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

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Corresponding Editor: J.P. Gustafson

Received January 17, 1994
Accepted June 16, 1994


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.


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 dissymetric 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 circonscrire 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...
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 F₁ and F₂ 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, F₁ and F₂ populations was evaluated subjectively. Chromosome associations were examined in meioocytes of one F₂ hybrid individual from the cross of 4x S. commersonii × S. fendleri. The range of segregation for a specific characteristic and degree of acclimated frost hardiness (Stone et al. 1993) was also assessed in F₁ and F₂ hybrids of 4x S. commersonii × 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 F₁ hybrids were recorded (Table 1).

INSERT TABLE 1

Techniques

Hybrids were made through controlled pollinations on emasculated pistillate plants, either in greenhouse, greenhouse, or via the cut stem technique (Pelouquin and Hougas 1959). Relatively few pollinations were made to generate F₁ hybrids (Table 1). Since only a small number of F₁'s were expected to be needed to generate an adequate number of F₂ progeny. All F₂ hybrids were made by selfing the F₁'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 F₁ hybrids were tested for crossability to S. tuberosum, no seeds resulted, except for acaule hybrids (Table 2). With the exception of F₁ families involving stoloniferum, relatively few self-pollinations were required to obtain many F₂ seeds (although only some of the clones in the F₁ families readily selfed in this single attempt) (Table 3).

TABLES 2 & 3

When F₂ 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 F₁-s (from intermated F₁-s) were crossed with *S. tuberosum*, 833 pollinations resulted in 646 seeds with a high rate of germination.

**INSERT TABLE 4**

**Vigor and fertility**

F₁ and F₂ 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 F₁ and F₂ 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 F₂ 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 F₁ 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 F₁ 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 combinations was observed in the field, plants within F₂ families appeared to have more morphological variation than their F₁ parents. While no F₂-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 F₁. For example, some (4x-cmm × fen) F₂-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 F₁ and F₂ hybrids of these species was compared, the range of F₂ hardiness was nearly 50% greater than that of the F₁-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 F₁ hybrids with disomic 4x species (Table 1). Although these F₁-s did not generally cross to *S. tuberosum* directly, synthesis of F₂ 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 F₁. Thus, this step would allow selection for desirable segregating types from a large population.

F₂ 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*.

**Acknowledgement**

The authors thank the University of Wisconsin Peninsula Agricultural Research Station program and staff for their cooperation in this work.


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

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


### 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*

<table>
<thead>
<tr>
<th>F1 families&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Clones tested</th>
<th>Pollinations</th>
<th>Fruit</th>
<th>Seeds</th>
<th>6x hybrid plants/seeds planted</th>
</tr>
</thead>
</table>

**Male tester: S. tuberosum cv. Katahdin or ssp. andigena**

<table>
<thead>
<tr>
<th>Female</th>
<th>4x-cmm × acl</th>
<th>17</th>
<th>328</th>
<th>177</th>
<th>200</th>
<th>66/200</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4x-cmm × fen</td>
<td>8</td>
<td>179</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4x-cmm × hjt</td>
<td>1</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4x-cmm × pta</td>
<td>5</td>
<td>84</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4x-cmm × sto</td>
<td>6</td>
<td>102</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Female tester: S. tuberosum cv. Butte, Hudson, Katahdin, or ssp. andigena**

<table>
<thead>
<tr>
<th>Male</th>
<th>4x-cmm × acl</th>
<th>21</th>
<th>1211</th>
<th>113</th>
<th>45</th>
<th>23/45</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4x-cmm × fen</td>
<td>8</td>
<td>97</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4x-cmm × hjt</td>
<td>1</td>
<td>66</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4x-cmm × pta</td>
<td>5</td>
<td>74</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4x-cmm × sto</td>
<td>6</td>
<td>131</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>See 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

<table>
<thead>
<tr>
<th>Selfed</th>
<th>No. of clones that selfed/total</th>
<th>Pollinations</th>
<th>Fruit</th>
<th>Seeds&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 families&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4x-cmm × acl</td>
<td>13/21</td>
<td>610</td>
<td>326</td>
</tr>
<tr>
<td></td>
<td>4x-cmm × fen</td>
<td>4/8</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>4x-cmm × hjt</td>
<td>1/1</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>4x-cmm × pta</td>
<td>1/5</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>4x-cmm × sto</td>
<td>1/5</td>
<td>19</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup>See Table 1 for key to family codes.

<sup>b</sup>Assumed to be 4x; germination >90%.
**Table 4.** Results of crosses made to test crossability of \( F_2 \) hybrids of 4x \( S. \) commersonii \( \times S. \) acaule and 4x \( S. \) commersonii \( \times \) Ser. LON species with \( S. \) tuberosum

<table>
<thead>
<tr>
<th>( F_2 ) family*</th>
<th>No. of clones tested</th>
<th>Pollinations</th>
<th>Fruit</th>
<th>Seeds</th>
<th>6x hybrid plants seeds planted</th>
</tr>
</thead>
<tbody>
<tr>
<td>4x-cmm ( \times ) acl</td>
<td>50</td>
<td>172</td>
<td>92</td>
<td>65</td>
<td>14/65</td>
</tr>
<tr>
<td>4x-cmm ( \times ) fen</td>
<td>58</td>
<td>1645</td>
<td>420</td>
<td>197</td>
<td>43/54</td>
</tr>
<tr>
<td>4x-cmm ( \times ) hjt</td>
<td>16</td>
<td>267</td>
<td>48</td>
<td>1</td>
<td>1/1</td>
</tr>
<tr>
<td>4x-cmm ( \times ) pta</td>
<td>42</td>
<td>959</td>
<td>165</td>
<td>21</td>
<td>17/20</td>
</tr>
<tr>
<td>4x-cmm ( \times ) sto</td>
<td>5</td>
<td>72</td>
<td>20</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

*Note: Male tester was \( S. \) tuberosum cv. Superior, Norland, Katahdin, or ssp. andigena

*See Table 1 for key to family codes.