MEIOTIC MUTANTS IN POTATO BREEDING
(haploids, 2n gametes, germplasm transfer)

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SUMMARY

Three factors provide unusual opportunities for potato improvement:
(1) The wild and cultivated tuber-bearing relatives of the potato represent
a large source of valuable germplasm. This genetic diversity can be incor­
porated into commercial cultivars. The germplasm is valuable in providing
both specific desirable traits, such as disease and insect resistance, and
for broadening the genetic base; (2) Haploids (2n=24) of cultivars (2n=48)
can be readily obtained and used. They offer the advantages of simpler
inheritance patterns (disomic vs. tetrasomic), and more important, a direct
approach to germplasm transfer from the numerous 24-chromosome, tuber-bearing
relatives of the potato. They provide us with a unique means of capturing
genetic diversity; and (3) The discovery of meiotic mutants which give rise
to 2n gametes provide unique and exciting opportunities to increase yield
and genetic diversity. It is estimated that 2n gametes formed by first
division restitution (FDR) transfer intact 80% of the genotype of diploids
to their tetraploid progeny in 4x-2x and 2x-2x crosses. The meiotic mutant
parallel spindles (ps) is an FDR mechanism that accomplishes this transfer
in the production of 2n pollen. Recently, two new meiotic mutants, sy2 and
sy3, have been discovered. They prevent normal crossing over, and thus the
result is mainly univalents at metaphase I and high male sterility. How­
ever, when a synaptic mutant is combined with the parallel spindles mutant,
fertile 2n pollen is produced. The genetic significance of this combination
of meiotic mutants is that it makes possible the incorporation of the intact
genotype of the parent into the gamete. Thus, a truly exceptional opportu­
nity is possible - transmitting 100% of the heterozygosity and epistasis of
the parent to the offspring. The meiotic mutants provide, therefore, a
powerful breeding method for maximizing heterozygosity and epistasis, and a
very efficient method of transferring germplasm from diploids to tetraploids.

INTRODUCTION

Several features pertaining to the genetics and reproductive biology of the potato provide background information and the rationale for the use of meiotic variations in breeding and
(1) The cultivated potato of North America and Europe, *Solanum tuberosum* Group Tuberosum is a tetraploid (2n=4x=48); the main cultivated form in South America, *S. tuberosum* Group Andigena is also tetraploid. Genetic and cytological evidence overwhelmingly supports the concept that the potato is a tetrasomic polyploid - four sets of similar chromosomes, tetrasomic genetic ratios, and the occurrence of multivalents at meiosis (HOUgas & Peloquin 1958).

(2) The potato is fortunate in having many wild and a few cultivated, tuber-bearing relatives. The more than 140 species form a polyploid series from diploids through hexaploids, with about 60% of the species being diploids. They are distributed from southern United States to southern Chile, from sea level to 4,000 meters, and the largest number of species and the greatest diversity occur in the Andean regions of Peru and Bolivia (Hawkes 1958). A worldwide collection of *Solanum* germplasm is available in the collection of the Inter-regional Potato Introduction Project, at Sturgeon Bay, Wisconsin (Ross & Rowe 1969). Most important, this germplasm represents an immense source of genetic variability for specific desirable traits such as disease and insect resistance, and abundant allelic diversity for obtaining maximum heterozygosity (Ross 1979).

(3) Haploids (2n=2x=24) of cultivars (2n=4x=48) are relatively easy to obtain through 4x x 2x crosses. They behave as normal diploids with bivalent pairing and disomic genetics.

(4) Haploids can be easily hybridized with most 24-chromosome, tuber-bearing species. The hybrids are vigorous, fertile, variable and possess improved tuberization compared to the wild species. The latter do not normally tuberize in the field under long-day conditions.

(5) Haploid-species hybrids generally have normal chromosome pairing and crossing over. Cytological investigations indicate very little differentiation between the chromosomes of the cultivated potato and those of most wild tuber-bearing relatives. Thus, germplasm transfer from wild to cultivated forms is relatively easy (Peloquin et al. 1966).

(6) Triploids are difficult to obtain following crosses between 4x cultivated potatoes and 2x species. The basis of the triploid block resides in faulty endosperm development. Normal seed development is dependent on a 2 maternal:1 paternal ratio of Endosperm Balance Numbers in the endosperm. This ratio is upset following either 4x x 2x (4 maternal:1 paternal) or 2x x 4x (1 maternal:1 paternal) crosses where normal n gametes function (Johnston et al. 1980).

(7) The potato of commerce is propagated clonally from tubers. Decrease in yield due to virus infection is prevented through the maintenance and increase of cultivars free of virus by foundation tuber programs.
The use of botanical seed to grow the crop is being explored. This method appears particularly promising in developing countries that either cannot maintain clean tubers (and thus have very low yields) or where the cost of importing clean tubers would be 60-80% of the cost of production. The viruses are not transmitted through botanical seed, and the cost of botanical seed is very low, so the previous problems are alleviated (UPADHYA 1979).

The genetic variance for yield is almost entirely nonadditive. Therefore, intralocus interactions (heterozygosity) and interlocus interactions (epistasis) are very important. Thus, one must strive to obtain maximum heterozygosity, more than two alleles per locus, to increase these possible interactions (MENDIBURU et al. 1974).

RESULTS AND DISCUSSION

Our overall breeding strategy involves three main components; the species are the source of genetic diversity, haploids of Tuberosum and Andigena are effective tools to utilize in capturing the genetic diversity, and 2n gametes, gametes with the sporophytic chromosome number, are the basis of an efficient method to transmit genetic diversity. This discussion will focus primarily on meiotic mutants that result in the formation of 2n gametes.

The principal objectives in obtaining a large number of haploids from many 4x parents were to; 1) work with less complicated genetics, disomic rather than tetrasomic ratios, and 2) be able to cross Tuberosum directly with the many 24-chromosome species. The variability available and the simpler, diploid genetics of the species-haploid hybrids have significantly accelerated our knowledge of potato genetics. Equally important is the improved tuberization of species-haploid hybrids as compared to both the species and the haploids. In retrospect all the effort devoted to obtaining haploids was worthwhile even if they were only used to put wild species germplasm in a usable form.

An unexpected and pleasant surprise was the large tuber yields of some hybrids between haploids and Phureja, a cultivated, 24-chromosome Group from Columbia and Peru. A few hybrids had tuber yields equivalent to standard cultivars (PELOQUIN et al. 1966). These large yields suggest that one could breed potatoes at the diploid level. The predominance of nonadditive genetic variance for yield, however, precludes the success of this breeding approach, since intra- and interlocus interactions are not transmitted from parent to offspring at the diploid level. Further, the possibility of four alleles per locus in the tetraploid versus only two in the diploid greatly increases the number of interactions, so that tetraploids are superior to diploids.
UNILATERAL SEXUAL POLYPLOIDIZATION

An alternate breeding approach is to obtain tetraploids directly from 4x - 2x crosses. One can obtain desirable, highly heterozygous, 2x parents with disomic genetics, and then return to the 4x level with 4x-2x crosses when 2n gametes function in the 2x parent. To test this breeding method 22 4x cultivars or advanced breeding selections were crossed reciprocally with 108 haploid-Phureja hybrids. The average number of seeds/fruit following more than 10,000 4x-2x and 2x-4x crosses was less than two, due to the triploid block and low frequency of 2n gametes. The progeny were very vigorous, and most, 93% were tetraploid. An encouraging result was the tuber yields; the mean yield of many 4x families exceeded the yield of the cultivar parents (HANNEMAN & PELOQUIN 1969).

Three of the Phureja-haploid hybrid 2x parents were exceptional in several respects; (1) high seed set, 30-50 seeds/fruit, (2) more than 99% of the progeny were 4x, (3) they were the parents of the highest yielding families, (4) the previous three points were true only when they were used as males, and (5) the progeny were relatively uniform; this was unexpected, since both the 4x and 2x parents are highly heterozygous.

It was obvious that a systematic alteration in meiosis must be occurring in these three clones. Cytological observations of microsporogenesis provided the answer to the unusual results (MOK & PELOQUIN 1975a). Normally, the first meiotic division is not followed by cytokinesis, and in the second division the two spindles are oriented such that their poles define a tetrahedron. Following cytokinesis a tetrad of four, n microspores is formed. The first division was normal in the exceptional 2x clones, but in some sporocytes the second division spindles were parallel leading to the formation of two, 2n microspores (dyads) following cytokinesis. The genetic significance of parallel spindles is that it is essentially a first division restitution (FDR) mechanism. Therefore, all parental, heterozygous loci between the centromere and the first crossover are heterozygous in the gamete, and one-half the heterozygous loci between the first and second crossovers are also heterozygous. With FDR, it is estimated that approximately 80% of the heterozygosity is transmitted from parent to offspring. This also means that a large fraction of the epistasis is intact and transmitted to the progeny. The parallel orientation of second division spindles is inherited as a simple Mendelian recessive, ps (MOK & PELOQUIN 1975b).

Further cytological analysis of meiosis in other 2x clones that gave high seed set in 4x x 2x crosses, revealed another meiotic alteration. The first meiotic division was followed by cytokinesis, no second division occurred, and a dyad of two, 2n microspores was formed (MOK & PELOQUIN 1975a). Genetically, this is a second division restitution (SDR) mechanism. All the loci from the centromere to the first crossover will be homozygous in the gamete, and all the loci between the first and second crossovers, that are heterozygous in the parent, will be heterozygous in the gamete. In contrast to FDR less
than 40% of the heterozygosity of the parent will be transmitted to the offspring with SDR.

The breeding value of 2x FDR, 2x SDR, and 4x gametes was compared by crossing nine cultivars with four FDR clones, four SDR clones, and eight cultivars. The superiority of the FDR clones was clearly demonstrated. The average yield in lbs/hill of the means of 36 families from 4x x 2x FDR was 7.7 compared to 5.1 for 36 families from 4x x 2x SDR, and 4.6 from 76 families from 4x x 4x (MOK & PELOQUIN 1975c).

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The breeding method used to obtain 4x progeny from 4x x 2x FDR crosses are illustrated in Figure 1. This method has been used to obtain 4x hybrids that outyield standard cultivars (DE JONG et al. 1981; MENDIBURU & PELOQUIN 1977a; MOK & PELOQUIN 1975c). A further advantage is its wide application, since the best locally adapted cultivars from an area can be used in crosses with the most heterotic, FDR 2x hybrids. It is also a good method for the production of botanical seed due to the excellent seedling vigor, and the yield and uniformity of the 4x hybrids (PELOQUIN 1979).

**HAPLOID (2n=24) x SPECIES (2n=24)**
- fertility
- 2n gametes

**CULTIVAR (2n=48) x HAPLOID-SPECIES HYBRIDS (2n=24)**
- adaptation
- 2n gametes

**TETRAPLOID HYBRID**
- desired trait
- type

Fig. 1. Breeding method to obtain 4x hybrids from 4x x 2x FDR crosses.

### BILATERAL SEXUAL POLYPLOIDIZATION

Tetraploids have also been obtained from matings between diploids that produce 2n pollen and 2n eggs. Significantly, the 4x progeny are more vigorous and outyield, by 30-50%, their 2x "full-sibs," but only if 2n pollen is formed by FDR. Further, the yields of many of the 4x from 2x-2x crosses are as good as standard cultivars (MENDIBURU & PELOQUIN 1977b). In contrast 4x obtained from 2x via colchicine doubling have approximately the same yields as their undoubled counterparts (ROWE 1967). The 4x from colchicine doubling can have only two alleles per locus in contrast to the opportunity for three or four per locus in 4x obtained from intermating unrelated 2x. The concept of maximum heterozygosity and the previous results have important genetic, breeding, and evolutionary implications.

The synthesis of 4x from 2x-2x crosses provides a breeding
method with significant potential (CHASE 1963; MENDIBURU et al. 1975). This method (Figure 2) involves the development of superior, unrelated, 2x hybrids. These hybrids are selected for adaptation, yield, other desired characteristics, and 2n gamete formation by FDR. Tetraploids with near maximum interactions, plus other desired characteristics are obtained from intermat­ing 2x hybrids. The 2x clones, Phureja-haploid hybrids, pro­ducing 2n pollen by FDR are available. But it is important to identify unrelated, 2x clones that produce high frequencies of 2n eggs by FDR.

![Diagram](image)

Fig. 2. Breeding method to obtain 4x hybrids from 2x FDR x 2x FDR crosses.

Recently, hybrids with adaptation and 2n gametes have been obtained from crosses between haploids and the wild, 24-chromosome species, *S. chacoense* (LEUE & PELOQUIN 1981). This species from Argentina is a source of genetic diversity, vegetative vigor, high fertility, and both disease and insect resistance. *S. chacoense* does not tuberize under long-day conditions, has very long stolons, and selection for tuberization has not proved effective. However, some hybrids between *S. chacoense* and particular haploids have tuber yields two to four times that of the haploids. This again illustrates the value of haploids in putting exotic germplasm in a workable form. Another surprising result with the haploid-*S. chacoense* hybrids was the early maturity of some clones; they were as early as the earliest cultivars. The frequency of 2n eggs, as determined by seeds/fruit following 2x x 4x crosses, is good in the haploid- *S. chacoense* hybrids. Several clones have been identified that produce from 20-90 2n eggs/ovary.

The major problem is to determine the mode of 2n egg for­mation. Currently, four approaches are being used: (1) Cytological observations of megasporogenesis in clones that produce high frequencies of 2n eggs. This is difficult and time con­suming, but results have been obtained. Two clones were found that had high frequencies of univalents only in megasporogenesis. The abnormal first division sometimes resulted in restitution nuclei being formed which could lead to FDR 2n eggs (this result was confirmed genetically). This meiotic mutant is inherited as a recessive, *sy* (IWANAGA & PELOQUIN 1979); (2)
Genetic through the use of half-tetrad analysis (MENDIBURU & PELOQUIN 1979). The location of a gene in relation to the centromere is determined by 4x x 2x FDR crosses. Genes found to be relatively close to the centromere can then be used to detect whether 2n eggs are formed by FDR or SDR. A 2x clone heterozygous for the marker gene is crossed with a monoallelic 4x clone (A/a x aaaa), and the frequency of 4x monoallelic progeny is determined. Through the use of the markers Y (yellow tuber flesh) and E\textsuperscript{B} (esterase is basic gel) two clones that produce 2n eggs by FDR, and two that form 2n eggs by SDR have been identified (IWANAGA & PELOQUIN 1980; MOK 1981). Unfortunately, the two FDR clones produce a low frequency of 2n eggs, so we have yet to identify a clone that systematically produces a high frequency of 2n eggs by FDR; (3) Through the analysis of pollen fertility of the 4x progeny from 2x x 4x crosses. The concept involved is that male gametophyte viability (pollen stainability) is related to the level of heterozygosity of the sporophyte. Since 4x progeny obtained from 2x FDR x 4x crosses are more heterozygous than 4x progeny from 2x SDR x 4x crosses, the pollen stainability distributions of the FDR derived progeny would be significantly higher than SDR derived progeny. Differences in pollen stainability distributions have been detected, but these need to be correlated with either cytological or genetic evidence of the mode of 2n egg formation; and (4) The yield and uniformity of 4x progeny from 2x x 4x matings could also be used as an indirect indication of the mode of 2n egg formation. It is known that 4x progeny from 4x x 2x FDR crosses significantly outyield and are more uniform than progeny from 4x x 2x SDR. This approach, however, is very laborious and time consuming, and again the results must be correlated with either genetic or cytological evidence.

COMBINATIONS OF MEIOTIC MUTANTS

A new meiotic mutant, ay3, was recently discovered that adds significantly to the value of the 4x-2x and 2x-2x breeding methods (OKWUAGWU & PELOQUIN 1981). It is characterized by lack of chiasmata (thus no crossing over), only univalents at metaphase I, and the univalents are distributed at random to the telophase I nuclei. Ordinarily, this behavior would lead to almost complete male sterility, since the chance of the telophase I nuclei receiving a haploid set of chromosomes is (1/2)\textsuperscript{12}. However, when the synaptic mutant is combined with the parallel spindles mutant, a high frequency of functional 2n pollen is produced. No matter how unequal the distribution of chromosomes is in the first division, parallel spindles in the second division ensures the incorporation of two sets of 12 chromosomes in each pair of 2n microspores. The genetic significance of this combination of meiotic mutants resides in the unusual opportunity they provide - the incorporation of the intact genotype of the parent into all the 2n male gametes. Thus, 100% of the heterozygosity and epistasis of the parent can be transmitted to the progeny, a powerful breeding tool. Preliminary results indicate the progeny from 4x x 2x (no crossing over + FDR) are high yielding and uniform. Yield trials will determine how they compare with progeny from 4x x 2x (FDR) crosses.
It is revealing to indicate the genotype of two Phureja-haploid hybrids for four meiotic mutants (Table 1).

Table 1. Genotypes of W5337.3 and W5295.7 for meiotic mutants.

<table>
<thead>
<tr>
<th>Meiotic mutant</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parallel spindles</td>
<td>(W5337.3)</td>
</tr>
<tr>
<td></td>
<td>ps/ps</td>
</tr>
<tr>
<td>Premature cytokinesis</td>
<td>(W5295.7)</td>
</tr>
<tr>
<td></td>
<td>ps/ps</td>
</tr>
<tr>
<td>Univalents in megasporogenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sy1/sy1</td>
</tr>
<tr>
<td>High univalent frequency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sy3/sy3</td>
</tr>
</tbody>
</table>

The screening of new, 2x hybrid clones for these meiotic mutants is greatly facilitated by the ability to tentatively determine the genotype by screening pollen for size and stainability (Table 2).

Table 2. Screening for meiotic mutants via pollen morphology.

<table>
<thead>
<tr>
<th>Pollen types</th>
<th>Genotypes for meiotic mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>only n</td>
<td>Ps/ , Po/ , Sy3/</td>
</tr>
<tr>
<td>n and 2n</td>
<td>ps/pa, Po/ , Sy3/ or</td>
</tr>
<tr>
<td>2n stainable n aborted</td>
<td>ps/pa, Po/ , sy3/sy3</td>
</tr>
<tr>
<td>2n stainable 2n aborted</td>
<td>ps/pa, po/po, sy3/sy3</td>
</tr>
</tbody>
</table>

Another valuable meiotic mutant, sy2, was discovered in S. commersonii, a 2x wild species from Argentina (JOHNSTON et al. 1981). It is characterized by all univalents in the first division, and very poor fertility. A low frequency of stainable 2n pollen occurs probably as a result of the formation of restitution nuclei in the first division followed by a normal second division. Plants with this mutant are being crossed with clones with parallel spindles in an attempt to obtain, with further crossing, plants homozygous for both sy2 and ps, which are expected to have a high frequency of fertile 2n pollen. While determining the inheritance of sy2, a new meiotic variation was detected in a few plants (HANNEMAN & RHUDE unpublished). Again, only univalents occur, but
not even an abortive spindle is formed, so neither anaphase I, anaphase II or cytokinesis occur. All 24 univalents are included in the nucleus in most microspore nuclei leading to the formation of 2n pollen with the same genotype as the parent. It is obvious, from the previous discussion, that we have a wealth of meiotic variation in the tuber-bearing Solanums to utilize in genetic and breeding investigations.

A long range breeding method based on the most beneficial combination of meiotic mutants, from the breeding standpoint, is outlined in Figure 3. Two types of unrelated, 2x hybrids are needed to derive maximum benefits from this method. One 2x hybrid must produce highly heterozygous male gametes which are all of the same genotype. A meiotic system with no crossing-over followed by FDR accomplishes this goal; this system is available in the sy3, ps clones. The other 2x clone must produce a high frequency of 2n eggs, all highly heterozygous and of the same genotype. This would occur with either apospory, the development of the female gametophyte from a nucellar cell, or with no crossing-over followed by FDR. This type of 2x clone is not available, but one should be reasonably optimistic that even modest research efforts would be very productive in identifying the appropriate meiotic variants.

**Fig. 3.** Breeding method to obtain identical 4x hybrids from crossing two highly heterozygous 2x parents through the use of combinations of meiotic mutants.

ACKNOWLEDGMENTS

Paper No. 2578 from the Laboratory of Genetics. Research supported by the College of Agricultural and Life Sciences, International Potato Center, SEA, USDA CRGO 5901-0410-8-0180-0, and Frito-Lay, Inc.
LITERATURE CITED


