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MEIOSIS IN DIFFERENT F_1-HYBRIDS OF *SOLANUM ACAULE* BITT. × *S. BULBOCASTANUM* DUN. AND ITS BEARING ON GENOME RELATIONSHIP, FERTILITY AND BREEDING BEHAVIOUR

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SUMMARY

Meiosis was studied in some triploid, tetraploid and hexaploid F_1-hybrids from *Solanum acaule* × *S. bulbocastanum* and in a triploid F_1-hybrid from *S. acaule* × *S. tuberosum*-haploid.

The only anomaly found was stickiness at metaphase I, the degree of which appeared to be related to the proportion of the *S. bulbocastanum* chromosomes in the hybrids. No stickiness occurred at pre- and post-metaphase stages.

A clear allosyndetic pairing between chromosomes of the two *S. acaule* genomes was observed in all triploid and tetraploid hybrids. These genomes apparently are closely related and little differentiated. The triploids *S. acaule* × *S. bulbocastanum* had 0–1 trivalent per cell, whereas 2–5 trivalents per cell were observed in the triploid *S. acaule* × *S. tuberosum*-haploid. Therefore *S. acaule* is more closely related to *S. tuberosum* L. than to *S. bulbocastanum*. A small amount of pairing between *S. acaule* and *S. bulbocastanum* is apparent from the occurrence of multivalents in all hybrids.

Hexaploid F_1's ($2n = 72$) showed a nearly complete homologous pairing of chromosomes (35.2 bivalents per cell) and generally a normal separation of chromosomes at anaphase: 36–36. This offers an explanation for their high fertility. Triploid F_1's from *S. acaule* × *S. bulbocastanum* have a high frequency of univalents leading to irregular separation of chromosomes at anaphase and consequently to unbalanced gametes and extreme sterility. In the tetraploid F_1's ($2n = 48$) nearly complete bivalent pairing was observed, 50% expectedly being homologous and 50% homoeologous pairing. Separation of chromosomes at anaphase was generally normal 24–24. In spite of this normal behaviour and allowing for tight stickiness at metaphase the tetraploids are very sterile. A satisfactory explanation cannot yet be given.

Selfing and intercrossing hexaploid F_1's gives normal berry set and many seeds per berry. However crosses with *S. tuberosum* and even those with the fertile hexaploid hybrid from 8x–*S. acaule* × 4x–*S. tuberosum* are little successful: berry set is far below normal and the berries are either parthenocarpic or contain only one or two seeds. These rather unexpected results warrant further investigation. Large-scale selfings and intercrosses of triploid and tetraploid hybrids have not been successful as yet. Among the female gametes of tetraploid hybrids a few appeared to be functional in crosses with hexaploid hybrids and in those with *S. bulbocastanum*.

INTRODUCTION

Genes, which are of great value for breeding potato, *Solanum tuberosum*, have accumulated in many wild diploid Mexican *Solanum* species. Immunity from *Phytophthora infestans* is of particular importance. It is present in *S. bulbocastanum* to a very high degree as was recognized even before the thirties (REDDICK, 1930). This valuable character should be introduced into *S. tuberosum* in order to raise the level of polygenic resistance to late blight. Attempts by several researchers to cross *S. bulbocastanum* with *S. tuberosum* (LIVERMORE and JOHNSTONE, 1940, GRAHAM et al., 1959) failed.

DIONNE's discovery (1963) of the crossability of *S. bulbocastanum*, and also *S. pinnatisectum*, with the South-American tetraploid species *S. acaule* was a step forward, because, as DIONNE pointed out, *S. acaule* might serve as a bridge between *S. bulbocastanum* and *S. tuberosum* owing to its crossability with both species. Recently LEBEDEVA (1966) reported successful crosses of 4x-*S. bulbocastanum* with 4x-*S. vernei*, complex polyploid hybrid species of the series *Acaulia* and complex hybrid seedlings of *S. tuberosum* respectively. TOXOPEUS and VERDENIUS (personal communications) in 1963 and 1964 made 538 cross combinations between 20 introductions of *S. acaule* and 28 clones of *S. bulbocastanum* and obtained 150 different F_1-hybrids. HERMSEN (1966) studied 72 of these F_1-clones for their morphology, crossability, fertility and chromosome numbers. The accessions of both parent species could be divided into distinct groups on the basis of the crossability of the species. The average berry set was 11 % (range 0–24 %), the average number of seeds per berry 0.7 (range 0–4). Most F_1's (59) were triploid. Some however were tetraploid (13 F_1's) and clearly more *bulbocastanum*-like as the triploid F_1's. Therefore it was assumed that the tetraploids originated from fertilization of *S. acaule* by 'unreduced' male gametes from *S. bulbocastanum*. Both the triploids (pollen stainability less than 5 %) and the tetraploids (stainable pollen about 12 %) were highly sterile. Doubling the chromosome number of triploid F_1's by colchicine resulted in very fertile hexaploids (85 % stainable pollen), as was already found by DIONNE (1963). However doubled tetraploid F_1's ($2n = 96$) remained sterile although pollen stainability increased from 12 % to 29 %. HERMSEN (1966) ascribed these different stainability percentages of the 3x-, 4x-, 6x- and 8x-F_1's to their different genomic constitution, assuming alloploidy for *S. acaule* and no pairing between *S. bulbocastanum*- and *S. acaule*-chromosomes. On the same basis HERMSEN expected meiosis to be regular in the hexaploids, but increasingly irregular in octoploids, tetraploids and triploids respectively.

This hypothesis had to be tested by studying meiosis in a number of F_1's with different levels of ploidy. This cytological study was carried out by the junior author in 3x-, 4x- and 6x-plants of *S. acaule* × *S. bulbocastanum* and for comparison, in a triploid F_1-plant from *S. acaule* × *S. tuberosum*-haploid. The results – some of them were unexpected – will be reported in this article and discussed in relation with breeding for late blight resistance in potato.

MATERIAL AND METHODS

The F_1's investigated are presented in Table 1.

Table 1. Survey of the material used in this study.

Cross	Ploidy of F_1 (x = 12)	Code number
S. acaule CPC 2440 × *S. bulbocastanum* H 1938	3x	AB 64-532-2
S. acaule CPC 3498 × *S. bulbocastanum* H 1588-23	3x	AB 64-574-1
S. acaule CPC 3502-2 × *S. bulbocastanum* H 1594-10	3x	AB 65-16-2
S. acaule CPC 2525-3 × *S. bulbocastanum* H 1594-10	4x	AB 65-27-1
S. acaule CPC 2525-8 × *S. bulbocastanum* H 1594-10	4x	AB 65-30-1
S. acaule CPC 2525 × *S. bulbocastanum* H 1595-9	6x	AB 64-543-1
S. acaule H 82-3 × *S. tuberosum*-haploid (US-W 42)	3x	–

Meiosis was studied in young anthers during microsporogenesis, using the simple propiono-carmine method as described by SWAMINATHAN et al. (1954). All observations and photomicrographs were made using a Carl Zeiss microscope with phase contrast equipment.

RESULTS

Diakinesis and metaphase I

An unusual feature of meiosis is observed in all *S. acaule* × *S. bulbocastanum* hybrids, but does not show up in the hybrid *S. acaule* × *S. tuberosum*-haploid. It is the tendency of the chromosomes to clump together during metaphase I (Fig. 1) whereas pre- and post-metaphase stages are relatively normal and can clearly be analyzed. At pachytene and diakinesis only one nucleolus is observed in each pollen mother cell of both triploid, tetraploid and hexaploid F_1's. In triploids one bivalent +

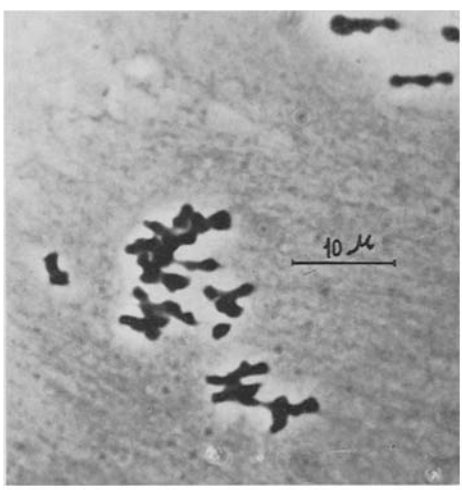

Fig. 1. Metaphase I of tetraploid F_1 showing stickiness.

Table 2. Chromosome associations in triploid, tetraploid and hexaploid F_1's from *S. acaule* × *S. bulbocastanum* and in triploid *S. acaule* × *S. tuberosum*-haploid.

Cross	Ploidy of F_1	Number of cells analyzed	Chromosome associations			
			IV	III	II	I
AB 64-532-2	3x	23	0	0.39	12.30	9.91
AB 64-574-1	3x	25	0	0.44	12.40	10.20
AB 65-16-2	3x	62	0	0.48	11.50	11.28
S. acaule × *S. tuberosum*-haploid (US-W42)	3x	82	0	3.25	8.81	8.58
AB 65-27-1	4x	56	0.11	0.16	23.28	0.44
AB 65-30-1	4x	48	0.13	0.06	22.91	0.31
AB 64-543-1	6x	57	0.19	0.14	35.19	0.56

one univalent, in tetraploids two bivalents and in hexaploids three bivalents are generally attached to the nucleolus. In all F_1's an analysis of different chromosome associations can readily be carried out at diakinesis. In triploids a considerable number of metaphases could be analyzed by examining a large number of preparations.

However in tetraploids it was impossible to obtain a single well-spread metaphase I, although more than 65 preparations were studied. Obtaining good metaphases in hexaploids is not so difficult as in tetraploids. Both in tetraploid and hexaploid F_1's only diakinesis stages were analyzed.

Stickiness does not occur in the triploid F_1 *S. acaule* × *S. tuberosum*-haploid at all. Therefore its chromosome associations were all studied at metaphase I.

The frequency of multivalents, bivalents and univalents in each of the F_1's is presented in Table 2 which shows:

1. A very low frequency of trivalents and a high frequency of bivalents and univalents in the triploid *S. acaule* × *S. bulbocastanum* hybrids. Slight differences were found and may be ascribed to the different numbers of cells that could be scored in each triploid. It is noteworthy that the maximum number of trivalents per cell is only one.

2. A relatively high frequency of bivalent and trivalent associations in *S. acaule* × *S. tuberosum*-haploid as compared with *S. acaule* × *S. bulbocastanum*. The number of trivalents per cell may vary from 2 to 5 per cell.

3. Practically all chromosomes in tetraploid F_1's at diakinesis pairing as bivalents, multivalent associations being rarely found. Not a single cell was found to contain more than one quadrivalent.

4. The situation in hexaploid F_1's is similar to that in tetraploids.

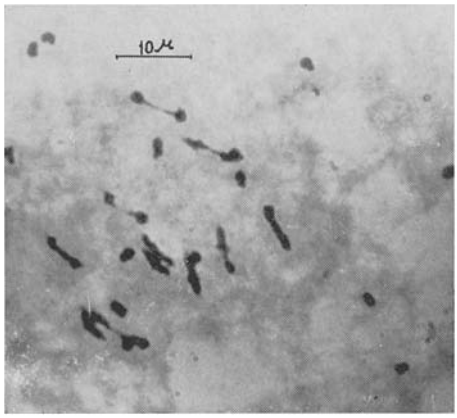

Fig. 2. Metaphase I of triploid F_1 of *S. acaule* × *S. bulbocastanum* showing 11 univalents, 11 bivalents and a trivalent.

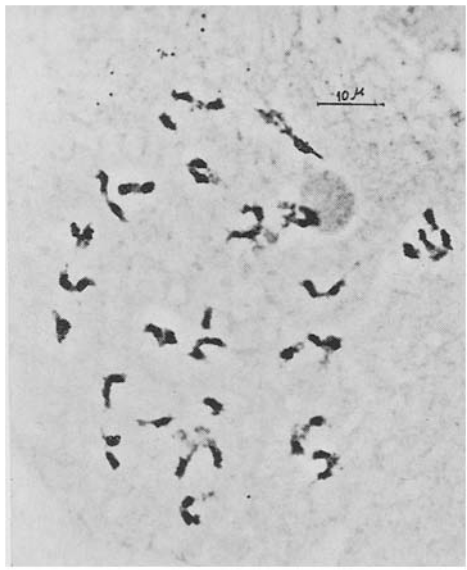

Fig. 3. Diakinesis of tetraploid F_1 showing 4 univalents and 22 bivalents.

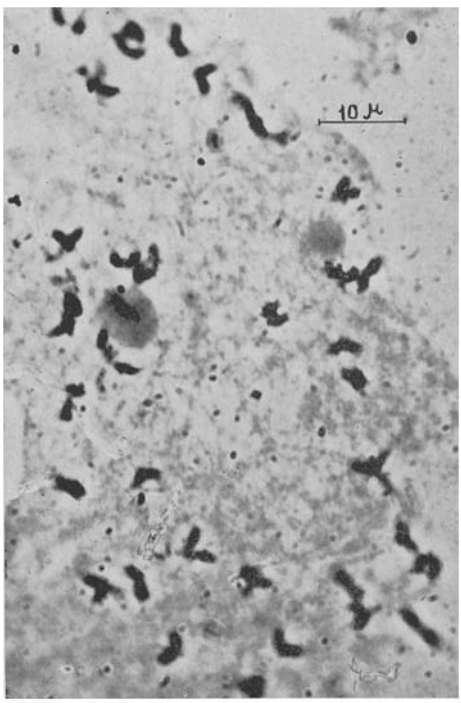

Fig. 4. Diakinesis of hexaploid F_1 showing 36 bivalents.

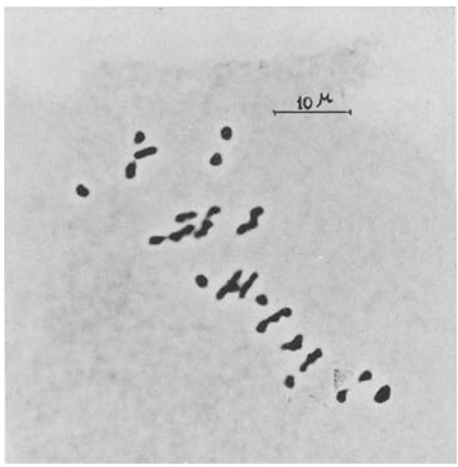

Fig. 5. Metaphase I of triploid F_1 of *S. acaule* × *S. tuberosum*-haploid showing 6 univalents, 9 bivalents and 4 trivalents.

Anaphase I

Anaphase separation of chromosomes is rather irregular in all triploid F_1's owing to a large number of univalents.

In many cells the univalents lag and divide precociously. Therefore in most cases the frequencies of different types of separation cannot be determined precisely. The frequencies of the types mentioned in Table 3 were established in cells with a clear-cut separation to two poles.

Table 3. Anaphase separation in triploid plants from *S. acaule* × *S. bulbocastanum*.

	Anaphase separation						
	12–24	13–23	14–22	15–21	16–20	17–19	18–18
Number of cells	0	2	7	16	18	26	23

A normal separation of chromosomes at anaphase I was generally observed both in tetraploid F_1's (24–24) and in hexaploid F_1's (36–36).

Only the pollen from hexaploid F_1's is normally viable, whereas the triploids and also the tetraploids produce highly sterile pollen.

DISCUSSION

a. Genomic relationship of S. acaule, S. bulbocastanum and S. tuberosum

The triploid F_1's *S. acaule* × *S. bulbocastanum* show a very low frequency of trivalents and a high frequency of bivalents and univalents (table 2). This finding might be explained in two ways:

1. The *S. acaule* genomes, indicated as A_2 and A_3 by HAWKES (1958), pair allosyndetically in the hybrid, leaving the chromosomes of *S. bulbocastanum* unpaired.
2. The *S. bulbocastanum* genome pairs with either A_2 or A_3. If this happens to be true, the univalents would belong to the unpaired *S. acaule* genome.

The first explanation is more obvious, since it is known from previous studies, that the two genomes of *S. acaule* pair to a high degree if present in haploid condition. Therefore these genomes have not differentiated or if so, only to a small extent (PROPACH, 1937; BAINS, 1951; SWAMINATHAN, 1954; MATSUBAYASHI, 1961).

Further evidence for this hypothesis may be derived from the meiotic pairing in tetraploid F_1's, which contain two probably identical *S. bulbocastanum* genomes + the genomes A_2 and A_3 from *S. acaule*. Nearly the maximum number of bivalents is observed, viz. 23.38 and 22.91 respectively. Twelve bivalents must result from homologous pairing between *S. bulbocastanum* chromosomes and the remaining bivalents from homoeologous pairing between the A_2- and A_3-chromosomes of *S. acaule*.

The occasional trivalents and quadrivalents in *S. acaule* × *S. bulbocastanum* suggest a certain amount of pairing and chiasma formation between the genomes of *S. acaule* and *S. bulbocastanum*. However the relatively high frequency of trivalents in *S. acaule* × *S. tuberosum*-haploid indicates a rather close relationship between the genomes of *S. acaule* and *S. tuberosum*.

b. Meiotic behaviour and fertility

The high fertility of hexaploid F_1's ($2n = 72$) is explained by the nearly complete pairing of chromosomes (35.19 bivalents per cell), which obviously takes place between homologous chromosomes. The amount of allosyndetic pairing is rather low, as evidenced by the low frequency of multivalents. The only meiotic anomaly observed in the hexaploid is stickiness at metaphase. Apparently this does not affect the subsequent stages of meiosis, since anaphase is normal (36 chromosomes to each pole) in most cells. Also pollen formation is normal, as expected.

Although in tetraploid F_1's both the relatively high frequency of bivalents (23.28 and 22.21 per cell) and the normal anaphase separation (24 chromosomes to each pole) agree with the situation in the hexaploid F_1, the tetraploids are highly sterile. The only difference with hexaploids (and triploids) is a much more pronounced stickiness at metaphase I which apparently runs parallel with the proportion of *S. bulbocastanum* chromosomes in the hybrids, viz. 50% in the tetraploids against 33% in hexaploids and triploids. The complete absence of stickiness in triploid *S. acaule* × *S. tuberosum*-haploid is in line with this explanation. However a satisfactory explanation for the high sterility in the tetraploids cannot yet be given.

The extremely low fertility of the triploid hybrids can be understood from the presence of many univalents, leading to irregular separation of chromosomes to the poles and consequently to unbalanced gametes.

c. The practical significance of pairing between S. acaule and S. bulbocastanum

As pointed out earlier the pairing between chromosomes of *S. acaule* and *S. bulbocastanum* does take place to some extent. Therefore transfer of genes or parts of chromosomes from *S. bulbocastanum* into *S. acaule* may be expected. Since there is a rather good pairing between chromosomes of *S. acaule* and *S. tuberosum*, genes from *S. bulbocastanum* may be introduced into the *tuberosum*-genome via *S. acaule*.

DIONNE (1966) studied backcrosses of *S. acaule* × *S. bulbocastanum* F_1's with *S. tuberosum* varieties. He found that after several backcrosses the hybrids still show an allopolyploid breeding behaviour. His conclusion is that characters from *S. bulbocastanum* must have been transferred into *S. tuberosum* via whole chromosomes or large chromosome fragments, carrying genes controlling chromosome pairing. Then the way from *S. bulbocastanum* to a commercial variety would be very long, many backcrosses to varieties being required. DIONNE and HODGSON (1966) have now obtained fertile, late blight resistant genotypes, but point out that these genotypes need further improvement by continued backcrossing with varieties.

d. Crossability of hexaploid S. acaule × *S. bulbocastanum*

Hexaploid F_1's of *S. acaule* × *S. bulbocastanum* can readily be selfed and intercrossed. Berry set and number of seeds per berry are such, that both male and female gametes must be normally functional. However crosses between these hexaploid hybrids (or their progeny) and *S. tuberosum* are little successful: berry set is far below normal and the berries contain either very few seeds or no seeds at all. Many crosses have to be made in order to get sufficiently large populations for further breeding work.

The use of hexaploid F_1's of 8x-*S. acaule* × 4x-*S. tuberosum* instead of pure *S.*

tuberosum, increases the success of crosses with 6x-*S. acaule* × *S. bulbocastanum* only to a very small extent, although both parents in these crosses are highly fertile. Cytological investigations in this type of hybrids appear to be desirable in order to clarify this problem. A first indication of irregular cytological behaviour was obtained by the observation, that the somatic chromosome number of one hybrid plant from 6x-(*S. acaule* × *S. bulbocastanum*) × 6x-(*S. acaule* × *S. tuberosum*) proved to be 66 instead of the expected number 72.

e. Crossability of triploid and tetraploid S. acaule × *S. bulbocastanum*

Large-scale selfings and intercrosses of triploid and tetraploid hybrids have not been successful yet. Crosses of triploids with other species (parents included) never produced a single berry. Crossing tetraploids (30 flowers) as a female with hexaploids gave ten small berries, which together contained only one seed. This seed did not germinate. Pollinating 82 flowers of tetraploids (♀) with a pollen mixture of *S. bulbocastanum* resulted in eight small berries with a total number of two seeds, of which one germinated. This backcross-plant had 36 chromosomes and was very much like *S. bulbocastanum*. Some female gametes of tetraploids appear to be functional.

f. Concluding remarks

S. bulbocastanum, although highly valuable for potato breeding, is a very difficult species in crossing. Its treasures are hardly accessible. The transfer of polygenically determined characters from this species to *S. tuberosum* need not be considered impossible. However the many backcrosses required to nobilize the hybrid material may render it impossible to keep the desired characters at the level, at which they are present in the original donor-species, i.e. *S. bulbocastanum*. Nevertheless it is worthwhile trying to exploit the species, because its genes for desired polygenic characters may be quite different from those in other species and therefore may finally raise the level of these characters in our varieties.

The necessity to use *S. acaule* as a bridge to *S. tuberosum* presents the opportunity to choose those *acaule*-genotypes which possess genes for immunity from virus X, frost resistance and resistance to *Heterodera rostochiensis*.

The basic studies reported in this and a former publication (HERMSEN, 1966) will be continued in order to solve fundamental problems, to facilitate the choice of starting material and to create genotypes, which in breeding programmes could serve as intermediary between the wild species and our potato varieties.

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