Spontaneous Structural Rearrangements in *Solanum phureja* Juz. et Buk. 3. Gene-centromere mapping of *ym* (yellow margin) by half-tetrad analysis

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In diploid ($2n=2x=24$) potatoes, several meiotic restitution mechanisms which can give rise to $2n$ gametes have been found (Ramanna 1974, 1979, Mok and Peloquin 1975). Based on the genetic consequences, two distinct modes of $2n$ gamete formation are distinguished: first division restitution (FDR) and second division restitution (SDR). Basically FDR $2n$ gametes include non-sister chromatids, whereas SDR $2n$ gametes comprise sister chromatids. Once the mode of $2n$ -gamete formation has been determined cytologically, half-tetrad analysis (HTA) can be used to map single genes with respect to the centromere (Mendiburu *et al.* 1974). HTA takes advantage of $2n$ -gamete formation in the diploid parent in $4x-2x$, $2x-4x$ or $2x-2x$ matings.

In potato, a number of loci have been mapped by establishing the map distance to the centromere through interploidy crosses. A summary of relative map distances of morphological characters and isozymes reported in the literature has been presented by Wagenvoort and Zimnoch-Guzowska (1992). The morphological marker *ym* (yellow margin) was mapped 34.16 centimorgans (cM) relative to the centromere via $4x-2x$ crosses, and 36.08 cM via $2x-4x$ crosses (Jongedijk *et al.* 1991). The diploid parents used in these HTAs were synaptic and produced $2n$ pollen by FDR or $2n$ eggs by SDR.

The diploid clones of *Solanum phureja* Juz. et Buk. designated 1931 and 1936 are heterozygous for an interchange between chromosome 3 and chromosome 12 and for one or two paracentric inversions (Wagenvoort 1988, 1995). Clone 1936 produced seeds after selfing. This result suggested that the *S* locus (gametophytic incompatibility) was involved in the interchange and had become inactive. Trisomics ($2n=2x+1=25$) were found in the first inbred generation of 1936 and were identified as tertiary trisomics or primaries being homozygous for the interchange (Wagenvoort 1995).

To establish the genetic constitution of both the normal chromosomes 3 and 12 and the interchanged chromosomes 3^{12} and 12^3 concerning the loci *S* and *ym*, and with respect to the breakpoints (T) of the interchange, it was necessary to ascertain the recombination rate of *ym* in the interchange heterozygote. If this recombination value could be compared with the estimate made by Jongedijk *et al.* (1991), who used a different approach, conclusions regarding the degree of interference, if any between *ym* and the centromere, could be drawn. In that case the relative map distance of *ym* to the centromere estimated in the interchanged chromosome can be used in further genetic analysis of progenies of 1931 and 1936. Thus, $4x \times 2x$ testcrosses were performed for mapping the recessive gene *ym* by HTA using the genotypes 1931 and 1936.

In this paper the relative distance of *ym* to the centromere in the interchange situation is estimated.

Materials and methods

Plant material

The diploid siblings *S. phureja* 75-1136-1931 and *S. phureja* 75-1136-1936, for the sake of brevity designated 1931 and 1936, were used in test-crosses and in HTA-mapping. Their pedigree has been described earlier in Wagenvoort (1988). The genotypes of these clones have been determined using the observed phenotype and the presence or absence of segregation in diploid progenies (Wagenvoort, unpublished results). Heterozygosity at the *ym*-locus in 1931 and 1936 was checked in the first inbred generation of 1936 and in an F_1 progeny of both clones. The diploid mutant for yellow margin, viz. Ym76-1-15 was selected from *S. phureja* (Wagenvoort 1982). From this clone a tetraploid ($2n=4x=48$) was produced through tissue culture (Wagenvoort, unpublished results) using the method by Roest and Bokelmann (1976).

Crosses were made on plants grafted onto tomato root stocks and on plants grown on bricks in a temperature-conditioned glasshouse.

Cytological methods and method of estimating gene-centromere map distance

Ploidy level of $4x-2x$ progenies and of plants from tissue culture was determined by counting the number of plastids in the stomatal guard cells after staining with iodine-potassium iodine. Whenever in doubt the number of chromosomes was counted in root meristems according to the method described by Wagenvoort and Lange (1975). Study of various stages of meiosis in the diploids were carried out as described by Wagenvoort and Ramanna (1979) in order to ascertain the mode of $2n$ -pollen formation.

For estimating the map distance of *ym* to the centromere in the interchange situation the $4x-2x$ cross was used since 1931 and 1936 produced $2n$ gametes only at the male side.

Mendiburu and Peloquin (1979) assumed only single-exchanges to occur in potato. Under that assumption the proportion of single-exchange tetrads is a linear function of the map distance between the locus in question and the centromere. In case of FDR the gene-centromere map distance = $2 \times (\text{frequency of nulliplex progeny}) \times 100 \text{ cM}$ (Mendiburn and Peloquin 1979). The binomial confidence interval was calculated using the expression

$$\hat{p} - 2\sqrt{\frac{\hat{p}\hat{q}}{N}} < p < \hat{p} + 2\sqrt{\frac{\hat{p}\hat{q}}{N}}$$

giving approximately 95% limits to p , where $\hat{p}$ and $\hat{q}$ are the frequencies of plants with and without the trait, respectively, and N is the total number of plants (Clarke 1982).

Results

Half-tetrad analysis with ym

The diploid clones 1931 and 1936 were studied at MII and later stages of meiosis in order to study the mode of $2n$ -pollen formation. In Table 1 the frequencies of normal spindles, fused spindles and parallel spindles at MII are presented along with the distribution of the chromosomes at AII/TII and the dyad, triad and tetrad frequencies at the sporad stage. Fig. 1 shows fused spindles at MII. The relative contribution of fused spindles to the pool of big pollen grains in the clones 1931 and 1936 was 52.9 and 66.7% respectively, whereas the dyad frequency exceeded the frequency of fused spindles. Fig. 2 shows a part of a PMC with dyads only. Triads were found in 3–5% of the PMCs analysed at the sporad stage. They will give rise to SDR or FDR $2n$ pollen depending on whether one of the equational walls has failed to form or the reductional wall is partly formed respectively (Ramanna 1974). Variation in the frequency of dyads occurred within and between anthers of the same plant. Therefore, because

Table 1. Frequencies of normal spindles (NS), parallel spindles (PS) and fused spindles (FS) at MII, chromosome distribution at AII/TII and the dyad, triad and tetrad frequency at the sporad stage in the diploid clones 1931 and 1936 from *S. phureja* Juz. et Buk.

Plant No.	Number of cells	MII (%)			Number of cells	AII/TII (%)			Number of cells	Sporad stage (%)		
		NS	PS	FS		12-12-12-12	12-12-24	24-24		tetrad	triad	dyad
1931	305	61	21	18	548	71	4	25	836	61	5	34
1936	979	70	20	10	1400	70	9	21	1491	82	3	15

Fig. 1. PMCs showing spindles with normal orientation (N), parallel orientation (P) and fused spindles (arrows) at MII.

Fig. 2. Part of a PMC, showing dyads only.

no other aberrations were observed, it was reasonable to conclude that fused and parallel spindles predominantly contributed to the pool of $2n$ pollen of 1931 and 1936, and consequently gave rise to FDR $2n$ pollen.

The $4x$ mutant for yellow margin was crossed to 1931 and 1936, the crosses being $ymymymym \times Ymym$. The triploid offspring of these $4x-2x$ crosses segregated for ym in 20 normal versus 26 mutant, which was consistent with the expected ratio 1:1 ($\chi^2=0.78$, $P>0.30$). In the tetraploid offspring the ratio 249 normal to 47 mutant was found. In the case of FDR $2n$ pollen all mutants originate from crossing-over between ym and the centromere. Using the formula of Mendiburu and Peloquin (1979) a map distance of 31.7 cM was calculated with a 95% binomial confidence interval of 27.5–35.9 cM.

Discussion

Parallel orientation of MII spindles may, but need not necessarily result in $2n$ -pollen formation (cf. Mok and Peloquin 1975, Ramanna 1979, Wagenvoort 1986). Parallel spindles found in 1931 and 1936 in this study may have partly contributed to the pool of $2n$ -pollen, indicated by the strong correlation between the occurrence of fused/parallel spindles, and dyads (Table 2).

The relative distance of ym (31.7 cM) to the centromere as found in this study was similar to that (viz. 34.16 cM) reported by Jongedijk *et al.* (1991) who in the $4x-2x$ testcross used the same $4x$ mutant and a synaptic diploid clone not carrying an interchange. The similarity of the map distances of ym assessed in both studies suggests that crossing over in 1931 and 1936 was not influenced by the presence of the interchange. This outcome also demonstrates that the position of the locus relative to the centromere has not been changed much owing to the interchange. Therefore, this map distance can be used in further genetic analysis of progenies from 1931 and 1936.

The relative map distance of ym was successfully estimated by half-tetrad analysis and the mode of $2n$ -pollen formation by cytological analysis. This map distance can be used for the placement of the gene on a gene map of potato.

Summary

A diploid ($2n=2x=24$) interchange heterozygote of *Solanum phureja* Juz. et Buk. produced first division restitution-equivalent $2n$ pollen by abnormal meiosis II spindle orientation. A relative map distance of 31.7 centimorgans was estimated by a half-tetrad analysis for the distance between ym (yellow margin) and the centromere. A similar distance was estimated for the non-interchange situation. These results suggest that crossing over in the interchange heterozygote is not influenced by the presence of the interchange.

Key words: interchange heterozygote, gene-centromere mapping, $2n$ pollen, *Solanum phureja* Juz. et Buk.

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