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Potato germ plasm enhancement with disomic tetraploid *Solanum acaule*. II. Assessment of breeding value of tetraploid F_1 hybrids between tetrasomic tetraploid *S. tuberosum* and *S. acaule*

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Abstract The breeding value of tetraploid F_1 hybrids between tetrasomic tetraploid *S. tuberosum* and the disomic tetraploid wild species *S. acaule* was examined. The F_1 hybrids showed a tuber yield and appearance comparable to those of their cultivated parent, indicating a potential as acceptable breeding stocks despite the 50% contribution to their pedigree from wild *S. acaule*. The cytological behavior of the tetraploid F_1 hybrids was examined to determine the probability of recombination for the introgression of *S. acaule* genes. The majority of the meiotic configurations at metaphase I was bivalents and univalents with mean frequencies of 17.6 and 9.9, respectively. Further, a low frequency of trivalents and quadrivalents was observed. An acceptable low level of meiotic irregularities were observed at the later stages of microsporogenesis, and a reasonable level of pollen stainability was obtained. Therefore, these hybrids could likely be employed for further introgression. From the cytological observations, the following speculations were drawn: (1) some genomic differentiation exists between the *S. acaule* genomes, (2) at least one of the *S. acaule* genomes may be homoeologous to the *S. tuberosum* genomes, (3) intergenomic recombination would likely occur due to the nature of the genomic constitution of the hybrids, and (4) the nature of sesquiploidy of the hybrids may facilitate efficient introgression and establishment of unique aneuploid and euploid recombinant genetic stocks.

Key words Disomic tetraploid · Tetrasomic tetraploid · Introgresion · Tuber-bearing *Solanum* · Germ plasm enhancement

Introduction

There are nearly 200 wild species in tuber-bearing *Solanum* with a ploidy series ranging from 2x to 6x. High levels of resistance to diseases and pests have been identified in many accessions of these species (Ross 1986), and some of these have been used in potato breeding. It has been mainly the diploid wild species that have been used since germ plasm enhancement at the diploid level is easier than at the tetraploid level due to simple diploid inheritance rather (Chase 1963; Peloquin et al. 1989). Some reproductive characters such as haploidization from tetraploid cultivars and 2n gamete formation in diploids also facilitate the utilization of these diploid wild species (Peloquin et al. 1989).

Some tetraploid wild species such as *Solanum acaule* and *S. stoloniferum* have also been used in breeding, as they have extremely high levels of virus resistance and other attributes that do not exist in the cultivated potato gene pool (Cockerham 1970; Ross 1986). While these species could not be crossed with tetraploid potatoes, they could be crossed with diploid potatoes due to the existence of a genetic mechanism called Endosperm Balance Number (EBN) (Ehlenfeldt and Hanneman 1988; Johnston et al. 1980). However, the efficiency of the genetic enhancement of cultivated potato with these tetraploid species was not initially appreciated, and it has taken decades to incorporate disease resistance from

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these wild species into potato cultivars (reviewed in Iwanaga et al. 1991; Ross 1986; Watanabe et al. 1992a).

The cytogenetic behavior of the above-mentioned tetraploid wild species is different from that of cultivated tetraploid potatoes: the former are disomic tetraploids (or allo-tetraploids) and regularly form 24 bivalents during meiosis. Potato cultivars, on the other hand, are tetrasomic tetraploids that can form several quardrivalents during meiosis (reviews in Dvořák 1983; Howard 1970; Matsubayashi 1991; Swaminathan 1954).

The mechanism of disomic behavior in the tetraploid species is not fully understood. Dvořák (1983) proposed that the exclusive disomic behavior of these tetraploid species may be due to a genetic control of chromosome pairing between heterogenetic chromosomes that are similar to each other in some degree rather than to highly differentiated genomes.

Matsubayashi (1991) reviewed the meiotic behavior of tetraploid hybrids and their hexaploid derivatives from crosses between disomic tetraploid species, *S. acaule*, and the diploid wild species that are closely related to tetraploid cultivated potatoes. He developed a hypothesis for the origin of *S. acaule* and of its genomic constitution through a series of investigations of chromosome pairing in such hybrids. His proposal was that the disomic tetraploid species have partially homoeologous genomes that pair preferentially, resulting in disomic behavior. He designated *S. acaule* as a segmental allo-tetraploid.

Tetraploid *S. acaule* has reproductive/genetic characters such as 2 EBN and disomic behavior that differentiate it from tetraploid cultivated potatoes with 4 EBN and tetrasomic behavior (Watanabe and Peloquin 1991). Intercrosses between *S. acaule* and cultivated tetraploid potatoes are very difficult due to differences in EBN (Johnston et al. 1980). Thus, it has been necessary to carry out complicated ploidy manipulations to overcome the crossing barriers and to introgress useful genes from disomic tetraploid wild species into cultivated tetraploid potatoes (reviews in Brown and Adiwilaga 1990; Iwanaga et al. 1991; Watanabe et al. 1992a). It has taken a long time and tedious manipulations by conventional methods to introgress even simply inherited and easily scored traits such as resistances to potato virus X and Y (Cockerham 1970; Ross 1986).

Direct F_1 hybrids between tetrasomic tetraploid potatoes and disomic tetraploid *S. acaule* have recently been obtained by an innovative approach (Iwanaga et al. 1991). This method consists of combining a second compatible pollination and embryo rescue, and provides a new opportunity to utilize the gene pool of disomic tetraploid species more efficiently for potato breeding (Watanabe et al. 1991, 1992a). The breeding value of these direct tetraploid F_1 hybrids is substantial since high levels of resistances to diseases and pests which do not exist in the present cultivated potatoes are made available. This paper elucidates the potential of the F_1 hybrids between cultivated potatoes and *S. acaule* in breeding and the evolution of *S. acaule*.

Materials and methods

Six tetraploid potato genotypes were used in this study (Table 1). 'Atzimba', 'LT-7', and '7XY.1' have been widely used in breeding programs worldwide and by the International Potato Center (CIP) as both breeding stocks and varieties. These genotypes are widely adapted to growing in subtropical and tropical regions in developing countries. AA-1, AA-2 and AA-3 are tetraploid F_1 hybrids between *S. tuberosum* and *S. acaule*. They were obtained at CIP from the combination of a second compatible pollination and embryo rescue, and their maternal parent is '7XY.1' (Iwanaga et al. 1991).

A completely randomized block design with three blocks was used for a preliminary comparison of yield components. Ten tubers or tuber pieces of commercial planting size were planted for each replication at the end of September, 1990, at Lima, Peru. The plants were harvested at the beginning of January, 1991. The growing environments are similar to those of temperate zones, with a day-length of 12–14 h during the planting season. The growing period was 100 days. Tubers from each hill were classified prior to analysis, and only tubers of marketable size were used. The replication mean over 10 plants were used for the analysis of variance for tuber yield.

Flower bud samples were obtained from the greenhouse-grown plants. It should be noted that under these conditions all of control plants (2x and 4x cultivated potatoes) showed normal meiosis and pollen fertility. Samples were fixed in 100% ethanol:glacial acetic acid (3:1) for 48 h at room temparature and stored in 70% ethanol at 4 °C until used. Observations on microsporogenesis were made using a standard acetocarmine squash technique. Pollen stainability with acetocarmine glycerol jelly (Marks 1954) was used for estimating pollen viability and abnormality.

Results and discussion

Performance of F_1 hybrids

The appearance of the tubers of the three F_1 hybrids was comparable to those of the three potato lines. A representative comparison is shown in Fig. 1. Tubers from these hybrids had acceptable tuber shape, eye depth, and color.

Significant differences in the tuber yield of single plants were observed among the six genotypes by an analysis of variance (Table 1). Two F_1 hybrids, AA-2 and AA-3, had the same level of tuber yield as their maternal potato breeding line, '7XY.1', while the two other potato varieties outyielded these two F_1 hybrids. This is not unexpected since the F_1 hybrids have 50% of their genomic content from *S. acaule*, which is a wild

Table 1 Pedigree and tuber yield of three tetraploid potato breeding stocks and three *S. tuberosum* × *S. acaule* tetraploid F_1 hybrids

Genotype	Pedigree	Yield per plant (G)
Atzimba	US133.3 × 52AT1	1959.2 a[a]
LT-7	CIP 378017.2	1953.6 a
7XY.1	N-550.420P	1292.8 b
AA-3	7XY.1 × 954.3CA (*S. acaule*)	1245.2 b
AA-2	7XY.1 × 954.3CA (*S. acaule*)	1016.8 b
AA-1	7XY.1 × 954.3CA (*S. acaule*)	495.9 c
c.v. (%) = 12.7		

[a] Duncan's multiple range test; significant at the 5% level $F_{(\text{genotype},df\,5,10)} = 33.486$**; significant at the 1% level $F_{(\text{replication},df\,2,10)} = 9.385$**; significant at the 1% level

species and highly adapted to cool highland conditions, while the two potato varieties were bred for adaptation to a range of environments. AA-1, a full sib of AA-2 and AA-3 showed a significantly lower single-plant tuber yield. This would be due to segregation of female gametes in the *S. tuberosum* parent, '7XY.1', which is highly heterozygous in contrast to highly homozygous *S. acaule*. It should be noted, however, that the tubers of the F_1 hybrids were similar in appearence to those of the cultivated potato (Fig. 1).

The F_1 hybrid AA-3 carries resistance to potato virus X, potato virus Y, and potato tuber spindle viroid (Iwanaga et al. 1991). It has been reported to possess a higher level of resistance to potato leaf roll virus, with this higher level of resistance being due to transgressive segregation (CIP 1992). Culinary characters of the tubers such as tuber flesh color and texture were acceptable to local consumers (unpublished data). In view of this preliminary information on yield and tuber characteristics and disease resistances, it may be possible to select potential cultivars directly from F_1 hybrids between *S. tuberosum* and disomic tetraploid wild species, although the stability of yield performance has not yet been confirmed by repeated field trials. Commercial use of the F_1 hybrids should do away with the necessity of time-consuming evaluation of introgression lines in order to identify those with a desirable confirmation of characters.

Meiotic behavior of F_1 hybrids, genome differentiation, and their potential for generating genetic stocks and breeding lines

Meiotic behavior of F_1 hybrids

The results on the meiotic behavior of the F_1 hybrids are presented in Tables 2 and 3. Only AA-2 and AA-3 were employed for cytological observations as AA-1 did not form enough flower buds. Both AA-2 and AA-3 had a high frequency of bivalents at metaphase I (Table 2), but a low frequency of multivalents was also observed. A vast majority of the bivalents observed in the hybrids were rod shaped. Since AA-2 and AA-3 had similar levels of chromosome pairing (Table 2), their data were combined. Data on the occurrence of irregularities during anaphase I and anaphase II are presented in Table 3. AA-2 and AA-3 had similar frequencies of abnormalities at anaphase I, anaphase II, the sporad stage and pollen grain development. As reported by Swaminathan and Magoon (1961), *S. tuberosum* cultivars form a high frequency of bivalents and some quadrivalents during meiosis. The frequency of bivalents and quadrivalents in the hybrids as shown in Table 2 was different from that observed in the potato parent '7XY.1'.

Table 2 Average chromosomal configuration at metaphase I in microsporocytes of two tetraploid hybrids between tetrasomic tetraploid cultivated potatoes and disomic tetraploid *S. acaule*

Hybrid		Chromosomal configuration at metaphase I of meiosis IV	 III	 II	 I
AA-2	Mean frequency	0.7	0.4	17.1	10.0
	SD	(0.76)	(0.64)	(2.54)	(4.55)
	Range	0–2	0–2	12–23	2–16
	n = 44[a]				
AA-3	Mean frequency	0.4	0.3	17.8	9.8
	SD	(0.69)	(0.50)	(2.39)	(3.85)
	Range	0–2	0–2	12–23	2–24
	n = 79				
Combined[b]					
	Mean frequency	0.5	0.3	17.6	9.9
	SD	(0.73)	(0.56)	(2.47)	(4.12)
	Range	0–2	0–2	12–23	2–24
	n = 123				
7XY.1 (*S. tuberosum*)					
	Mean frequency	5.3	3.1	11.5	3.6
	SD	(1.21)	(1.08)	(2.63)	(1.45)
	Range	3–8	1–5	8–14	2–6
	n = 98				

[a] *n*, Number of meiocytes observed

[b] $r_{(AA-2/AA-3\ \text{meiotic configuration},\ df=2)} = 0.999^{**}$; significant at the 1% level

Tetraploid *S. tuberosum* is regarded as being an autotetraploid with four homologous genomes. This classification is based upon decades of genetic and

Fig. 1 Comparison of representative tuber appearance among a cultivated tetrasomic tetraploid potato '7XY.1', wild disomic tetraploid *S. acaule*, and their tetraploid F_1 hybrid AA-3

Table 3 Meiocytes with abnormalities observed at anaphase I and anaphase II, and abnormal sporads and pollen grains in two tetraploid F_1 hybrids between tetrasomic tetraploid *S. tuberosum* and disomic tetraploid *S. acaule*

	Number observed			Frequencies of abnormality (%)		
Stage	AA-2	AA-3	Combined	AA-2	AA-3	Combined[a]
Anaphase I	300	350	650	32.1	29.8	31.0
Anaphase II	350	241	591	25.4	36.3	31.0
Sporads	678	1521	2199	13.1	17.8	16.4
Pollen grain	2364	2537	4901	85.5	87.9	86.7

[a] $r_{(AA-2/AA-3\ \text{frequency of abnormality},\ df=2)} = 0.985^{*}$; significant at the 5% level

cytological observations: (1) haploids ($2n = 2x = 24$) of tetraploid *S. tuberosum* usually form 12 bivalents (Irikura 1976; Yeh et al. 1964), (2) tetraploid *S. tuberosum* forms up to several quadrivalents (Swaminathan 1954; Swaminathan and Magoon 1961), (3) tetrasomic ratios have been observed for morphological traits (Cadman 1942; Lunden 1960; Peloquin et al. 1989), (4) duplication of isozyme loci (Douches and Quiros 1987), and (5) duplication of restriction fragment length polymorphism (RFLP) loci (Gebhardt et al. 1989). On the basis of the these facts, it was expected that tetraploid F_1 hybrids between tetraploid *S. tuberosum* and disomic tetraploid *S. acaule* would have a high frequency of bivalents.

Matsubayashi (1982, 1991) analyzed meiosis in triploid hybrids between *S. acaule* and diploid wild species that are closely related to cultivated potatoes with homologous genomes. The triploids formed trivalents at an unexpectedly high frequency, and Matsubayashi (1982, 1991) came to the conclusion that one of the *S. acaule* genomes in homoeologous to the genomes of the cultivated potatoes. These results also suggest that some chromosome pairing would occur between *S. tuberosum* and *S. acaule* chromosomes in the tetraploid F_1 hybrids reported here.

The present results support the expectations with a high bivalent frequency of 17.6. Further, haploids from *S. acaule* have an average of 6.7 bivalents and 10.6 univalents (Camadro et al. 1992). This would also back up our observation of a high frequency of bivalents in the present tetraploid F_1 hybrids. On the other hand, a relatively high frequency of univalents (9.9) (Table 2) and high frequencies of meiotic irregularities at microsporogenesis (Table 3) would indicate some genomic imbalance between *S. tuberosum* and *S. acaule*. This could be attributed mainly to some genome differentiation within *S. acaule* and between *S. tuberosum* and *S. acaule*.

Allo-tetraploid, genome differentiation, and evolutionary significance

Two major questions concerning *S. acaule* have not yet been answered clearly (Dvořák 1983; Matsubayashi 1982, 1991):(1) what is the nature of tetraploidy, and (2) what causes disomic bahavior? Previous chromosome pairing analyses have been on triploids and their colchicine-doubled hexaploids to examine whether one of the *S. acaule* genome shares affinity with the *S. tuberosum* genomes (reviews in Dvořák 1983; Matsubayashi 1991). The occurrence of a high frequency of trivalents in the triploid hybrids that was found in previous research has led to a proposal that one of *S. acaule* genomes is homoeologous to that of cultivated potatoes. This information is of importance with respect to evolutionary aspects and to chromosome manipulation in germ plasm enhancement of potato with disomic tetraploid *S. acaule*.

Some alternative approaches have been taken to elucidate the nature of tetraploidy in *S. acaule*. One is the extraction of haploids from the disomic tetraploid *S. acaule*. If two genomes in a haploid are highly homoeologous, a high frequency of bivalent formation would be expected (Howard 1970). Camadro et al. (1992) obtained parthenogenetic haploid plants ($2x = 24$) from *S. acaule* ($4x = 48$) using a haploid inducer (Hermsen and Verdenius 1973). The mean meiotic configuration in microsporogensis of these haploid plants was 6.72 bivalents. It seems that while chromosome pairing in these haploids is high a high frequency of univalents also exists, causing Camadro et al. (1992) to propose that *S. acaule* is a segmental allo-tetraploid. This has been supported by a lack of segregation of the fixed heterozygosity at the dimeric enzyme aspartate aminotransferase (AAT) loci in these haploids (Camadro et al. 1992).

The approach taken in the present study was to use direct F_1 hybrids between tetrasomic tetraploid potatoes and disomic tetraploid *S. acaule* (Howard 1970; Iwanaga et al. 1991; Matsubayashi 1991). The occurrence of multivalents and an average of 17.6 bivalents (mainly rod bivalents) in each meiocyte (Table 2) suggests that: (1) at least one of the *S. acaule* genomes shares high affinity with *S. tuberosum* genoomes and (2) two genomes of *S. acaule* are partially homoeologous. On the other hand, an average of 9.9 univalents is (Table 2) an indication of genome differentiation in some degree between two genomes of *S. acaule* and between *S. acaule* and *S. tuberosum*. A similar conclusion was made by Camadro et al. (1992) on the basis of their haploids from *S. acaule*. The present findings indicate that *S. acaule* is likely to be a segmental allo-tetraploid. This supports previous proposals by Hawkes (1958), Irikura (1976) and Matsubayashi (1982, 1991), which were made based on morphological and/or cytological observations.

A further question to be answered is what causes that disomic behavior in tetraploid *S. acaule*. Homoeologous chromosomes in a disomic tetraploid do not pair as a consequence of either structural differentiation or the functioning of pairing, regulating genes (Hermsen 1984).

Disomic behavior or allo-tetraploidization would be created by inducing differential pairing affinity between genomes (Sybenga 1969, 1973; Doyle 1979, 1990). Matsubayashi (1982, 1991) attributed his observations to segmental allopolyploidy, so that differential pairing affinity based on genomic differentiation would be the mechanism of the disomic behavior. This conclusion may be supported by Dvořák and McGuire (1981) who suggested that even a small change in nucleotide sequence would be recognized by the chromosome pairing process resulting in preferential pairing with more "homologous" chromosomes. On the other hand, Dvořák (1983) claimed that the presence of a genetic system would alter the chromosome pairing in *S. acaule* genomes. Conclusive evidence is not yet available on this question. Thus, it will be essential to test if a presence of a genome or a dose of gene(s) can change the genetic control and alter heterogenetic pairing when interspecific hybridizations are made between disomic tetraploid species and diploid species. This would be comparable to the genetic control system to homoeologous chromosome pairing in other genera such as *Triticum* (Riley and Chapman 1958; Sears 1976).

Disomic behavior in a polyploid has several advantages over polysomic polyploids under a highly autogamous condition (MacKay 1970). A built-in heterozygosity and conserved genetic diversity would be highly favorable for the maintenance of high fitness under variable conditions. Although vegetative propagation is is possible in *S. acaule*, it is essential for *S. acaule* in the wild to have stable sexual propagation with high genetic flexibility and stability under the harsh habitats where it usually exists. Since disomic tetraploid *S. acaule* is known to be a highly autogamous species and can be regarded as a segmental allotetraploid, it fits the scheme of MacKay (1970).

Generation of unique genetic stocks for breeding

A haploid set of the genomes of tetraploid *S. tuberosum* can be regarded as a 2n complement of a diploid species due to high genetic affinity between its genomes (Irikura 1976; Peloquin et al. 1989; Yeh et al. 1964). The tetraploid F_1 hybrids described in this paper can be regarded as being sesquiploid, since a haploid set of the genomes of *S. acaule* exists with the 2n complement from diploid *S. tuberosum*. Sesquiploids possess the sporophytic or 2n complement of one species and the gametic or 1n set of another species (DeVerna et al. 1987; Chavez et al. 1988; Rick et al. 1986).

One of the difficulties in the introgression of genes of the disomic tetraploid species to the cultivated tetraploid gene pool has been the low efficiency of recombination. Previous approaches (reviewed in Iwanaga et al. 1991, Ross 1986, and Watanabe et al. 1992a) employed ploidy manipulation of the disomic tetraploid which resulted in higher ploidy and higher chances of homologous recombination in the initial hybrids, and which did not facilitate homoeologous recombinations.

Brown and Adiwilaga (1990) commented that a triploid between *S. acaule* and cultivated diploid potatoes has one copy each genome and that no chromosome set has a completely homologous pairing partner; thus intergenomic exchange would occur between two genomes of *S. acaule* that are homoeologous and the cultivated potatoes. However, these triploid hybrids had inevitable genetic loss due to an unbalanced meiosis with different genomes and the nature of the triploidy. On the other hand, the concept of a sesquiploid would provide more opportunity in utilizing the disomic tetraploid species. The sesquiploid would be able to have enhanced interspecific recombination since the two genomes from *S. tuberosum* would maintain some normal chromosomal behavior at meiosis, while the genomic difference within the two genomes of *S. acaule* would enhance heterogenetic chromosome pairing with the chromosomes of *S. tuberosum*.

It seems that chromosome pairings between more similar partners are not always favored in colchicine-doubled *Secale* hybrids (Benavente and Orellana 1991). This would support the probability of heterogenetic recombination between *S. acaule* and *S. tuberosum*: competitions in chromosome pairing among all potentially homoeologous partners would likely occur, and the more homologous chromosomes would not always be preferably paired.

The occurrence of multivalents and the high frequency of bivalents in the sesquiploid-like F_1 hybrids examined here support the expectation that heterogenetic chromosome pairing and recombination could occur between genomes of *S. tuberosum* and *S. acaule*. Although a high frequency of meiotic irregularity was seen in this experiment, a reasonable level of pollen fertility was also observed (Table 3). Further, the seeds obtained from the crosses between the tetraploid F_1 hybrids and diploid breeding lines are viable (Watanabe et al. 1992b), and the seedlings generated consisted of euploids and aneuploids such as trisomics. DeVerna et al. (1987) also observed the occurrence of aneuploid and euploid recombinant types from sesquiploids from *Lycopersicon* and *Solanum* intergeneric hybridization. It appears that the occurrence of aneuploid and euploid recombinant genotypes in the progeny could provide an unique opportunity to generate genetic stocks with *S. acaule* chromosome segments. These aneuploid and euploid recombinant genetic lines would be valuable for further characterization during introgression, particularly for genes of interest for potato breeding.

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