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THE INHERITANCE OF THREE MECHANISMS OF DIPLANDROID (2*n* POLLEN) FORMATION IN DIPLOID POTATOES

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SUMMARY

Genetic data obtained from seven test-crosses (159 progeny) substantiated an earlier hypothesis that all three mechanisms of diplandroid formation (parallel spindles, *ps*; premature cytokinesis-1, *pc-1*; and premature cytokinesis-2, *pc-2*) are controlled by simple recessive mutations. Results of tests for allelism suggested that *pc-1* and *pc-2* are not allelic. The expression of *ps*, *pc-1* and *pc-2* at the tetraploid level was observed when the tetraploid is nulliplex at the corresponding locus.

1. INTRODUCTION

THREE mechanisms of 2*n* pollen (diplandroids) formation in diploid potatoes have been discovered (Mok and Peloquin, 1975). In each, microsporogenesis is modified and leads to the production of diplandroids (male gametophytes and gametes with the somatic chromosome number). The genetic consequences of these mechanisms are distinctly different and of two types. Parallel spindles (*ps*) result in diplandroids which are genetically equivalent to first division restitution (FDR) gametes (Mok and Peloquin, 1974). The cytological event that leads to diplandroid formation is the parallel orientation of Anaphase II spindles during microsporogenesis, in contrast to normal *n* microspore formation where Anaphase II spindles are at an angle of about 60 degrees. Nuclei at each pole restitute, and following cleavage furrow formation, dyads are formed instead of tetrads. The other two mechanisms of diplandroid formation, premature cytokinesis-1 (*pc-1*) and premature cytokinesis-2 (*pc-2*), give meiotic products which are genetically equivalent to second division restitution (SDR) gametes. Both premature cytokinesis-1 and premature cytokinesis-2 are characterised by the occurrence of cytokinesis after the first meiotic division (which does not normally occur), and the ommission of the second meiotic division. They differ in that in premature cytokinesis-1 there is irregular movement of bivalents to and from the Metaphase I plate and the chromatids fall apart at Telophase I. The premature cytokinesis occurs after Telophase I in premature cytokinesis-1 and at Prophase II in premature cytokinesis-2.

These meiotic mutants are of interest not only for cytological studies, but they also provide excellent materials for various genetic investigations and new, exciting approaches to plant breeding. It is important to know the

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mechanism of diplandroid formation so that the genetic consequences can be determined. For example, FDR gametes provide a powerful tool in breeding since the parental genotypes, especially with regard to intra- and inter-locus interactions, are largely transmitted from parent to offspring.

In order to exploit fully the potential offered by diplandroids, the inheritance of mechanisms leading to their formation must be determined. Our earlier studies (Mok and Peloquin, 1975), suggested that parallel spindles, premature cytokinesis-1 and premature cytokinesis-2 are controlled by simple recessive mutation. This paper deals with the results of further genetic studies including: (1) testing our earlier hypothesis of simply inherited mutants; (2) test of allelism between *pc-1* and *pc-2*; and (3) expression of these three mutants at the $4x$ level.

2. Materials and methods

Hybrids between *Solanum tuberosum* Group Phureja ($2n = 2x = 24$) introductions, and haploids ($2n = 2x = 24$) of *S. tuberosum* Group Tuberosum ($2n = 4x = 48$) provided the plant material. The initial studies centred on six parental diploid clones (W1268.8, W5293.3, W5293.6, W5295.7, W5337.3 and W5853.2) and 147 progeny obtained from crosses

Table 1

Proposed genotypes of parental clones based on progeny distribution of initial crosses with regard to three mechanisms of diplandroid formation

Clone			
W1268·8	*PS/ps*	*PC-1/PC-1*	*PC-2/PC-2*
W5288.2	*ps/ps*	—	—
W5293.3	*ps/ps*	*pc-1/pc-1*	—
W5293.6	*PS/ps*	*PC-1/PC-1*	*PC-2/PC-2*
W5295.7	*ps/ps*	*PC-1/pc-1*	*PC-2/pc-2*
W5337.3	*ps/ps*	*PC-1/pc-1*	—
W5853.2	*PS/ps*	*PC-1/PC-1*	*pc-2/pc-2*

between them. Meiosis in each parental clone was examined to detect the presence and method of diplandroid formation. All progeny obtained from matings between parental clones were classified according to the mechanism of diplandroid formation after cytological examination of more than 250 microsporocytes from each clone. Tentative genotypes of each parental clone, with regard to the mechanisms of diplandroid formation, were assigned based on the results of initial progeny distributions and phenotypes of these parental clones (table 1).

To test the genetic hypothesis of simple recessive mutation controlling these mechanisms of diplandroid formation, seven test-crosses were made. Microsporogenesis in 159 clones from the seven families was analysed for mechanism of diplandroid formation. The expected distribution of progeny in each family, according to the mechanisms of diplandroid formation, was calculated based on the tentatively assigned genotypes of the parents and was compared with the actual distribution from these test-crosses.

Diplandroid formation by premature cytokinesis-1 and premature cytokinesis-2 is similar in that cytokinesis is premature and there is no second division. These two mechanisms could either be controlled by separate loci both affecting the premature occurrence of cytokinesis, or premature

cytokinesis-1 could be under the regulation of two very closely linked loci; one locus affecting premature cytokinesis which is allelic with the gene *pc-2*, and the other irregular chromosome movement at first meiotic division. To distinguish between these two possibilities, microsporogenesis in progeny obtained from the following crosses was examined: W5853.2 (*pc-2*/*pc-2*) × W5293.3 (*pc-1*/*pc-1*) and L1-12 (*pc-2*/*pc-2*) × 1J-25 (*pc-1*/*pc-1*).

Microsporogenesis in tetraploids generated from diploid-diploid matings was examined in order to study the expression of *ps*, *pc-1* and *pc-2* at the tetraploid level. The parental diploid clones were all homozygous for the locus being tested. Tetraploid progeny derived from bilateral sexual polyploidisation (from 2*n* eggs and 2*n* pollen) from the following crosses were examined for mechanism of diplandroid formation: (1) W5293.3 (*ps*/*ps*) × W5295.7 (*ps*/*ps*); (2) W5293.3 (*pc-1*/*pc-1*) × 1J-25 (*pc-1*/*pc-1*); and (3) W5853.2 (*pc-2*/*pc-2*) × L1-12 (*pc-2*/*pc-2*).

3. Results

(i) *Parallel spindles*

In our previous studies, all matings between two parents with parallel spindles had yielded progeny with parallel spindles (W5295.7 × W5293.3 and W5295.7 × W5337.3). About one-half of the progeny obtained from matings between parents with and without parallel spindles (W1268.8 × W5295.7, W5295.7 × W5293.6 and W5853.2 × W5295.7) had the same phenotype.

The results of new test-crosses between clones with and without parallel spindles are presented in table 2. The test-crosses 1, 2, 4, 6, 7, all agree with a 1:1 ratio. All offspring obtained from matings between parents with parallel spindles (cross 3) have parallel spindles. The progeny from a cross (cross 5) between parents without parallel spindles, but thought to be heterozygous for the gene *ps*, on the basis of initial crosses, fit a 3: 1 ratio. Thus, data obtained from new crosses support our previous hypothesis that parallel spindles are controlled by a single recessive mutation.

(ii) *Premature cytokinesis-1*

The earlier discovery of progeny with premature cytokinesis-1 from a mating between parents without premature cytokinesis (W5295.7 × W5337.3) suggests that the gene determining this phenotype is recessive. According to the progeny distributions in initial crosses, it appeared that a simple recessive mutation causes the observed cytological abnormality. This hypothesis was tested by crossing clones of tentatively assigned genotypes and determining the frequency of clones with premature cytokinesis-1 in the progeny. Diplandroids were formed by premature cytokinesis-1 in half of the offspring from the mating between W5337.3 × W5293.3, which is as expected (table 2). Premature cytokinesis-1 was not observed in other matings involving at least one parent tentatively identified as homozygous dominant for *pc-1* (crosses 2, 4 and 5). The limited data confirm the hypothesis that premature cytokinesis-1 is due to a recessive mutation.

(iii) *Premature cytokinesis-2*

Premature cytokinesis-2 was previously observed only in microsporogenesis of W5853.2 and one-half the progeny from W5853.2 × W5295.7.

TABLE 2

Distribution of progeny by mechanism of diplandroid formation from test-crosses: obtained/(expected)

Clone		No. of Fls	Normal	Parallel spindles	Parallel spindles + premature cyto-kinesis-1	Parallel spindles + premature cyto-kinesis-2	Premature cyto-kinesis-2	Parallel spindles + premature cyto-kinesis-3	Premature cyto-kinesis-3
1. W5288.2 (*ps/ps* — —)	× W1268.8 (*PS/ps PC-1/PC-1 PC-2/PC-2*)	12	5 (6)	7 (6)					
2. W5293.3 (*ps/ps pc-1/pc-1 PC-2/PC-2*)	× W1268.8 (*PS/ps PC-1/PC-1 PC-2/PC-2*)	27	9 (13·5)	10 (13·5)				4 (0)	4
3. W5337.3 (*ps/ps PC-1/pc-1 PC-2/PC-2*)	× W5293·3 *ps/ps pc-1/pc-1 PC-2/PC-2*)	23		13 (11·5)	10 (11·5)				
4. W5337.3 (*ps/ps PC-1/pc-1 PC-2/PC-2*	× W1268.8 (*PS/ps PC-1/PC-1 PC-2/PC-2*)	39	20 (19·5)	19 (19·5)					
5. W5853.2 (*PS/ps PC-1/PC-1 pc-2/pc-2*)	× W1268.8 (*PS/ps PC-1/PC-1 PC-2/PC-2*)	14	11 (10·5)	3 (3·5)					
6. W5853.2 (*PS/ps PC-1/PC-1 pc-2/pc-2*)	× W5293.3 (*ps/ps pc-1/pc-1 PC-2/PC-2*)	24	10 (12)	14 (12)					
7. W5853.2 (*PS/ps PC-1/PC-1 pc-2/pc-2*)	× K1-17 (*PS/ps PC-1/pc-1 PC-2/pc-2*)	20	6 (5)	6 (5)			5 (5)	3 (5)	

Offspring obtained from matings between W5853.2 × K1-17 (crosses 7) were examined. If our tentatively assigned genotypes to each parental clone are correct, then K1-17, which forms diplandroid by parallel spindles only and is an F_1 clone from W5853.2 (*pc-2/pc-2*) × W5295.7 (*PC-2/pc-2*), must be heterozygous for *pc-2*. Eight out of 20 clones obtained produce diplandroids by premature cytokinesis-2. The evidence of this phenotype being controlled by a simple recessive mutation is supported by the result of this test-cross.

(iv) *Test of allelism between* pc-1 *and* pc-2

The results of tests for allelism are presented in table 3. Neither premature cytokinesis-1 nor premature cytokinesis-2 was observed in progeny obtained from the cross W5853.2 (*pc-2/pc-2*) × W5293.3 (*pc-1/pc-1*). This finding indicates that *pc-1* and *pc-2* are not allelic. Progeny obtained from the mating between L1-12 (*pc-2/pc-2*) and 1J-25 (*pc-1/pc-1*) provide additional

TABLE 3

Results of tests of allelism of pc-1, pc-2.

Cross	Number of progeny	Normal	Premature cytokinesis	
W5853.2 × W5293.3	24	24	0	Expected if *pc-1* and *pc-2* are not allelic
(*pc-2/pc-2*) (*pc-1/pc-1*)		0	24	Expected if *pc-1* and *pc-2* are allelic
	Obtained	24	0	
L1-12 × 1J-25	12 (4*x*)	12	0	Expected if *pc-1* and *pc-2* are not allelic
(*pc-2/pc-2*) (*pc-1/pc-1*)		0	12	Expected if *pc-1* and *pc-2* are allelic
	Obtained	12	0	

support for this view. Twelve clones were obtained; they are all tetraploids, which is not unexpected since L1-12 produces a high number of 2*n* eggs, and 1J-25 forms 98 per cent 2*n* pollen. Premature occurrence of cytokinesis was not observed in any of these progeny. This observation again indicated the non-allelism of *pc-1* and *pc-2* based on the assumption that premature cytokinesis-1 and premature cytokinesis-2 are also expressed at the tetraploid level. This assumption appears to be valid based on the studies of microsporogenesis of tetraploids synthesised from interdiploid matings (next section).

(v) *Expression of parallel spindles, premature cytokinesis-1 and premature cytokinesis-2 at the tetraploid level*

Five tetraploids derived from crossing W5293.3 (*ps/ps*) with W5295.7 (*ps/ps*) were found to produce diplandroids (in this case the gametes have the chromosome number of 48) by parallel spindles. The frequency of diplandroids varied from 3 to 20 per cent. Since both parents are homozygous for *ps*, it is apparent that tetraploids obtained are nulliplex for *ps* and that the expression of parallel spindles is not altered at the tetraploid level. Six tetraploids generated from the cross W5293.3 (*pc-1/pc-1*) × 1J-25 (*pc-1/pc-1*) were found to produce diplandroids by premature cytokinesis-1. The frequency of diplandroid formation ranged from 14 to 34 per cent. Two

tetraploids obtained from mating two diploids homozygous for *pc-2* (W5853.2 × L1-12) were found to form diplandroids by premature cytokinesis-2 and the frequencies were 14 and 18 per cent, respectively. Thus both premature cytokinesis-1 and premature cytokinesis-2 are expressed at the tetraploid level when nulliplex.

(vi) *The discovery of premature cytokinesis-3*

A mechanism of diplandroid formation previously not detected was discovered when microsporogenesis was examined in progeny obtained from the mating between W5293.3 × W1268.8 (table 2, cross 2). This mechanism appears to affect microsporogenesis in a similar fashion to *pc-2*, and it was termed premature cytokinesis-3. If premature cytokinesis-3 and premature cytokinesis-2 are identical, premature cytokinesis should have been observed in offspring obtained from W1268.8 × W5295.7 since W5295.7 is heterozygous for *pc-2* and W1268.8 must be " heterozygous " for premature cytokinesis-3, but it was not found. In addition, cytological analysis of microsporogenesis in 14 clones obtained from the mating of W5853.2 × W1268.8 (table 2, cross 5) did not reveal the occurrence of premature cytokinesis. These two observations suggest that premature cytokinesis-3 is different from premature cytokinesis-2; however, the exact nature of inheritance of premature cytokinesis-3 is not known.

4. Discussion

Genetic data obtained from initial and test-crosses indicate that the three mechanisms of diplandroid formation are controlled by simple recessive mutants. The frequency of diplandroids ranged from 3 to 99 per cent. This varying degree of expressivity of these mutants could be due to the highly variable genotypes of the different clones. Clones that are homozygous recessive at more than one of these three loci can produce diplandroids by more than one mechanism. However, only one mechanism is functional in an individual pollen mother cell.

Many meiotic mutants have been found in *Drosophila* (Sandler, Lindsley, Nicoletti and Trippa, 1968) and yeast (Hartwell, Culotti and Reid, 1970). These mutants can affect pairing of homologous chromosomes, the frequency of genetic exchange, cytokinesis and fertility. Most of these mutations reported are simple recessives. Mutants which modify meiosis and lead to $2n$ gamete formation were reported in corn: the elongate gene (Rhoades and Dempsey, 1966), and *Datura*, the dyad gene (Satina and Blakeslee, 1935). Premature cytokinesis-1 and -2 are similar to these mutants in that second meiotic division is omitted and SDR gametes are formed. However, limited results obtained from studying $2n$ egg formation suggested that *pc-1* and *pc-2* do not affect megasporogenesis. Although gametes with the unreduced chromosome number have been reported in a number of plant species such as *Fragaria* (Bringhurst and Gill, 1970), *Dichanthium* (de Wet and Harlan, 1970), *Medicago* (Bingham, 1969), and possibly in ferns (Hickock and Klekowski, 1973), the mechanism of $2n$ gamete formation was not investigated. Parallel spindles is the only reported mechanism whereby first division restitution gametes are formed in higher plants.

Formation of first division restitution gametes by parallel spindles in diploids represents a unique situation. All heterozygous loci from the centromere to the first cross-over on each chromosome in the parent will be heterozygous in the gametes (Mendiburu, 1971). Thus not only heterozygosity, but also most of the interactions between loci are transferred to the offspring by FDR gametes. On average, it is estimated that approximately 75 per cent of the parental genotype is transmitted intact to the offspring through FDR gametes. It is obvious that FDR gametes provide an exciting opportunity for exploring new methods of plant breeding.

The successful formation of polyploids requires two conditions: the means of achieving polyploidisation, and advantage of the newly arisen polyploids. The formation of diplandroids under simple genetic control has significant implications in the evolution of polyploids in solanums. It suggests that clones capable of generating diplandroids may occur quite frequently in nature. Since diploid *Solanum* species are mostly cross compatible, the formation of polyploids from diploids through the functioning of $2n$ gametes is a most direct and simple method of polyploidisation. Furthermore, the result of the participation of FDR gametes from superior diploids in sexual polyploidisation is increased vegetative vigour of the newly arisen polyploid. Introgression of diploids into polyploids can also be maintained through $2n$ gametes from diploids. The occurrence of diplandroids in a number of diploid *Solanum* species (Quinn, Mok and Peloquin, 1974), and the discovery of a naturally occurring triploid *S. chacoense* which is homozygous for *ps* (Mok, Peloquin and Tarn, in press), provide initial evidence for the possible role of $2n$ gametes in evolution of the tuber-bearing solanums.

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