Use of half-tetrad analysis to discriminate between two types of 2n egg formation in a potato haploid

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The ratio of the first division restitution (FDR) to second division restitution (SDR) 2n eggs was estimated in 4182t, a haploid (2n = 2x = 24) of Solanum tuberosum L. that produces 2n eggs by the two modes. The segregation of three genes previously mapped relative to their centromeres, Pgm-2 (2.0 cM), Mdh-1 (33.5 cM), and 6-Pgdh-3 (30.1 cM) was analyzed in the tetraploid offspring of a 2x × 4x cross. Based on the segregation of the Pgm-2 locus, 39.7% of the progeny originated from FDR 2n eggs and 60.3% from SDR. Segregation patterns of the two distal loci within the FDR-derived 4x subpopulation indicated that the gene–centromere recombination rate during meegasporogenesis was significantly reduced for Mdh-1 when compared with a previous estimate during microsporogenesis. In the SDR-derived 4x subpopulation, the gene–centromere recombination rates for Mdh-1 and 6-Pgdh-3 were not significantly different from previous estimates. Tetraploid progeny generated from one 2x × 4x cross where the 2x parent produces 2n gametes by two modes can be used to make an unbiased comparison of the potential breeding value of FDR and SDR gametes.

Key words: potato, meegasporogenesis, first division restitution, second division restitution, isozyme.


Chez l’haploïde 4182t (2n = 2x = 24) de Solanum tuberosum L., laquelle possède deux modes de production d’oösphères 2n, le rapport entre la première division de restitution (PDR) et la seconde division de restitution (SDR) des oösphères 2n a été évalué. Chez les descendants tétraploïdes issus d’un croisement 2x × 4x, la ségrégation de trois gènes antérieurement cartographiés en fonction de leur position par rapport au centromère, soit les Pgm-2 (2.0 cM), Mdh-1 (33.5 cM) et 6-Pgdh-3 (30.1 cM), a été analysée. Sur la base de la ségrégation du locus Pgm-2, 39.7% des descendants sont issus d’oösphères 2n dérivées de la PDR et 60,3% de la SDR. Les patrons de ségrégation des deux locus distaux de l’intérieur de la sous-population 4x dérivée de la PDR indiquent que le taux de recombinant gène–centromère au cours de la gynosporogenèse a été significativement réduit pour le locus Mdh-1 par comparaison aux estimés antérieurs établis lors de l’androsporogenèse. Chez les sous-populations 4x dérivées de la SDR, les taux de recombinant pour les locus Mdh-1 et 6-Pgdh-3 n’ont pas différé significativement des estimés antérieurs. Les descendants tétraploïdes résultant d’un croisement 2x × 4x, dont les parents 2x produisent des gamètes 2n de façon bimodale, peuvent être utilisés pour établir une comparaison non biaisée de la valeur potentielle d’amélioration des gamètes PDR et SDR.

Mots clés : pomme de terre, gynosporogenèse, première division de restitution, seconde division de restitution, isozyme.

[Traduit par la rédaction]

Introduction

Occurrence of 2n gametes (gametes with the sporophytic chromosome number) has been well documented in potato, Solanum tuberosum (2n = 4x = 48) (den Nijs and Peloquin 1977; Watanabe and Peloquin 1989; Werner and Peloquin 1991). The identification of a high frequency of both 2n gamete producing plants and 2n gametes per se among many wild species and haploids extracted from the cultivated tetraploids indicates the importance of sexual polyplidization in the origin and evolution of the polyploid series in potato. These findings led to the development of modern breeding methods of the cultivated tetraploid potato; production of 4x progeny via 4x-2x (2x × 4x, 4x × 2x) and 2x-2x crosses as a means to effectively exploit the abundant genetic diversity of the diploid Solanum species (Peloquin et al. 1989).

There are two main modes of 2n gamete formation in potato: first division restitution (FDR) and second division restitution (SDR). Determination of the mode is important, since FDR and SDR differ in their genetic consequences. FDR 2n gametes transmit greater heterozygosity from the 2x parents to the 4x progeny than SDR 2n gametes; higher levels of heterozygosity have been considered to contribute to the higher yields of 4x progeny generated from 4x × 2x (FDR) in comparison to 4x from 4x × 2x (SDR) crosses (Mok and Peloquin 1975; Mediburu and Peloquin 1977; Peloquin et al. 1989). Various methods have been employed to determine the mode of 2n gamete production. Cytological and genetic analyses have revealed that 2n pollen in potato is predominantly formed owing to the parallel spindles mechanism which leads to FDR-type gametes (Mok et al. 1976), while SDR is the principal mode in the formation of 2n eggs (Stelly and Peloquin 1986; Werner and Peloquin 1991). The last authors, using microscopic observations, reported the occurrence of 2x potato clones (haploids of S. tuberosum and wild species) that produced 2n eggs by more than one mechanism representing the two genetic modes.
Diploid clones that form 2n gametes provide a means to perform half-tetrad analysis (HTA) in 4x progeny from 2x-4x crosses as a genetic test, either for gene mapping in relation to the centromere when the mode is known, or to determine the mode(s) of 2n gamete formation when gene-centromere relationships are estimated. The basis for HTA is that two strands of a bivalent from the diploid parent are recovered together in tetraploid progeny. HTA has been applied to a number of organisms that form 2n gametes, e.g., maize (Rhoades and Dempsey 1966; Nel 1975), rainbow trout (Thorgaard et al. 1983), paradise fish (Gervai and Csanyi 1984). In potato, gene-centromere linkages have been estimated for a yellow tuber flesh marker (Y) (Mok et al. 1976; Stelly and Peloquin 1986; Douches and Quiros 1987), blue pigmentation locus (P) (Mendiburu and Peloquin 1979), and 11 isozyme loci (Douches and Quiros 1987, 1988). Because of their codominant expression, isozyme markers offer unbiased estimations compared with morphological markers. In addition, the linkage data indicate that these markers are distributed over at least 8 of 24 chromosome arms in the potato genome with a wide range of distances from the centromeres (0.9–33.5 cM).

A marker tightly linked to the centromere is of special value for HTA when a 2x clone producing 2n gametes heterozygous for a marker is crossed to a monoallelic 4x clone (Aa × aaaa). In this case, no recombination is expected between the centromere and the locus. If 2n gametes are formed by FDR, all progeny would be simplex for the marker (Aaaa); on the other hand, for 2n gametes formed by SDR, half of the progeny would be duplex (A4aa) and the other half nulliplex (aaa). If a 2x clone produces 2n gametes by both modes, the segregation of a tightly linked marker in a 2x×4x cross would provide an estimate of the ratio of FDR and SDR 2n eggs within a 2x parent. The situation is more complex for a gene distal to the centromere (single exchange tetrad is expected), since the differences between the two modes of 2n egg formation are less pronounced (25% of nulliplex with FDR and 0% with SDR) (Mendiburu and Peloquin 1979).

The objective of this study was to apply HTA to determine the frequency of FDR and SDR 2n eggs in a haploid of *Solanum tuberosum* that had been cytologically determined to produce 2n eggs by both modes. Cytological classification alone can often lead to a biased estimation, while HTA takes into account only those 2n gametes which contribute to the offspring.

### Materials and methods

A haploid 4182t (2n = 2x = 24) extracted from the advanced breeding line W231 of *Solanum tuberosum* has been cytologically determined to produce 2n eggs by three mechanisms: delayed first meiotic division and omission of the second meiotic division (Warner and Peloquin 1991) and a synaptic variant (Werner and Peloquin 1987). This clone was cytologically reexamined to determine the frequencies of the different mechanisms by the whole ovule clearing technique as described by Stelly et al. (1984).

The haploid has been determined to be heterozygous for three unlinked isozyme loci: *Pgm-2*, 6-*Pgdh*-3, and *Mdh-1*, previously mapped 2.0, 30.1, and 33.5 cM from their centromeres, respectively (Douches and Quiros 1987). The tetraploid used as a pollen parent in 2x × 4x crosses, NDD277-2, is homozygous (nulliplex) for all three markers. The 2x × 4x crosses were performed in the greenhouse during the winter months with supplemental lighting (16-h daylight). The average greenhouse temperature was 25°C.

### Table 1. Segregation of 4x progeny from the 4182t × NDD277-2 (2x × 4x, *Pgm-2*^2^ × *Pgm-2*^2^ × *Pgm-2*^2^ × *Pgm-2*^2^) cross for the *Pgm-2* locus

<table>
<thead>
<tr>
<th>Locus genotype</th>
<th>Mode</th>
<th>No. of 4x progeny</th>
<th>% of 4x progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pgm-2</em>^2^<em>Pgm-2</em>^2^<em>Pgm-2</em>^2^<em>Pgm-2</em>^2^ (duplex)</td>
<td>SDR</td>
<td>98</td>
<td>33.6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>292</td>
<td>100</td>
</tr>
</tbody>
</table>

The leaf tissue from 4-week-old greenhouse seedlings (2n = 4x) was sampled for electrophoretic analyses according to Quiros (1981) and Douches and Ludlam (1991). *x*^2^ tests and 95% confidence intervals were calculated to determine the gene-centromere recombination rates for both FDR and SDR 2n eggs. The segregation of the *Pgm-2* locus (2.0 cM from the centromere) was used to discriminate between FDR- and SDR-derived progeny. All simplex progeny (*Pgm-2*^2^*Pgm-2*^2^*Pgm-2*^2^*Pgm-2*^2^) were designated FDR-derived, while duplex and nulliplex progeny (*Pgm-2*^2^*Pgm-2*^2^*Pgm-2*^2^*Pgm-2*^2^, respectively) were SDR-derived. The segregation of the distal loci (*Mdh-1* and 6-*Pgdh*-3) was examined in each of the subpopulations defined by the segregation of the *Pgm-2* locus to estimate recombination rates in the 2x parent.

### Results

Cytological analysis indicated that in the 4182t haploid, there were 56.2 and 56.4% 2n megaspores and 2n megagametophytes, respectively. The rate of aborted ovules was 29.2%. Among a total of 421 ovules observed, 108 represented stages of meiosis leading to 2n megaspores that allow classification of a developing megaspore into either FDR or SDR mode: 15% of the cells analyzed at meiosis displayed delayed first meiotic division (both FDR and SDR), 36.4% synaptic variant (FDR), and 48.6% omission of the second meiotic division (SDR).

Two hundred and ninety-two seedlings were electrophoretically examined. Figure 1 presents the banding patterns for the PGM system of segregating 4x progeny from the 2x × 4x cross. The segregation ratio of the *Pgm-2* locus in the 4x offspring indicated that 116 plants were simplex (*Pgm-2*^2^*Pgm-2*^2^*Pgm-2*^2^*Pgm-2*^2^), 98 duplex (*Pgm-2*^2^*Pgm-2*^2^*Pgm-2*^2^*Pgm-2*^2^), and 78 nulliplex (*Pgm-2*^2^*Pgm-2*^2^*Pgm-2*^2^*Pgm-2*^2^). There was no significant difference between the frequency of duplex and nulliplex progeny (*x^2^ = 2.27, p = 0.52). The results indicate that 39.7% of the progeny originated from FDR 2n eggs and 60.3% from SDR (Table 1).

The segregation data for the FDR-derived subpopulation are summarized in Table 2. The observed recombination rates for the two distal loci (21.6% for *Mdh-1* and 25.9% for 6-*Pgdh*-3) were lower than expected (33.5 and 30.1%, respectively); however, the gene–centromere recombination rate for the 6-*Pgdh*-3 locus was not significantly different from the expected rate. The expected values represent the distance of the locus in relation to the centromere based on the frequency of recombinants as previously determined by Douches and Quiros (1987). The segregation of the SDR-derived subpopulation is summarized in Table 3. According to paired *t*-tests, the observed gene–centromere recombination rate for the *Mdh-1* locus (28.7%) was not significantly lower (p > 0.05) than expected (33.5%), whereas the observed recombination rate for the 6-*Pgdh*-3 locus (35.0%) was not greater than expected (30.1%).

<table>
<thead>
<tr>
<th>TABLE 1. Segregation of 4x progeny from the 4182t × NDD277-2 (2x × 4x, <em>Pgm-2</em>^2^ × <em>Pgm-2</em>^2^ × <em>Pgm-2</em>^2^ × <em>Pgm-2</em>^2^) cross for the <em>Pgm-2</em> locus</th>
<th>Locus genotype</th>
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<td></td>
<td>292</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Segregating 4x progeny from the cross 4182t × NDD277-2 (2x × 4x) for the Pgm-2 locus (two lower bands). The nulliplex, duplex, and simplex genotypes are designated by N, D, and S, respectively.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Progeny size</th>
<th>Genotypic classification</th>
<th>Gene–centromere recombination rate (%)</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nulliplex</td>
<td>Duplex</td>
<td>Simplex</td>
<td></td>
</tr>
<tr>
<td>Mdh-1</td>
<td>116</td>
<td>12</td>
<td>13</td>
<td>91</td>
<td>21.6±3.8</td>
</tr>
<tr>
<td>6-Pgdh-3</td>
<td>116</td>
<td>15</td>
<td>104</td>
<td>—</td>
<td>25.9±4.1</td>
</tr>
</tbody>
</table>

aFrom Douches and Quiros (1987).

bSum of both duplex and simplex genotypes.

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<tr>
<th>Locus</th>
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<td></td>
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<td>Nulliplex</td>
<td>Duplex</td>
<td>Simplex</td>
<td></td>
</tr>
<tr>
<td>Mdh-1</td>
<td>176</td>
<td>34</td>
<td>41</td>
<td>101</td>
<td>28.7±3.4</td>
</tr>
<tr>
<td>6-Pgdh-3</td>
<td>173</td>
<td>26</td>
<td>147</td>
<td>—</td>
<td>35±0±3.6</td>
</tr>
</tbody>
</table>

aFrom Douches and Quiros (1987).

bSum of both duplex and simplex genotypes.

Discussion
The mode of 2n egg formation is of practical significance. For 2x clones producing gametes with the unreduced chromosome number exclusively by one mechanism, cytological analysis is a quick and sufficient method to determine the mode of 2n gamete formation. However, when a 2x parent produces 2n eggs by both modes, microscopic examination may be inconclusive. Cytological estimation does not take into consideration the number of ovules that abort owing to improper chromosome distribution or nuclear restitution events necessary for the formation of FDR 2n eggs. The percentage of aborted ovules in the reported haploid accounts for the discrepancy in the number of FDR and SDR 2n eggs between cytological and genetic analyses. Both delayed meiotic division and the synaptic variant have been associated with abortion of ovules (Werner and Peloquin 1991). On the other hand, clones producing 2n eggs owing to omission of the second meiotic division, a mechanism that has balanced divisions during megagametogenesis, usually have a low number of aborted ovules (Werner and Peloquin 1991). HTA together with cytological analysis allows for unbiased estimation of viable FDR and SDR 2n gametes that contribute to the 4x progeny in interploidy crosses.

The separation into SDR and FDR subpopulations was based on one proximal locus (Pgm-2; 2 cM). Misclassification is occasionally expected owing to recombination. With a population size of 292, assuming a 2010 recombination rate, approximately two FDR-derived and four SDR-derived progeny would be misclassified. Douches and Quiros (1987) used two other proximal loci (Got-1 and Sdh-1) to discriminate between FDR and SDR 2n eggs in 2x × 4x crosses. Examination of these additional loci would reduce the misclassification, but these loci were not heterozygous in the haploid parent. Since the number of misclassifications would have been small relative to the total population size, the observed ratio between FDR- and SDR-derived progeny was not distorted and should not have significantly altered the observed gene–centromere recombination rate estimates for Mdh-1 and 6-Pgdh-3.

FDR 2n gametes transmit more heterozygosity from the 2x parent to the 4x progeny than SDR gametes (Peloquin
et al. 1990). The level of heterozygosity may be important in tetrasomic polyploids; maximum heterozygosity leads to heterosis for polygenic traits for which inter- and intra-locus interactions are important, e.g., total tuber yield in potato. Based on the results reported herein, the cited superiority of FDR 2n gametes per se does not correlate to the superiority of 4x FDR-derived progeny. There is no evidence for gametophytic selection or developing seed competition between embryos that would favor FDR over SDR 2n gametes. Survival of the particular mode of 2n gametes seems to be unaffected by the mechanism of 2n gamete formation.

Lower gene–centromere recombination rates than previously estimated were observed for the distal loci in the FDR subpopulation (Table 2), whereas recombination rates in the SDR subpopulation were not conclusive (Table 3). These results may be attributed to the synaptic variant that is expressed at meiosporegenesis. Several synaptic variants have been identified in potato that affect microsporogenesis (Johnston et al. 1981; Okwagwu and Peloquin 1981; Iwanaga 1984; Ramanna 1983). In general, cytological observations of these mutants indicate a lack of pairing during diplonem and diakinesis stages, followed by random distribution of the chromosomes during the first division. For example, the sy3sy3 genotype leads ultimately to pollen sterility unless accompanied by the parallel spindle mechanism (Okwagwu and Peloquin 1981). Douches and Quiros (1988) estimated 89% reduction in recombination in a synaptic mutant of the potato (sy3sy3, psps) that produced FDR 2n pollen. The reduction in recombination for the distal loci reported herein was considerably less pronounced (35.5% for Mdh-1, 13.6% for 6-Pgdh-3). This may in part be due to the facts that two mechanisms lead to FDR 2n eggs in 4182t haploid and it is unknown whether the delayed meiotic division mechanism affects recombination. To estimate the effect that the synaptic variant has on recombination during meiosporegenesis, one needs to measure the recombination rate in diploid clones that produce 2n eggs solely by this mechanism. Jongedijk et al. (1991) determined that sex differences in recombination did not occur in desynaptic (ds-ids-1) diploid potato clones. Under these assumptions that the same level of recombination occurs in the synaptic variant affecting meiosporegenesis as in the synaptic variant expressed in microsporegenesis (sy3sy3), and the delayed meiotic mechanism does not reduce recombination, one could estimate that about 40% of the viable FDR 2n eggs were derived from the synaptic variant. Cytological analysis, however, indicated 70.8% of FDR 2n eggs to be produced by the synaptic variant and 29.2% owing to delayed meiotic division. Therefore, considering low viability of the 2n eggs derived from delayed meiotic division (Werner and Peloquin 1991), it is unlikely that the synaptic variant reduced recombination in meiosporeogenesis to the same extent as those variants observed in microsporeogenesis.

The predominant mode of 2n egg formation in potato is SDR (omission of the second meiotic division). However, Werner and Peloquin (1991) reported that of the 2n egg producing clones, 42% of the haploids and 47% of the wild species formed 2n eggs by two modes, mainly owing to synaptic variant and omission of second division. The mixture of FDR and SDR 2n eggs in one clone was also indicated by Stelly and Peloquin (1986) based on genetic analysis, and by Conicella et al. (1991) from cytological observation. Furthermore, based on the number of mechanisms described for 2n gametes in potato, 2n eggs are formed by more divergent mechanisms than 2n pollen. Genes controlling 2n egg formation are less exposed to selection pressure than genes controlling 2n pollen production owing to both the course of gametogenesis and the biology of fertilization. Occurrence of FDR 2n eggs among SDR 2n eggs might be also an evolutionary means to introduce more heterozygosity from the parent to the progeny in a manner similar to occasional outcrossing that was found in otherwise self-pollinated plants.

From the practical point of view, occurrence of 2n potato clones producing 2n gametes by two modes creates the ideal conditions for unbiased comparison of the value of both FDR and SDR 2n gametes. In the experiments conducted to date, owing to unavailability of desired clones, the breeding value of FDR and SDR 2n gametes has been partially confounded with allelic diversity of the 2x parents since different 2x clones producing either FDR or SDR 2n pollen were used in 4x–2x crosses (Mok and Peloquin 1975). When clones that form 2n gametes by two genetically different modes are utilized, the 4x progeny obtained from 2xFDR × 4x and 2xSDR × 4x crosses would be generated from the same genotypes. Moreover, the mode of 2n gamete formation in the 2x parent that led to 4x progeny can be determined by HPA. Therefore, the differences detected between 4x progeny from such a cross could be attributed to the differences in transmission of heterozygosity through 2n gametes.

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