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Using disomic 4x (2EBN) potato species' germplasm via bridge species *Solanum commersonii*

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The cultivated potato *Solanum tuberosum* Dunal has many wild related species with desirable traits. Some of these wild tetraploids have disomic chromosome pairing, ready selfing with little inbreeding depression, but have strong crossing barriers with cultivars. They hybridize most easily with 2EBN forms (which include most diploid species). Chromosome doubling to the 8x level, use of 2n gametes, use of 2n gametes of 4x-2x triploid hybrids, and embryo rescue have been proposed to overcome the crossability barrier of these species with *S. tuberosum*. In this study, 2x *S. commersonii* (cmm) was used as a bridge species with *S. acaule* and series *Longipedicellata* species. Synthetic tetraploid 4x-cmm crossed readily to disomic 4x species, resulting in fertile F₁ and F₂ hybrids. Some of these had 2n gametes, which enabled direct crossing to *tuberosum*, resulting in 6x hybrids. The benefits of this scheme are (i) hybrids are relatively fertile, so many progeny may be produced for selection at each step, (ii) hybridization with cmm results in 2n gametes needed for crossing to *tuberosum*, and breaks up restricted recombination within disomic genomes, and (iii) simple techniques and tools are employed.

Key words: *Solanum*, potato, germplasm, crossability.

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Le *Solanum tuberosum* Dunal, la pomme de terre cultivée, est relié à plusieurs espèces indigènes possédant des caractères désirables. Certaines de ces espèces tétraploïdes indigènes présentent un appariement chromosomique disomique et sont prêtes à l'autogamie avec peu de dépression due à l'isogamie, mais elles offrent de fortes barrières pour les croisements avec d'autres cultivars. Ces plantes s'hybrident plus facilement avec les formes 2EBN, lesquelles incluent la majorité des espèces diploïdes. Le doublement des chromosomes au niveau de 8x à l'aide de gamètes 2n, de gamètes 2n d'hybrides triploïdes 4x-2x et de la récupération d'embryons, a été proposé pour circonvenir les barrières de croisement de ces espèces avec le *S. tuberosum*. Dans la présente étude, le *S. commersonii* (cmm) 2x a été employé comme une espèce-pont avec les espèces *S. acaule* et Ser. LON. L'espèce tétraploïde synthétique 4x-cmm s'est croisée facilement avec l'espèce disomique 4x, ce qui s'est traduit par des hybrides fertiles F₁ et F₂. Certains de ces hybrides avaient des gamètes 2n, rendant possible le croisement direct avec le *S. tuberosum* et la production d'hybrides 6x. Les bénéfices de cette stratégie sont : (i) les hybrides sont relativement féconds, de sorte que plusieurs descendants peuvent être obtenus pour la sélection à chaque étape; (ii) l'hybridation avec le cmm produit des gamètes 2n, qui sont nécessaires pour les croisements avec le *S. tuberosum* et annulent la recombinaison restreinte avec les génomes disomiques; et (iii) des techniques et des outils simples sont utilisés.

Mots clés : *Solanum*, pomme de terre, plasma germinal, possibilité de croisement.

[Traduit par la rédaction]

Introduction

Cultivated potato (*Solanum tuberosum* L.) has many related wild species, encompassing a range of ploidy and crossing barriers. These species often exhibit economically desirable traits, which are either absent or at lower levels of expression in adapted breeding stocks, making these species desirable for breeding purposes (Hanneman 1989; Plaisted and Hoopes 1989).

Groups of species that are taxonomically distinct can often be grouped by ploidy, crossing, and breeding behavior. For the purposes of this study, the tetraploid species of series

Acaulia (ACA) and *Longipedicellata* (LON) (Hawkes 1990) were so grouped. These species are associated by ready crossability with most wild and cultivated diploids of series *Tuberosa*, resulting in sterile triploids, while having a strong direct crossability barrier with 4x cultivated *S. tuberosum* (Estrada 1980; Rammana and Abdalla 1970; Vavilova 1973). These species have relatively few functional 2n gametes (Swaminathan 1951; Watanabe et al. 1992; Watanabe and Peloquin 1991). Meiotic chromosome pairing, breeding behaviour, and genetic studies suggest that these are disomic or allopolyploid species (Matsubayashi 1982, 1991; Hawkes

1979; MacKey 1970; Singit and Hanneman 1987; Everhart and Rowe 1974). Since these species are ready selfers, the reproductive fitness associated with uniformly self-compatible gametes may explain their relatively low levels of $2n$ gametes (Watanabe and Peloquin 1991).

These species are known to have traits of particular interest to those intent on improving cultivated potato. *Solanum acaule* from series ACA is one of the most frost resistant of potato species and also has heat tolerance. Some accessions are immune to potato virus X and others highly resistant to potato leafroll virus, aphids, nematodes, and ring rot. Series LON species exhibit notable *Verticillium* resistance. Raw tuber flesh of *S. hjertingii* will not darken after cooking, and *S. stoloniferum* has immunity to viruses A and high resistance to a variety of insects (Hanneman and Bamberg 1986).

Crossing these species directly to $4x$ *S. tuberosum* cultivars is rarely successful because of the difference in endosperm balance number (EBN), of the parents. EBN crossability theory (Johnston and Hanneman 1980) predicts that the EBN (and therefore the ploidy) of these $4x$ (2EBN) disomic species must be doubled to match the EBN and crossability group of $4x$ (4EBN) *S. tuberosum* cultivars. Two basic approaches to this have been investigated: sporophytic chromosome doubling to the $8x$ (4EBN) level (Lam 1953; Swaminathan 1951; Brown 1988) or selecting $4x$ clones that produce the equivalent (unreduced with respect to ploidy) $2n$ gametes (Camadro and Espinillo 1990; Brown 1988; Von Wagenheim 1954). Alternately, $4x$ clones of these species may first be crossed to $2x$ (2EBN) diploids, resulting in abundant but sterile triploid progeny. Fertility is then restored, and the EBN requirement for crossing to *S. tuberosum* met by induced sporophytic chromosome doubling to the $6x$ (4EBN) level (Brown 1988; Watanabe et al. 1992) or $3x$ clones are identified, which produce the equivalent ($3x$) $2n$ gametes (Estrada 1980; Brown 1988; Brown and Adiwilaga 1990). A third alternative circumvents the EBN requirement by directly crossing to *S. tuberosum* with the aid of mentor pollinations, followed by in vitro embryo rescue (Watanabe et al. 1992).

Although any means of incorporation of these species into breeding lines is a welcome advance, certain considerations would make a breeding scheme for these species more desirable. To make selection practical, large numbers of progeny should be obtained with minimal input. Here potential inputs are laboriously induced ploidy manipulations, microscopic screening and selection for acceptable levels of $2n$ gametes, and the need for specialized techniques and equipment. It is also crucial that recombination among the homeologous genomes of the disomic species and between these genomes and that of *S. tuberosum* is facilitated (Brown 1988).

These advantages are features of the scheme proposed here, in which artificial tetraploid *S. commersonii* is used as a bridge between disomic species and cultivated tetraploid *S. tuberosum*.

Materials and methods

Crosses were made to test the usefulness of *S. commersonii* as a bridge species to enhance crossability of disomic tetraploid species to *S. tuberosum*. The method consisted of making F_1 and F_2 hybrids of $4x$ *S. commersonii* ($4x$ -cmm) with five tetraploid disomic *Solanum* species (*acaule*, *stoloniferum*, *fendleri*, *papita*, *hjertingii*). Artificial tetraploid *S. commersonii* was used as the

female parent. Components of practical success of the method were then assessed as follows.

Crossability

Crossing efficiency of parents used to make the hybrids and of F_1 and F_2 hybrids to *S. tuberosum* was measured by tallying and comparing number of pollinations and the resulting fruit, seed, and hybrid plants.

Vigor and fertility

Relative vine vigor, flowering, and pollen shed of the hybrids was subjectively assessed. Fertility was judged by seed production parameters in controlled crosses and (in some cases) OP berries of field grown plants. Male fertility was also estimated by percent grains stained with acetocarmine (Marks 1954).

Evidence of recombination

The general appearance of field-grown plants within parental species, F_1 and F_2 populations was evaluated subjectively. Chromosome associations were examined in meiocytes of one F_2 hybrid individual from the cross of $4x$ *S. commersonii* \times *S. fendleri*. The range of segregation for a specific characteristic and degree of acclimated frost hardiness (Stone et al. 1993) was also assessed in F_1 and F_2 hybrids of $4x$ *S. commersonii* \times *S. acaule*.

Identity of materials used

The *S. commersonii* used was a population of selfed progeny from a single colchicine induced clone of PI 243503 (ssp. *commersonii*). This clone resulted from colchicine treatment of $2x$ true seeds. All other unselected disomic tetraploid species were obtained from the Inter-Regional Potato Introduction Project (NRSP-6), Sturgeon Bay, Wis. *Solanum tuberosum* used were fertile tetraploids of ssp. *andigena* or U.S. ssp. *tuberosum* cultivars. Although parents and testers were bulked, the PI numbers of accessions used to make F_1 hybrids were recorded (Table 1).

INSERT TABLE 1

Techniques

Hybrids were made through controlled pollinations on emasculated pistillate plants, either in screenhouse, greenhouse, or via the cut stem technique (Peloquin and Hougas 1959). Relatively few pollinations were made to generate F_1 hybrids (Table 1), since only a small number of F_1 s were expected to be needed to generate an adequate number of F_2 progeny. All F_2 hybrids were made by selfing the F_1 s. Hybridity was confirmed by ploidy and physical appearance. Ploidy was determined by counting mitotic metaphase chromosomes in root tip cells.

Results

Crossability

The disomic species used do not cross to $2x$ *S. commersonii* (cmm), but raising cmm to the tetraploid level ($4x$ -cmm), resulted in relatively easy crossing to each of these species, although seed germination was sometimes poor (Table 1). When the resulting F_1 hybrids were tested for crossability to *S. tuberosum*, no seeds resulted, except for *acaule* hybrids (Table 2). With the exception of F_1 families involving *stoloniferum*, relatively few self-pollinations were required to obtain many F_2 seeds (although only some of the clones in the F_1 families readily selfed in this single attempt) (Table 3).

TABLES 2 & 3

When F_2 families were tested for crossability with $4x$ *S. tuberosum*, crossing success was acceptable but low by intraspecific standards (Table 4). Moreover, the resulting $6x$ hybrids crossed relatively easily to $4x$ *S. tuberosum* in

both directions. When hexaploid F_2 s (from intermated F_1 s) were crossed with *S. tuberosum*, 833 pollinations resulted in 646 seeds with a high rate of germination.

INSERT TABLE 4

Vigor and fertility

F_1 and F_2 families had larger vines when compared with parent species grown under similar field conditions. Flowering in these hybrids was also more abundant and long lasting. Pollen shed was typically excellent in both F_1 and F_2 tetraploid hybrids, and percentage of acetocarmine stained pollen grains was consistently greater than 50%. Hybrids grown in the field produced numerous OP seeds. These were sampled from a bulk of the F_2 hybrid families represented by 28 plants. Approximately 500 berries were produced, which contained about 10 000 seeds. These seeds had a high rate of germination (>80%). Hexaploid hybrids resulting from crosses of (4x-cmm × disomic species) × *S. tuberosum* were vigorous and produced ample flowering and pollen shed. Eighty-eight intermating pollinations among a bulk of 6x F_1 clones from each family resulted in over 450 seeds with a high rate of germination (>80%).

Evidence of recombination

When meiocytes were examined in one of the F_2 hybrid individuals and its disomic parent, *S. fendleri*, quadrivalent associations were observed in the hybrid, whereas none could be seen in the pure disomic parent. When variability of each of the interspecific populations was observed in the field, plants within F_2 families appeared to have more morphological variation than their F_1 parents. While no F_2 s could be described as absolute parental types, plants that favored the parental species were noted. In addition, certain characteristics were observed, which were not seen in either the parental species or F_1 . For example, some (4x-cmm × fen) F_2 s had spurlike projections from the base of the calyx, and others exhibited the unusual tendency to form underwater adventitious roots in the pollination bottles. Both 4x *commersonii* and *acaule* are very cold hardy and the former is particularly able to acclimate (i.e., become more hardy after exposure to chilling temperatures). When acclimated cold hardiness of F_1 and F_2 hybrids of these species was compared, the range of F_2 hardiness was nearly 50% greater than that of the F_1 s.

Discussion

When considering methods for making disomic tetraploid wild species accessible to breeding, it is desirable to have simple, inexpensive techniques, which result in many fertile hybrid breeding stocks. The scheme should also allow maximum recombination and selection before the cross to *S. tuberosum* is made. The method proposed here uses artificial tetraploid *S. commersonii* as a bridge between disomic tetraploid wild species and *S. tuberosum*. *Solanum commersonii* is atypical for a South American diploid by virtue of its 1EBN (Hanneman and Bamberg 1986). To make this species 4x (2EBN) like disomic tetraploids, it was chromosome doubled, the only step in this scheme requiring specialized techniques or equipment. Tetraploid *S. commersonii* is vigorous and fertile, and easily produced F_1 hybrids with disomic 4x species (Table 1). Although these F_1 s did not

generally cross to *S. tuberosum* directly, synthesis of F_2 selfs was easy for at least some of each interspecific hybrid (Table 3) and probably a useful step with respect to breeding. In contrast with diploids, all possible allelic combinations are not normally obtainable in gametes of an F_1 . Thus, this step would allow selection for desirable segregating types from a large population.

F_2 individuals from different (4x-cmm × disomic species) families are highly interfertile (data not shown). With two homeologous sets of chromosomes and two sets of homologous *commersonii* chromosomes, highly fertile tetraploids must result from free interspecific and (or) interhomeologous pairing. The limited microscopic evidence provided here and review of others' observations on similar crosses (Dvořák 1983) indicate that hybrids of disomic and polysomic polyploids are generally polysomic (i.e., have no pairing restrictions), presumably because the polysomic genome cancels pairing control characteristic of the disomic genome. Even if this explanation is rejected in favor of true genome differences, the constituent species used here all possess the "A" genome or a minor variation, suggesting that recombination could freely occur (Matsubayashi 1991).

In this crossing scheme, only some individuals produced seeds, and seed set and germination would be considered very poor by intraspecific standards. However, considering that these are wide interspecific hybrids, a relatively low investment per seedling was required.

Recurrent selection could be practiced at the tetraploid level on combinations of various (4x-cmm × disomic species) hybrids for segregants with fixed maximum genetics for traits of interest. Since crosses of these hybrids with *S. tuberosum* resulted exclusively in 6x progeny, presumably via 2n eggs, prebreeding at the 4x level would also provide an opportunity to select for higher frequencies of 2n gametes to improve the rate of crossing to *S. tuberosum*. Progeny of desirable 6x selections backcrossed to *S. tuberosum* would be expected to quickly move through 5x back to the 4x level (Black 1943). Also, since selection at the diploid level appears to have unique advantages (Ortiz et al. 1991), 2x maternal haploids could be extracted from 5x backcross hybrids, desirable recombinants selected, and directly crossed with *S. tuberosum* breeding stocks (4x-2x) via 2n gametes (Ehlenfeldt and Hanneman 1988).

Conclusions

This scheme for accessing disomic wild tetraploid species germplasm is simple and inexpensive and has other desirable attributes. Disomic species are crossed with a single synthetic autotetraploid bridge species, which promotes heterogenetic chromosome pairing and recombination and high fertility of the initial hybrids. Prebreeding can thus be performed on advanced generations of these interwild species hybrids before crossing to *S. tuberosum*.

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TABLE 1. Results of crosses made to generate F₁ hybrids of 4x *S. commersonii* × *S. acaule* and 4x *S. commersonii* × Ser. LON species

Male	Pollinations	Fruit	Seeds	4x hybrid plants/ seed planted	Family code
acl bulk	123	29	75	22/75	4x-cmm × acl
fen bulk	17	5	44	8/44	4x-cmm × fen
hjt bulk	4	2	4	1/4	4x-cmm × hjt
pta bulk	8	1	17	5/17	4x-cmm × pta
sto bulk	7	1	7	5/7	4x-cmm × sto

NOTE: Female parent was 4x *S. commersonii* PI 243503. acl, *S. acaule*; cmm, *S. commersonii*; fen, *S. fendleri*; hjt, *S. hjerlingii*; pta, *S. papita*; sto, *S. stoloniferum*. PI numbers of bulks: acl: 175396, 195160, 210029, 210033, 217449, 320276, 365307, 472641. fen: 275156, 275157, 275158, 275162, 275163, 283101. hjt: 186559, 251063, 251065, 275174, 283103. pta: 265895, 275227, 275740, 275741, 283143. sto: 160224, 186544, 275244, 310964, 338617.

TABLE 2. Results of crosses made to test crossability of F₁ hybrids of 4x *S. commersonii* × *S. acaule* and 4x *S. commersonii* × Ser. LON species with *S. tuberosum*

F ₁ families ^a	Clones tested	Pollinations	Fruit	Seeds	6x hybrid plants/ seeds planted
Male tester: <i>S. tuberosum</i> cv. Katahdin or ssp. <i>andigena</i>					
Female					
4x-cmm × acl	17	328	177	200	66/200
4x-cmm × fen	8	179	0	—	—
4x-cmm × hjt	1	15	0	—	—
4x-cmm × pta	5	84	0	—	—
4x-cmm × sto	6	102	0	—	—
Female tester: <i>S. tuberosum</i> cv. Butte, Hudson, Katahdin, or ssp. <i>andigena</i>					
Male					
4x-cmm × acl	21	1211	113	45	23/45
4x-cmm × fen	8	97	0	—	—
4x-cmm × hjt	1	66	0	—	—
4x-cmm × pta	5	74	0	—	—
4x-cmm × sto	6	131	0	—	—

^aSee Table 1 for key to family codes.

TABLE 3. Results of crosses made to generate F₂ hybrids of 4x *S. commersonii* × *S. acaule* and 4x *S. commersonii* × Ser. LON species

Selfed F ₁ families ^a	No. of clones that selfed/ total	Pollinations	Fruit	Seeds ^b
4x-cmm × acl	13/21	610	326	15,000
4x-cmm × fen	4/8	40	14	206
4x-cmm × hjt	1/1	8	5	100
4x-cmm × pta	1/5	23	14	650
4x-cmm × sto	1/5	19	2	10

^aSee Table 1 for key to family codes.

^bAssumed to be 4x; germination >90%.

TABLE 4. Results of crosses made to test crossability of F₂ hybrids of 4x *S. commersonii* × *S. acaule* and 4x *S. commersonii* × Ser. LON species with *S. tuberosum*

F ₂ family ^a	No. of clones tested	Pollinations	Fruit	Seeds	6x hybrid plants seeds planted
4x-cmm × acl	50	172	92	65	14/65
4x-cmm × fen	58	1645	420	197	43/54
4x-cmm × hjt	16	267	48	1	1/1
4x-cmm × pta	42	959	165	21	17/20
4x-cmm × sto	5	72	20	0	—

NOTE: Male tester was *S. tuberosum* cv. Superior, Norland, Katahdin, or ssp. *andigena*
^aSee Table 1 for key to family codes.