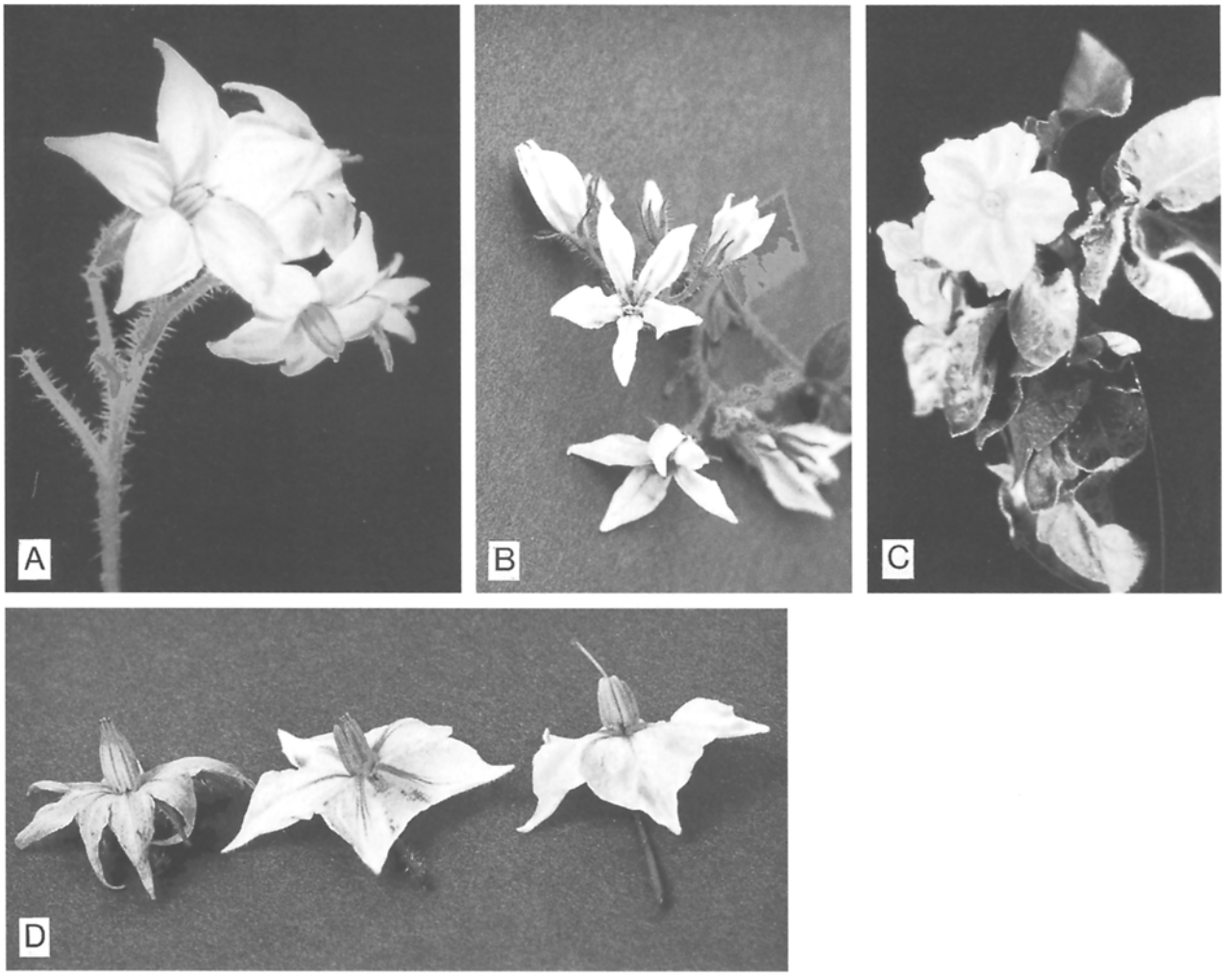

Fig. 4A–D. Flowers of **A** allotriploid somatic hybrid G7-82A, **B** allotriploid somatic hybrid A7-146D, **C** allotetraploid somatic hybrid G7-101B, **D** (from *left to right*) flower morphology of tomato, allotriploid G7-82A and diploid potato: tomato and allotriploid anthers have sterile tips

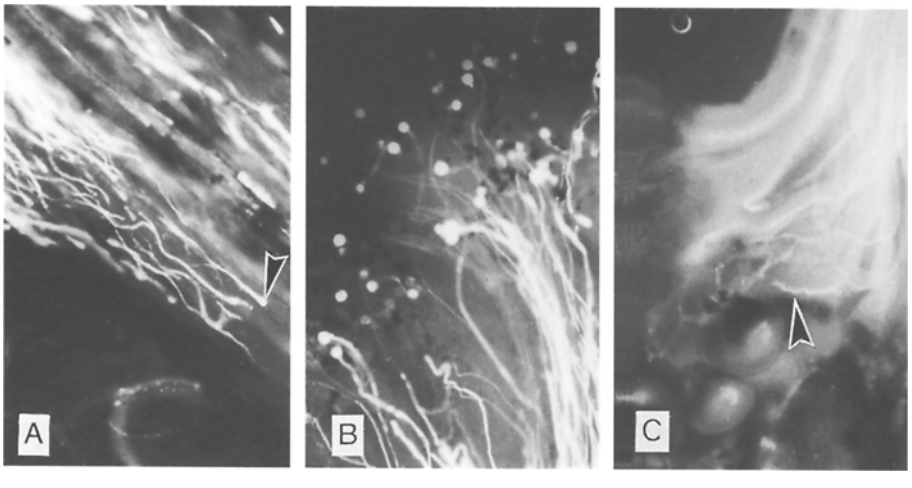

Fig. 5A–C. A Tomato pollen-tube growth in the pistil of A7-82A (2n = 36). Tube growth was inhibited in the style, where callose was formed at the top (◀). **B, C** *Lycopersicon pennellii* pollen-tube growth in the pistil of A7-82A. Tube growth was inhibited near the ovules, where callose was formed (◀) and where tubes sometimes spirulated

tubes grew further into the styles of the hybrids, tube growth was arrested near the ovules (Fig. 5b, c). Allotriploids A7-74B and A7-82A spontaneously produced berries with some immature seeds. Embryos from these seeds, cultured on HLH medium (Neal and Topolenski 1983), did not develop further.

Discussion

Altogether, 25 allotriploids were selected from 450 somatic hybrids. From flow cytometric histograms of parental protoplast populations it was expected that a large fraction of both somatic hybrid populations would be allotriploid. However, allotriploids were rarely found, while allotetraploids were present at a high frequency. Several explanations are possible for this: (1) monoploid potato protoplasts did not take part in the fusion to the same extent as diploid potato protoplasts; (2) the fusion treatment yielded many multiple fusions (this also explains the presence of allopentaploids); (3) polyploidization of allotriploid cells upon fusion resulted in hexaploid somatic hybrids (these were also found at a high frequency); and (4) allotriploid fusion products displayed a low fitness during cell and tissue culture as compared with, for example, tetraploid hybrids. This lower fitness might have been caused by, for example, the difficult combination of potato chloroplasts with a prevailing tomato genome (Wolters et al. 1991).

As compared to asymmetric somatic hybrids of tomato and potato that have been produced with the aid of gamma irradiation of potato protoplasts prior to fusion (H. C. H. Schoenmakers et al. 1993), these fusion experiments demonstrated a far better and quicker regeneration of phenotypically normal-appearing somatic hybrid plants.

Only 6 of the 25 allotriploids produced full-grown flowers, but vital pollen was seldom found. Apart from aneuploidy of some of the allotriploids, the arrest of floral development and the male sterility of flower-producing allotriploids can partly be explained by assuming recombination between mitochondrial DNAs of tomato and potato in the somatic hybrids. Recombination between parental mitochondrial DNAs in somatic hybrids, which has been described for several related combinations (Derks et al. 1991; Kofer et al. 1991), is suggested as the cause of cytoplasmic male sterility. Kofer et al. (1991) further postulated that nuclear-mitochondrial interaction occurs at several stage in tobacco floral development and that the expression of several mitochondrial genes is essential for normal stamen and corolla development.

Male (and also female) sterility of allotriploid somatic hybrids might further be influenced by homoeologue pairing between tomato and potato chromosomes and the formation of trivalents during meiosis. Lee and Power (1988) reported the complete sterility of, presumably trivalents-forming, autotriploid somatic hybrids and the complete fertility of allotriploid somatic hybrids in *Petunia*. Rick et al. (1986) described a very low fertility of tomato autotriploids and a higher fertility of allotriploids that contained two tomato genomes and one *S. lycopersicoides* genome. In these latter hybrids the tomato chromosomes exhibited strong preferential pairing, whereas the *S. lycopersicoides* chromosomes remained unpaired and got lost during meiosis. Since *S. tuberosum* is more distantly related to tomato than *S. lycopersicoides* is, we expect that homoeologue pairing is very limited and that, as a consequence, a fair number of gametes with a balanced tomato genome are formed. The observed homoeologue pairing in some hypotetraploid somatic hybrids of *L. esculentum* (+) *S. tuberosum* described by de Jong et al. (1993) must also be considered as incidental. Homeologue crossingover would offer further possibilities for introgression of potato traits in tomato. We assume that only gametes either without or with only a small number of potato chromosomes are sufficiently balanced genetically to be able to take part in crosses.

Apart from the observed male and possible female sterility, hybridization might also be hampered by a genetic incongruity between the allotriploids and its crossing partners. (Unilateral) incongruity in *Lycopersicon* and *Solanum* species, expressed at the level of pollen-tube growth and zygotic abortion, has been described by Chetelat and Deverna (1991), Chetelat et al. (1989), DeVerna et al. (1987), Gadish and Zamir (1986), Jacobsen et al. (1992), de Nettancourt et al. (1974), Rick (1963) and Smith and Desborough (1986). The suppressed pollen germination, the arrested pollen-tube growth and the observed embryo abortion indicate incongruity between the allotriploids and tomato and potato. Possibly, crosses with *L. pennellii*, followed by embryo rescue, will ultimately give progeny.

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