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Pollen markers for gene-centromere mapping in diploid potato

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Abstract The utility of two pollen genetic markers for estimating the extent of meiotic recombination between the centromere and a marker gene was tested in 2n pollen of diploid potato clones. One of these markers was the distal locus *amylose-free* (*amf*) on chromosome 8 and the other was the isozyme locus *alcohol dehydrogenase* (*Adh-1*) on chromosome 4. In the case of the *amf* locus, the gene-centromere distance was estimated in a normal synaptic and a desynaptic genotype. In both cases the genetic analysis was confined to: (1) a direct estimation of the phenotypic (blue vs red) segregation ratios in FDR (first-division restitution) 2n pollen and (2) a classification of the 4x progeny from 4x (nulliplex *amf*) × 2x (*Amf/amf*) crosses into duplex, simplex and nulliplex classes. The recombination frequency between the centromere and the *amf* locus in the normal synaptic genotype B92-7015-4 corresponded to a gene-centromere distance of 48.8 cM, whereas this distance amounted to 13.3 cM in the desynaptic genotype RS93-8025–1. Hence desynapsis reduced crossing-over by 73%. The observed genetic distance of 48.8 cM in the normal synaptic clone, B92-7015–4, is the highest gene-centromere distance reported so far in potato and this could be explained on the assumption of absolute chiasma interference. For the *Adh-1* locus, it was found that heterozygous 2n pollen grains could be detected in pollen samples of the diploid clones, because of the occurrence of a heterodimeric band of the isozyme. Unlike the *amf* locus, the gene-centromere distance for the *Adh-1* locus was estimated only on the basis of the duplex, simplex and nulliplex classes in the progenies from 4x (nulliplex $Adh\text{-}1^2$) × B92-7015–4 ($Adh\text{-}1^1/Adh\text{-}1^2$) crosses and was found to be 19.4 cM. Because the accurate positions of centromeres in relation to other loci are not available in the existing genetic maps of potato, which are saturated with molecular markers, half-tetrad analysis is a promising additional approach to the basic genetics of this crop.

Key words 2n gametes · Crossing-over · Desynapsis · Amylose-free starch · Alcohol dehydrogenase · *Solanum tuberosum*

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Introduction

As in many other crops, genetic maps of the diploid (2n=2x=24) cultivated potato, *Solanum tuberosum*, have become available in recent years (Bonierbale et al. 1988; Gebhardt et al. 1991; Tanksley et al. 1992; Jacobs et al. 1995; Van Eck et al. 1995). Despite the localization of numerous molecular and some morphological markers on the 12 possible linkage groups of potato, the maps do not accurately indicate the positions of the centromeres in relation to these marker loci. Information about centromere position is important for providing fixed points in the linkage groups of genetic maps, distinguishing both chromosome arms, identifying proximal and distal marker genes, and investigating interference. One method of localizing centromeres in relation to marker loci is through 'half-tetrad analysis' (HTA), or 'gene-centromere mapping', which has been successfully used in some plants (Rutishauser 1956; Nel 1975; Qu and Hancock 1995) as well as in fishes (Thorgaard et al. 1983; Allendorf et al. 1986; Seeb and Seeb, 1986; Liu et al. 1992; Johnson et al. 1996) and mammals (Eppig et al. 1983; Jarrell et al. 1995). However, the application of this method requires that the organisms studied produce numerically unreduced (2n) gametes or that their first-meiotic-division products can be isolated, and that the cytological mode of origin of 2n gametes is clearly known. In potato, 2n gametes occur frequently (Den Nijs and Peloquin 1977), and their cytological modes of origin have been elucidated as first-division restitution (FDR), second-division restitution (SDR), or a mixture of both types (Peloquin et al. 1989). One of these modes of origin

is fused spindle formation during metaphase-II and anaphase-II which gives rise to FDR gametes (Mok and Peloquin 1975; Ramanna 1979).

So far, HTA has been carried out in potato by monitoring segregation ratios in the tetraploid (2n=4x=48) progenies derived from crosses between 4x (nulliplex) × 2x (heterozygotes), or vice versa (Mendiburu and Peloquin 1979; Douches and Quiros 1987, 1988; Jongedijk et al. 1991 a; Wagenvoort and Zimnoch-Guzowska 1992; Werner et al. 1992; Barone et al. 1995). Instead of analysing the 4x progenies, it would be more efficient to monitor segregation for marker genes in the 2n gametes themselves. The combination of pollen-specific markers in diploid clones that produce 2n pollen through fused spindle formation offers the possibility of analyzing large samples of FDR 2n gametes in potato.

In the present investigation we have tested the utility of two pollen markers for such an approach. The first involves the monogenic recessive mutant *amylose-free* (*amf*), with a modified potato starch composition (Jacobsen et al. 1989). There are a number of advantages in using this genetic marker: (1) iodine-potassium iodide staining of the microspores enables unambiguous scoring of the wild-type (blue) and mutant (red) phenotypes; (2) large samples of both n and 2n microspores can be easily scored (Jacobsen et al. 1991): (3) at the tetraploid level, duplex, simplex, and nulliplex genotypes can be identified (Flipse et al. 1996), and (4) its locus is assigned to the distal part of chromosome 8 of potato (Gebhardt et al. 1991; Jacobs et al. 1995). The second genetic marker is the dimeric isozyme alcohol dehydrogenase (ADH), which is expressed in the pollen of potato and is found to be a proximal marker (Douches and Quiros 1987). In tomato, the ADH isozyme in mature pollen grains (Tanksley et al. 1981) is known to be encoded by the *Adh-1* locus on chromosome 4 (Tanksley et al. 1992). In the present investigation, the nomenclature *Adh-1* used in tomato has been retained for potato, because the locus that encodes the ADH isozyme in the mature pollen of potato has also been localized on chromosome 4 (Jacobs et al. 1995).

In order to test the effectiveness of gene-centromere mapping, the segregation data from 2n microspores in diploid heterozygotes were compared with those from progenies of 4x (nulliplex) × 2x (heterozygotes) crosses. The specific aims of the HTA were: (1) to estimate the genetic distance between the *amf* locus and the centromere; (2) to determine the effect of desynapsis (*ds-1*) (Jongedijk and Ramanna 1989) on the extent of crossing-over between *amf* and the centromere; and (3) to verify the proximal position of the *Adh-1* locus.

Materials and methods

Plant material

Three diploid (2n=2x=24) and five tetraploid (2n=4x=48) genotypes were generated from complex crosses between diploid *S. tuberosum* (*tbr*), *S. phureja* (*phu*), *S. microdontum* (*mcd*), and *S. verrucosum* (*ver*). The choice of the diploid genotypes was based on the following criteria: (1) allelic differences for amylose-free (*amf*) starch resulting from the defective granule-bound starch synthase (*GBSS*) locus on chromosome 8 (Gebhardt et al. 1991; Jacobs et al. 1995), (2) normal (*Ds-1/.*) versus desynaptic (*ds-1/ds-1*) chromosome pairing behaviour, and (3) the presence or absence of 2n pollen. The genotypes at the *Adh-1* locus on chromosome 4 (Tanksley et al. 1992; Jacobs et al. 1995) of the three selected clones were identified based on differences in the electrophoretic migration of ADH-1 proteins, demonstrating the presence of the alleles $Adh\text{-}1^1$, $Adh\text{-}1^2$, and $Adh\text{-}1^3$. The genotypes and parentages of each of the three diploids are as follows:

1. HB93-7108-08: *Amf/amf*; $Adh\text{-}1^2/Adh\text{-}1^3$; *Ds-1/.* This clone is an interspecific hybrid between clone B16 of *mcd* (BGRC 18568) and clone 87-1031-29 of *tbr* (Jacobsen et al. 1989), and produces only n pollen. It was used as a control for monitoring the segregation of the *amf* and *Adh-1* markers in n pollen.
2. B92-7015-4: *Amf/amf*; $Adh\text{-}1^1/Adh\text{-}1^2$; *Ds-1/.*. This clone produces a high frequency of 2n pollen. It is a hybrid between clone 880004-2 of predominantly *tbr* origin (Jacobsen et al. 1991) and clone IVP101 of predominantly *phu* origin (Hutten et al. 1994). B92-7015-4 was used for monitoring the segregation of the *amf* and *Adh-1* markers in both n and 2n pollen.
3. RS93-8025–1: *Amf/amf*; $Adh\text{-}1^2/Adh\text{-}1^2$; *ds-1/ds-1*. This clone was derived from the cross between clones 880004-11 and EC322, both of which are heterozygous for desynapsis (*Ds-1/ds-1*) (Jacobsen et al. 1991). In a population with 112 normal synaptic (*Ds-1/.*) and 28 desynaptic (*ds-1/ds-1*) plants, the clone RS93-8025-1 was the only one that expressed the desired four characters, viz., heterozygosity for *amf*, homozygosity for *ds-1*, male fertility, and the formation of 2n pollen.

The five tetraploid genotypes were all nulliplex for the *amf* marker. The clones J90-6001-25, J90-6011-3, J90-6020-17, J90-6020-22 were derived from *amf/amf/amf/amf* × *Amf/amf* crosses (Jacobsen et al. 1991), whereas clone HB93-7133-3 was derived from the selfing of a duplex genotype, involving the diploid clone BE1050 (Hutten et al. 1995) and the tetraploid clone J90-6011-3. Among the tetraploid clones, only HB93-7133-3 was homozygous for the *Adh-1* marker ($Adh\text{-}1^2/Adh\text{-}1^2/Adh\text{-}1^2/Adh\text{-}1^2$); all the others were heterozygous.

Plants were grown in the greenhouse either on bricks or as grafts on tomato root-stocks in order to induce flowering. The tetraploid female parents were emasculated 2–3 days before the opening of the flowers and pollinated when stigma were receptive. Berries were harvested about 6 weeks after pollination. The 4x progenies derived from 4x (nulliplex) × 2x (heterozygous) crosses were used to classify the duplex, simplex and nulliplex genotypes. Hereafter, these 4x progenies will be indicated as 4x.2x progenies.

Cytological analysis of 2n pollen formation

For the study of n and 2n microsporogenesis young anthers were fixed in a 3:1 mixture of ethanol and propionic acid (saturated with ferric acetate), and squashed in a 2% aceto-carmine solution. In these preparations, the number of bivalents was estimated in at least 50 cells. Since fused spindles give rise to FDR 2n pollen formation in both normal synaptic and desynaptic clones (Jongedijk et al. 1991 b), the frequency of fused spindle formation was investigated in 100–600 cells per clone. For the estimation of pollen stainability and the frequency of 2n pollen, 500–1000 mature pollen grains were counted after staining with lactophenol acid fuchsin. Within the sample of stainable pollen, 2n pollen was distinguished from normal reduced pollen on the basis of grain size and the number of germ pores (Jacobsen 1976; Ramanna 1979).

Iodine staining for starch phenotypes (*amf*-marker)

Anthers of flower buds were collected about 1 day before anthesis, and two anthers per bud were squashed in a drop of Lugol/chloral hydrate (1:2) (Jacobsen et al. 1991; Flipse et al. 1996). Microspores

with blue or red starch were counted. In the clones B92-7015-4 and RS93-8025–1, the number of blue and red microspores were determined separately in the large 2n and in the smaller n microspores. In tetraploids, about 50 microspores were scored for the confirmation of nulliplex (100% red) genotypes, and 100–500 microspores were scored to distinguish the simplex (blue: red=1:1) and duplex (blue: red=5:1) genotypes. Segregation data of similar and homogeneous ($P_{homogeneity}>0.05$) 4x.2x progenies were pooled. Chi-square tests were used to test the goodness of fit to the expected ratios, significant at the 0.001 level.

Isozyme analysis of ADH-1

For ADH-1 isozyme electrophoresis mature anthers of three flowers were collected and ground in a 1% solution of 2-mercaptoethanol in order to extract proteins. Electrophoresis through a 12.5% polyacrylamide gel (Phast System) and staining were both carried out according to Jacobs et al. (1995). Based on the electrophoretic mobility of ADH-1, three different homodimeric variants were detected. In order of decreasing mobility, these variants were designated as ADH-$1^{1/1}$, ADH-$1^{2/2}$, and ADH-$1^{3/3}$, encoded by the alleles *Adh-1*^{1}, *Adh-1*^{2}, and *Adh-1*^{3} respectively. In tetraploids, the simplex genotypes were distinguished from the duplex genotypes on the basis of the intensity of the homodimeric bands (Douches and Quiros 1987).

Gene-centromere mapping

Assuming complete chiasma interference, gene-centromere distances were estimated according to the formulae of Mendiburu and Peloquin (1979) using the frequencies (f) of the different genotypes in the segregating population. For the *amf* marker gene in FDR 2n pollen the gene-centromere distance was estimated as 2.f(*amf/amf*).100 cM. From the analysis of 4x.2x progenies, the gene-centromere map distance was estimated as f(nulliplex+duplex). 100 cM, for both the *Adh-1* and the *amf* marker. The 95% binomial confidence interval (f_i ,f_j) for the frequency (f) of nulliplex genotypes in a population of *n* individuals was calculated according to Fruend (1971) as:

$$(f_i, f_j)=n/(n+(1.96)^2)\cdot\{f+[(1.96)^2/2n] \pm 1.96\cdot\sqrt{[f(1-f)/n+(1.96)^2/4n^2]}\}.$$

The corresponding confidence interval for the estimated gene-centromere distance was derived by substituting f_i and f_j in the relevant mapping formulae.

Results

Microsporogenesis in diploids

Microsporogenesis and pollen formation were examined in the three diploid clones to investigate the level of chromosome pairing and the restitution mechanism of 2n pollen formation. The control diploid, HB93-7108-08, and the 2n pollen-forming clone, B92-7015-4, exhibited normal bivalent pairing and a high percentage of pollen stainability (Table 1). Unlike these, the desynaptic clone RS93-8025-1 had an average of about three bivalents per cell and, consequently, pollen stainability was very low. The characteristic feature of the control genotype, HB93-7108-08, was that fused spindles were completely absent at the metaphase-II stages and only stainable (n) pollen grains of uniform size (±23 μm diameter) were formed. On the other hand, B92-7015-4 produced both n and 2n pollen grains. The 2n pollen grains were distinctively large in size (far exceeding 23 μm) and amounted to 63% of the stainable pollen. In the desynaptic clone RS93-8025-1, only the 2n pollen grains were stainable. There was a correspondence between fused spindle and functional 2n pollen formation in both B92-7015-4 and RS93-8025-1 (Table 1). This indicates that the 2n pollen in both clones originated through FDR.

Segregation for the *amf* marker in diploids and estimates of its map distance to the centromere

Based on starch phenotypes and size, the microspores of both diploid and tetraploid clones could be unambiguously classified (Fig. 1 A–J). All three diploid clones segregated for the blue and red microspore phenotypes. The n pollen of the control clone, HB93-7108-08, segregated 1 blue : 1 red (Fig. 1 D), as expected. In B92-7015-4, which formed both n and 2n pollen, the segregation ratios for the two classes were different (Fig. 1 E). Whereas the n microspores segregated approximately 1 blue : 1 red, as expected, the 2n microspores showed a segregation ratio of 3 blue : 1 red (Table 2). This 3:1 segregation of 2n microspores could be explained by assuming one cross-over between the centromere and the *amf* locus in all pollen mother cells, followed by random assortment of cross-over and non-cross-over chromatids during the restitutional (fused spindle) anaphase-II stage. As a result of this, the three genotypes of 2n microspores, viz., *Amf/Amf*; *Amf/amf* and *amf/amf*, were expected to occur with a ratio of 1:2:1 respectively, which is in agreement with the observed value of 3 blue (*Amf/.*) to 1 red (*amf/amf*). Based on the microspore segregation data of this clone, the gene-centromere

Table 1 Meiotic behaviour (bivalent formation and percentage of fused spindles), and pollen formation (percentages of stainable pollen and 2n pollen) in three diploid clones HB93-7108-08 (control), B92-7015-4 (normal synaptic) and RS93-8025-1 (desynaptic)

Diploid clone (*Amf/amf*):	Average number of bivalents per cell	Percentage of		
		Fused spindles (per 100 pollen mother cells)	Stainable pollen (per 100 pollen grains)	2n pollen (per 100 stainable pollen grains)
HB93-7108-08 (*Ds-1/.*)	12.0	0	90	0
B92-7015-4 (*Ds-1/.*)	12.0	75	72	63
RS93-8025-1 (*ds-1/ds-1*)	2.7	12	12	100

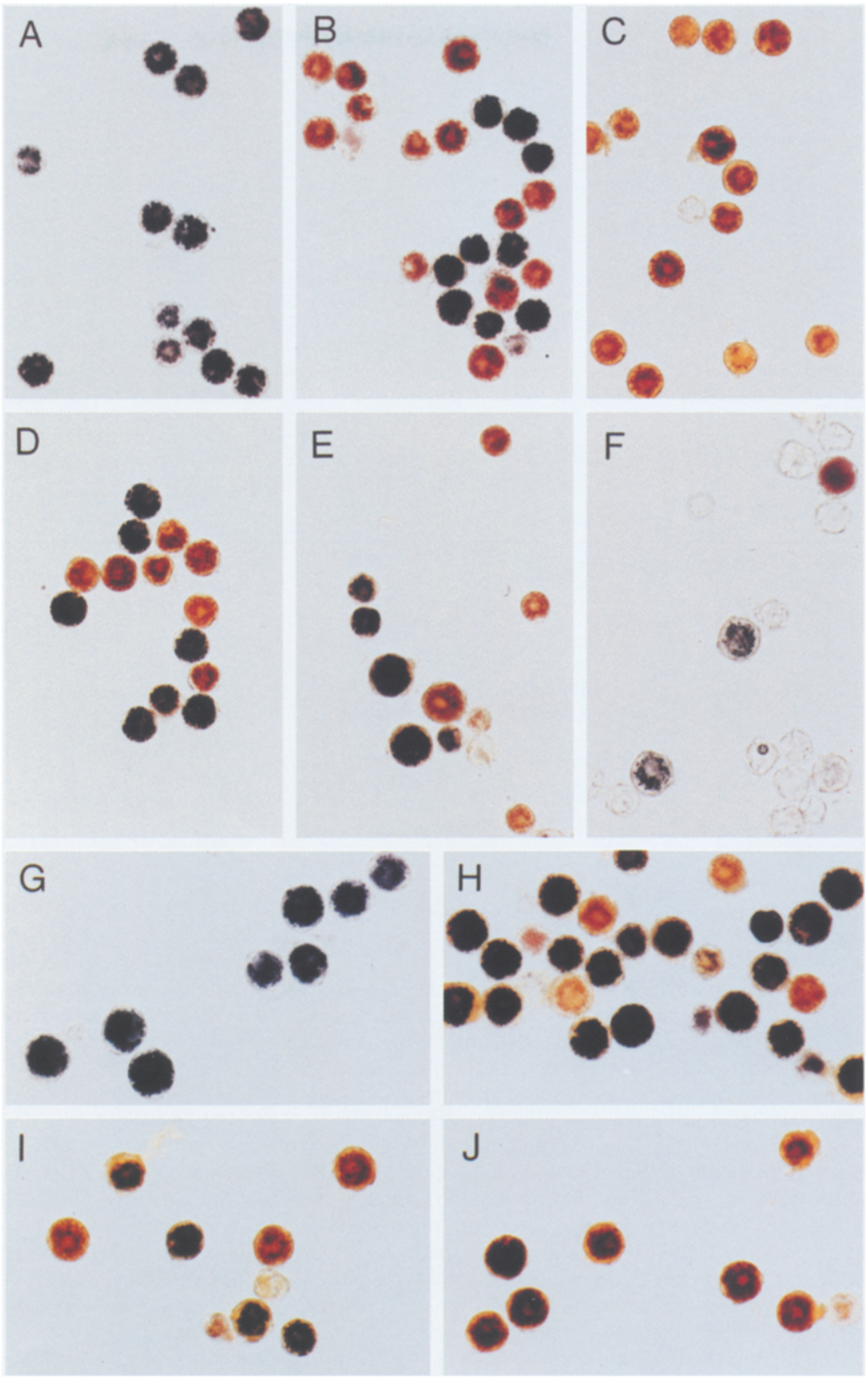

Fig. 1 A–J Starch phenotypes of blue and red in n and 2n microspores in diploid (**A–F**) and tetraploid (**G–I**) genotypes. **A–C** n microspores in three diploid genotypes; **A** = wild-type *Amf/Amf*; **B** = heterozygous genotype *Amf/amf*; and **C** = mutant genotype *amf/amf*. Note the 1 blue : 1 red segregation in **B** as compared to solely blue and red in **A** and **C** respectively. **D–F** n and 2n microspores in heterozygous diploid clones. **D** shows the microspores of the control clone, HB93-7108-08, containing only small (n) microspores with blue and red starch phenotypes. **E** shows the segregation for small (n) and large (2n) microspores in the normal synaptic clone, B92-7015-4, in which both types segregate for blue and red phenotypes. **F** shows the microspores of the desynaptic clone, RS93-8025-1, showing large functional 2n microspores (blue and red) and the aborted ones. **G–J** microspore segregation pattern in the wild-type tetraploid genotype (**G**) and in the three genotypic classes of 4x progenies derived from 4x (nulliplex *amf*) × 2x (*Amf/amf*)-crosses (**H–J**). **G** = quadruplex (*Amf/Amf/Amf/Amf*) genotype, all blue; **H** = duplex (*Amf/Amf/amf/amf*), 5 blue : 1 red; **I** = simplex (*Amf/amf/amf/amf*), 1 blue : 1 red and **J** = nulliplex (*amf/amf/amf/amf*), all red

distance for the *amf* locus was estimated as 48.8 cM (42.5–55.6 cM). Although the desynaptic clone RS93-8025-1 also produced 2n microspores through fused spindles (i.e. FDR as in B92-7015–4), the segregation of blue:red microspores deviated significantly from the expected 3:1 ratio (Table 2; Fig. 1 F). This result could be explained on the basis of a reduction of chiasma formation due to desynapsis in this clone. The map distance between the centromere and the *amf* locus in RS93-8025-1 was estimated as 13.3 cM (10.9–16.2 cM).

Table 2 Segregation ratios of n and 2n microspores for blue and red phenotypes in three diploid clones HB93-7108-08 (control), B92-7015-4 (normal synaptic) and RS93-8025-1 (desynaptic)

Diploid clone (*Amf/amf*)	n Microspores			2n Microspores		
	Blue (*Amf*)	Red (*amf*)	$P_{1:1}$	Blue (*Amf/.*)	Red (*amf/amf*)	$P_{3:1}$[a]
HB93-7108-08 (*Ds-1/.*)	232	222	>0.6	–	–	
B92-7015-4 (*Ds-1/.*)	365	296	>0.005	496	160	>0.6
RS93-8025-1 (*ds-1/ds-1*)	–	–		1305	93	<0.0005

[a] Assuming FDR and complete chiasma interference

Table 3 Genotype classes of duplex, simplex and nulliplex 4x progenies derived from 4x (nulliplex *amf*) × 2x (*Amf/amf*) crosses. Both the normal synaptic diploid parent B92-7015-4 and the desynaptic diploid parent RS93-8025-1 produced 2n pollen through fused spindles (FDR)

4x female parent	2x male parent	4x.2x progeny (4x)			
(*amf/amf/amf/amf*)	(*Amf/amf*)	# Duplex (%) (*Amf/Amf/amf/amf*)	# Simplex (%) (*Amf/amf/amf/amf*)	# Nulliplex (%) (*amf/amf/amf/amf*)	Total
HB93-7133-3	B92-7015–4	8 (17)	28 (60)	11 (23)	47
J90-6001-25	(*Ds-1/.*)	32 (26)	62 (51)	27 (22)	121
J90-6011-3		19 (15)	84 (65)	27 (21)	130
J90-6020-22		24 (15)	102 (65)	32 (20)	158
Total 4x.2x-progenies[a]		83 (18)	276 (61)	97 (21)	456
HB93-7133-3	RS93-8025–1	1 (2.0)	45 (90)	4 (8.0)	50
J90-6011-3	(*ds-1/ds-1*)	14 (8.6)	134 (83)	13 (8.1)	161
J90-6020-17		1 (4.5)	21 (95)	0 (0.0)	22
J90-6020-22		9 (4.0)	202 (90)	13 (5.8)	224
Total 4x.2x-progenies[a]		25 (5.5)	402 (88)	30 (6.6)	457

[a] 4x.2x progenies are homogeneous, $P(\chi^2$ homogeneity)>0.05

Classification of 4x.2x progenies for *amf* marker segregation

The segregation of the *amf* marker in 2n microspores allowed a distinction between only two phenotypic classes, viz., blue (*Amf/.*) and red (*amf/amf*). In order to distinguish all three expected classes, *Amf/Amf*; *Amf/amf* and *amf/amf*, it was necessary to generate 4x progenies from 4x (nulliplex *amf*) × 2x (*Amf/amf*) crosses and classify these 4x.2x progenies into duplex, simplex, and nulliplex genotypes. Such a classification, based on the segregation for starch phenotypes of microspores in tetraploids (Figs. 1 G–J), was carried out for two population types in which the normal synaptic (B92-7015-4) and the desynaptic (RS93-8025-1) clones, respectively, were used as male parents on a set of four different female parents (Table 3). In both types of population, about 450 plants of the 4x.2x progenies could be classified into duplex, simplex and nulliplex (Figs. 1 H–J) genotypes. Since both the duplex and nulliplex genotypes originated from a crossover between the centromere and the *amf* locus, followed by FDR in pollen mother cells, these two classes were expected to occur at equal frequencies. The observed numbers of duplex and nulliplex genotypes (83 and 97 in the 4x.2x progenies of B92-7015-4; 25 and 30 in the 4x.2x progenies of RS92-8025-1) in both cases fit a 1:1 ratio (χ^2=1.09 and 0.45 respectively). The map distance between *amf* and the centromere was estimated as 39.5 cM (35.1–44.0 cM) in the normal synaptic clone B92-7015-4, and as 12.0 cM (9.2–15.2 cM) in the desynaptic clone RS93-8025-1.

A heterodimeric band of ADH-1 as an indication of FDR 2n pollen

Isozyme analysis of the *Adh-1* marker gene in the diploid clones that were used for the analysis of n and 2n pollen revealed different numbers of bands per clone (Fig. 2). The control HB93-7108-08 (*Adh-1*^{2}/*Adh-1*^{3}) showed two homodimeric bands encoded by the alleles *Adh-1*^{2} and *Adh-1*^{3}. Since the desynaptic clone RS93-8025-1 was homozygous (*Adh-1*^{2}/*Adh-1*^{2}), it showed only the homodimer encoded by the *Adh-1*^{2} allele. The characteristic feature of the normal synaptic clone B92-7015-4 (*Adh-1*^{1}/*Adh-1*^{2}) was that it showed not only the two homodimeric bands ADH-1$^{1/1}$ and ADH-1$^{2/2}$, but also an extra band of the heterodimeric protein ADH-1$^{1/2}$. The presence or absence of the heterodimer was investigated in relation to pollen production in these clones. The control clone was not able to produce any 2n pollen and showed only the homodimeric

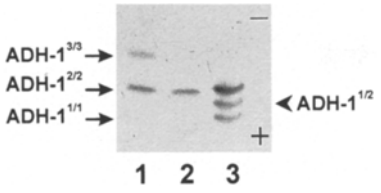

Fig. 2 Zymogram of pollen samples of the three diploid clones. *Lane 1* shows the homodimers ADH-$1^{2/2}$ and ADH-$1^{3/3}$ in a pollen sample of the control clone HB93-7108-08 (*Adh-1²/Adh-1³*). *Lane 2* shows the homodimer ADH-$1^{2/2}$ in a pollen sample of the desynaptic clone RS93-8025-1 (*Adh-1²/Adh-1²*). *Lane 3* shows the homodimeric bands ADH-$1^{1/1}$ and ADH-$1^{2/2}$, and the extra band of the heterodimer ADH-$1^{1/2}$ in a pollen sample of the normal synaptic clone B92-7015-4 (*Adh-1¹/Adh-1²*), indicating the presence of 2n pollen grains of genotype *Adh-1¹/Adh-1²*

bands. Obviously, the pollen mixture comprised only the haploid genotypes *Adh-1²* and *Adh-1³*, so the two subunits of the isozyme were never present in the same cell to form a heterodimer. The clone B92-7015-4 produced 63% of 2n pollen and was able to produce the heterodimeric protein ADH-$1^{1/2}$ (Fig. 2) in all heterozygous FDR 2n pollen, as identified by the presence of both *Adh-1¹* and *Adh-1²* alleles in the same cell. Since the heterodimeric band was strong, the pollen mixture had to include a lot of 2n pollen with the *Adh-1¹/Adh-1²* genotype. Assuming *Adh-1* to be a proximal marker gene, these genotypes confirmed the expected FDR origin of the 2n pollen.

Classification of 4x.2x progeny for *Adh-1* marker segregation

Pollen of 36 tetraploid offspring plants from the 4x.2x cross between HB93-7133-3 (*Adh-1²/Adh-1²/Adh-1²/Adh-1²*) and B92-7015-4 (*Adh-1¹/Adh-1²*) was used for the classification of the genotype for the isozyme marker *Adh-1*. The nulliplex (*Adh-1²/Adh-1²/Adh-1²/Adh-1²*) genotypes were identified based on the absence of the homodimer ADH-$1^{2/2}$, while the simplex (*Adh-1¹/Adh-1²/Adh-1²/Adh-1²*) genotypes were distinguished from the duplex (*Adh-1¹/Adh-1¹/Adh-1²/Adh-1²*) genotypes based on the intensity of each of the bands of the homodimers ADH-$1^{1/1}$ and ADH-$1^{1/1}$. The 4x.2x progeny segregated into three genotypic classes in the ratio of nulliplex:simplex:duplex = 5:29:2 respectively. The gene-centromere map distance of the *Adh-1* marker in clone B92-7015-4 was estimated as 19.4 cM (9.7–35.0 cM).

Discussion

The utility of the *amf* marker for studying the extent of crossing-over

This investigation demonstrates the usefulness of the pollen marker *amf* for a study of basic genetics in the potato. In addition to the clear-cut identification of phenotypes in large populations of n microspores (Fig. 1), it is possible to estimate the extent of crossing-over between the *amf* locus and the centromere by the segregation patterns in 2n microspores. For example, a clear difference between the segregation ratios of n and 2n microspore samples was observed in clone B92-7015-4 (Table 2). Its n microspores segregated 1 blue:1 red (monogenic), as in the control clone HB93-7108-08, whereas the 2n microspores displayed a ratio fitting 3 blue : 1 red. Because the 2n microspores of clone B92-7015-4 were produced after fused-spindle formation, they have a FDR origin. Therefore, the occurrence of 24.4% red 2n microspores in this clone should be interpreted as due to a high frequency of pollen mother cells with one cross-over between the *amf* locus and the centromere, resulting in a gene-centromere map distance of 48.8 cM (42.5–55.7 cM). Indeed, this marker has been localized to the most distal part of chromosome 8 in genetic linkage maps of potato (Gebhardt et al. 1991; Jacobs et al. 1995). However, the latter investigations revealed no information about the gene-centromere distance. The 3:1 ratio of blue (*Amf/.*) and red (*amf/amf*) 2n microspores was also found by Jacobsen et al. (1991) in 2n microspores of three normal synaptic diploid clones, which are closely related to clone B92-7015-4. For the parental clone 880004-2, which forms fused spindles, they observed 178 blue (*Amf/.*) and 56 red (*amf/amf*) microspores. From their data a gene-centromere map distance of 47.9 cM (37.8–59.6 cM) can be calculated. This is very close to the estimation of 48.8 cM in the present investigation.

In 2n microspores, only two of the expected three genotypic classes can be detected, because *amf/amf* genotypes are red, and both the *Amf/Amf* and *Amf/amf* genotypes are blue. The only method of classifying all three genotypes of 2n microspores is via identification of duplex, simplex and nulliplex genotypes in 4x progenies from nulliplex tetraploids × heterozygous diploids, as demonstrated in Table 3. The numbers of nulliplex and duplex genotypes fit the expected 1:1 ratio, but a slight shortage of duplex genotypes is observed. Owing to this shortage, the *amf*-centromere distance of 39.5 cM, as calculated from the 4x.2x progenies data of clone B92-7015-4 (Table 3), is lower than the distance of 48.8 cM calculated from the 2n microspores (Table 2). The shortage of duplex genotypes may be due to misclassification of simplex and duplex genotypes in the 4x.2x progeny, rather than to any other putative cause. The rationale of this assumption is the fit of 359 (duplex+simplex) : 97 (nulliplex) to 3 blue : 1 red (χ^2=3.38), as observed also in 2n microspores (Table 2). Pooling of the simplex and duplex genotypes resulted in a gene-centromere distance of 42.5 cM (35.5–50.7 cM). In view of the overlapping confidence intervals, the estimates of *amf*-centromere map distances derived from 2n microspore segregations and those from the segregations in 4x.2x progenies are not significantly different.

Indications for one cross-over per arm

Assuming complete chiasma interference in FDR 2n gametes, a distal marker (*A/a*) is expected to segregate *A.*:*aa*=3:1 or *AA*:*Aa*:*aa*=1:2:1 and, therefore, its gene-centromere distance is estimated as 2.[f(*aa*)=1/4].100 cM= 50 cM (Mendiburu and Peloquin 1979). Assuming random multiple exchanges per chromosome arm, the segregation of a distal marker is expected to be independent of the centromere, and should be *A.*:*aa*=5:1 or *AA*:*Aa*:*aa*=1:4:1 (Mather 1935). In this model, the frequency of *aa* gametes will not exceed 1/6 for distal genes. In the model of Mendiburu and Peloquin (1979), a frequency of *aa* gametes of 1/6 corresponds to a gene-centromere distance of 2.[f(*aa*)=1/6].100 cM=33.3 cM. Therefore, estimates of gene-centromere distances exceeding 33.3 cM are in favour of the complete chiasma interference model of Mendiburu and Peloquin (1979). However, marker genes with a gene-centromere distance of more than 33.3 cM are rarely found in HTA experiments in potato (Douches and Quiros 1987; Jongedijk 1991 a; Wagenvoort and Zimnoch-Guzowska 1992). Consequently, Jongedijk (1991 a) supported a genetic model for random multiple exchanges per chromosome arm (Mather 1935). Since our estimates of the gene-centromere distance of the *amf* marker in FDR 2n pollen and 4x.2x progenies clearly exceeded 33.3 cM, our data indicate that multiple cross-overs do not occur between the centromere and the *amf* locus. This corroborates the model of Mendiburu and Peloquin (1979). The occurrence of one cross-over per arm has been further confirmed through RFLP analysis of 2x.4x progenies in potato (Bastiaanssen et al., in preparation). This genetic evidence for a high degree of chiasma interference agrees with cytological observations of 13.3–14.0 chiasmata per cell (or 1.2 per bivalent) in diploid potato (Jongedijk and Ramanna 1989) and 17.1±2 chiasmata per cell in tomato (Sherman and Stack 1995). In line with this situation, gene-centromere mapping in different fish species (Thorgaard et al. 1983; Allendorf et al. 1986; Seeb and Seeb 1986; Liu et al. 1992) revealed evidence for complete chiasma interference.

Reduction of crossing-over due to desynapsis

A genotype combining pollen fertility, FDR 2n microspore formation, the *amf* marker gene in a heterozygous condition (*Amf/amf*), and desynapsis (*ds-1/ds-1*), could be used for demonstrating the effect of desynapsis on the extent of crossing-over through HTA. In the population RS93-8025 with 140 plants only one such genotype was selected. The fertile pollen (12%) of this selected desynaptic clone RS93-8025-1 comprised only FDR 2n pollen (Table 1). This FDR origin of 2n microspores enabled a comparison of the extent of crossing-over between the centromere and the *amf* locus in the desynaptic clone RS93-8025-1 with that in the normal synaptic clone B92-7015-4. Whereas the gene-centromere distance was found to be 48.8 cM in the normal synaptic clone, it was only 13.3 cM in the desynaptic clone. This was confirmed both in 2n microspores and in 4x.2x progenies. This reduction of crossing-over (73%) nearly equals the observed reduction (78%) in bivalent formation from 12.0 bivalents per cell in the normal synaptic clone to 2.7 bivalents per cell in the desynaptic clone (Table 1). Severe reduction of recombination of more than 70% in desynaptic potato clones was also found in other HTA studies in which different markers were used (Douches and Quiros 1988; Jongedijk et al. 1991 a).

Segregation of *Adh-1* in 2n pollen and in 4x.2x progenies

Although the isozyme ADH-1 can be used as a pollen marker, the phenotypic classes of 2n pollen grains cannot be distinguished individually in the present material. However, this isozyme is helpful for an assessment of the occurrence of FDR 2n pollen because of the presence of heterodimeric bands. Such heterodimeric bands occur only in cells where two different alleles are expressed, as in the case of heterozygous 2n pollen grains. Owing to its relatively proximal position, the large amount of heterozygous 2n pollen found in clone B92-7015-4 (Fig. 1) and the 80.6% of simplex (*Adh-1*^{1}/*Adh-1*^{2}/*Adh-1*^{2}/*Adh-1*^{2}) 4x.2x progeny plants confirmed the FDR origin of this 2n pollen. The corresponding map distance between the centromere and the *Adh-1* locus was estimated to be 19.4 cM (9.7–35.0 cM) and is comparable with the distance of 15.8 cM that was found by Douches and Quiros (1987) for the ADH isozyme which is expressed in the pollen of potato.

Gene-centromere mapping through pollen markers

If a null allele at the *Adh-1* locus becomes available, it will be possible, as was demonstrated in tomato (Wisman et al. 1991), to distinguish individual pollen phenotypes just as in the case of the *amf* marker. Also transformation of diploid potato clones producing FDR 2n pollen with reporter genes that are expressed in the pollen, like the cloned wild-type *Amf* allele (Flipse et al. 1996) or the β-glucuronidase (GUS) gene, can be used to increase the number of genetic loci for the identification of individual pollen phenotypes. The possibility of using transgenics with pollen-specific marker loci at all 12 chromosomes would be of great importance for efficient gene-centromere mapping and defining the extent of crossing-over in potato. Although tomato has been used for extensive genetic mapping, HTA has not been exploited in this model plant species because 2n gametes occur very rarely, if ever (Ramanna, unpublished). Information about the positions of centromeres in relation to molecular marker loci in tomato is only available for chromosomes 6, 7 and 9, obtained from mapping in deletion and trisomic lines (Van Wordragen et al. 1994; Frary et al. 1996). In view of the synteny of the genetic maps of potato and tomato (Bonierbale et al. 1988; Tanksley et al. 1992), the exploitation of 2n gametes for gene-centromere mapping in potato may be highly relevant to tomato as well.

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References

Allendorf FW, Seeb JE, Knudsen KL, Thorgaard GH, Leary RF (1986) Gene-centromere mapping of 25 loci in rainbow trout. J Hered 77:307–312

Barone A, Gebhardt C, Frusciante L (1995) Heterozygosity in 2n gametes of potato evaluated by RFLP markers. Theor Appl Genet 91:98–104

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosome evolution in potato and tomato. Genetics 120:1095–1103

Den Nijs TPM, Peloquin SJ (1977) 2n gametes in potato species and their function in sexual polyploidization. Euphytica 26:585–600

Douches DS, Quiros CF (1987) Use of 4x-2x crosses to determine gene-centromere map distances of isozyme loci in Solanum species. Genome 29:519–527

Douches DS, Quiros CF (1988) Genetic recombination in a diploid synaptic mutant and a *Solanum tuberosum* × S. *chacoense* diploid hybrid. Heredity 60:183–191

Eppig JT, Eicher EM (1983) Application of the ovarian teratoma mapping in the mouse. Genetics 103:797–812

Flipse E, Keetels CJAM, Jacobsen E, Visser RGF (1996) The dosage effect of the wild-type GBSS-allele is linear for GBSS activity but not for the amylose content: absence of amylose has a distinct influence on the physico-chemical properties of starch. Theor Appl Genet 92:121–127

Frary A, Presting GG, Tanksley SD (1996) Molecular mapping of the centromeres of tomato chromosomes 7 and 9. Mol Gen Genet 250:295–304

Fruend JE (1971) Mathematical statistics, 2nd edn. Prentice Hall, Englewood Cliffs, New Jersey

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schachtschabel U, Kaufmann H (1991) RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor Appl Genet 83:49–57

Hutten RCB, Schippers MGM, Hermsen JGTh, Ramanna MS (1994) Comparative performance of FDR and SDR progenies from reciprocal 4x-2x crosses in potato. Theor Appl Genet 89:545–550

Hutten RCB, Schippers MGM, Hermsen JGTh (1995) Comparative performance of diploid and tetraploid progenies from 2x.2x crosses in potato. Euphytica 81:187–192

Jacobs JME, van Eck HJ, Arens P, Verkerk-Bakker B, te Lintel Hekkert B, Bastiaanssen HJM, El-Kharbotly A, Pereira A, Jacobsen E, Stiekema WJ (1995) A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons, and classical markers. Theor Appl Genet 91:289–300

Jacobsen E (1976) Cytological studies of diplandroid production in a dihaploid potato clone and its correlation with seed set in 4x-2x crosses. Z Pflanzenzücht 77:10–15

Jacobsen E, Hovenkamp-Hermelink JHM, Krijgsheld HT, Nijdam H, Pijnacker LP, Witholt B, Feenstra WJ (1989) Phenotypic and genotypic characterization of an amylose-free starch mutant of the potato. Euphytica 44:43–48

Jacobsen E, Ramanna MS, Huigen DJ, Sawor Z (1991) Introduction of an amylose-free (*amf*) mutant into breeding of cultivated potato, *Solanum tuberosum* L. Euphytica 53:247–253

Jarrell VL, Lewin HA, Da Y, Wheeler MB (1995) Gene-centromere mapping of bovine DYA, DRB3, and PRL using secondary oocytes and first polar bodies: Evidence for four-strand double crossovers between DYA and DRB3. Genomics 27:33–39

Johnson SL, Gates MA, Johnson M, Talbot WS, Horne S, Baik K, Rude S, Wong JR, Postlethwait JH (1996) Centromere-linkage analysis and consolidation of the zebra fish genetic map. Genetics 142:1277–1288

Jongedijk E, Ramanna MS (1989) Synaptic mutants in potato *Solanum tuberosum* L. II. Concurrent reduction of chiasma frequencies in male and female meiosis of *ds-1* desynapsis mutants. Genome 32:1054–1062

Jongedijk E, Hutten RCB, van der Wolk JMASA, Schuurmans-Stekhoven SIJ (1991 a) Synaptic mutants in potato, *Solanum tuberosum* L. III. Effect of the *Ds-1/ds-1* locus (desynapsis) on genetic recombination in the male and female meiosis. Genome 34: 121–130

Jongedijk E, Ramanna MS, Sawor Z, Hermsen JGTh (1991 b) Formation of first-division restitution FDR 2n megaspores through pseudohomotypic division in *ds-1* desynapsis mutants of diploid potato: routine production of tetraploid progeny from 2x FDR-2x FDR crosses. Theor Appl Genet 82:645–656

Liu Q, Goudie CA, Simco BA, Davis KB, Morizot DC (1992) Gene-centromere mapping of six enzyme loci in gynogenetic Channel Catfish. J Hered 83:245–248

Mather (1935) Reductional and equational separation of the chromosomes in bivalents and multivalents. J Genet 30:53–78

Mendiburu AO, Peloquin SJ (1979) Gene-centromere mapping by 4x-2x matings in potato. Theor Appl Genet 54:177–180

Mok DWS, Peloquin SJ (1975) Three mechanisms of 2n pollen formation in diploid potatoes. Can J Genet Cytol 17:217–225

Nel PM (1975) Crossing-over and diploid egg formation in the elongate mutant of maize. Genetics 79:435–450

Peloquin SJ, Yerk GL, Werner JE (1989) Ploidy manipulations in the potato. In: Adolph KW (ed) Chromosomes: eukaryotic, prokaryotic and viral, volume II. CRC press Inc, Boca Raton, Florida, pp 168–177

Qu L, Hancock JF (1995) Nature of 2n gamete formation and mode of inheritance in interspecific hybrids of diploid *Vaccinium darrowi* and tetraploid *V. corymbosum*. Theor Appl Genet 91:1309–1315

Ramanna MS (1979) A re-examination of the mechanisms of 2n gamete formation in potato and its implications for breeding. Euphytica 28:537–561

Rutishauser A (1956) Chromosome distribution and spontaneous chromosome breakage in *Trillium grandiflorum*. Heredity 10: 367–407

Seeb JE, Seeb LW (1986) Gene-mapping of isozyme loci in chum salmon. J Hered 77:399–402

Sherman JD, Stack SM (1995) Two-dimensional spreads of synaptonemal complexes from Solanaceous plants. VI. High-resolution recombination-nodule map for tomato (*Lycopersicon esculentum*). Genetics 141:683–708

Tanksley SD, Zamir D, Rick CM (1981) Evidence for extensive overlap of sporophytic and gametophytic gene expression in *Lycopersicon esculentum*. Science 213:453–455

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Thorgaard GH, Allendorf FW, Knudsen KL (1983) Gene-centromere mapping in rainbow trout; high interference over long map distances. Genetics 7:524–530

Van Eck HJ, Rouppe van der Voort J, Draaistra J, van Zandvoort P, van Encevort E, Segers B, Peleman J, Jacobsen E, Helder J, Bakker J (1995) The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. Mol Breed 1:397–410

Van Wordragen MF, Weide R, Liharska T, Van Der Steen A, Koornneef M, Zabel P (1994) Genetic and molecular organization of the short arm and pericentromeric region of tomato chromosome 6. Euphytica 79:169–174

Wagenvoort M, Zimnoch-Guzowska E (1992) Gene-centromere mapping in potato by half-tetrad analysis: map distances of *H1*, *Rx*, and *Ry* and their possible use for ascertaining the mode of 2n pollen formation. Genome 35:1–7

Werner JE, Douches DS, Freyre R (1992) Use of half-tetrad analysis to discriminate between two types of 2n egg formation in a potato haploid. Genome 35:741–745

Wisman E, Koornneef M, Chase T, Lifshytz E, Ramanna MS, Zabel P (1991) Genetic and molecular characterization of an *Adh-1* null mutant in tomato. Mol Gen Genet 226:120–128