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Identification and mapping of three flower colour loci of potato (*S. tuberosum* L.) by RFLP analysis

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Summary. The inheritance of flower colour in diploid potato ($2n = 2x = 24$), was found to be controlled by three unlinked loci *D*, *F* and *P*. To determine the allelism with previously described loci and to dissect this oligogenic trait, a set of tester clones with well-defined genotypes was developed. By backcrossing the mapping population with these tester clones it was possible to obtain monogenic segregation ratios. These were required to detect linkage with RFLP loci and, despite distorted Mendelian ratios, the inheritance and mapping of the *D*, *F* and *P* loci could be unambiguously determined. Locus *D*, involved in the biosynthesis of red anthocyanins, was mapped on chromosome 2, while locus *P*, involved in the production of blue anthocyanins, was mapped on chromosome 11. Locus *F*, involved in the flower-specific expression of gene(s) accommodated by the *D* and *P* loci, was mapped on chromosome 10. The tester clones and the map position of the *D*, *F* and *P* loci may be of considerable value in simplifying the genetics of anthocyanin pigmentation.

Key words: *Solanum tuberosum* – Allelism – RFLP map – Anthocyanin markers – Tester clones

Introduction

Knowledge of the inheritance of many morphological and agronomically important traits in potato is poor. Tetrasomic inheritance complicates genetic studies. Dihaploids extracted from cultivars are predominantly male-sterile and possess many lethal genes. This may explain the scarcity both of genetic information and of usable genetic marker stocks as compared to other important crops. Most of the classical genetic research on potato has focused on the inheritance of anthocyanin pigmentation in flower, stem and tuber skin. This work has been reviewed by Black (1933), Swaminathan and Howard (1953), Howard (1960, 1970), and more recently by de Jong (1991). From the beginning of the century various authors have postulated over 20 genes to explain all the different phenotypes.

Since excellent RFLP maps of the potato genome are now available (Bonierbale et al. 1988; Gebhardt et al. 1989, 1991), the many morphological and anthocyanin biosynthesis genes can be readily mapped on the genome. Information on the linkage relations of these markers would be useful for future genetic studies and for marker-assisted selection in plant breeding.

Our objectives in this paper were: (1) to study the inheritance of flower colour, (2) to localize the relevant loci on the potato genome with RFLP markers, (3) to prove allelism between the mapped loci and previously postulated loci, and (4) to differentiate whether a locus is involved in the biosynthesis of anthocyanins or in the regulation of the tissue-specific expression of genes involved in anthocyanin biosynthesis.

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Materials and methods

Nomenclature of loci

The nomenclature of the loci used in this study is based on the genetic system developed for the tetraploid Group Tuberosum (Salaman 1910; Lunden 1937, 1960, 1974). In this genetic model for anthocyanin pigmentation five main loci are postulated to explain all different phenotypes. Loci *D*/*d* and *P*/*p* are basic factors which are necessary for the development of red and/or blue pigmentation in various plant parts. Loci *E*/*e*, $R/R^f/r$ and *F*/*f* have a tissue-specific role in the expression of these basic factors for anthocyanin biosynthesis. Tuber skin colour is a trait which is not present in the plant material used in this study

indicating recessiveness for the loci *R* and *E*. In combination with a dominant allele of the basic factors *D* or *P*, the dominant locus *F* is involved in the expression of flower colour. Dominant alleles of the basic factors *D* or *P* give, independent of loci *E*, *R* and *F*, coloured sprout tips and some background pigmentation in both stem and inflorescence.

Observation of flower colour

Assessment of flower colour was according to the Nickerson Color Fan, (1957, Published and Distributed by Munsell Color Co., Inc., 2441 N. Calvert Street, Baltimore, Maryland 21218, USA). For the genetic analysis of flower colour three classes are distinguished: BLUE, RED and WHITE. The class RED ranged from Nickerson code 10P to 5P comprising the pink and red to purple flowering phenotypes. The flower colour class BLUE ranged from code 10PB to 7.5PB comprising the very distinct phenotype of pale-blue flowers.

Plant material: pedigree of tester clones

The tester clones used in this study carry alleles which are identical by descent to those involved in the previous study of Lunden (1974). From the tetraploid ($2n=4x=48$) cultivar Gineke with red tubers and flowers having the genotype *ppppDDddR*^{f}*rrrFfffeeee* (Lunden 1974), the dihaploid clone G254 ($2n=2x=24$) with white tubers and red flowers was extracted by Hermsen et al. (1978). The white tubers of clone G254 indicate recessiveness at the *R* locus. The three white-flowering and white-tubered tester clones are inbreds from clone G254, which were selected and kindly provided by Dr. J. G. Th. Hermsen. I_1G254-83 is the double recessive for the *D* and *F* loci, whereas I_1G254-26 and I_1G254-77 have complementary genes.

Plant material: pedigree of the RFLP mapping population ($2n=2x=24$)

The RFLP map is based on a backcross population USW5337.3 × 77.2102.37. Clone USW5337.3 (referred to as clone C), selected by Hanneman and Peloquin (1967), is a hybrid between *S. phureja* PI 225696.1 and the dihaploid US-W42, extracted from cv Chippewa. Clone 77.2102.37 (referred to as clone E), selected by Jacobsen (1980), is a hybrid between a *S. vernei*-*S. tuberosum* backcross, $VH^3$4211, and clone C. Flower colour in clone C is light-purple (code 5P7/7 = class RED); clone E has a light-purple to light-violet pigmented flower (code 2.5P6/7 = class RED). The reciprocal crosses C × E and E × C and their offspring are coded CE and EC respectively. Flower colour could be assessed in 256 CE clones. RFLP analysis was performed on 90 plants consisting of 67 CE clones and 23 EC clones.

Molecular techniques

Isolation of genomic DNA from young leaves and shoots was as described by Bernatzky and Tanksley (1986). Digestions were performed with the enzymes: *Dra*I (BRL), *Eco*R1, *Eco*RV, *Hin*DIII and *Xba*I (Amersham) using 5 units per µg of DNA, according to the manufacturer's instructions. Fragments, 6–8 µg of DNA per lane, were separated on an 0.8% TAE-buffered agarose gel and transferred onto a Genescreen plus membrane (NEN). Blots were prehybridized, hybridized with radiolabelled inserts from RFLP clones, and washed according to Bernatzky and Tanksley (1986). Membranes were placed on X-ray film (Kodak X-Omat) for 1–5 days.

Tomato genomic DNA clones (TG-clones) were kindly provided by S. D. Tanksley, Cornell University, USA. Because of the similarity between the molecular maps of the tomato and potato genomes (Bonierbale et al. 1988), these tomato markers with known positions were selected as a basis for assigning potato loci to their respective linkage groups. All other markers were developed in our labs.

Segregation, linkage analysis and map construction

Whenever appropriate, a distinction was made between alleles segregating from parent C or parent E, to avoid erroneous linkage. Pairwise recombination frequencies were calculated with the computer program Linkage-1 (Suiter et al. 1983). This program is capable of combining 1:1:1:1, 3:1, 1:2:1 and 1:1 types of segregation within one cross, and does not require typical F_2 or testcross data sets. Maps were constructed with the computer program JoinMap (Stam, unpublished). Recombination frequencies were converted into map units according to Kosambi (1944).

Results

The genotypic identification of tester clones

The segregation ratios for flower colour in the progeny from G254-selfed, and from crosses between the three tester lines I_1G254-26, I_1G254-77 and I_1G254-83 (Table 1 A), fit the genetic model of two complementary genes postulated for tetraploid potato by Lunden (1937) and Salaman (1910). The phenotypes of G254 and I_1G254-77, comprising red-coloured sprout tips and a brownish-red anthocyanin pigmentation of the stem and inflorescence, indicate the presence of a dominant *D* allele. The white flowering I_1G254-26 and I_1G254-83 never showed pigmentation of any organ or tissue, indicating recessiveness at the *D* and *P* loci. Therefore, clone I_1G254-26 is identified as a tester homozygous for the *F*-locus, clone I_1G254-77 as a tester homozygous for the *D*-locus, and I_1G254-83 as a tester double recessive at both loci. Blue-pigmented flowers are not observed in either the testers or their offspring indicating recessiveness at the *P*-locus.

For any unknown clones it is now possible to deduce the genotype and to test for allelism by crossing them with the tester genotypes *DDff*, *ddFF* and *ddff*. New, non-allelic loci involved in anthocyanin pigmentation will also be recognized.

Segregation of flower colour in the mapping population

The observed ratio BLUE:RED:WHITE = 6:135:115 for flower colour in the cross C × E did not allow genetic interpretation. Therefore, it was necessary to unravel the phenotypes and the underlying genotypes of the parents C and E and their offspring using the three diploid testers. Additionally, this approach would identify allelic relationships between the *D* and *F* loci in the tester clones and the pigmentation loci expressed in the mapping population. The observed ratios and the χ^2 test for goodness of fit with expected ratios for flower colour from crosses between clones C, E and CE-clones are presented in Table 1 B, C.

Table 1. Segregation for flower pigmentation

Parents		Phenotype[a]		Observed[a]			Expected[b]		Parental genotypes[c]	
Female	Male	Female	Male	BLUE	RED	WHITE	Ratio (B:R:W)	χ^2	Female	Male
Part A: crosses between tester genotypes										
G254	selfed	RED			20	15	9:7	0.01	*pp Dd Ff*	*pp Dd Ff*
I_1G254-26	I_1G254-77	WHITE	WHITE		33	0	1:0	0.00	*pp dd FF*	*pp DD ff*
I_1G254-77	I_1G254-83	WHITE	WHITE		0	26	0:1	0.00	*pp DD ff*	*pp dd ff*
I_1G254-83	I_1G254-26	WHITE	WHITE		0	38	0:1	0.00	*pp dd ff*	*pp dd FF*
Part B: crosses between parents C and E and tester genotypes										
C	G254	RED	RED		78	37	9:7	6.26*	*pp Dd Ff*	*pp Dd Ff*
C	I_1G254-26	RED	WHITE		24	22	1:1	0.09	*pp Dd Ff*	*pp dd FF*
I_1G254-26	C	WHITE	RED		10	1	1:1	7.36**	*pp dd FF*	*pp Dd Ff*
C	I_1G254-77	RED	WHITE		15	19	1:1	0.47	*pp Dd Ff*	*pp DD ff*
I_1G254-77	C	WHITE	RED		11	18	1:1	1.69	*pp DD ff*	*pp Dd Ff*
C	I_1G254-83	RED	WHITE		13	30	1:3	0.63	*pp Dd Ff*	*pp dd ff*
E	G254 (1990)	RED	RED	9	150	73	3:18:11	10.96**	*Pp Dd Ff*	*pp Dd Ff*
E	G254 (1991)	RED	RED	9	45	27	3:18:11	0.28	*Pp Dd Ff*	*pp Dd Ff*
G254	E	RED	RED	11	127	90	3:18:11	6.77*	*pp Dd Ff*	*Pp Dd Ff*
E	I_1G254-26	RED	WHITE	13	56	33	1:2:1	8.82*	*Pp Dd Ff*	*pp dd FF*
I_1G254-26	E	WHITE	RED	10	36	20	1:2:1	3.58	*pp dd FF*	*Pp Dd Ff*
E	I_1G254-77	RED	WHITE	0	25	19	0:1:1	0.82	*Pp Dd Ff*	*pp DD ff*
I_1G254-77	E	WHITE	RED	0	25	30	0:1:1	0.45	*pp DD ff*	*Pp Dd Ff*
E	I_1G254-83	RED	WHITE	7	17	28	1:2:5	1.89	*Pp Dd Ff*	*pp dd ff*
I_1G254-83	E	WHITE	RED	1	5	19	1:2:5	2.42	*pp dd ff*	*Pf Dd Ff*
C	E	RED	RED	6	135	115	3:18:11	22.35**	*pp Dd Ff*	*Pp Dd Ff*
Part C: crosses between some CE and EC clones and tester genotypes										
CE16	I_1G254-26	WHITE	WHITE	0	23	0	0:1:0	0.00	*·· DD ff*	*pp dd FF*
CE16	I_1G254-77	WHITE	WHITE	0	0	18	0:0:1	0.00	*·· DD ff*	*pp DD ff*
CE17	I_1G254-26	WHITE	WHITE	8	13	2	1:2:1	3.52	*Pp Dd ff*	*pp dd FF*
CE17	I_1G254-77	WHITE	WHITE	0	0	19	0:0:1	0.00	*Pp Dd ff*	*pp DD ff*
CE18	I_1G254-83	BLUE	WHITE	9	0	29	1:0:3	0.04	*Pp dd Ff*	*pp dd ff*
CE24	I_1G254-83	BLUE	WHITE	7	0	30	1:0:3	0.73	*Pp dd Ff*	*pp dd ff*
CE60	I_1G254-26	WHITE	WHITE	9	11	5	1:2:1	1.64	*Pp Dd ff*	*pp dd FF*
CE60	I_1G254-77	WHITE	WHITE	0	0	20	0:0:1	0.00	*Pp Dd ff*	*pp DD ff*
CE67	I_1G254-83	RED	WHITE	1	8	30	1:2:5	4.69	*Pp Dd Ff*	*pp dd ff*
CE69	I_1G254-83	RED	WHITE	0	8	38	0:1:3	1.42	*pp Dd Ff*	*pp dd ff*
CE70	I_1G254-83	RED	WHITE	0	16	24	0:1:1	1.60	*pp D· F·*	*pp dd ff*
CE77	I_1G254-26	WHITE	WHITE	0	0	20	0:0:1	0.00	*pp dd ··*	*pp dd FF*
CE77	I_1G254-83	WHITE	WHITE	0	0	23	0:0:1	0.00	*pp dd ··*	*pp dd ff*
CE84	I_1G254-26	WHITE	WHITE	0	0	22	0:0:1	0.00	*pp dd ff*	*pp dd FF*
CE84	I_1G254-77	WHITE	WHITE	0	0	21	0:0:1	0.00	*pp dd ff*	*pp DD ff*
CE102	I_1G254-83	RED	WHITE	0	5	15	0:1:3	0.00	*pp Dd Ff*	*pp dd ff*
CE110	I_1G254-26	WHITE	WHITE	0	23	0	0:1:0	0.00	*pp DD ff*	*pp dd FF*
CE110	I_1G254-77	WHITE	WHITE	0	1?	20	0:0:1	–	*pp DD ff*	*pp DD ff*
CE111	I_1G254-26	WHITE	WHITE	0	12	11	0:1:1	0.04	*pp Dd ff*	*pp dd FF*
CE111	I_1G254-77	WHITE	WHITE	0	0	21	0:0:1	0.00	*pp Dd ff*	*pp DD ff*
EC601	I_1G254-83	RED	WHITE	13	14	36	1:2:5	3.82	*Pp Dd Ff*	*pp dd ff*
EC602	I_1G254-83	RED	WHITE	12	31	15	1:2:1	0.59	*Pp Dd FF*	*pp dd ff*

[a] According to colour classes based on Nickerson colour fan
[b] Ratio (BLUE:RED:WHITE)
[c] Postulated: BLUE = *PpddF·*/RED = *··D·F·*/WHITE = *····ff* or *ppdd··*
*, $P<0.05$; **, $P<0.01$

The genotype of parental clone C. The data in Table 1 B support heterozygosity for both the *D* and the *F* loci of clone C, although the ratios found in C × G254 and I_1G254-26 × C deviated significantly from expectation by an excess of RED individuals. However, the ratio in the reciprocal cross C × $I_1$254-26 fitted 1:1 unambiguously. As descendants with blue pigments were not observed, clone C is recessive at locus *P*. Therefore, the genotype of clone C is proposed to be *ppDdFf*, whereby loci segregating from clone C show allelism to those postulated by Lunden for tetraploid cultivars.

The genotype of parental clone E. Like clone C, the flower colour of clone E is RED according to our classification. However, clone E gave pale-blue, purple, red, pink and white flowering descendants in diverse crosses, and in ambiguous ratios. To explain these ratios it is necessary to consider epistatic relations between the loci involved in red and blue pigmentation. As a clear distinctive class of BLUE descendants is found segregating from the RED clone E, it is concluded that locus *D*, giving the RED phenotype, is epistatic to a third genetic factor, giving the BLUE phenotype. This third genetic factor, locus *P*, is present in clone E in a heterozygous condition, since white flowering descendants were found as well. Descendants with white flowers are also observed when clone E is crossed with either tester clone I_1G254-26 or I_1G254-77. This indicates heterozygosity of clone E for the *D* and *F* loci. However, when clone E has the genotype *PpDdFf*, some of the ratios shown in Table 1 B do not support this hypothesis. There is a striking deficit of light-blue-pigmented flowers in the offspring of the crosses C × E, E × G254 (made in 1990), G254 × E, E × I_1G254-26, and I_1G254-26 × E. This deficit seems to be associated with clone E, as CE-clones showed unbiased ratios for blue-pigmented flowers. Biased segregation of alleles of clone E was also observed at the RFLP level. The RFLP marker Ssp75, closely linked with the *P* locus, segregated 8:54, whereas a 1:1 ratio was expected. The scarcely present RFLP allele was in coupling phase with the dominant allele of the *P* locus. The restriction fragments hybridizing with Ssp75 transmitted from parent C showed a correct 1:1 ratio. Therefore, blue pigmentation is a monogenic trait, and the genotype of clone E is *PpDdFf*. The loci *D* and *F* for flower colour postulated by Lunden for Group Tuberosum show allelism to those found in clone E.

The genotypes of the mapping population. The flower colour genotype of CE- or EC-clones cannot be assessed from the phenotype. Neither is it possible to detect heterozygosity because of dominance. By crossing CE- and EC-clones with the testers I_1G254-26, 77 and 83 it was possible to deduce individual CE or EC flower-colour genotypes. Examples of the results are shown in Table 1 C. The genotypes of CE70 and CE77 are not fully unravelled because a conclusive combination with I_1G254-77 is lacking in both cases. To determine the genotype of CE18, CE24, CE67, and CE69, one cross was already decisive. The genotype of clone CE16 cannot be established with respect to locus *P* because locus *D*, being homozygous dominant, will mask the effect of the *P* locus. The finding of one red-flowering seedling in the offspring of CE110 × I_1G254-77 might be due to a contamination during pollination, during seed harvesting, or in growing the seedlings. The presence of a dominant *D*- or *P*-allele in a certain genotype, as determined genetically with the testers, was always confirmed with the phenotypic observation of a weak brownish-red, or purplish pigmentation in other parts of the plant. Several CE-clones with fully classified genotypes may serve as testers themselves, as we were able to identify clones with any possible genotypic combination of the *D*, *F* and *P* loci.

Linkage analysis

Cosegregation between RFLPs and the *P* locus was detected with the markers Ssp75, TG30 and TG47. TG47 and TG30 are reference markers previously localized on chromosome 11 in tomato (Tanksley et al. 1987). TG30 was mapped to the same position in potato (Bonierbale et al. 1989). The location of Ssp75 on chromosome 11 was confirmed in a different cross (Kreike et al., manuscript in preparation). Linkage with the *F*-locus was detected with the markers TG63 and TG43, both placed on chromosome 10 (Bonierbale et al. 1989; Gebhardt et al. 1991). Locus *D* is linked with the markers TG20(b) and TG48, previously mapped on chromosome 2 (Bonierbale et al. 1989; Gebhardt et al. 1991), and with marker STF13. No recombinant was found between the *D* locus and a locus hybridizing with a *Petunia hybrida* cDNA clone, pVip5043, for chalcone synthase (kindly provided by Dr. Ronald Koes, Amsterdam Free University).

Pairwise recombination frequencies were calculated with the computer program LINKAGE-1 and JoinMap. Each computer program has a different approach. The pairwise recombination percentages and the LOD score (Risch 1992) indicating the significance of linkage between the flower colour loci *D*, *F* and *P* and the linked RFLP markers are shown in Table 2.

Linkage maps of chromosomes 2, 10 and 11 were calculated with JoinMap using the mapping function of Kosambi (1944). The computer program JoinMap calculates the most likely map configuration from all pairwise recombination frequencies within a linkage group, and the level of significance of these linkages. This may result in a map distance which is different from the direct distance between two loci. For instance, without having observed a recombinant, JoinMap calculated 4.2 cM distance between the *D* and *CHS* loci. The calculated maps are shown in Fig. 1.

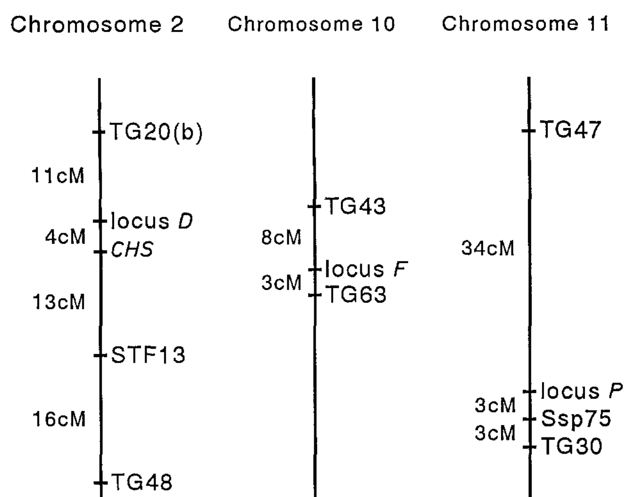

Fig. 1. Maps of the potato chromosomes 2, 10 and 11 showing the positions of the loci *D*, *F* and *P*. Map distances (cM) were calculated with JoinMap using the Kosambi mapping function

Table 2. Pairwise recombination percentage (half matrix above diagonal) and the LOD score indicating the level of significance (below diagonal) between molecular markers and the loci *D*, *F* and *P* for anthocyanin pigmentation

Chromosome 2, locus *D*					
	TG20(b)	locus *D*	*CHS*	STF13	TG48
TG20(b)	–	6.3%	16.1%	24.4%	38.1%^{ns}
locus *D*	4.1	–	0.0%	13.8%^{ns}	21.8%^{ns}
CHS	3.4	2.2	–	12.1%	26.5%^{ns}
STF13	2.5	1.5^{ns}	4.6	–	14.0%
TG48	1.0^{ns}	0.3^{ns}	1.7^{ns}	6.3	–
Chromosome 10, locus *F*					
	TG63	locus *F*	TG43		
TG63	–	4.6%	10.8%		
locus *F*	9.2	–	4.8%		
TG43	5.6	4.7	–		
Chromosome 11, locus *P*					
	TG47	locus *P*	Ssp75	TG30	
TG47	–	29.5%	33.3%^{ns}	39.2%^{ns}	
locus *P*	1.7	–	2.0%	6.1%	
Ssp75	1.0^{ns}	12.6	–	2.3%	
TG30	0.5^{ns}	9.8	22.1	–	

^{ns} Not significant

Discussion

Distorted segregation

This study deals with a case of potato genetics which is typical for that crop: segregations often do not fit Mendelian ratios. Such distorted segregation ratios have complicated the genetic interpretation of data on flower colour. Gebhardt et al. (1991) found aberrant ratios on many chromosomes of up to 40% of the loci mapped. Aberrant Mendelian segregation ratios may be the result of selection processes taking place during sporogenesis, gametogenesis, pollination, fertilization, seed development and germination. It is apparent that the potato harbours many deleterious alleles which are easily sexually transmitted in a tetraploid genome and maintained in a vegetatively propagated plant species.

The distorted segregation ratios require special efforts to make genetic interpretations plausible. Firstly, this study shows that the aid of simply inherited and fitness-neutral RFLP markers provided evidence for the distorted segregation of the *P* locus. RFLP loci linked to locus *P* on chromosome 11 showed a similar distortion, shifting an expected 1:1 ratio to a misleading 1:3 ratio. Secondly, crosses between plants of the mapping population and tester clones demonstrated clear cut segregations which confirmed the postulated genetics of flower colour.

The value of diploid tester clones

The possibility of deriving dihaploids from tetraploid cultivars allows for the development of diploid genetic stocks of Group Tuberosum. With the diploid tester clones I_1G254-26, I_1G254-77 and I_1G254-83 two problems could be solved. Firstly, it is demonstrated that an unknown genotype of a clone can be identified by crossing it with the testers and analyzing segregation ratios in the progeny. The identified genotypes of the mapping population are now also available as testers. Secondly, the alleles in the testers, which are identical by descent with the loci *D* and *F* defined by Lunden, and the loci segregating in the mapping population were proven to be allelic.

A genetic approach and a map-based approach to show allelism

The localization and identification of loci *D*, *F* and *P* is an important step in the further development of the classical genetics of potato. A consensus on the nomenclature of these classical loci is of great importance in order to avoid new symbols being assigned to previously described traits. While map position and phenotype are powerful indications of the identity of a segregating locus, they cannot provide proof of allelism of the kind demonstrated by crossing experiments which show genetic complementation.

The possible biochemical role of loci D, P and F

The *D* and *P* loci are assumed to be basic factors involved in the biosynthesis of red and blue anthocyanins respec-

tively, because their effect can be seen throughout the plant. In this study it is shown that blue flower colour can be observed in the absence of a dominant *D*-allele, and red flower colour can be observed in the absence of *P*. This indicates that the *P* and *D* loci are not involved in the same branch of the biochemical pathway. In addition, neither locus *P* nor locus *D* are involved in that part of the biochemical pathway where dihydrokaempherol is produced, which is the last intermediate before the branching point to red or blue anthocyanins.

The effect of locus *F* appeared to be localized in petals and did not affect the pigmentation of other parts of the plant. Therefore, a regulatory role of locus *F* in the flower-specific expression of the *D* and *P* loci is plausible.

The epistatic relations between loci D, F and P

According to Lunden (1937, 1974) locus *P* is epistatic to locus *D* as it is able to alter flower colour from violet into blue-purple. The segregation ratios observed in this study showed that red flower colour is due to the action of locus *D*, which is epistatic to the action of locus *P*. This difference may be regarded as an effect of defining the flower colour classes. Since we have observed a very clear distinction in our material between the BLUE flower colour class ($P \cdot ddF \cdot$) and the RED class, but no clear distinction within the RED class between $ppD \cdot F \cdot$ and $P \cdot D \cdot F \cdot$, it is justified to include both the $ppD \cdot F \cdot$ and the $P \cdot D \cdot F \cdot$ genotypes into the class RED. Other authors mention the same inconvenience in classifying flower colour. Dodds and Long (1955) conclude that epistasy is incomplete in the flower.

Mapping of loci D, F and P

The linkage analysis allowed us to map the *D*, *F* and *P* loci on chromosomes 2, 10 and 11, respectively, whereby the order of the RFLP loci on the chromosomes and their relative distances are in agreement with previously published maps (Bonierbale et al. 1989, Gebhardt et al. 1991). This makes flower colour the first non-monogenic morphological trait mapped on the potato genome.

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References

Bernatzky R, Tanksley SD (1986) Genetics of actin-related sequences in tomato. Theor Appl Genet 72:314–321

Black W (1933) Studies on the inheritance of tuber colour in potatoes. J Genet 27:319–339

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

Dodds KS, Long DH (1955) The inheritance of colour in diploid potatoes I. Types of anthocyanidins and their genetic loci. J Genet 53:136–149

Gebhardt C, Ritter E, Schachtschabel U, Walkemeier B, Uhrig H, Salamini F (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78:65–75

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

Hanneman RE, Peloquin SJ (1967) Crossability of 24-chromosome potato hybrids with 48-chromosome cultivars. Eur Potato J 10:62–73

Hermsen JGTh, Taylor LM, Van Breukelen EWM, Lipsky A (1978) Inheritance of genetic markers from two potato dihaploids and their respective parent cultivars. Euphytica 27:681–688

Howard HW (1960) Potato cytology and genetics, 1952–1959. Biblio Genet 19:87–216

Howard HW (1970) Genetics of the potato *Solanum tuberosum*. Logos Press, London

Jacobsen E (1980) Increase of diplandroid formation and seed set in 4x × 2x crosses in potatoes by genetical manipulation of dihaploids and some theoretical consequences. Z Pflanzenzüchtg 85:110–121

Jong H de (1991) Inheritance of anthocyanin pigmentation in the cultivated potato: a critical review. Am Potato J 68:585–593

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lunden AP (1937) Arvelighetsundersökelser i potet. Meldinger fra Norges Landbrukshogskole 30:1–48

Lunden AP (1960) Some more evidence of autotetraploid inheritance in the potato (*Solanum tuberosum*). Euphytica 9:225–234

Lunden AP (1974) Inheritance of tuber and flower colour in the potato (*Solanum tuberosum*). Report No. 190. Meldinger fra Norges Landbrukshogskole 53:1–19

Risch N (1992) Genetic linkage: interpreting Lod scores. Science 255:803–804

Salaman RN (1910) The inheritance of colour and other characters in the potato. J Genet 1:7–46

Suiter KA, Wendel JF, Case JS (1983) LINKAGE-1: a PASCAL computer program for the detection and analysis of genetic linkage. J Hered 74:203–204

Swaminathan MS, Howard HW (1953) The cytology and genetics of the potato (*Solanum tuberosum*) and related species. Biblio Genet 16:1–192

Tanksley SD, Mutschler MA, Rick CM (1987) Linkage map of the tomato (*Lycopersicon esculentum*) (2n = 24) In: O'Brien SJ (ed) Genetic maps 1987. A compilation of linkage and restriction maps of genetically studied organisms. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 655–669