Localisation of morphological traits on the genetic map of potato using RFLP and isozyme markers.

Localisatie van morfologische eigenschappen op de genetische kaart van de aardappel door middel van RFLP en isozym merkers.

Ontvangen

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Cover: Reproduction of Plate XXIX showing tubers of 23 descendants which segregate for tuber shape and skin colour. From: Salaman R.N. (1910) The inheritance of colour and other characters in the potato. Journal of Genetics Vol. 1 No 1. p:7-46

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Key-words: *Solanum tuberosum*, genetics, RFLP mapping, QTL, Plant breeding

Bibliographic Abstract: The thesis describes the construction of a genetic linkage map of the potato genome, comprising molecular, isozyme and morphological markers. The linkage map is based on the offspring from non-inbred parents. The computer program JoinMap allowed to combine the maternal and paternal linkage groups, which are a consequence of using non-inbred parents. The map was applied for the analysis and localisation of morphological and agronomically important traits, with special emphasis on the inheritance of anthocyanin pigmentation. The presence of multiple alleles was demonstrated at the *Ro* locus which harbours a QTL involved in tuber shape. The accurate analysis of quantitative genetic variation caused by the segregation of a QTL with multiple alleles requires molecular markers which can distinguish all possible four allele combinations.

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Stellingen

1 De locus *D* (Salaman, 1910; Lunden, 1937) welke betrokken is bij de produktie van rode anthocyaan pigmentatie is allelisch met de locus R/R^{pw} (Dodds en Long, 1956), en is niet allelisch met de schilkleur locus *I* zoals beweerd door Howard (1970) en de Jong (1991).

Dit proefschrift.
Dodds K.S., Long D.H. (1956) J. Genet. 54:27-41.
Howard H.W. (1970) Genetics of the potato, *Solanum tuberosum*, Logos Press, NY.
De Jong H. (1991) Am. Potato J. 68:585-593.
Lunden A.P. (1937) Særtrykk av Meldinger fra Norges Landbrukshøiskole, Norges Landbrukshøiskoles Åkervekstforsøk, pp.1-156.
Salaman R.N. (1910) J. Genet. 1:7-46.

2 Multipele allelie vormt een onderschatte bron van kwantitatief genetische variatie.

3 Het effect van een allel van een *quantitative trait locus* (QTL) wordt mede bepaald door inter- en intralocus interacties.

Rasmusson J. (1934) Hereditas 18:245-261.

4 De situatie dat een kwantitatieve eigenschap louter gebaseerd is op polygenen met slechts twee allelen, of de situatie dat waarin sprake is van slechts één locus met multipele allelie, zijn allebei te beschouwen als de extremen van een continuüm.

Sirks M.J. (1929) Multiple allelomorphs versus multiple factors. Proc. of the Int. Congr. of Pl. Sci., Ithaca, New York 1:803-814.

5 De door Sirks (1929) waargenomen splitsingsverhoudingen wijken minder af van de theoretische verwachtingen, dan volgens het toeval verwacht mag worden.

Sirks M.J. (1929) Genetica 11:293-328.

6 De evolutietheorie is afhankelijk van een aantal uiterst creatieve momenten.

7 Alvorens biologisch-dynamische produkten te kopen, dient men zich af te vragen of men achter het antroposofisch gedachtengoed van Rudolf Steiner kan staan.

8 De theorie van de morphogenetische velden van Rupert Sheldrake gaat ook op voor het ontstaan van BC1 nakomelingen van somatische hybriden tussen aardappel en tomaat via zaadknopcultuur.

9 Tegenover een recht om te sterven kan nooit een plicht om te doden staan.
Dr. G. Manenschijn. De cultivering van hulp bij zelfdoding. Trouw, 5 november 1994.

10 De verantwoordelijkheid voor het creëren van deeltijd-functies ligt niet alleen bij werkgevers en vakbonden, maar ook bij mensen die thans een volledige baan hebben.

11 Duurzame ontwikkeling is een utopie.

12 Hoe harder de muziek, hoe duidelijker de teksten.

13 Een assistent in opleiding moet soms meerdere assistenten opleiden.

Stellingen behorende bij het proefschrift getiteld "**Localisation of morphological traits on the genetic map of potato using RFLP and isozyme markers**", door Herman J. van Eck.

Wageningen, 10 januari 1995.

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Chapter 1

General introduction

Potato breeding

On a world scale potato is a widely grown crop and ranks fourth in food production (261 x 10^9 kg), following wheat (550 x 10^9 kg), rice (519 x 10^9 kg), and maize (478 x 10^9 kg) (FAO Production Yearbook, 1991). The crop belongs to the plant family *Solanaceae* and is related to several other well-known crops like tomato, egg-plant, tobacco, and chili peppers. The genus *Solanum* comprises of 225 wild tuber-bearing species according to the latest taxonomic interpretation by Hawkes (1990). Nine *Solanum* species with different ploidy levels, are cultivated. Only the cultivated potato *Solanum tuberosum* L. is being grown worldwide. It is a highly heterozygous tetraploid species with $2n=4x=48$ chromosomes. At the diploid level potato species are obligatory outbreeders due to gametophytic self-incompatibility. At the tetraploid level potato is self-compatible and a high degree of spontaneous self-fertilisation can be found. However, at both the diploid and the tetraploid level it is hardly possible to obtain homozygous lines because severe inbreeding depression is observed in inbred progenies.

At present, the breeding method consists of crossing and selection of descendants. This is not basically different from the method applied by Geert Veenhuizen or Klaas de Vries who selected cv. Eigenheimer in 1893 and cv. Bintje in 1910, respectively. A typical breeding scheme, as presently applied by commercial Dutch breeding companies, is clonal selection among 70,000-150,000 seedlings per year, derived from 150-300 different crosses. Selection is performed on more than 50 traits, which can be grouped in (1) yield, (2) resistance to pathogens and environmental factors and (3) quality traits with respect to different utilisation purposes. Classical potato breeding consists of crossing, evaluation and selection, official variety tests, healthy maintenance and clonal propagation of seed potatoes and marketing. It takes 10 - 15 years to generate a new variety.

During the last 3-4 decades new approaches to accelerate and improve classical

potato breeding have been attempted, such as: ploidy manipulation using parthenogenesis and unreduced gametes, breeding of parental lines at the 2x and 4x level and utilisation of valuable genes from wild and primitive *Solanum* species (Chase, 1963; Wenzel *et al.*, 1979; Ross, 1986; Hermsen, 1994). At present plant breeding is being enriched by new and promising molecular genetic tools for the diagnosis of valuable genetic variation and for the synthesis of new variation by genetic modification. The contribution of molecular diagnostics with DNA markers in the advancement of plant breeding and genetics is described in the next paragraph.

Plant breeding, genetics and DNA polymorphisms

Improvement and adaptation of crop species have been achieved by a centuries old process of selection of individuals with a superior performance. The process of sexual recombination, and offspring selection applied by the breeder is aiming at genetic improvement of the crop by an accumulation of beneficial alleles and elimination of detrimental alleles. Until the beginning of this century the only information about the results of his breeding efforts, was obtained by the breeder through phenotypic assessment of the selected varieties.

The advancement of 'genetics' as a new science in the beginning of this century (Bateson, 1905) was based on the rediscovery of the work by Mendel (1875). This new discipline provided plant breeding with a scientific base and allowed the development of rational selection methods to complement the intuitive selection through the breeder's eye. Additionally, genetics gave a better understanding of the relation between the phenotype and the underlying genotype. When dealing with a qualitative character, the relation between the phenotype and the genotype of the parents is easily recognised from the simple numerical proportions observed in the segregating progeny, as pointed out by Mendel. For agronomically important traits qualitative inheritance seems not to occur generally, and may even be considered as exceptional. Quantitative traits cannot be described in discrete phenotypic classes, but are described through the trait values of single individuals, which are conceived as aselect drawings from a continuous distribution. The relation between the phenotypic value and the genotype for most quantitative traits has remained obscure. Common

unanswered questions have been: How many genes influence the trait? How much does each separate gene contribute to the trait? Is there additive or non-additive interaction between alleles at the same locus, or epistatic interaction between loci?

Genetic markers can be used to study these questions. However, morphological markers may adversely affect the investigated traits and are, like isozyme markers, limited in number. Molecular biology has recently provided genetics with a new class of markers, which are based upon the presence of variation at the DNA sequence level. These DNA sequence variations can be monitored as changes in the length of DNA fragments produced by restriction endonucleases. The method has, therefore, been termed 'Restriction Fragment Length Polymorphisms' (RFLPs) (Grodzicker *et al,*. 1974; Botstein *et al.*, 1980). When the potential of this tool was recognised for identification of genetic variability, construction of genetic maps, genetic analysis of economic traits, and breeding methodologies (Beckman and Soller, 1983; Burr *et al.*, 1983) it was soon followed by other molecular techniques to visualise DNA sequence variation. Polymorphic DNA fragments can also be generated with the PCR approach using short (10-mer) oligonucleotide primers (RAPDs) (Williams *et al.*, 1990). At present, many types of molecular markers with different useful properties have emerged and can be utilised for genetic analysis (See review Rafalski and Tingey, 1993)

The most fundamental characteristic of codominant molecular markers is the possibility to probe an unlimited amount of loci whereby detailed information about the genetic variation in the nuclear genome can be obtained at the DNA level. This means that genetic variation can be assessed genome-wide from the genotype, rather than from the phenotype. This results in a one-to-one relationship between genotypic variability and phenotypic variability, irrespective of the question whether or not the mode of inheritance of a trait is known. Essentially the differences have disappeared between monogenic or polygenic inheritance (Paterson *et al.*, 1988), and between qualitative or quantitative traits (Robertson, 1985). The dominant, epistatic or heterotic interactions between alleles from one or more loci can be estimated (Fatokun *et al.*, 1992, Stuber *et al.* 1992). The shift from a genetics based on the

inference of genotype from phenotype, as pioneered by Mendel, to a genetics based on the direct analysis of DNA sequence variation has been indicated as a change in genetic paradigm by Beckmann (1988). He introduced the term "Genomic genetics" to mark this pervasive research technique to study the structure and organisation of the nuclear genome in contrast to "Mendelian genetics" which targets the inheritance of individual traits.
The plant breeder may presently use molecular markers to relate superior phenotypical performance to the presence of specific genomic regions. Subsequently the RFLP probes marking these regions can be used in indirect selection for agronomic traits.

Application of molecular markers in potato breeding and genetics
For maximum performance of a potato cultivar, tetraploidy and a high degree of heterozygosity are required. However, these very requirements severely hamper progress in breeding along the classical lines of hybridisation and selection, and also greatly complicate genetic analysis of the crop. But still, a profound knowledge of its genetic composition is the basic requirement for developing more efficient breeding methods, including techniques of gene cloning and genetic modification.
For this reason the Netherlands Technology Foundation (STW) decided in 1988 to grant a project aimed at enhancing the genetic knowledge of potato. This project was on the construction of a genetic map of the potato genome using molecular, classical and cytogenetical methods. A genetic map based on Restriction Fragment Length Polymorphisms (RFLPs) was considered to be a powerful tool to unravel the heredity of agronomically important traits and to develop marker-assisted selection methods. The linkage groups as identified with genetic markers should be related to the potato chromosomes as cytogenetically identified by Ramanna and Wagenvoort (1976) at pachythene stage. The development of a protocol for *in situ* hybridisation of potato chromosomes with labelled DNA probes allowing chromosome recognition, and/or the use of a series of trisomics was therefore considered to be required.
Since 1988 the features of potato genetics have remarkably changed through the use of RFLPs. Before that, the genetics of potato was poorly developed due to

tetrasomic inheritance and distorted segregations caused by a high genetic load. Only a few genetic markers were described. The development of potato genetics was greatly stimulated by research on the diploid level and by the transfer of molecular-genetic achievements from tomato to potato. Bonierbale *et al.* (1988) published the first molecular map of potato using tomato RFLP markers and demonstrated co-linearity between the potato and tomato genomes. On the basis of this co-linearity further exchange of results of genome projects is possible and may be benefical to genetic improvement of both crops.

The first genetic map (Bonierbale *et al.* 1988) was based on 1:1 segregating polymorphisms from only one parent. The second genetic map of potato (Gebhardt, 1989) was constructed on the basis of three linkage groups per chromosome. The first linkage group consists of alleles from the female parent, segregating in a 1:1 ratio. The second linkage group comprises 1:1 segregating alleles from the male parent. Since the mapping population was a backcross, both parents had at least one allele in common. When this common allele is polymorphic, the third linkage group can be based on both parents and includes alleles segregating 3:1. Finally, these three linkage groups have been merged according to the positions of common loci (Gebhardt and Salamini, 1992).

During the last years several monogenic loci for resistance to nematodes, viruses, and fungi have been localised on the potato genome using molecular markers (Table 1). The information on the map position allows further identification of genes for resistance from different donor species to different pathotypes. Information on the map position of resistance genes can be used for marker assisted selection, but is also being used in attempts to clone these genes using transposon tagging (Pereira *et al.*, 1992). Transposon tagging was successfully applied by Jones *et al.* (1994) to isolate the *Cf9* gene from tomato. An alternative approach for the isolation of resistance genes using position information, such as map-based cloning has recently resulted in cloning of the *Pto* gene from tomato (Martin *et al.*, 1993).

Contrary to the tremendous emphasis on localisation of resistance genes only few efforts have been made to map morphological marker genes. Agronomically important traits predominantly display a polygenic and/or quantitative inheritance. Therefore, these traits are less amenable to genetic analysis. Only

recently, the localisation of Quantitative Trait Loci (QTLs) in diploid potato has been demonstrated by Kreike *et al.* (1993, 1994), Leonards-Schippers *et al.* (1994) and Van Eck *et al.* (1994). These publications are also the first to describe QTL mapping in allogamous species. Therefore, potato can be regarded at this moment as a model species for QTL mapping experiments to other allogamous crops.

Table 1: Monogenic trait loci mapped on the potato genome using RFLPs.

Trait	Symbol of loci	Chromosome	Reference
Classical			
Gametophytic self-incompatibility	*S*	*1*	Gebhardt *et al.* (1991)
Yellow flesh colour	*Y*	*3*	Bonierbale *et al.* (1988)
Purple skin colour	*PSC*	*10*	Gebhardt *et al.* (1989, 1991)
Nematode resistance			
Globodera rostochiensis	*Gro1*	*7*	Barone *et al.* (1990)
Globodera rostochiensis	*H1*	*5*	Pineda *et al.* (1993)
" "	*H1*	*5*	Gebhardt *et al.* (1993)
Virus resistance			
PVX adg CPC 1673	*Rx1*	*12*	Ritter *et al.* (1991)
PVX acl MPI 44.1016/10	*Rx2*	*5*	Ritter *et al.* (1991)
Fungus resistance			
Phytophthora infestans	*R1*	*5*	Leonards-Schippers *et al.* (1992)
Phytophthora infestans	*R3*	*11*	El-Kharbotly *et al.* (1994)

Scope of this thesis

The aim of the investigations described in this Ph.D. thesis has been the construction of a genetic map of potato including morphological, biochemical and molecular markers. This resulted into the integrated genetic map of potato as described in chapter 2. This map was constructed on the basis of polymorphisms which segregated from both heterozygous parents. The method

is described to merge the two maps reflecting the female and male recombination into one chromosome map.

Most of the morphological marker loci on this map are involved in anthocyanin pigmentation. The genetics of anthocyanin pigmentation in potato has been a matter of controversy for many decades, and two genetic models have been postulated in the literature. For a long period arguments were put forward to indicate that loci involved in pigmentation were different in tetraploid *Solanum tuberosum* Group Tuberosum on one hand and in diploid cultivated species on the other. Using diploid tester clones representing *Solanum tuberosum* Group Tuberosum germplasm, and information on the map position of skin colour loci, it appeared to be possible to bridge the two genetic models. The nomenclature of the loci involved in anthocyanin pigmentation is related now to a genomic position. The localisation of the loci *D*, *F* and *P* involved in flower pigmentation is described in chapter 3. The localisation of factors involved in skin colour as well as allelic relations between the genetic models on anthocyanin pigmentation are described in chapter 4.

The results of a mapping study of another morphological trait, tuber shape, is described in chapter 5, and provides evidence for the existence of multiple alleles at QTLs. The effects of multiallelism and heterozygosity of both parents have led to important generalised suggestions for QTL mapping in outbreeding crops which are dealt with in this chapter.

The thesis ends in chapter 6 in a general discussion to evaluate the results and the utility of RFLP mapping in potato.

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CHAPTER 2

A MOLECULAR MAP OF POTATO FROM NON-INBRED PARENTS INCLUDING ISOZYME AND MORPHOLOGICAL TRAIT LOCI

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Summary

A genome map of diploid *Solanum tuberosum* L. ($2n=2x=24$) was constructed using segregation data of 197 RFLP, 9 isozyme and 11 classical genetic markers in a 67 backcross progeny generated from non-inbred parents. A mixture of five types of singe-locus segregations was observed. Seventy 1:1♀, sixty-five 1:1♂, nine 3:1, seven 1:2:1 and sixty-six multi-allelic 1:1:1:1 segregating marker loci were (sub)divided to construct a separate maternal map, based on 152 marker loci heterozygous in the female recurrent parent, and a separate paternal map, based on 147 loci segregating in the male hybrid parent. The separate parental maps, and the combined genome map, were constructed with the computer program JoinMap, using the 3:1, 1:2:1 and multiallelic 1:1:1:1 segregating loci as 'allelic bridges' to align the parental linkage groups. This method of map construction is compared with presently employed strategies to overcome obstacles encountered during map construction, caused by the non-inbred nature of the parental clones. Skewed segregations, found at 38 loci ($p < 0.05$) could be ascribed to gametic selection at 28 loci. Zygotic selection, detected with 1:1:1:1 segregating markers, was found at 9 loci.

Introduction

Linkage maps are a valuable tool in genetic studies and plant breeding. The accurate localization of monogenic as well as polygenic traits, within an extensive map framework, allows the identification, efficient introgression and selection of individuals with specific traits. Marker assisted selection is one of

the most promising methods to accelerate the time consuming breeding process. In cultivated potato (*Solanum tuberosum* L.), genetic analysis of morphological and agronomically important traits, and the mapping of the relevant loci, is difficult, because of its autotetraploid nature (2n=4x=48) which is accompanied with a high degree of heterozygosity and tetrasomic inheritance. The use of diploid wild species and especially the development of techniques to obtain dihaploid clones of *S. tuberosum* (*eg*. Hermsen and Verdenius, 1973), have simplified inheritance studies and made the study of potato genetics more feasible. However, the development of specific breeding parents and tester lines with interesting traits, is time-consuming and hindered by severe inbreeding depression and by self-incompatibility of *S. tuberosum* at the diploid level. Therefore, despite the development of diploid clones, only a few classical genetic markers have been mapped to date. These include isozyme loci, tuber flesh colour (Bonierbale *et al.*, 1988) and tuber skin pigmentation (Gebhardt *et al.*, 1989).

The development of molecular marker techniques has dramatically increased the number of marker loci mapped in the potato genome. This has resulted in two RFLP maps of potato to date, both based on diploid populations. One is based on the homology of the potato and tomato genomes, using tomato RFLP markers in interspecific crosses between diploid wild potato species and *S. tuberosum* (Bonierbale *et al.*, 1988; Tanksley *et al.*, 1992). The other makes use of potato RFLP markers in an intraspecific cross of *S. tuberosum* (Gebhardt *et al.*, 1989). Both maps consist almost exclusively of molecular markers. The development of such a genetic map, including isozyme and classical markers can provide a valuable tool for marker assisted selection of desired genotypes.

The aim of this research is to construct a genetic map of potato that can serve as a basis for genetic studies as well as for breeding purposes. In this paper we describe a general method for the construction of a genetic map, using the progeny of non-inbred parents. This allowed the development of two independent parental maps that integrate molecular markers with morphological and isozyme markers. These parental maps were merged into a combined map,

based on the computer program JOINMAP (Stam, 1993). The resulting linkage map thus fully exploits the different types of single locus segregation ratios observed in a highly heterozygous crop like potato. This map is being used as a reference framework for further studies including the mapping of quantitative trait loci and disease resistance genes. Furthermore, to enable targeted transposon tagging of resistance genes, this map is also being used for determining the integration sites of T-DNA's and transposable elements in the potato genome in a large set of independent potato transformants.

Materials and methods

Plant material and genetic structure of the mapping population.

Two diploid potato clones, coded C (originally named USW5337.3; Hanneman and Peloquin, 1967) and E (originally named 77.2102.37; Jacobsen, 1980) were crossed. Clone C is a hybrid between *S. phureja* PI 225696.1 and the *S. tuberosum* dihaploid USW42. Clone E was obtained from a cross between clone C and the *S. vernei* - *S. tuberosum* backcross clone $VH^3$4211 (Jacobsen, 1978). The diploid mapping population (C x [C x $VH^3$4211]), is therefore a backcross population, with a size of 67 descendants.

As a consequence of using non-inbred parents, five single locus segregation classes can be observed. The consequence of using a backcross from non-inbred parents, is that two or three alleles can segregate at a locus, and that the parental clones C and E always have one allele in common. The clones C and E and their offspring have been used in previous genetic studies (Jongedijk *et al.*, 1989; Jongedijk *et al.*, 1990). The diploid potato clone J92-6400-A16 was used to generate the many independant transformants with single integrations of T-DNA constructs containing maize transposable elements (El-Kharbotly *et al.*, in preparation).

Molecular techniques

DNA isolation, Southern blotting, hybridisation, and autoradiography were performed as previously described by Van Eck *et al.* (1993). Survey blots with DNA from both parental clones C and E, digested with *Dra*I, *Eco*RI, *Eco*RV, *Hin*DIII, and *Xba*I, were used for finding polymorphisms. Whenever possible

the most informative probe/enzyme combination was selected, displaying both alleles from both parental clones in the mapping population.

RFLP markers

Different sources of RFLP markers were used to detect segregating loci. From a potato leaf cDNA library of c.v. Bintje (Nap *et al.*, 1993) **ST** markers (*Solanum tuberosum*) were developed. **TDs**, **TAc** and **TI** markers are derived from potato genomic DNA sequences flanking the integration sites of T-DNA constructs containing a Ds, Ac or I transposable element. **Ac** markers originate from potato genomic DNA flanking the reintegration site of transposed Ac elements. The flanking potato DNA sequences were obtained via plasmid rescue by electroporation (Dower *et al.*, 1988) or were isolated by the inverse polymerase chain reaction (IPCR) (Triglia *et al.*, 1988). **Ssp** markers originated from a genomic PstI library of the wild species *Solanum spegazzinii* (Kreike *et al.*, 1993). **TG** markers, genomic markers from tomato with known chromosomal positions in potato (Bonierbale *et al.*, 1988) and **GP** markers, genomic potato markers (Gebhardt *et al.*, 1989) enabled alignment with other potato maps and an analogous chromosome numbering of our map. In addition, cDNA probes of cloned potato genes were used as probes for mapping: GBSS (Granule bound starch synthase) and BE (Branching enzyme) were provided by R. Visser; CHS*St* (Chalcone synthase, *S. tuberosum*) and STF13 (*S. tuberosum* flower specific cDNA) were provided by DLO-CPRO; a CHS*Ph* cDNA clone pVIP5043 from *Petunia hybrida* was provided by R. Koes. Duplicate loci detected by a single probe are indicated by the addition of a letter at the end of the locus name, eg. TAc13A and TAc13B.

Isozymes

Ten different enzyme systems were assayed for enzyme activity, for gel type giving optimal resolution and consistent appearance of bands, and for enzyme polymorphisms. Young leaf tissue was used to prepare samples for the following enzyme systems: 6-Phosphogluconate dehydrogenase (6-PGDH), Diaphorase (DIA), and Glutamate oxaloacetate transaminase (GOT), Phosphoglucomutase (PGM), Shikimate dehydrogenase (SKDH), Malate

dehydrogenase (MDH), Triose phosphate isomerase (TPI) and Acid phosphatase (APS). Tuber tissue was used in case of Esterases (EST) and Alcohol dehydrogenase (ADH) (*Adh-1* locus) and anthers for the *Adh-2* locus.
For sample preparation, tissues were ground on ice with an equal volume of cold 0.05 M Tris-HCl extraction buffer (pH 6.9) containing 1 % β-mercaptoethanol. Immediately after brief centrifugation to remove debris, clear supernatant was applied on the gel using 1 μl wells. Electrophoresis was carried out on precast polyacrylamide PhastGels (Pharmacia). The gels were buffered with 0.112 M Tris-acetate (pH 6.4). The native buffer strips contained 0.25 M Tris and 0.88 M L-alanine (pH 8.8) in 2% agarose IEF (Pharmacia).
The electrophoresis conditions for the 10-15 % and 8-25 % gradient gels, programmed on the control unit of the PhastSystem, were as follows: (1) pre-run 400 V, 10 mA, 2.5 W, 10 Vh; (2) sample-application run 400 V, 1 mA, 2.5 W, 2 Vh; (3) separation 400 V, 10 mA, 2.5 W, 268 Vh. Conditions for the 12.5 % homogeneous gel were (1) pre-run 400 V, 10 mA, 2.0 W, 10 Vh; (2) sample-run 400 V, 1 mA, 2.0 W, 10 Vh; (3) separation 400 V, 10 mA, 2.0 W, 125 Vh. For the homogeneous 20 % and the high density gel the conditions for electrophoresis were (1) Pre-run 500 V, 10 mA, 3.0 W, 40 Vh; (2) sample-run 500 V, 1 mA, 3.0 W, 10 Vh: (3) Separation 500 V, 10 mA, 3.0 W, 400 Vh. The temperature was maintained at 15°C. Separation was obtained in about 75 min for all gel types. The gels were stained using standard procedures (Vallejos, 1983).
The nomenclature to indicate loci and alleles is based on the electrophoretic mobility of the enzymes. The fastest migrating locus is named *locus-1* and the fastest allele within a locus is named *locus-1*^{1}. However, in several cases it was preferred to use locus names which correspond to previous publications.

Classical genetic markers

Classical genetic markers used in this study, along with their locus symbol and methods of analysis are summarized in Table 1. Analyses of morphological traits have been previously described in this mapping population for loci involved in anthocyanin pigmentation of flowers *F*, *D* and *P* (Van Eck *et al.*, 1993; Chapter 3), and tuber shape locus *Ro* (Van Eck *et al.*, 1994; Chapter 5).

Table 1.
Description of the classical genetic trait loci with respect to the origin of the locus symbol and the method of analysis.

Trait	Symbol	Scoring method and reference
Flower colour	*F/f* (Lunden, 1937)	crosses with tester lines (Van Eck *et al.*, 1993)
Red anthocyanins	*D/d* (Salaman, 1910)	
Purple anthocyanins	*P/p* (Salaman, 1910)	
Tuber shape	*Ro/ro* (Masson, 1985)	phenotypic observation (Van Eck *et al.*, 1994)
Tuber flesh colour	*Y/y* (Howard, 1970)	phenotypic observation (Jongedijk *et al.*, 1990)
Yellow margin	*Ym/ym* (Simmonds, 1965; Hermsen *et al.*, 1978)	test-crosses (this paper)
Crumpled	*Cr/cr* (Jongedijk *et al.* 1990)	test-crosses (this paper)
Desynapsis	*Ds1/ds1* (Jongedijk *et al.*, 1988)	Cytogenetic observations (Jongedijk *et al.*, 1988)
Metribuzine sensitive	*Me/me* (De Jong, 1983)	Nutrient solution test (De Jong, 1983)
Self-incompatibility	*S* (Cipar *et al.*, 1964)	Stylar glycoprotein assay (Thompson *et al.*, 1991)
Earliness	*El/el* (This paper)	Field test (this paper)

Tuber flesh colour data were recorded according to the Dutch Descriptive List of Varieties (Anonymous, 1988). The trait was observed in tubers from three replicated field plots. In comparison to the flesh yellowness of standard varieties, the clones of the mapping population could be classified on an ordinal scale ranging from 3 to 9. Although tuber flesh colour displayed a continuous variation, clones were indicated white (*yy*) when the average trait value over three replications was below 5½, and yellow (*Y-*) when the trait value was ≥5½ (Jongedijk *et al.*, 1990).

The recessive morphological leaf mutant 'yellow margin' (*ymym*) is described by Simmonds (1965) and Hermsen *et al.* (1978). Yellow margin did not segregate in the mapping population (Jongedijk *et al.*, 1991), because the parental clone C is homozygous dominant. Backcrosses of the mapping population to the parental clone E (*Ymym*) were made to establish the genotype at the *Ym* locus of each CE clone. For each backcross, 25 seedlings were observed to detect segregants with yellow margins.

The recessive morphological mutation 'crumpled' (*crcr*) segregated in a 3:1 ratio in the CE population, since both parents are heterozygous (Jongedijk *et al.*, 1990). Crumpled descendants are easily identified by their stunted phenotype. However, these sublethal plantlets could not be used in RFLP analysis. Segregation at the *Cr* locus was determined in the non-mutant part of the C×E offspring. Backcrosses to both parental clones were used to differentiate between the *CrCr* or *Crcr* individuals.

A mutation at the *Ds1* locus affects pairing and recombination of homologous chromosomes during the meiotic prophase I. The mutant phenotype desynapsis segregated in a 3:1 ratio in the mapping population, because both parental clones are heterozygous *Ds1ds1* (Jongedijk *et al.*, 1990). Cytogenetic assessment of the phenotypes was carried out according to Jongedijk and Ramanna (1988). Fertile plants with a normal meiosis were scored *Ds1-*; desynaptic individuals, lacking fertility, were scored homozygous recessive *ds1ds1*. Sensitivity to the herbicide metribuzin (Sencor®) was screened by the growth of cuttings in a metribuzin containing nutrient solution (De Jong, 1983). Parental clones and the CE offspring were tested in three replications, each with five cuttings.

Segregation at the *S* locus, involved in gametophytic self-incompatibility, was assessed by isoelectric focusing of the stylar glycoproteins involved in the incompatibility reaction (Thompson *et al.*,1991). The *S*-alleles of the parental clones were identified by using standard clones provided by R. Eijlander.
Several agronomically important traits, including earliness, were assessed by planting the mapping population, together with the parental clones, in a field experiment. This involved a complete randomized block design with four hill plots in three replications. Earliness data were recorded on an ordinal scale ranging from 2 to 9 according to the Dutch Descriptive List of Varieties (Anonymous, 1988). The cultivars Pimpernel (late = 3.5) and Bintje (medium early = 6.5) were used as standards. Clones which had a score below the average of the population were indicated as late maturing types, provisionally indicated with the homozygous genotype *elel* of a putative locus involved in earliness. Clones, more early than average, were indicated with *El*-.

Data collection, storage and preliminary analysis

Data on the genotypes of the parents and the offspring clones were recorded according to strict rules, to be able to handle the mixture of five types of singe-locus segregations (see Table 2) in a genetically consistent way. This type of segregation was established on the basis of the parental genotypes, the number of offspring classes, and the segregation ratio between the offspring classes. The genotypes of the individual descendants were recorded using the two-letter codes as shown in Table 2. The first position of the code indicates the maternally derived allele, and the second position indicates the paternal allele. Segregation distortion was analyzed using the Chi-square test for goodness-of-fit. At 1:1♀, 1:1♂, and 1:1:1:1 segregating loci skewness, due to gametic selection was tested ($\chi^2_{(df=1)}$) using the gametic classes segregating from clone C and clone E separately. Zygotic selection was tested using the Chi-square contingency test (df=1) only at 1:1:1:1 segregating loci. Input files for linkage analysis and map construction with the computer programs Linkage-1 (Suiter *et al.*, 1983) and JOINMAP (Stam, 1993) were composed from these raw data. Linkage-1 can use two-letter codes. However, the commonly available software for map construction allows only one-letter codes (A, H and B) per genotype.

Table 2. Possible types of single locus segregation ratios in the BC progeny from non-inbred parents, and types and numbers of markers examined in this study.

Parental genotypes[1]	Offspring genotypes	Expected ratio's	Parental map		Marker type											
					RFLP									Isozyme	Classical	
					ST	Ssp	TG	GP	TDs	TAc	TI	Ac	genes			total
AH × *HH*	*AH*:*HH*	1:1	♀	-	5	6	16	-	21	3	1	7	3	4	3	69
HH × *HB*	*HH*:*HB*	1:1	-	♂	3	3	10	-	29	3	1	7	1	5	3	65
Cc × *Cc*	*C-*:*cc*	3:1	♀	♂	2	-	-	-	1	1	-	-	-	-	4	8
AB × *AB*	*AA*:*AB*:*BB*	1:2:1	♀	♂	3	-	2	-	-	-	-	-	-	-	1	6
AH × *HB*	*AH*:*AB*:*HH*:*HB*	1:1:1:1	♀	♂	6	5	17	2	18	1	4	11	3	-	-	67
unassigned					2	2	1	-	6	2	-	6	2	1	-	22
mapped					17	12	44	2	63	6	6	19	5	8	11	193
Totals					19	14	45	2	69	8	6	25	7	9	11	215

[1] *A*, *H* and *B* are symbols to indicate different alleles in the parental and offspring genotypes, whereby the letter *H* always refers to the common allele shared by the parents of this backcross, and the letters *A* and *B* are used to indicate the alternative allele.

Although originally designed for mapping studies in species with inbred parents, JoinMap is suitable for linkage analysis and map construction using the offspring from non-inbred parents. For that purpose the population descending from the non-inbred parents was regarded as two independent populations of female and male gametes. Two-letter coded genotypes were converted to single-letter codes, so as to meet the requirements of the JoinMap program. As shown in Table 2, the marker displaying the parental genotypes *AH* x *HB*, will segregate into the classes *AH*, *AB*, *HH*, *HB*. The maternal gametes are *A* and *H*. Gamete *A* is recognized in the two classes *AH* + *AB* and gamete *H* in *HH* + *HB*, while the paternal gametes *H* and *B* do not hinder the recognition of the gametes segregating from the female parent. Similarly, the segregation of the paternal gametes *H* and *B* is inferred from the classes (*AH* + *HH*) and (*AB* + *HB*), respectively. The consequence of a backcross is that the parental clones have one allele in common. Information about linkage or repulsion phase is stored within the data set, when this common allele is consequently indicated with *H*, and the alternative maternal and paternal allele with *A* and *B*, respectively. The above also applies to loci which show the 1:1♀ or 1:1♂ single-locus type of segregation. Loci at which the maternal gametes *A* and *H* segregate in a 1:1 ratio, are indicated with the JoinMap code 'A×H', whereas loci at which the paternal gametes *H* and *B* segregate, are indicated with 'H×B'.

At marker loci where the parental clones have the same heterozygous genotype, the parents have both alleles in common. At these loci, the offspring genotypes do not reveal the parental origin of the alleles. Therefore the loci segregating *AA*:*AB*:*BB* in a 1:2:1 ratio, or *C*-:*cc* in a 3:1 ratio, have to be re-coded in a different way. At codominant loci the offspring genotypes *AA*, *AB* and *BB* are converted into one-letter codes A, H and B, respectively. At dominant loci the genotypes *C*- and *cc* are converted into the one-letter codes C and A. These loci are indicated with the JoinMap code 'H×H'.

With this procedure the segregation data of the mapping population were split into separate maternal and paternal data sets. Such a one-parent data set contained only those loci which were heterozygous in the respective parent.

Data of the loci segregating in 1:2:1 and 3:1 ratios were present in both data sets. These separate maternal and paternal data sets, containing the information on gametic segregation, are regarded as data sets generated by two independent crosses.

Linkage analysis and map construction

Linkage groups were established using a LOD threshold of 3.0. The genetic map of each chromosome of the female parental clone C was constructed by JOINMAP using the maternal data set. The genetic distances between those loci which were heterozygous in clone C correspond to the amount of recombination in the female meiosis. Correct estimation of recombination values between maternal loci with different type of segregation ('A×H' and 'H×H') is ensured by JOINMAP. Similarly, on the basis of the paternal data set the genetic map of clone E was constructed.

When the order of the marker loci along the parental maps turned out to be different, the results were re-examined. Differences in marker order between parental maps possibly result from (a) scoring errors, (b) random differences between the parental data sets, or (c) chromosome rearrangements. To distinguish between these possibilities, all recombination events were localized in the data set. It was considered unlikely, that two recombination events had occurred on both sides of a locus. For these potential scoring errors the autoradiograms were re-examined, and, if necessary, the data were corrected. Furthermore, the map position of a locus was considered unlikely, when many individuals displayed double recombinations at both sides of that locus. Alternative positions were only considered if these resulted in a decrease of double recombination events.

In case of ordering differences between the parental maps, the parental marker order with the smallest number of unlikely recombination events was considered most reliable. The alternative marker order, taken from the more likely parental map, was submitted to JOINMAP using the option 'fixed orders' to reconstruct the parental map. The new map was found correct when the Chi-square value,

indicating the overall goodness-of-fit of the map, showed a negligible increase. When the alternative marker order was in contradiction with the data set, it was impossible to overrule JOINMAP with 'fixed sequences'. This situation suggests the presence of chromosome rearrangements between the genomes of the parental clones.
Another method to verify or to improve the ordering of loci along the map relies on the relatedness of the parental clones. For all chromosomes, one of the chromatids in clone E was inherited from clone C. This chromatid is easily recognized in clone E, because it accommodates the alleles clone C and E have in common. Along the chromatids of clone E these common alleles are linked in coupling phase, since these alleles were transmitted to clone E through the gamete of clone C. Along the chromatids of clone C the common alleles are also in coupling phase, except for those loci flanking the cross-overs which occurred during the meiotic development of the gamete that gave rise to clone E. Therefore, graphical genotypes (Young and Tanksley, 1988) were made of the chromatids of parental clones C and E. Especially in regions with high marker density, ordering problems were solved under the assumption that three adjacent recombination events were unlikely.

The construction of the combined map was performed by combining the maternal and paternal data sets into one JOINMAP input file, as if we were dealing with two separate crosses. The maternal and paternal maps should have at least two loci in common per chromosome to join the separate maps in the correct orientation. These so called 'allelic bridges' (Ritter *et al.*, 1990) are the loci which are heterozygous in both parental clones, and preferably segregating in a 1:1:1:1 manner. Allelic bridges based on markers segregating with 1:2:1 and 3:1 ratios may result in less accurate ordering of marker loci, because of the larger standard error of the estimated recombination values. The combined map is based on recombination values between pairwise loci. For loci which were heterozygous in both parental clones recombination values correspond to the weighted average of recombination value estimated in the female and male meiosis. This can be viewed at, as stretching the shorter interval and compressing the longer interval. However, parental differences in recombination

frequency are levelled only at the intervals between allelic bridges, leaving recombination values of other intervals unchanged. In a few cases, this stretching effect caused artifacts in marker ordering along the combined map, which were corrected using the JOINMAP option 'fixed orders'.
Conversion of recombination values to map units (cM) was performed according to Kosambi (1944). Graphic representation of the map was performed with the computer programme Drawmap (Van Ooijen, 1994).

Results

Polymorphic loci in the mapping population

RFLP loci: DNA Polymorphisms segregating in the progeny were easily detected with the RFLP probes, reflecting the high degree of heterozygosity of the potato genome. As indicated in Table 2, the majority of the RFLP markers (61 %; 119/195) revealed heterozygosity for DNA polymorphisms in only one parental clone, with a similar frequency in clone C (31 %; 62/195) and clone E (29 %; 57/195). About 39 % (76/195) of the RFLP probes detected loci which were heterozygous in both parental clones, of which the multiallelic RFLP loci (34 %; 67/195) were much more frequent than the loci which segregated in a 1:2:1 ratio (3 %; 5/195) or 3:1 ratio (2 %; 4/195).

In total 94 RFLP loci were mapped (from the 108 loci detected) with the probes derived from genomic DNA sequences flanking the integration sites of T-DNA's or transposable elements (Table 2). The TDs, TAc and TI markers detected loci on all chromosomes and were evenly spread over the entire potato genome. This indicates that integration of T-DNA's does not seem to have any preference for particular chromosomes and/or chromosomal regions in the genotype mainly used for transformation. However, as anticipated, some RFLP probes derived from genomic DNA sequences flanking the re-integration sites of Ac elements mapped in a cluster around the initial T-DNA integration site. This is, for example, shown by the markers Ac11-13 and Ac11-17 on chromosome *4* indicating reintegration sites of the Ac element in offspring plants from the original transgenic plant of which TAc11 was isolated.

Isozyme markers:

Optimal appearance and separation of enzyme polymorphisms was obtained with 10-15 % gradient gels for 6-PGDH, DIA and GOT. For PGM, SKDH, MDH and TPI the 8-25% gradient gels were optimal. Using homogenous gels good separation was obtained for ADH (12.5 %), APS (20 %) and Esterases (high density gel). Seven out of the ten enzyme systems tested revealed segregating isozyme loci in the CE-population (Table 3). The isozyme loci mapped are displayed in Fig. 1. No enzyme polymorphisms were found for SKDH and PGM. Complex EST patterns were unfit for genetic analysis. Four out of the nine segregating isozyme loci, *Dia-1*, *Got-2*, *Mdh-2* and *Adh-1*, were heterozygous in clone C, and five loci, *6-Pgdh-2*, *Got-1*, *Tpi-1*, *Adh-2* and *Aps-2*, were heterozygous in clone E.

For the enzyme system 6-PGDH the *6-Pgdh* locus with the lowest electrophoretic mobility segregated. Close linkage was observed with several RFLP markers on chromosome *12*. On the basis of its position on chromosome *12* the locus is indicated *6-Pgdh-2* in analogy to other Solanaceous genome maps (Tanksley and Kuehn, 1985; Bernatzky and Tanksley 1986; Bonierbale *et al.*, 1988). Segregation was absent for other *6-Pgdh* loci with higher electrophoretic mobilities.

Among the complex banding pattern of DIA one polymorphic locus was observed. In our mapping population this locus mapped on chromosome *5*. Although the bands have an intermediate electrophoretic mobility the locus is named *Dia-1* analogous to the locus on the potato map published by Bonierbale *et al.* (1988).

Two loci with GOT activity were observed and both segregated in the mapping population. The locus with the highest electrophoretic mobility *Got-1* mapped on chromosome *8*, whereas the other locus, *Got-2*, mapped on chromosome *7*. With close linkage to the loci TG69 and *Dia-1* a polymorphic dimeric MDH locus was mapped on chromosome *5*. On the basis of its electrophoretic mobility this locus is indicated with *Mdh-2*.

Two zones with enzyme activity were observed indicating the presence of two dimeric *Tpi* loci. Only the fastest locus segregated. Linkage was detected between this *Tpi-1* locus and an unassigned RFLP marker TAc4. The *Adh-1*

Table 3. Separation of isozymes and localisation of polymorphic loci on the potato genome.

Enzyme system	Gel type	polymorphic loci	parental genotypes C × E	Chromosome	linked marker[2]	LOD score
	gradient gels					
6-Phosphogluconate dehydrogenase	10-15 %	*6-Pgdh-2*	$2^1 2^1 \times 2^1 2^2$	12	TDs1-19	6.5
Diaphorase	10-15 %	*Dia-1*	$1^1 1^2 \times 1^1 1^1$	5	TG69	4.0
Glutamate oxaloacetate transaminase	10-15 %	*Got-1*	$1^1 1^1 \times 1^1 1^2$	8	*Aps-2*	4.6
		Got-2	$2^n 2^2 \times 2^2 2^2$	7	TG143	5.4
Phosphoglucomutase	8-25 %	not polymorphic				
Shikimate dehydrogenase	8-25 %	not polymorphic				
Malate dehydrogenase	8-25 %	*Mdh-2*	$2^1 2^2 \times 2^1 2^1$	5	*Dia-1*	10.1
Triose phosphate isomerase	8-25 %	*Tpi-1*	$1^2 1^2 \times 1^1 1^2$	unassigned	Tac4	5.6
	homogeneous gels					
Alcohol dehydrogenase	12.5 %	*Adh-1*	$1^1 1^2 \times 1^2 1^2$	6	TG254	2.9
		Adh-2	$2^2 2^2 \times 2^1 2^2$	4	TDs428	9.1
Acid phosphatase	20 %	*Aps-2*	$2^2 2^2 \times 2^1 2^2$	8	Ac3-28	4.0
Esterase	high density	--[1]				

[1] Complex patterns were unfit for genetic analysis.

[2] The most significantly linked marker is presented to indicate the reliability of its assignment to a linkage group

locus responsible for alcohol dehydrogenase activity in roots and tubers, as well as the *Adh-2* locus, active in pollen, segregated in the mapping population. The *Adh-1* locus showed linkage to the chromosome *6* marker TG253. The *Adh-2* locus is mapped on chromosome *4*, significantly linked to the RFLP TDs428. Only the slow migrating *Aps-2* locus segregated in the CE-population. The *Aps-2* locus co-segregated with chromosome *8* RFLP markers, with most significant linkage to Ac3-28.

The localization of classical trait loci.

All eleven classical trait loci studied in this mapping population could be localized. The observed segregations of the classical trait loci and their linkage groups are shown in Table 4, as well as the RFLP locus linked to the classical marker locus with the highest LOD score. The positions of the classical traits on the genetic map are shown in Fig. 1. With the exception of earliness, the inheritance of the classical traits is well documented in literature and in most cases the parental genotypes were known from previous genetic studies (see materials and methods). On the basis of these parental genotypes expected ratios were proposed. In most cases the observed segregations were in agreement with the expected ratios. The segregations observed at locus *P* (χ^2 = 32.97; p $\leq$ 0.001) and locus *D* (χ^2 = 4.78; p $\leq$ 0.05) did not fit the proposed genetic model, but flanking RFLP loci showed similar segregation distortion.

With isoelectric focusing of stylar glycoproteins the parental genotypes were identified. The genotypes of clones C and E are S_1S_2 and S_2S_3, respectively. In the CE population the genotypes S_1S_3 and S_2S_3 were observed in a correct 1:1 ratio. It was also observed that individuals of the mapping population having the genotype S_1S_3 were compatible to clone E, whereas no backcross offspring to clone E was obtained from S_2S_3 individuals. The *S* locus was mapped on chromosome *1*.

Clear differences were observed among the genotypes in their sensitivity to the herbicide metribuzin. The parental clone C was tolerant to metribuzin, whereas the parental clone E was sensitive (*meme*). The mapping population segregated in a 1:1 ratio, which is in agreement with the heterozygosity of parent C (*Meme*),

Table 4. Segregation of classical genetic trait loci and their localisation on the potato genome.

Trait	Parental Genotypes C × E	Segregation		Chromosome	linked marker[1]	LOD score
		Observed	Expected			
Self-incompatibility	$S_1S_2 \times S_2S_3$	$S_1S_3 : S_2S_3$ = 28:31	1:1 ♀	1	TG24	4.8
Metribuzine sensitive	*Meme* × *meme*	*Me-* : *meme* = 26:38	1:1 ♀	2	TDs33	8.5
Red anthocyanins	*Dd* × *Dd*	*D-* : *dd* = 58: 9[2]	3:1	2	TG20B	4.1
Tuber flesh colour	*Yy* × *yy*	*Y-* : *yy* = 37:30	1:1 ♀	3	TDs258	7.1
Earliness	-[3]	*El-* : *elel* = 39:24	--[3]	5	GP21	10.9
Yellow margin	*YmYm* × *Ymym*	*YmYm* : *Ymym* = 20:10	1:1 ♂	5	TG23	5.6
Desynapsis	*Ds1ds1* × *Ds1ds1*	*Ds1-* : *ds1ds1* = 44:22	3:1	8	*Got-1*	2.8
Crumpled	*Crcr* × *Crcr*	*CrCr* : *Crcr* = 12:17	1:2[4]	10	TDs277A	2.7
Tuber shape	*Roro* × *Roro*	*Ro-* : *roro* = 49:17	3:1	10	TG303	5.5
Flower colour	*Ff* × *Ff*	*F-* : *ff* = 44:19	3:1	10	TG63	9.2
Purple anthocyanins	*pp* × *Pp*	*Pp* : *pp* = 10:57[2]	1:1 ♂	11	Ssp75	12.6

[1] The most significantly linked marker is presented to indicate the reliability of its assignment to a linkage group.

[2] Segregation is significantly different from the expected ratio.

[3] The inheritance of earliness is unknown.

[4] Expected ratio in the non-mutant part of the mapping population.

Fig. 1: Linkage map of potato from the backcross population C × E. For each chromosome the female (C♀), male (E♂) and combined map (CE) are given. Linkage groups are established with LOD > 3.0, except for three intervals indicated in the figure. Individual markers mapped with a LOD < 3.0 are underlined. Distorted segregation is indicated with asterisks following the marker name on the parental chromosomes in the case of gametic selection and on the combined maps in the case of zygotic selection. Chromosome numbering and orientation is according to Bonierbale *et al.* (1988).

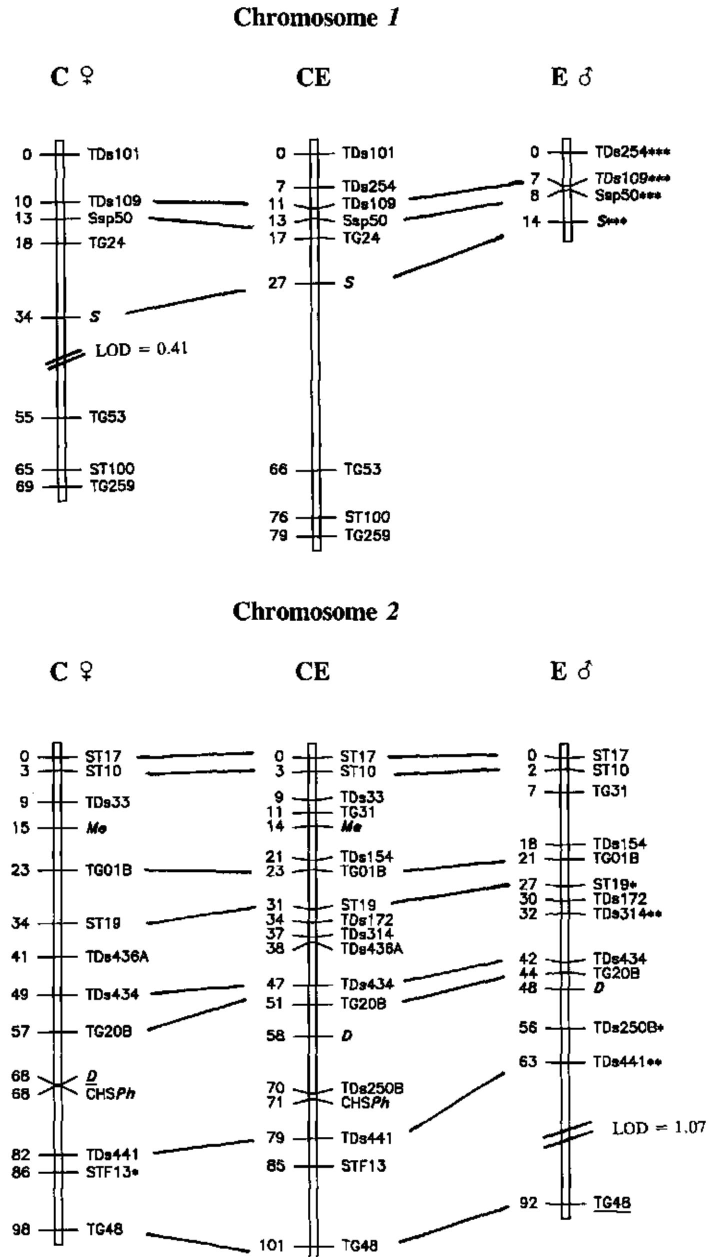
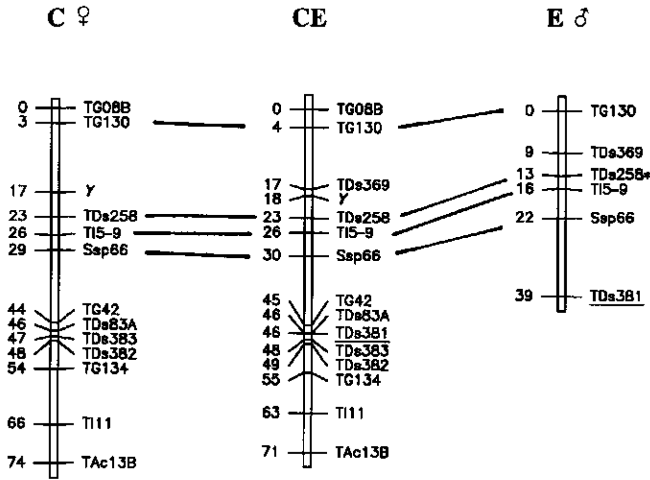


Figure 1, continued

Chromosome *3*



Chromosome *4*

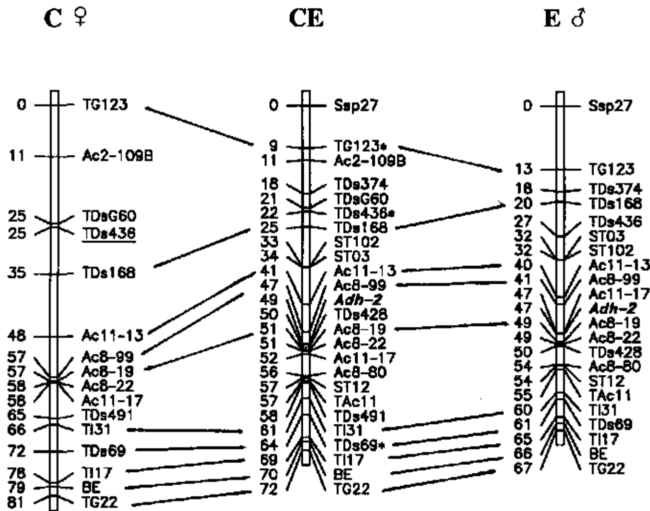
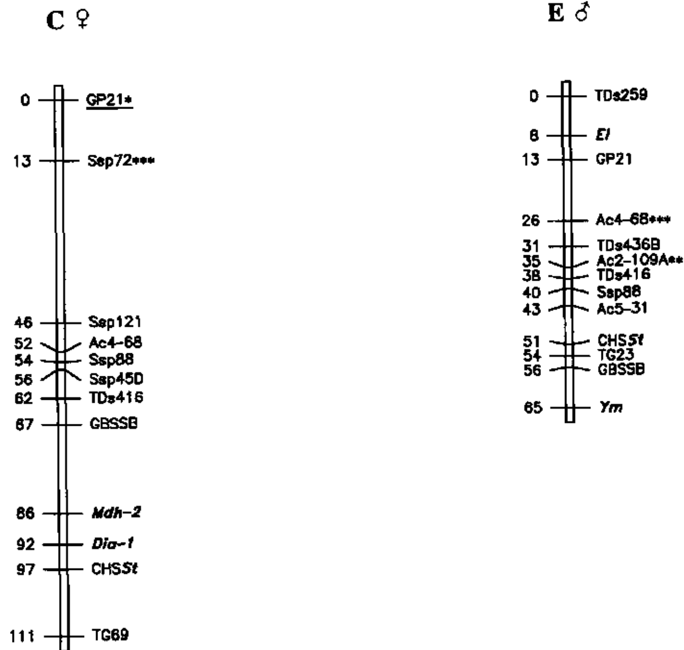


Figure 1, continued

Chromosome *5*



Chromosome *6*

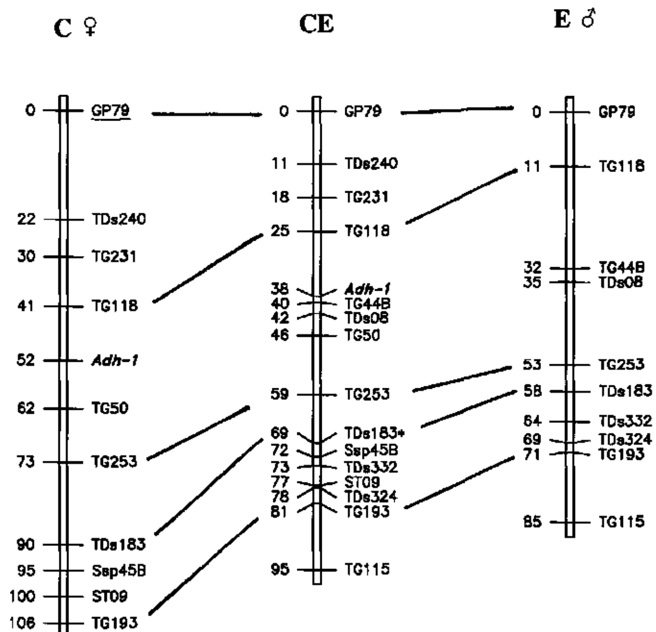
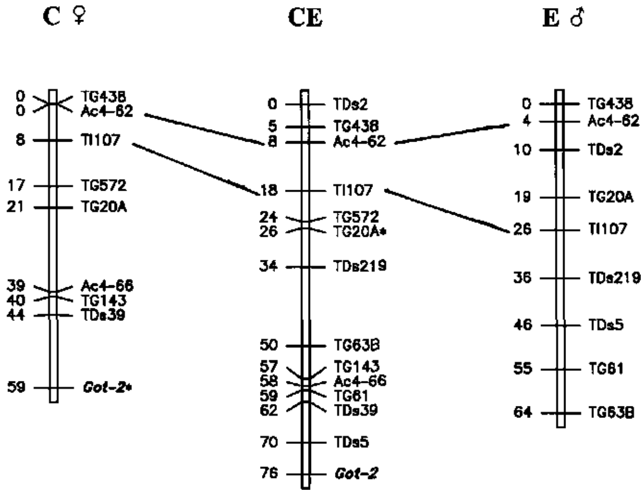


Figure 1, continued



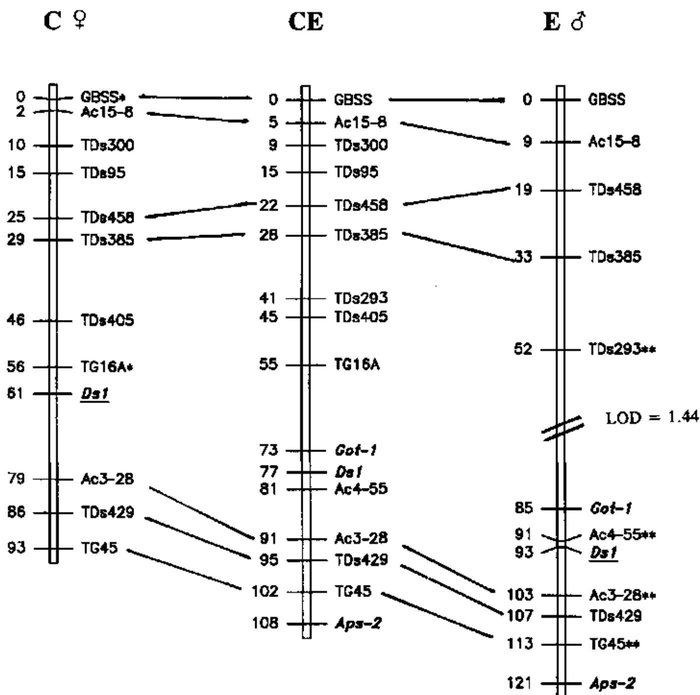
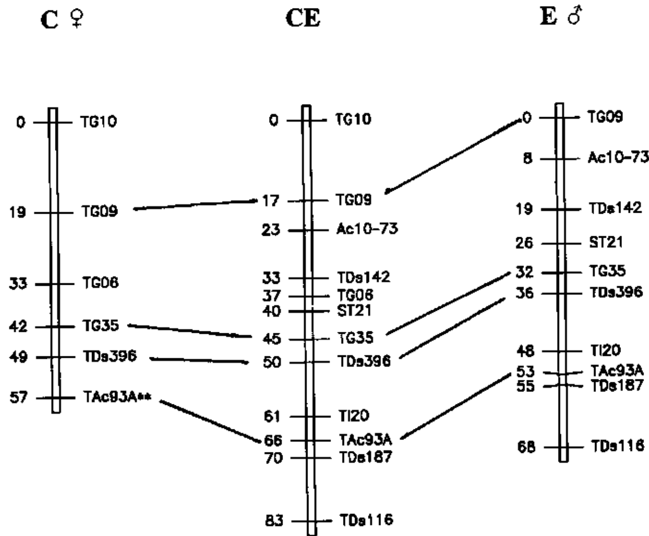


Figure 1, continued



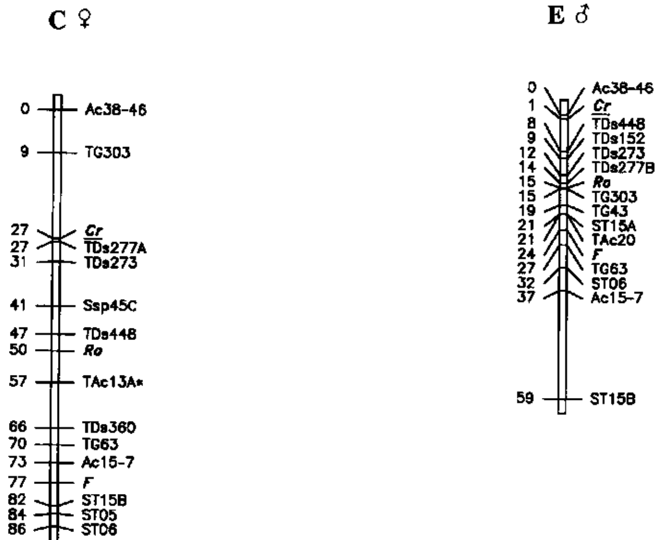
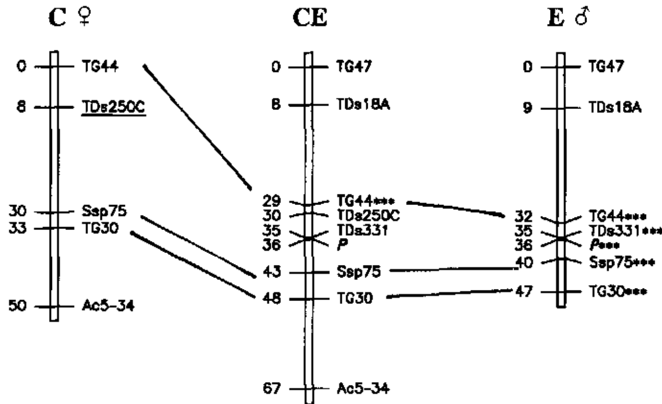
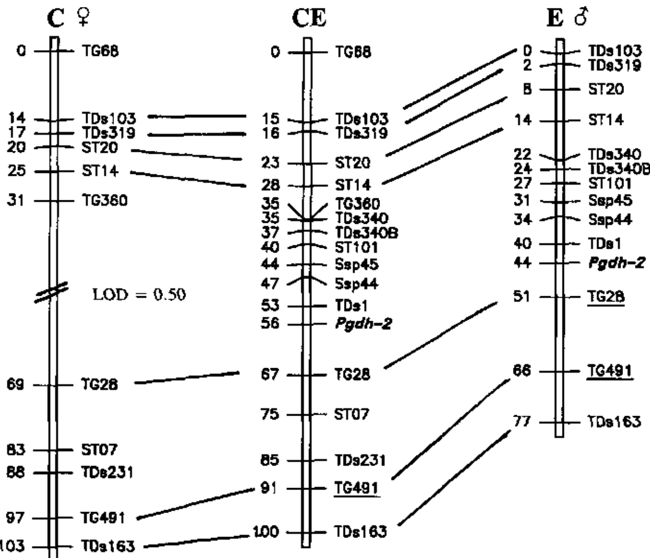


Figure 1, continued

Chromosome *11*



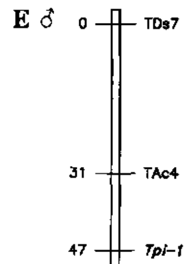
Chromosome *12*



Unassigned markers:

ST04, ST11,
Ssp27D, Ssp45C, TG16B,
TDs18, TDs219, TDs222,
TDs436B, TDs454, TDsXX
TAc13X, TAc93D,
Ac5-55C, Ac5-55D, Ac5-55E,
Ac5-55J, Ac5-55K, Ac8-80

Unassigned linkage group:



as indicated by De Jong (1983). The observed segregation allowed mapping of the *Me* locus on chromosome 2.
For flesh colour the parental clones C and E scored 8.5 (yellow) and 4.0 (white) respectively. In the mapping population flesh colour showed a continuous distribution, ranging from 3 (white) to 9 (bright yellow). Thirty clones with a trait value below 5.5 were putatively indicated with *yy*, and 37 clones with a trait value ≥ 5.5 were assigned *Yy*. The *Yy* : *yy* segregation fit the monogenic 1:1 ratio. Flesh colour was mapped on chromosome *3*.
The average value for earliness over three replications of the parental clones C and E was 4.5 and 6.8, respectively. The average value of the CE-clones ranged from 2 (very late maturing) to 9 (very early). The population mean was 6.0. Earliness of the mapping population showed a bimodal distribution, which easily allowed classification into early and late types. The CE offspring segregated into 39 early types (trait value > 6) provisionally indicated with *El-* and 24 late types (trait value ≤ 6) indicated with *elel*. Co-segregation between this putative *El* locus and several RFLP markers on chromosome *5* was highly significant. Therefore, we conclude the presence of a major locus involved in earliness with closest linkage to RFLP marker GP21.
With respect to yellow margin, the backcrosses to clone E resulted in the identification of 20 *YmYm* clones and 10 *Ymym* clones. Although the number of observations was low, linkage on chromosome *5* to TG23 was highly significant, as indicated by the LOD score.
Low LOD scores were obtained for linkage between desynapsis (*Ds1*) and chromosome *8* markers. The cytogenetic observations were in agreement with fertility, since only clones with normal synapsis gave high seed-set in the compatible backcrosses to the parental clones (data not shown).
Twelve *CrCr* and seventeen *Crcr* CE-clones were identified by backcrosses. Although the number of observations is low, linkage to TDs277A was found with a LOD score of 2.71. This allowed the mapping of crumpled on chromosome *10*.
The *Ro* locus involved in tuber shape was localized on chromosome *10* on the basis of the 3:1 segregation obtained by visual classification of clones with round or long tuber shape. A detailed quantitative analysis of tuber shape is

described in Chapter 5 (Van Eck *et al.* 1994a). The loci *D*, *F* and *P*, involved in anthocyanin pigmentation of flowers and tubers, were placed on chromosomes *2*, *10* and *11*. respectively. Further description of the inheritance and mapping of loci involved in anthocyanin pigmentation of flowers and is found in chapter 3 (Van Eck *et al.*, 1993). The inheritance of skin colour pigmentation is described in detail in chapter 4 (Van Eck *et al.*, 1994b).

Segregation distortion

Significant deviations ($p < 0.05$) from the expected Mendelian ratios were found at 38 loci (38/215 = 18 %) segregating from clone C and/or clone E, as the result of gametic or zygotic selection. Gametic selection was identified at 1:1 or 1:1:1:1 segregating loci on the basis of under- or over-representation of one of the gametic classes segregating from the female or male parent. The skewed segregations of the gametes are listed in Table 5, disregarding the normal segregating gametes contributed by the other parent. Skewed ratios of maternal gametes were observed at nine loci distributed over seven chromosomes. Skewed ratios of paternal gametes were observed at 20 loci in clusters on chromosomes *1*, *2*, *8* and *11*. Zygotic selection affected the segregation of eight 1:1:1:1 segregating markers, mostly located on chromosomes *4* and *5*.

Table 5. Distorted segregation ratios and Chi-square values for loci which show significant gametic or zygotic selection ($p < 0.05$).

Chr#	Locus	Expected	Observed				χ^2-value	Selection
		1:1♀	*A_*		*H_*			
		1:1♂	*_H*	*_B*				
		1:1:1:1	*AH*	*AB*	*HH*	*HB*		
1	TDs109	1:1 ♂	53	4			22.04***	gametic
1	Ssp50	1:1 ♂	44	2			38.34***	gametic
1	TDs254	1:1 ♂	41	8			22.22***	gametic
2	STF13	1:1♀	18		32		3.92	gametic
2	ST19	1:1 ♂	15	28			3.93	gametic
2	TDs314	1:1 ♂	15	34			7.36**	gametic
2	*D*	3:1	D-:dd = 58:9				4.78	---
2	TDs250B	1:1 ♂	17	32			4.59	gametic
2	TDs441	1:1 ♂	15	34			7.36**	gametic
3	TDs258	1:1 ♂	18	32			3.92	gametic
4	TG123	1:1:1:1	20	10	10	15	3.91	zygotic
4	TDs436	1:1:1:1	19	9	13	18	3.98	zygotic
4	TDs69	1:1:1:1	8	14	11	3	6.12	zygotic
5	Ssp72	1:1♀	42		12		16.66***	gametic
5	GP21	1:1♀	36		21		3.94	gametic
5	Ac2-109A	1:1 ♂	10	28			8.52**	gametic
5	Ac4-68	1:1 ♂	10	37			15.51***	gametic
5	Ac4-68	1:1:1:1	10	15	0	21	10.73**	zygotic
5	TDs416	1:1:1:1	20	14	9	19	4.39	zygotic
6	TDs183	1:1:1:1	16	9	8	15	4.09	zygotic
7	TG20A	1:2:1	AA:AB:BB = 5:30:8				7.14	zygotic
7	*Got-2*	1:1♀	25		42		4.31	gametic
8	GBSS	1:1♀	32		13		6.14	gametic
8	TG16A	1:1♀	33		17		5.12	gametic
8	TDs293	1:1 ♂	35	14			9.00**	gametic
8	Ac4-55	1:1 ♂	14	34			8.33**	gametic
8	Ac3-28	1:1 ♂	34	15			7.36**	gametic
8	TDs429	1:1 ♂	37	16			8.32**	gametic
8	TG45	1:1 ♂	30	10			10.00**	gametic
9	TAc93A	1:1 ♀	15		33		6.75**	gametic
10	TAc13A	1:1 ♀	30		15		5.00	gametic
10	TG63	1:1:1:1	14	8	6	14	4.75	zygotic
11	TG44	1:1 ♂	39	11			15.68***	gametic
11	TG44	1:1:1:1	12	10	26	1	12.13***	zygotic
11	TDs331	1:1 ♂	50	8			15.21***	gametic
11	P	1:1 ♂	57	10			32.97***	gametic
11	Ssp75	1:1 ♂	43	6			27.93***	gametic
11	TG30	1:1 ♂	46	12			19.93***	gametic

**, ***: $p < 0.01, 0.001$ resp.

Construction and comparison of two separate parental maps

Linkage maps were constructed by using the data sets as described in the materials and methods. Fifteen maternal and fourteen paternal linkage groups were obtained. Homologous maternal and paternal linkage groups were aligned using markers segregating in both parents. Chromosome numbers were assigned on the basis of TG and GP markers. By lowering the LOD threshold twelve chromosomes were identified, leaving one paternal linkage group and 21 markers (10 %) unassigned. The marker order was compared between the maternal and paternal maps, with respect to those markers which were heterozygous in both parents. For every linkage group of this preliminary map differences were observed in marker order between the parental chromosomes, except for chromosomes *1*, *6* and *9* (not shown). For the chromosomes *2*, *3*, *4*, *7*, *8*, *11* and *12* these ordering differences reflect merely (*a*) a sample difference between the paternal and maternal gametes, and (*b*) a sample difference between the joint segregation of pairwise loci using a data set where the volume of missing datapoints is about 30 %. Critical examination of the raw data revealed that a difference in marker order between the parental maps almost invariably coincided with unlikely recombination events in one of the parental maps. New maps were constructed with JOINMAP using the 'fixed order' option. For the chromosomes *5* and *10* the cause of ordering differences between the parental could not be ascribed to random differences alone. At these chromosomes marker orders remained ambiguous. The 987 cM linkage map constructed on the basis of the maternal data set consisted of 136 markers (124 RFLP, 4 isozyme, 8 classical marker loci), whereas the 821 cM paternal linkage map consisted of 141 markers (128 RFLP, 5 isozyme, 8 classical marker loci). The maps are shown in Fig. 1. Fourteen RFLP probes detected 22 duplicate loci. Since the duplicate loci were not clustered to specific regions, this can not be taken as an indication for duplication in the potato genome.

Construction of a combined map

Merging of the maternal and paternal data set allowed the construction of the combined map shown in Fig. 1. The position of the loci and the genetic distances were calculated on the basis of recombination values observed in the

female and male meiosis. It should be realized that since a combined map calculated in this way reflects a statistical optimum, it may not represent the true biological situation in all detail. This is true for every calculated map, especially when combing data from distinct meioses which may have different recombination rates, such as between male and female meiosis. The map shown in Fig 1. represents the most likely marker order, as estimated by JOINMAP using the 'fixed order' option. Fixed orders were used for chromosomes *2*, *3*, *4*, *7*, *8*, *11* and *12* to align the marker orders of the maternal, paternal and combined map. Since the marker order for chromosomes *5* and *10* remained ambiguous, no combined maps are produced for these chromosomes.

Discussion

Mapping of molecular, isozyme and classical genetic markers

The linkage map of the potato genome comprising molecular, isozyme and classical genetic markers was constructed using a backcross population generated from diploid non-inbred parents. The genetic interpretation of segregating DNA polymorphisms and the mapping of RFLP loci was facilitated by common alleles, shared by the recurrent parent C and the F_1 parent E. In addition, whenever multiple bands, revealed by a single probe, suggested more than one locus, all maternal and paternal bands belonging to one and the same locus could be identified on the basis of co-segregation (in repulsion) with this common band. The majority of the molecular markers was generated by DNA probes originating from potato genomic DNA sequences flanking the integration sites of transposable elements or the T-DNA constructs containing these elements. These flanking DNA markers were equally usable for mapping as genomic or cDNA clones. Moreover, the flanking DNA markers not only represent loci on this map, they also reflect the genomic positions of transposable elements in an equally large population of independent transformants. A transposon mapped close to an interesting gene can be recombined in linkage phase to the desired allele and used for efficient transposon targeting due to preferential transposition to linked sites (Pereira *et al.*, 1992).

Most isozyme loci reported here mapped to similar genomic positions in either potato (Bonierbale *et al.*, 1988; Tanksley *et al.*, 1992) or tomato (Tanksley *et al.*, 1992). Isozymes are known to be useful genetic marker loci. However, we found it very difficult to give names to the segregating isozyme loci. Firstly, loci can be named on the basis of their electrophoretic mobility. However, buffer-pH or gel type may influence the relative mobilities of the loci, which affects the nomenclature. In this study, optimal separation of most enzyme systems was established with gradient gels where separation of the enzymes is mainly influenced by the size, rather than the charge of the molecule. Other separation systems are more difficult to compare, since the mobility of the molecule is influence by a combination of size and charge.
Secondly, isozyme loci can be named analogous to earlier studies in potato. However, this may contradict with results of mapping studies in tomato. In potato we localized the *Adh-1* locus on chromosome *6* on approximately the same position where the tomato *Adh-2* locus was mapped, and potato *Adh-2* is mapped on chromosome *4* where tomato *Adh-1* is found. These contradicting results can be explained on the basis of the original data reported in literature. Shortly after the assignment of the *Adh-1* locus, expressed in germinating seed and pollen, to the long arm of chromosome *4* (Tanksley, 1979), expression of a second more anodal alcohol dehydrogenase was detected in callus and root tissue (Tanksley and Rick, 1980). The nomenclature of these tomato enzymes is not in agreement with their electrophoretic mobility, but the potato loci are. The potato *Adh-2* gene (chr.*4*) is expressed in pollen, whereas potato *Adh-1* (chr.*6*) is expressed in roots and tubers (Martinez-Zapater and Olivier, 1985). Thus, alcohol dehydrogenase gene expression and map position is not different between tomato and potato.
Thirdly, names of isozyme loci on the genetic map of potato can be derived from the locus names on the genetic map of tomato. Up till now three *6-Pghd* loci have been mapped in tomato (Tanksley and Loaiza-Figueroa, 1985) and one in potato (Bonierbale *et al.*, 1988). The locus we localized on chromosome *12* was indicated with *6-Pgdh-2*, in agreement with previous tomato mapping studies, and disregarding its relative mobility. The same argumentation applies to locus *Dia-1* placed on chromosome *5*.

Lastly, the most accurate identification of isozyme loci is on the basis of allelism. In potato only two loci are described for GOT whereas four loci are mapped on the tomato genome. The position of *Got-2* on chromosome *7* in this study is in agreement with the positions of this locus on the potato map of Bonierbale *et al.* (1988) and the isozyme map of tomato (Tanksley and Rick, 1980). The locus of *Got-1* in our map coincides with the locus of *Got-4* of tomato. Evidence for allelism between potato *Got-1* and a tomato GOT locus, which is most likely *Got-4*, is provided by Jacobsen *et al.* (1994). They present a zymogram showing a new intralocus heterodimer formed between potato *Got-1* and tomato *Got-4* in potato-tomato somatic hybrids. We assume that the locus name *Got-4* on potato chromosome *8* (Bonierbale, 1988; Tanksley *et al.*, 1991) is synonymous to our potato *Got-1*.
The locus *Tpi-1* could not be assigned to a linkage group on the basis of comparative mapping. The only locus mapped in tomato is *Tpi-2* on chromosome *4* (Tanksley and Rick, 1980). Allelism between the *Tpi-1* and *Tpi-2* loci of tomato and the respective loci in potato was demonstrated by intergeneric heterodimerization in somatic hybrids (data not shown). Finally, the localisation of *Aps-2* on chromosome *8* was in agreement with its known position on the tomato genome.

Up till now, gene mapping in potato has resulted in the localization of only two morphological traits. The *Y* locus involved in yellow flesh colour on chromosome *3* (Bonierbale *et al.*, 1988), and the purple skin colour (*PSC*) locus involved in pigmentation of the tuber skin on chromosome *10* (Gebhardt *et al.*, 1991). The research described here has added several other morphological markers. The localization of locus *Y* on chromosome *3* is confirmed by our results. Indirectly the localization of the *PSC* locus on chromosome *10* is confirmed by the mapping of the loci *Ro* and *F* on chromosome *10*, since linkage of a flower and skin colour locus was reported by Dodds and Long (1956), and linkage of tuber shape and skin colour was reported by DeJong and Rowe (1972). The mapping of the loci *D*, *F* and *P*, involved in anthocyanin pigmentation, is discussed in chapter 3 (Van Eck *et al.*, 1993).

The *S*-locus for self-incompatibility could be mapped on the basis of polymorphic stylar glycoproteins involved in the self-incompatibility reaction (Thompson *et al.*, 1991). The presence of two genotypes, S_1S_3 and S_2S_3, found in the mapping population, and the absence of S_1S_2 and S_2S_2, corresponds with the expected action of the *S* alleles in a gametophytic one-locus system. CE-clones originating from male gametophytes which carry the S_2 allele were not found. Success or failure of backcrosses also corresponded with the action of the *S* alleles. Only CE-clones with genotype S_1S_3 produced offspring in backcrosses to parental clone E. The position of the *S* locus was in agreement with skewed segregations of male alleles in a region at chromosome *1*, and the localization of a cDNA probe coding for *Sr1*, a self-incompatibility allele of *Solanum tuberosum* (Gebhardt *et al.*, 1991).

The mapping of the *Ym* locus on chromosome *5* is not in agreement with its assignment to chromosome *12* (Wagenvoort, 1982) using trisomic analysis of this mutant phenotype. This contradiction may be explained by assuming different chromosomal mutations causing the same mutant phenotype. However, yellow margin is a well known and common morphological mutant in *Solanum phureja* germplasm. Since both our material and the clone used by Wagenvoort was derived from *Solanum phureja* germplasm, the *ym* allele may be identical by descent. Another explanation may be the highly similar morphology of potato chromosomes *5* and *12* in pachytene stage, which complicates trisomic identification (Ramanna and Wagenvoort, 1976).

The *Cr* locus was localized on chromosome *10*, while the *Ds1* locus and *Got-1* were mapped on chromosome *8*. However, a linkage group consisting of the loci *Cr*, *Ds1* and *Got-1* was proposed by Jongedijk *et al.* (1990). Moreover, when using the computer program Linkage-1 significant linkage was detected between the *Cr* locus and another three loci on chromosome *8* (*Cr* - *Aps-2*, $\chi^2_{(df=1)} = 7.67$, $p = 0.0056$; *Cr* - Ac3-28, $\chi^2_{(df=1)} = 6.04$, $p = 0.014$; *Cr* - TG45, $\chi^2_{(df=1)} = 9.67$, $p = 0.0019$). Nevertheless, these linkages were artifacts. Firstly, in our case linkage analysis was performed on the basis of co-segregation, while Jongedijk *et al.* (1990) assumed that the segregation

distortion they observed was the result of selection at the *Cr* locus. Secondly, linkage is excluded on the basis of the direction of the skewed segregation. At all chromosome *8* loci gamete selection in parent E is favouring those alleles which the parental clones have in common. Contrary to this, selection took place against the crumpled phenotype (*crcr*), where the recessive *cr*-allele is the allele that clones C and E have in common. Thirdly, the high χ^2 values we obtained, using the *CrCr* : *Crcr* segregation data, indicating linkage to *Aps-2*, Ac3-28 and TG45, were also artifacts. Diverging numbers of clones in the non-recombinant offspring classes caused the high χ^2 values.
When using JOINMAP no significant linkage was detected between the *Cr* locus and chromosome *5* loci. This is a result of the different test statistics used by these computer programs. The χ^2 test, as used by Linkage-1, is less conservative than the LOD score, which is used by JOINMAP (Gerber and Rodolphe, 1994). Additionally, the LOD score is not affected by the number of heterozygous *Crcr* individuals observed in the different offspring classes. These classes are not used in calculating the standard error of the maximum likelihood estimate in the case of 1:1 segregating loci.

Three morphological traits localized on the genetic map of potato have similar features. Yellow flesh colour, tuber shape and earliness can be regarded as a qualitative monogenic traits, as well as quantitative traits. Within the segregating classes genetic variation is ascribed to minor genetic factors. The major locus of each of those traits has been mapped on the basis of a single locus Mendelian segregation, although their monogenic inheritance is under discussion. Flesh colour has generally been regarded as a monogenic trait, but different degrees of yellowness are ascribed to modifying genes (Howard, 1970). Brown *et al.* (1993) demonstrated the presence of a third allele at the *Y* locus involved in orange coloration of tuber flesh due to carotenoids. Tuber shape is determined by a major gene at the *Ro* locus with multiple alleles (Van Eck *et al.*, 1994a). The inheritance of earliness is unclear, but up till now our results suggest also the presence of a major locus on chromosome *5*. The results for these three traits, as well as for quantitatively inherited nematode resistance (Kreike *et al.*, 1993, 1994) and PLRV resistance (Barker *et al.* 1994) may

indicate a general phenomenon in potato genetics. Traits previously regarded as polygenic appear to be based on surprisingly few major genes, where a part of the genetic variance can be explained on the basis of the dominance relation between multiple alleles. Many of the morphological traits described in potato (Ortiz and Huaman, 1994) have now been located on the potato genome. Elucidation of the hereditary basis of additional complex morphological traits, such as eye depth, leaf shape, and light sprout characters as well as more complex agronomical traits will indicate the universality of the above mentioned phenomenon.

Distorted segregation

Several chromosomal regions were identified showing distorted segregation. Especially the loci that segregate with a 1:1:1:1 ratio enabled us to explain skewness as the result of differential gametic or zygotic viability, or both. Unfortunately, with the exception of two regions, the real cause of the selection remains obscure. The gametic selection on chromosome *1* observed at the paternal side can be explained as the result of the *S* locus involved in self-incompatibility. The use of non-related parents might have prevented this cause of segregation distortion. At the region of chromosome *10* where the *Cr* locus was mapped zygotic selection was expected. The recessive *crcr* genotypes were omitted from RFLP analysis because of their sublethal phenotype, but no significant zygotic selection was found at RFLP loci closely linked to the *Cr* locus on chromosome *10*.

We tested for zygotic selection at 67 multiallelic loci and 268 times we tested for gametic selection. Therefore a number of 17 false positives is expected at a threshold for p of 0.05.

At chromosomes *1*, *5*, *8* and *11* segregation distortion was found at several linked loci. False positives might be those loci, mentioned in table 5, which do not belong to such a cluster with segregation distortion. Distorted segregations are not uncommon for potato (Bonierbale *et al.*, 1988; Gebhardt *et al.*, 1989, 1991; El-Kharbotly *et al.*, 1994). Vegetatively maintained and outcrossing polyploids are known to carry a high genetic load, which is buffered in the tetraploid genome. The effect of many deleterious recessive alleles, exposed by

inbreeding and in primary dihaploids (Howard, 1970), could be observed in the backcross, which is a form of inbreeding, used in this study.

Construction of a linkage map using non-inbred parents

The construction of a linkage map, using the offspring of non-inbred parents, has been accomplished in potato (Bonierbale *et al.*, 1988; Gebhardt *et al.*, 1989, 1991; Tanksley *et al.* 1992), and some few other species including citrus (Durham *et al.*, 1992; Jarrell *et al.*, 1992), alfalfa (Echt *et al.*, 1993), apple (Hemmet *et al.*, 1994), loblolly pine (*Pinus taeda* L.)(Devey *et al.*, 1994), and *Eucalyptus* (Grattapaglia and Sederoff, 1994). However, the method of map construction - from the collection of segregation data up to the ordering of marker loci along the map - is still under development. In contrast to the uniform type of di-allelic segregations in F_2, backcross or recombinant inbred populations from homozygous inbred parents, the different types of segregation in the offspring from non-inbred parents are a troublesome obstacle. In this paper we described a general method for map construction, different from previous methods employed in potato and other outbreeding crops. In the following, the solutions are discussed we proposed to overcome the obstacles encountered during map construction, caused by the non-inbred nature of the parental clones.

heterozygosity of the parental clones

Initially, the obstacle of heterozygosity of both parents was circumvented by using the segregation data from only one parent. In conifers haploid megagametophytes from a single tree have been used for genetic studies. This method was successfully applied to construct maps of white spruce (*Picea glauca*; Tulsieram *et al.*, 1992), maritime pine (*Pinus pinaster*; Gerber *et al.*, 1993) and Norway spruce (*Picea abies*, Binelli and Bucci, 1994). Another solution was applied by Bonierbale *et al.* (1988) and Durham *et al.* (1992). They observed the segregation of only those alleles which descend from one parent, disregarding the alleles segregating from the other parent. In a second mapping population Durham *et al.* (1992) used the three-point-test to construct a linkage map without the use of mapping software from pairwise recombination

frequencies estimated by Linkage-1 (Suiter *et al.*, 1983).

RAPD markers used for linkage mapping in *Eucalyptus* resulted almost exclusively in 1:1 segregating marker loci (Grattapaglia and Sederoff, 1994). Therefore, the use of RAPDs can also be considered as a simplification to avoid the mixture of different types of single locus segregation ratios. This "two-way pseudo-testcross" mapping strategy resulted into two parental maps which cannot be alligned.

Simplification of segregation data by lumping of offspring classes, from loci with three or four different alleles into di-allelic ratios, was performed by Jarrel *et al.* (1992) and Devey *et al.* (1994), to be able to use the MAPMAKER (Lander *et al.*, 1987) and GMENDEL 2.0 mapping software (Liu and Knapp, 1990), respectively. The method described in this paper is based on all segregation data that can be collected from loci in both parents. In this way we maximized the amount of the mapping information that can be obtained from one single population.

alignment of parental maps

In obligatory outbreeding species, two types of populations can be used for mapping studies. In this study a backcross population was used, whereas the F_1 from unrelated parents was used as mapping population for apple (Hemmet *et al.*, 1994) and loblolly pine (Devey *et al.*, 1994). In the method used in this study, to combine separated maternal and paternal data, several aspects are relying on the presence of a common allele shared by the parents of a backcross mapping population. In F_1 or BC_1 progenies, alignment of the separate parental maps relies on allelism between loci which are heterozygous in both parents. However, in contrast to what was suggested by Echt *et al.* (1993), segregating phenotypes in a 1:1:1:1 ratio can not be used to distinguish between allelic or duplicate loci, when maternal and paternal segregation of alleles is detected with the same RFLP probe. In our case, allelism is proven by the presence of a common allele shared by the parental clones. Lack of recombination between the other, dissimilar marker alleles in repulsion is, in

principle, the same argument (Gebhardt *et al.*, 1989; Ritter *et al.*, 1991).

different types of single-locus segregation

Loci segregating in 1:1, 1:2:1 or 3:1 ratios, with unknown linkage phase, combined within a single parent input file were no obstacle to JOINMAP. The estimated recombination frequencies, together with their standard error, could be used to produce a linkage map with the most likely marker order. The differences between the parental maps we observed were in most cases the result of random differences between the maternal and paternal data set. The method to check for frequent double cross-over events was very useful in finding the reason behind the differences in marker order between the parental maps. These were mainly introduced by missing data. With the option offered by JOINMAP to fix the order of markers, corrections were made only to those regions where ambiguities were the result of random differences between the parental data sets. The alternative marker order was allowed when the overall Chi-square value for mapping indicated a similar likelihood. These requirements were not met for the parental differences in the maps of chromosomes *5* and *10*, suggesting the presence of chromosome rearrangements. The slightly reduced map length of the paternal chromosomes *5* and *10* supports the hypothesis of chromosome rearrangements. During meiotic recombination rearrangements result in the formation of dicentric bridges and acentric fragments with low viability, implying selection against recombinant gametes.

joining of the female and male maps

For the construction of the combined map, newly estimated recombination frequencies and standard errors correspond to the weighted average of the recombination values estimated using the maternal and paternal data. In these combined maps the order of marker loci changed occasionally. Many of these re-orderings were found in regions with differences between female and male recombination. At those regions, the intervals between allelic bridges are 'stretched' or 'compressed', whilst the recombination distance of other intervals remained the same. Solving this problem with the 'fixed order' option is not the most elegant solution.

Other re-orderings observed in the combined map concerned predominantly the 1:2:1 and 3:1 segregating loci. These re-orderings were caused by the method we used. Joinmap was developed to integrate mapping information from independent sources. All pieces of information are combined into a single estimate with standard error, after weighing with the "amount of information" contained in the distinct pieces of information. In our case, the female and male data sets are not independent with respect to the 1:2:1 and 3:1 segregating loci. They are the same for both parents. A mapping population of 67 individuals was used to estimate the recombination values for the female and male map, but the double amount of observations (134) was available to estimate recombination in the combined map between two 1:1:1:1 segregating markers. When the data of 3:1 and 1:2:1 segregating loci are present in both the maternal and paternal data set during the construction of the combined map, this estimate is falsely based on 134 observations.

Furthermore, in some cases, biased estimates of the standard error of the recombination frequency are introduced when the dataset is simplified by analysis of the 1:1 segregation ratio of gametes, rather than the 1:1:1:1 segregation ratio of offspring genotypes. The standard error of the recombination frequency is calculated using the information function *Ip* (Allard, 1956). When linkage between two 1:1:1:1 segregating loci is analysed with our method, actually two intervals are analysed between two 1:1 segregating loci. By lumping the offspring classes of a 1:1:1:1 segregation type into a 1:1 type, the informative male gametes are discarded to obtain the maternal dataset and vice versa. In our method, the standard error of the recombination frequency is always calculated correctly, in the case of an interval flanked by 1:1:1:1 segregating markers. The 50 % reduction of the information, caused by lumping offspring classes, is compensated for by the doubling of the number of observations (double test-cross). This is also reflected by the value of the information function of complete classification ($Ip_{1:1:1:1 \text{ by } 1:1:1:1} = 2n/p(1-p)$), which is twice the value of the information fuction of the test-cross ($Ip_{1:1 \text{ by } 1:1} = n/p(1-p)$). However, when the interval is flanked by one 1:1:1:1 segregating locus and a 1:2:1 or 3:1 segregating locus, the lumping of offspring classes results in the loss of information, which is not compensated for by the doubled

number of observations. In these cases, the value of the information functions, which correspond to the original data, are more than twice the value of the information functions which correspond to the separated parental data sets. Our method may result into a fourfold larger standard error of the recombination value (see appendix).

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Appendix: Information funtions for the calculation of the standard error of the recombination frequency (p), whereby Ip = n.ip (Allard, 1957).

$ip_{1:1:1:1 \text{ by } 1:1:1:1} = 2/p(1\text{-}p)$
$ip_{1:1 \text{ by } 1:1} = 1/p(1\text{-}p)$

Ratio between $ip_{1:1:1:1 \text{ by } 1:1:1:1}$ and $ip_{1:1 \text{ by } 1:1} = 2$

$ip_{1:1:1:1 \text{ by } 1:2:1} = 2 + ¾(1\text{-}2p)^2/p(1\text{-}p) + (2p\text{-}1)^2/(2p^2\text{-}2p+1)$ (this thesis)
$ip_{1:1 \text{ by } 1:2:1} = 1/2p(1\text{-}p)$

Ratio between $ip_{1:1:1:1 \text{ by } 1:2:1}$ and $ip_{1:1 \text{ by } 1:2:1}$ is a function of p. With decreasing recombination frequencies, the ratio between the information functions increases to a maximum of 4.

$ip_{1:1:1:1 \text{ by } 3:1} = 2 + p^2/1\text{-}p^2 + (2p\text{-}1)^2/2(p^2\text{-}p+1) +$
$(1\text{-}p)^2/p(2+p) + (1\text{-}2p)^2/2p(1\text{-}p)$ (this thesis)
$ip_{1:1 \text{ by } 3:1} = (1+2p\text{-}2p^2)/2p(1\text{-}p^2)(2\text{-}p)$ (Ritter *et al.*, 1990)

Ratio between $ip_{1:1:1:1 \text{ by } 3:1}$ and $ip_{1:1 \text{ by } 3:1}$ is also a function of p. The ratio will reach its maximal value at recombination frequencies between 0.15 and 0.25.

CHAPTER 3

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 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 find monogenic segregation ratios. These were required to detect linkage with RFLP loci, and despite distorted Mendelian ratios, the inheritance and mapping of loci *D*, *F* and *P* could be elucidated unambiguously. 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 loci *D* and *P*, was mapped on chromosome 10. The tester clones and the map position of the loci *D*, *F* and *P* may be of considerable value in simplifying the genetics of anthocyanin pigmentation.

Introduction

Knowledge of the inheritance of many morphological and agronomically important traits 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

and 1970), and more recently by De Jong (1991). From the beginning of this century various authors have postulated over twenty genes to explain all 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 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 tissue specific expression of genes involved in anthocyanin biosynthesis.

Materials and methods

Nomenclature of loci

The nomenclature of the loci used in this study is based on the genetic system developed for 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 of 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). For 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. 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 by Lunden (1974). From the tetraploid (2n=4x=48) cultivar Gineke with red tubers and flowers having 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 locus *D* and *F*, whereas I_1G254-26 and I_1G254-77 have complementary genes.

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

The RFLP map is based on a backcross population USW5337.3 x 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 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 x E and E x 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 DNA, according to the manufacturer's instructions. Fragments, 6-8 μg DNA per lane, were separated on an 0,8 % TAE buffered agarose gel and transferred on 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 Dr. 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 markers with known positions were selected as a basis for assigning potato loci to the respective linkage groups. All other markers were developed in our labs.

Segregation, linkage analysis and map construction

Whenever appropriate, 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, 1993). 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 1A), 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, I_1G254-83 as a tester double recessive at both the loci. Blue pigmented flowers are not observed in either the testers or their offspring indicating recessiveness at the *P*-locus.

For any unknown clone it is now possible to deduce the genotype and to test 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 loci *D* and *F* 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 1B, C.

- *The genotype of parental clone C*

The data in Table 1B support heterozygosity for both the *D* and *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.

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	ppDdFf	ppDdFf
I_1G254-26	I_1G254-77	WHITE	WHITE		33	0	1:0	0.00	ppddFF	ppDDff
I_1G254-77	I_1G254-83	WHITE	WHITE		0	26	0:1	0.00	ppDDff	ppddff
I_1G254-83	I_1G254-26	WHITE	WHITE		0	38	0:1	0.00	ppddff	ppddFF
Part B: crosses between parents C and E and tester genotypes										
C	G254	RED	RED		78	37	9:7	6.26 *	ppDdFf	ppDdFf
C	I_1G254-26	RED	WHITE		24	22	1:1	0.09	ppDdFf	ppddFF
I_1G254-26	C	WHITE	RED		10	1	1:1	7.36 **	ppddFF	ppDdFf
C	I_1G254-77	RED	WHITE		15	19	1:1	0.47	ppDdFf	ppDDff
I_1G254-77	C	WHITE	RED		11	18	1:1	1.69	ppDDff	ppDdFf
C	I_1G254-83	RED	WHITE		13	30	1:3	0.63	ppDdFf	ppddff
E	G254 (1990)	RED	RED	9	150	73	3:18:11	10.96 **	PpDdFf	ppDdFf
E	G254 (1991)	RED	RED	9	45	27	3:18:11	0.28	PpDdFf	ppDdFf
G254	E	RED	RED	11	127	90	3:18:11	6.77 *	ppDdFf	PpDdFf
E	I_1G254-26	RED	WHITE	13	56	33	1:2:1	8.82 *	PpDdFf	ppddFF
I_1G254-26	E	WHITE	RED	10	36	20	1:2:1	3.58	ppddFF	PpDdFf
E	I_1G254-77	RED	WHITE	0	25	19	0:1:1	0.82	PpDdFf	ppDDff
I_1G254-77	E	WHITE	RED	0	25	30	0:1:1	0.45	ppDDff	PpDdFf
E	I_1G254-83	RED	WHITE	7	17	28	1:2:5	1.89	PpDdFf	ppddff
I_1G254-83	E	WHITE	RED	1	5	19	1:2:5	2.42	ppddff	PpDdFf
C	E	RED	RED	6	135	115	3:18:11	22.35 **	ppDdFf	PpDdFf

Table 1, continued

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	· · DDff	ppddFF
CE16	I_1G254-77	WHITE	WHITE	0	0	18	0:0:1	0.00	· · DDff	ppDDff
CE17	I_1G254-26	WHITE	WHITE	8	13	2	1:2:1	3.52	PpDdff	ppddFF
CE17	I_1G254-77	WHITE	WHITE	0	0	19	0:0:1	0.00	PpDdff	ppDDff
CE18	I_1G254-83	BLUE	WHITE	9	0	29	1:0:3	0.04	PpddFf	ppddff
CE24	I_1G254-83	BLUE	WHITE	7	0	30	1:0:3	0.73	PpddFf	ppddff
CE60	I_1G254-26	WHITE	WHITE	9	11	5	1:2:1	1.64	PpDdff	ppddFF
CE60	I_1G254-77	WHITE	WHITE	0	0	20	0:0:1	0.00	PpDdff	ppDDff
CE67	I_1G254-83	RED	WHITE	1	8	30	1:2:5	4.69	PpDdFf	ppddff
CE69	I_1G254-83	RED	WHITE	0	8	38	0:1:3	1.42	ppDdFf	ppddff
CE70	I_1G254-83	RED	WHITE	0	16	24	0:1:1	1.60	ppD · F ·	ppddff
CE77	I_1G254-26	WHITE	WHITE	0	0	20	0:0:1	0.00	ppdd · ·	ppddFF
CE77	I_1G254-83	WHITE	WHITE	0	0	23	0:0:1	0.00	ppdd · ·	ppddff
CE84	I_1G254-26	WHITE	WHITE	0	0	22	0:0:1	0.00	ppddff	ppddFF
CE84	I_1G254-77	WHITE	WHITE	0	0	21	0:0:1	0.00	ppddff	ppDDff
CE102	I_1G254-83	RED	WHITE	0	5	15	0:1:3	0.00	ppDdFf	ppddff
CE110	I_1G254-26	WHITE	WHITE	0	23	0	0:1:0	0.00	ppDDff	ppddFF
CE110	I_1G254-77	WHITE	WHITE	0	1?	20	0:0:1	-.--	ppDDff	ppDDff
CE111	I_1G254-26	WHITE	WHITE	0	12	11	0:1:1	0.04	ppDdff	ppddFF
CE111	I_1G254-77	WHITE	WHITE	0	0	21	0:0:1	0.00	ppDdff	ppDDff
EC601	I_1G254-83	RED	WHITE	13	14	36	1:2:5	3.82	PpDdFf	ppddff
EC602	I_1G254-83	RED	WHITE	12	31	15	1:2:1	0.59	PpDdFF	ppddff

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 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 is found 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 heterozygousity of clone E for the *D* and *F* loci. However, when clone E has the genotype *PpDdFf*, some of the ratios shown in Table 1B 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 locus *P*. 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 1C. 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 of locus *D* being homozygous dominant will mask the effect of locus *P*. 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 phenotypical 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 loci *D*, *F* and *P*.

Linkage analysis

Cosegregation between RFLPs and the locus *P* 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 on 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 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 locus *D* 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.

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

Linkage maps of the 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 Figure 1.

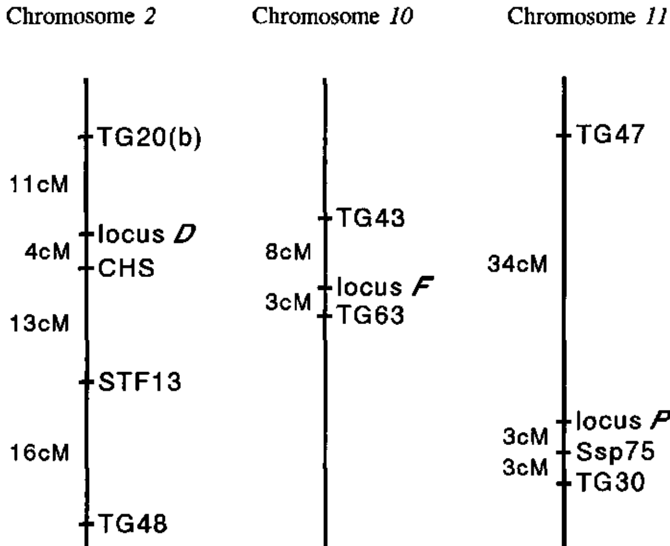

Figure 1. Maps of the potato chromosomes *2*, *10* and *11* showing the positions of the loci *D*, *F* and *P*. Map distances (cM) were calculated with JoinMap using the Kosambi mapping function.

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 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 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. Consensus on nomenclature of these classical loci is of great importance 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 loci *D* and *P* are assumed to be basic factors involved in the biosynthesis of red and blue anthocyanins respectively, because their effect can be observed 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 locus *P* and locus *D* are not involved in the same branch of the biochemical pathway. In addition, neither

locus *P* nor locus *D* are involved in the 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 pigmentation in other parts of the plant. Therefore, a regulatory role of locus *F* in the flower specific expression of the loci *D* and *P* 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 the effect of defining the flower colour classes. As we observed a very clear distinction in our material between the BLUE flower colour class (*P·ddF·*) and the RED class, and not a clear distinction within the RED class between *ppD·F·* and *P·D·F·*, it is justified to include both the genotypes *ppD·F·* and *P·D·F·* 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 loci *D*, *F* and *P* on the chromosomes 2, 10 and 11, respectively, whereby the order of the RFLP-loci on the chromosomes and the 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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CHAPTER 4

THE INHERITANCE OF ANTHOCYANIN PIGMENTATION IN POTATO (*Solanum tuberosum* L.) AND MAPPING OF TUBER SKIN COLOUR LOCI USING RFLPs.

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Summary

Two existing genetic models for anthocyanin pigmentation are compared: the genetic model as proposed by Lunden for tetraploid potato *Solanum tuberosum* Group Tuberosum and the model by Dodds and Long for diploid cultivated *Solanum* species. By crossing well defined genotypes from both genetic sources it was demonstrated that locus R/R^{pw} and locus *D* are allelic. Both loci are involved in the biosynthesis of red anthocyanins. We propose to maintain the symbol *D* for this locus. Tuber skin colour is expressed due to the complementary action of one locus involved in anthocyanin biosynthesis and a second locus involved in tissue specific regulation of anthocyanin expression. The inheritance of two phenotypes of skin colour was investigated in this study: pigmentation of the epidermis and pigmentation of the cortex. In two different populations the loci determining the two phenotypes were located with RFLPs on the map of the potato genome, at approximately the same position as the earlier mapped *PSC*-locus on chromosome 10. Lunden proposed the symbols *E* and *R* for the loci determining these phenotypes. Dodds and Long proposed only one locus *I* for tuber skin colour without describing these different phenotypes. The identity and nomenclature of the loci studied by the various authors are discussed.

Introduction

Genetic research on the potato started at the beginning of this century by Salaman (1910) with a study on the inheritance of tuber skin colour. Salaman postulated three unlinked loci to explain the phenotypes of purple, red and white

tubers. Red pigmentation of tubers is the result of the complementary action of the loci *D* and *R* which both show dominance. Together with factor *P* tubers have purple skin colour. Later, locus *E* was added to this model to explain another phenotype of skin colour pigmentation (Salaman, 1926; Lunden, 1937). Locus *E* is involved in the pigmentation of the epidermis and the eyes of the tuber, whereas locus *R* is expressed in the cortex. The studies on the inheritance of anthocyanin pigmentation in tetraploid European varieties in the first half of this century were reviewed by Von Rathlef and Siebeneick (1934) and by Swaminathan and Howard (1953) and is presently known as the genetic model for Group Tuberosum (2n=4x=48).

Without much reference to previous work, Dodds and Long (1955, 1956) developed another genetic model for the inheritance of anthocyanin pigmentation in diploid cultivated species (2n=2x=24). The loci *P*, R/R^{pw}, *B*, *I* and *F* were postulated to explain anthocyanin pigmentation in various parts of the plant. Reviews by Howard (1970) and De Jong (1991) tried to compare the loci postulated for Group Tuberosum and loci postulated for diploid cultivated species. Experimental evidence on assumed allelic relations between the two genetic models has not been presented until now.

Efficient methods for the production of dihaploid clones from tetraploid varieties (Hermsen and Verdenius, 1973) allow genetic studies of Group Tuberosum germplasm at the diploid level without the complication of tetrasomic inheritance. At the diploid level clones representing Group Tuberosum germplasm can be hybridised with diploid cultivated species. The development of RFLP technology and the publication of detailed genetic maps of the potato genome (Bonierbale *et al.*, 1988, Gebhardt *et al.*, 1989, 1991) have greatly stimulated and facilitated genetic studies in potato.

At this moment, several loci involved in the inheritance of classical morphological traits are mapped. Bonierbale *et al.* (1988) mapped locus *Y* involved in flesh colour on chromosome 3, and Gebhardt *et al.* (1989) mapped a purple skin colour locus (*PSC*) on chromosome 10. In our previous publications we showed the mapping of flower colour locus *F* on chromosome 10 and the loci *D* and *P* involved in anthocyanin biosynthesis on chromosome 2 and 11 respectively (Van Eck *et al.*, 1993), and the *Ro* locus for tuber shape

on chromosome 10 (Van Eck *et al.*, 1994).
This study had two objectives: (1) Demonstrating allelism for loci belonging to two different genetic models for anthocyanin pigmentation, which were postulated for Group Tuberosum and diploid cultivated species respectively. (2) Investigation of the inheritance of two different phenotypes for tuber skin colour and to place the loci involved on the genetic map of potato.

Materials and methods

Nomenclature in the genetic model for Group Tuberosum and description of the phenotypes.

The genetic model for Group Tuberosum germplasm was proposed by Salaman (1910) and extended by Lunden (1937). The postulated loci *D*, *P* and *F* have been located on the genetic map of potato (Van Eck *et al.*, 1993).

Locus *D*; Chromosome 2: A dominant locus involved in the biosynthesis of red pigments throughout the plant. Locus *D* is complementary to the flower and skin colour loci *F*, *E* and *R*. Without the presence of dominant alleles at these flower and skin colour loci, it is still possible to detect the presence of a dominant *D* allele by the weak brownish-red pigmentation of the sprout tips, stems, flower stalks and calyx (Lunden, 1937).

Locus *P*; Chromosome 11: A dominant locus involved in the biosynthesis of blue pigments throughout the plant, especially visible in hypocotyl and sprout tips. Like locus *D* the *P*-locus is complementary to the loci *F*, *E* and *R* in the expression of flower and tuber skin colour (Lunden, 1937).

Locus *E*: A dominant locus regulating the presence of red or purple pigmentation in the epidermis of the tuber in combination with locus *D* or *P* respectively. Plants with genotype *ppddE.* still have a weak, diluted reddish colour in the tubers, with stronger pigmentation in the eyes and at the basis of the sprouts.

Locus *R*: A dominant locus regulating the presence of pigmentation in the outer

layers of the cortex of the tuber; no colour in the epidermis. In combination with either locus *D* or *P*, red or blue-purple tubers are produced. Genotype *P.D.R.* has an intensely pigmented cortex of the tuber with a black appearance. The almost white tuber skin phenotype of *ppddR.* is difficult to distinguish from white skinned *D.rr* or *P.rr* genotypes (Lunden, 1937). Pigmentation of the tuber can be located by stripping off the epidermis of freshly harvested tubers.

Locus *F*; Chromosome 10: A dominant locus regulating the presence of flower colour. Genotype *ppD.F.* gives red-purple flowers, *P.ddF.* pale-blue flowers, *P.D.F.* blue-purple flowers and *ppddF.* or *....ff* gives white flowers (Lunden, 1937).

Nomenclature of the genetic model for diploid cultivated species and description of phenotypes.

Dodds and Long (1955, 1956) developed the genetic model for diploid cultivated species:

Locus R/R^{pw}: In the absence of *P*, this locus controls the production of red pigments in tubers, flowers and sprouts. The homozygous recessive genotype $R^{pw}R^{pw}$ has pink tubers and sprouts but white flowers. A recessive allele *r* giving complete absence of pigmentation has never been described in diploid cultivated species (Dodds and Long, 1955).

Locus *P*: This locus controls the formation of blue pigments throughout a diploid potato plant. In tubers locus *P* is epistatic to locus R/R^{pw}; in flowers epistasy is incomplete (Dodds and Long, 1955).

Locus *I*: The genotype *ii* at locus *I* causes absence of pigmentation of the tubers (Dodds and Long, 1956). Genotypes $P.R^{pw}R^{pw}ii$ and *ppR.ii* have, according to this model, purple and red sprouts respectively with white tuber skin.

Locus *F*: A locus with a flower specific expression involved in the contrast self-coloured versus flecked, homozygous recessives *ff* being flecked. Genotypes which are recessive at locus R/R^{pw} have white flowers (Dodds and Long, 1956).

Table 1. Description of the skin colour phenotypes and genotypes of the parental potato (*Solanum tuberosum* L.) clones.

		Genotypes nomenclature	
Potato clones	Phenotypes	Group Tuberosum	Diploid cultivated species
Parental clones:			
cv. Gineke[a]	Red (L2)[f]	*ppppDDddeeeeRrrrFfff*	
G254[b]	White	*ppDdeerrFf*	
I_1G254 - 26[c]	White	*ppddeerrFF*	
I_1G254 - 77[c]	White	*ppDDeerrff*	
I_1G254 - 83[c]	White		*ppddeerrff*
79-48[d]	Red (L1)		$ppR\ R^{pw}II$
7553-1[d]	Purple (L2)		$PpR\ R^{pw}Ii$
7506-1[d]	White		$ppR^{pw}R^{pw}ii$
Genotypes selected from the cross 79-48 x 7506-1[e]:			
88-405- 1	Red (L1)		$ppR\ R^{pw}Ii$
88-405- 3	Pink		$ppR^{pw}R^{pw}Ii$
88-405- 5	Red (L1)		$ppR\ R^{pw}Ii$
88-405- 8	Pink		$ppR^{pw}R^{pw}Ii$
88-405-12	Pink		$ppR^{pw}R^{pw}Ii$
88-405-38	Pink		$ppR^{pw}R^{pw}Ii$
genotypes selected from the cross 7553-1 x 7506-1[e]:			
88-402-12	Purple (L2)		$PpR^{pw}R^{pw}Ii$
88-402-25	White		$ppR\ R^{pw}ii$

[a] (Lunden, 1974) [b] (Hermsen *et al.*, 1978) [c] (Van Eck *et al.*, 1993) [d] (De Jong, 1987)
[e] This article [f] L1 and L2 refer to epidermis and cortex, respectively.
The genotype is given in the nomenclature belonging to the origin of the germplasm.

Pedigree and genotypes of plant materials:

Group Tuberosum germplasm is represented by three diploid tester genotypes I_1G254-26, I_1G254-77 and I_1G254-83 derived from the dihaploid G254 from the Group Tuberosum cultivar cv. Gineke (Van Eck *et al.*, 1993). The genotypes of these tester clones with white flowers and tubers are indicated in Table 1, following the nomenclature by Lunden. Alleles in this material are identical by descent to those involved in previous studies by Lunden (1974). The genotypes

of parental clones 7553-1, 79-48 and 7506-1 are described (De Jong, 1987) according to the nomenclature by Dodds and Long (1955, 1956). The clones are derived from an Agriculture Canada Breeding Project, Fredericton, N.B., involving the diploid species *S. stenotomum* and *S. phureja*. Seeds from 79-48 x 7506-1 and 7553-1 x 7506-1 were kindly provided by Dr. H. De Jong. Cross 79-48 x 7506-1 gave progeny 88-405 which segregated for red pigmentation of the epidermis of the tuber; cross 7553-1 x 7506-1 gave progeny 88-402 which segregated for blue-purple pigmentation of the cortex of the tuber. The genotypes of the clones, representing loci for anthocyanin pigmentation from diploid cultivated species, are also shown in Table 1. Individual genotypes of the progenies 88-405 and 88-402 were crossed as female parents with the earlier mentioned tester clones of Group Tuberosum used as male parents.

Procedures to detect DNA polymorphisms
Isolation of genomic DNA from young leaves and shoots was as described by Bernatzky and Tanksley (1986). Restriction digestions were performed with the enzymes: *Eco*RI, *Hin*DIII and *Xba*I using 2 units per μg DNA, according to the manufacturers instructions (Amersham). Fragments, 6-8 μg DNA per lane, were separated in a 0.8 % TBE buffered agarose gel and transferred to Hybond N membrane by capillary blotting using a 1M NH_4OH / 20mM NaOH transfer solution. Blots were hybridised against digoxigenin-dUTP labelled DNA probes. Hybridisation mixtures with labelled probe can be reused several times for a period of at least one year. Signal detection was performed with anti-dig-AP antibodies (Boeringer) and AMPPD (Tropix) as substrate for chemiluminescence according to Kreike *et al.* (1990). Luminograms were obtained within two hours by placing the membranes on X-ray film (Kodak X-Omat). After stripping the probe from the blot, reuse of the blots is possible for at least five times. When too much background signal limits further use of the blots with the chemiluminescence procedure, it is still possible to use the blot in a radioactive procedure.
The map positions of the RFLP marker loci are published and the RFLP DNA clones are kindly provided by Gebhardt *et al.* (1989, 1991: GP and CP clones); Bonierbale *et al.* (1988: TG clones); Kreike *et al.* (1993: Ssp clone).

Segregation, linkage analysis and map construction
Only the female parents 88-405-5 and 88-402-12 segregated at RFLP loci, and the male 'inbred' parent I_1G254-77 appeared to be homozygous at all loci tested. Consequently, the genetic maps are based on recombination events in the female meiosis. The computer program JoinMap (Stam, 1993) calculated the most likely map configuration, using the mapping function of Kosambi (1944). The computer program DrawMap (van Ooijen, 1994) was used to draw the maps from this study and previous data (Van Eck *et al.*, 1993, 1994) referring to the flower colour locus *F* (Lunden, 1937) and the tuber shape locus *Ro* (Masson, 1985). Raw RFLP data are loaded in the nightshade database which is available via anonymous ftp from: probe.nalusda.gov in the directory /pub/solgenes (For more information: Epaul@nightshade.cit.cornell.edu).

Results

Phenotypes of tuber skin colour in the segregating progenies 88-405 and 88-402
Progenies 88-405 of the cross 79-48 x 7506-1 and 88-402 of the cross 7553-1 x 7506-1 represent genetic material of diploid cultivated species (De Jong, 1987). The progeny 88-405 segregated into descendants with red and pink tubers in a 19:13 ratio (this fits an expected ratio of 1:1; $\chi^2_{(1)} = 1.13$) due to heterozygosity at the R/R^{pw} locus in the female parent 79-48. The clones of 88-405 with red tubers and red flowers have the genotype $ppRR^{pw}Ii$, and descendants with pink tubers and white flowers have the genotype $ppR^{pw}R^{pw}Ii$ (See Table 1). These observations are in agreement with the genetic model for diploid cultivated species (Dodds and Long, 1955) and with the parental genotypes postulated by De Jong (1987): the pistilate parent 79-48 with red flowers and red tubers has the genotype $ppRR^{pw}II$; the unpigmented staminate parent 7506-1 has the genotype $ppR^{pw}R^{pw}ii$.
The segregation observed in progeny 88-402, derived from 7553-1 x 7506-1, is also in agreement with the parental genotypes postulated by De Jong (1987) and the genetic model for diploid cultivated species. Heterozygosity of the pistilate parent 7553-1 at locus *P*, R/R^{pw}, and *I*, is confirmed by the observation of purple, red and white skinned descendants, and purple, red and white pigmentation of the sprouts within the descendants with white tuber skin colour.

The red skin colour of 88-405 descendants was observed in the epidermis of the tuber. In contrast to this phenotype the red or purple skin colour segregating in 88-402 clones is located in the outer cell layers of the cortex. The genetic model for anthocyanin pigmentation in diploid cultivated species (Dodds and Long, 1955) does not discriminate between these two phenotypes, whereas the genetic model for tetraploid Group Tuberosum has two different loci, *E* and *R*, to explain these phenotypes.
The phenotype of the offspring clones 88-405-.. with pink tubers and white flowers having genotype $ppR^{pw}R^{pw}Ii$ match well with the phenotype of *ddEe* as described by Lunden (1937). This phenotype is described as having a weak diluted reddish colour in the tubers, with stronger pigmentation in the eyes and at the base of the sprouts (Lunden, 1937). The resemblance suggests that skin colour locus *I* (Dodds and Long, 1955) in this material, derived from diploid cultivated species, may be allelic with the skin colour locus *E* (Lunden, 1937) from tetraploid Group Tuberosum germplasm. The segregation of pigmentation in the tuber epidermis as well as in the flower due to locus R/R^{pw} resembles the action of locus *D* (Lunden, 1937) which is involved in the production of red pigments throughout the plant (Van Eck *et al.*, 1993). Therefore, allelism between locus R/R^{pw} (Dodds and Long, 1955) and locus *D* (Lunden, 1937) is tested.

In 88-402 clones, where pigmentation was located in the cortex of the tubers, the effect of locus *I* (Dodds and Long, 1955) is similar to the effect of a second skin colour locus *R* as described by Lunden (1937). Among the descendants of 88-402 clones, the typical pink tubers with red eyes as observed in the previous cross due to skin colour locus *E* were not observed, indicating another difference between these skin colour loci. $ppR^{pw}R^{pw}Ii$ descendants from 88-402-12, had almost white tubers and white flowers, but could be distinguished from true white $ppR^{pw}R^{pw}ii$ genotypes on the basis of pigmentation of the sprout tips. These phenotypical observations suggest allelism between the skin colour locus *I* (Dodds and Long, 1955) and the skin colour locus *R* (Lunden, 1937) from tetraploid Group Tuberosum material.

The inheritance of flower and tuber skin colour in 88-405 genotypes

To examine allelic relations between the loci postulated in diploid cultivated species and Group Tuberosum, the 88-405-clones were hybridised with the tester clones I_1G254-26, I_1G254-77 and I_1G254-83 with white flowers and tubers. Clone I_1G254-77 with genotype *ppDDeerrff* will transmit to its progeny the dominant *D*-allele which is involved in the biosynthesis of red anthocyanins. Tester clone I_1G254-26 with genotype *ppddeerrFF* will transmit a dominant *F*-allele which is involved in the flower specific expression of pigmentation. The complementary action of the loci *D* and *F* will result in the presence of red flower colour. Clone I_1G254-83 is recessive at loci *P*, *D* and *F* (Van Eck *et al.*, 1993), and at the loci *E* and *R*. Observations on flower and tuber skin colour of hybrid progenies between 88-405 and the tester clones are presented in the Tables 2 and 3, respectively.

No difference in segregation was observed for either flower colour or tuber skin colour between descendants of clone I_1G254-26 and I_1G254-83. However, when tester I_1G254-77 was used, which contributes the *D*-allele to the progeny, the pink tuber skin and white flowers were found. Recessiveness at locus R/R^{pw} resulting in pink tubers and white flowers can be supplemented in diploid cultivated species with a dominant allele of locus *D* originating from Group Tuberosum. This clearly demonstrates allelism between locus R/R^{pw} and locus *D*.

From Table 2 the effect of a dominant *F*-allele can be inferred from hybrids between 88-405 clones and tester clone I_1G254-26 in comparison with hybrids between 88-405 and I_1G254-83. Because there is no difference in the presence or segregation ratio of flower colour in descendants of both I_1G254-83 and I_1G254-26 it is concluded that 88-405 individuals are uniformly homozygous dominant at the *F*-locus.

Although locus *I* is segregating in progenies from all 88-405 clones, the data in Table 2 demonstrate that flower colour, as expected, is not affected by the segregating locus *I* involved in tuber skin colour. The presence or absence of flower colour and the expected ratios for this trait can be adequately explained from the genotype at locus R/R^{pw} in the female 88-405 parent.

Table 2: Segregation of flower colour in crosses between germplasm of diploid cultivated species (88-405 clones) and Group Tuberosum (I_1G254-tester clones).

Parents		Phenotype		Observed		Expected[a]	Parental genotypes	
Female	Male	Female	Male	red *Dd..*	white[d] *dd..*	red:white	Female[b]	Male[c]
88-405-1	I_1G254-26	red	white	13	16	1:1	$R\,R^{pw}Ii$	*ddeeFF*
88-405-1	I_1G254-77	red	white	14	0	1:0	$R\,R^{pw}Ii$	*DDeeff*
88-405-3	I_1G254-26	white	white	0	23	0:1	$R^{pw}R^{pw}Ii$	*ddeeFF*
88-405-3	I_1G254-77	white	white	12	0	1:0	$R^{pw}R^{pw}Ii$	*DDeeff*
88-405-3	I_1G254-83	white	white	0	24	0:1	$R^{pw}R^{pw}Ii$	*ddeeff*
88-405-5	I_1G254-26	red	white	16	14	1:1	$R\,R^{pw}Ii$	*ddeeFF*
88-405-5	I_1G254-77	red	white	15	0	1:0	$R\,R^{pw}Ii$	*DDeeff*
88-405-5	I_1G254-83	red	white	11	7	1:1	$R\,R^{pw}Ii$	*ddeeff*
88-405-8	I_1G254-26	white	white	0	48	0:1	$R^{pw}R^{pw}Ii$	*ddeeFF*
88-405-8	I_1G254-77	white	white	22	0	1:0	$R^{pw}R^{pw}Ii$	*DDeeff*
88-405-8	I_1G254-83	white	white	0	27	0:1	$R^{pw}R^{pw}Ii$	*ddeeff*
88-405-12	I_1G254-26	white	white	0	6	0:1	$R^{pw}R^{pw}Ii$	*ddeeFF*
88-405-12	I_1G254-77	white	white	16	0	1:0	$R^{pw}R^{pw}Ii$	*DDeeff*
88-405-12	I_1G254-83	white	white	0	33	0:1	$R^{pw}R^{pw}Ii$	*ddeeff*
88-405-38	I_1G254-26	white	white	0	20	0:1	$R^{pw}R^{pw}Ii$	*ddeeFF*
88-405-38	I_1G254-77	white	white	12	0	1:0	$R^{pw}R^{pw}Ii$	*DDeeff*
88-405-38	I_1G254-83	white	white	0	13	0:1	$R^{pw}R^{pw}Ii$	*ddeeff*

a) all observations are in agreement with the expected ratios. b) genotype symbols according to the nomenclature common for diploid cultivated species (Dodds and Long, 1955, 1956). c) genotype symbols according to the nomenclature common for Group Tuberosum (Lunden, 1937)
d) the genotypes of the descendants belonging to the classes: red = *DdEeF.* / *DdeeF.*; white = *ddEeF.* / *ddeeF.* in the nomenclature common for Group Tuberosum; red = $R\,R^{pw}Ii$ / $R\,R^{pw}ii$; white = $R^{pw}R^{pw}Ii$ / $R^{pw}R^{pw}ii$ in the nomenclature common for cultivated diploid species.

Table 3: Segregation of tuber skin colour in crosses between germplasm of diploid cultivated species (88-405 clones) and Group Tuberosum (I_1G254-tester clones).

Parents		Phenotype		Observed				Expected[a]	Parental genotypes	
Female	Male	Female	Male	*DdEe* red	*Ddee* white	*ddEe* pink	*ddee* white[d]	r:w:p:w	Female[b]	Male[c]
88-405-1	I_1G254-26	red	white	21	20	13	32	1:1:1:1	$R\ R^{pw}Ii$	*ddeeFF*
88-405-1	I_1G254-77	red	white	13	37	0	0	1:1:0:0	$R\ R^{pw}Ii$	*DDeeff*
88-405-3	I_1G254-26	pink	white	0	0	45	36	0:0:1:1	$R^{pw}R^{pw}Ii$	*ddeeFF*
88-405-3	I_1G254-77	pink	white	41	38	0	0	1:1:0:0	$R^{pw}R^{pw}Ii$	*DDeeff*
88-405-3	I_1G254-83	pink	white	0	0	26	38	0:0:1:1	$R^{pw}R^{pw}Ii$	*ddeeff*
88-405-5	I_1G254-26	red	white	17	15	28	14	1:1:1:1	$R\ R^{pw}Ii$	*ddeeFF*
88-405-5	I_1G254-77	red	white	38	34	0	0	1:1:0:0	$R\ R^{pw}Ii$	*DDeeff*
88-405-5	I_1G254-83	red	white	21	18	21	13	1:1:1:1	$R\ R^{pw}Ii$	*ddeeff*
88-405-8	I_1G254-26	pink	white	0	0	35	45	0:0:1:1	$R^{pw}R^{pw}Ii$	*ddeeFF*
88-405-8	I_1G254-77	pink	white	34	37	0	0	1:1:0:0	$R^{pw}R^{pw}Ii$	*DDeeff*
88-405-8	I_1G254-83	pink	white	0	0	45	38	0:0:1:1	$R^{pw}R^{pw}Ii$	*ddeeff*
88-405-12	I_1G254-26	pink	white	0	0	48	34	0:0:1:1	$R^{pw}R^{pw}Ii$	*ddeeFF*
88-405-12	I_1G254-77	pink	white	45	49	0	0	1:1:0:0	$R^{pw}R^{pw}Ii$	*DDeeff*
88-405-12	I_1G254-83	pink	white	0	0	26	24	0:0:1:1	$R^{pw}R^{pw}Ii$	*ddeeff*
88-405-38	I_1G254-26	pink	white	0	0	48	44	0:0:1:1	$R^{pw}R^{pw}Ii$	*ddeeFF*
88-405-38	I_1G254-77	pink	white	40	40	0	0	1:1:0:0	$R^{pw}R^{pw}Ii$	*DDeeff*
88-405-38	I_1G254-83	pink	white	0	0	36	48	0:0:1:1	$R^{pw}R^{pw}Ii$	*ddeeff*

a) all observations are in agreement with the expected ratios. b) genotype symbols according to the nomenclature common for diploid cultivated species (Dodds and Long, 1955, 1956). c) genotype symbols according to the nomenclature common for Group Tuberosum (Lunden, 1937).
d) the genotypes assigned to descendants belonging to the classes: red, white, pink, white are in the nomenclature common for Group Tuberosum. The alternative notation would have been: red, white, pink, white = $R\ R^{pw}Ii$, $R\ R^{pw}ii$, $R^{pw}R^{pw}Ii$, $R^{pw}R^{pw}ii$, respectively.

Four phenotypes of tuber pigmentation could be observed in the progeny of the crosses 88-405-1 x I_1G254-26, 88-405-5 x I_1G254-26 and 88-405-5 x I_1G254-83 (Table 3). The tuber skin colour classes red, pink and white, are easily recognised. Subsequently, within the class with white tubers the segregation at locus *D* can be recognised by the presence of either the flower colour, or pigmentation of the inflorescence or the sprout. However, sprout pigmentation is also affected by locus *E*, but in a different way. Sprouts of *Ddee* genotypes are pigmented mainly at the tip (characteristic No.7; UPOV, 1986). *ddEe* genotypes have pigmentation of the sprout mainly at the base (characteristic No.4; UPOV, 1986). In *DdEe* genotypes, sprout colour is red both at the base, as well at the tip. The genotype may also be deduced from the sprout phenotype for the other crosses mentioned in Table 3.

The pink tuber skin colour is only observed in *ddEe* genotypes. This indicates that the pink pigments are the effect of a dominant *E*-allele (= *I*-allele) and not the result of a recessive *d*-allele (= R^{pw}-allele). According to the description by Dodds and Long (1955), the effect of locus *I* would be a recessive inhibitor of pigments produced by the recessive R^{pw}-allele. Genetically, this may be a correct interpretation, but from a molecular point of view, it is paradoxical to ascribe a positive function to a recessive allele.

In Table 3 it is shown that tuber skin colour is not influenced by locus *F*. This is in agreement with our expectations as locus *F* is involved in the expression of flower colour.

A few additional aspects to the observations in the Tables 2 and 3 need to be mentioned. Firstly, for a specific offspring the progeny size for flower colour is not equal to the progeny size for tuber skin colour. This is because not all seedlings flowered or set tubers. Secondly, the red flowering descendants segregated 1:1 for red and white tuber skin colour, and the white flowering ones segregated into pink and white tuber skin colour. Both segregations are due to the segregation of locus *I*.

RFLP mapping of the locus involved in skin colour in the epidermis

The progeny of 88-405-5 x I_1G254-77 comprised of 64 descendants was used to map skin colour. In this population cosegregation was observed between skin

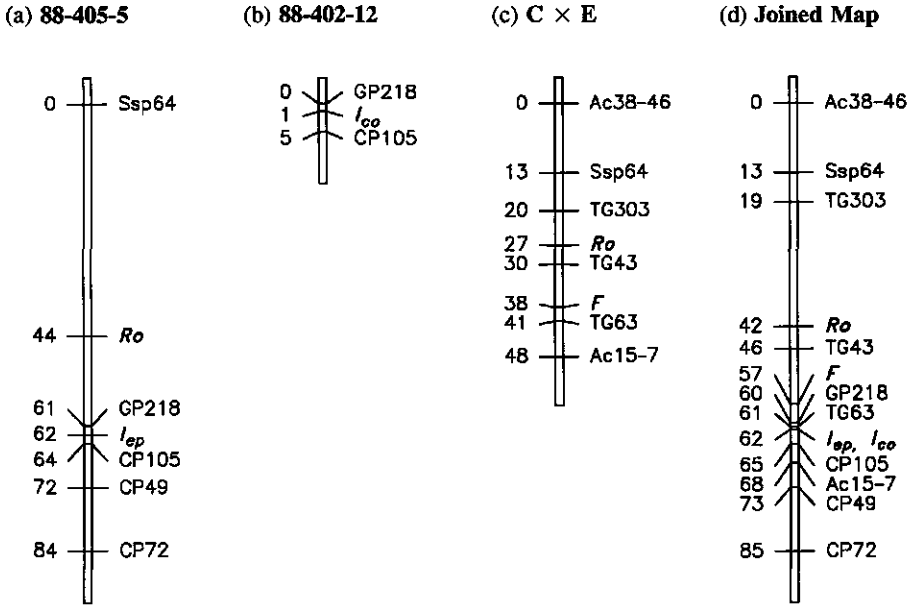

Fig. 1: Genetic maps of potato chromosome 10 (Kosambi distances in cM). (a) Genetic map of clone 88-405-5 showing the tuber skin colour locus I_{ep} involved in pigmentation of the epidermis. (b) Genetic map of clone 88-402-12 showing the tuber skin colour locus I_{co} involved in pigmentation of the cortex. (c) Genetic map of potato showing flower colour locus *F* (Van Eck, 1993) and tuber shape locus *Ro* (Van Eck, 1994). (d) Joined map of potato chromosome 10 showing the positions of morphological marker loci.

colour and tuber shape (17.2 ± 4,7 % recombination; $\chi^2_{(1)}$= 27.8; LOD = 6.5). The *Ro* locus involved in tuber shape is located on chromosome 10 (Van Eck *et al.*, 1994). Additionally, it was known that on potato chromosome 10 the *PSC* locus involved in purple skin colour was mapped (Gebhardt *et al.*, 1989). This information gave a strong indication about the possible map position of this tuber skin colour locus. Using RFLP markers from chromosome 10 it was possible to locate skin colour. In 63 observations only one recombination was found between the skin colour locus and RFLP marker GP218 at one side (1.59 % recombination; $\chi^2_{(1)}$= 59.1; LOD = 16.7) and another recombination between the skin colour locus and CP105 at the other side (1.59 % recombination; $\chi^2_{(1)}$= 59.1; LOD = 16.7). The resulting map of

cross 88-405-5 x I_1G254-77 including several other RFLP markers is shown in Fig. 1A. The tuber skin colour locus is indicated with symbol I_{ep} because the allele is descending from diploid cultivated species and allelism with locus *E* (Lunden, 1937) is not demonstrated. The suffix indicates that the effect of this allele is observed in the epidermis of the tuber.

The inheritance of flower and tuber skin colour in 88-402 genotypes

In this material skin colour is based on cortex pigmentation, not the epidermis, and therefore the tetraploid symbol *R/r* has to be used. Due to poor flowering, female sterility or low berry set only three combinations with the tester clones could be obtained. The segregations observed in these three progenies are presented in Table 4. The results of cross 7553-1 x 7506-1 are not fully described because sixteen (2^4) genotypes resulting in ten different phenotypes are expected. With respect to tuber skin colour alone, the observed segregation is in close agreement with the expected ratio.

The most striking observation in this material is the absolute correlation between flower and skin colour in the progenies 88-402-12 x I_1G254-77, 88-402-12 x I_1G254-83 and 88-402-25 x I_1G254-83 (Table 4). The correlation between flower and skin colour was particularly curious, because clones with pigmented tubers always had white flowers and clones with pigmented flowers always had white tubers. Initially, Lunden (1937) described independent loci for tuber and skin colour. Much later (Lunden, 1960), he discovered close linkage between skin colour locus *R* and flower colour locus *F*, although this conclusion was weakened in his latest publication (Lunden, 1974). The correlations reported in the literature always describe individuals where skin and flower colour are both present or absent. Dodds and Long (1956) described a linkage group comprising three loci *B-I-F*. However, the *F* locus as described by Dodds and Long (1956) is assumed to be involved in the contrast flecked versus self-coloured flowers.

To explain this correlation found in descendants of 88-402-12 we propose linkage in repulsion phase between the skin colour and flower colour locus. This explanation is a combination of the *B-I-F* linkage group postulated for diploid cultivated species (Dodds and Long, 1956) and the phenotype of locus

Table 4 Segregation for tuber skin colour and flower colour in crosses between 88-402 clones (diploid cultivated species) and tester clones (group Tuberosum)

Parents		Flower colour, skin colour		Parental genotype		Expected (assuming $R/R^{pw} = D/d$ locus)					Observed*
Female	Male	Female	Male	Female	Male	Genotypes and phenotypes				Ratio	
7553-1	7506-1			$PpRR^{pw}\frac{If}{if}$	$ppR^{pw}R^{pw}\frac{iF}{if}$	16 genotypes with 10 different phenotypes expected					
	Flowers	White	White						purple:blue:red:white	1:1:1:5	Not observed
	Skin	Purple	White						purple:blue:red:white	2:0:1:5	7:0:4:18
88-402-12	I_1G254-77			$PpR^{pw}R^{pw}\frac{If}{iF}$	$ppDD\frac{rf}{rf}$	$PpDd\frac{If}{if}$	$PpDd\frac{iF}{if}$	$ppDd\frac{If}{if}$	$ppDd\frac{iF}{if}$	1:1:1:1	
	Flowers	Purple	White			White	Purple	White	Red		(8:5:14:7)†
	Skin	Purple	White			Purple	White	Red	White		22:22:22:25
88-402-12	I_1G254-83			$PpR^{pw}R^{pw}\frac{If}{iF}$	$ppdd\frac{rf}{rf}$	$Ppdd\frac{If}{if}$	$Ppdd\frac{iF}{if}$	$ppdd\frac{If}{if}$	$ppdd\frac{iF}{if}$	1:1:1:1	
	Flowers	Purple	White			White	Blue	White	White		(16:14:14:6)
	Skin	Purple	White			Purple	White	White	White		22:17:14:8
88-402-25	I_1G254-83			$ppRR^{pw}\frac{if}{if}$	$ppdd\frac{rf}{rf}$		$ppDd\frac{if}{if}$		$ppdd\frac{if}{if}$	1:1	
	Flowers	White	White				White		White		(13:10)
	Skin	White	White				White (red sprouts)		White (white sprouts)		30:22

*All observations are in agreement with the expected ratios.
†Observed numbers of flower colour phenotypes within skin colour phenotypes.

F as described by Lunden (1937) and its complementary action with locus *D* (Lunden, 1937; Van Eck *et al.*, 1993). Flower and tuber skin colour are independent in 7553-1 x 7506-1 because the dominant alleles of locus *I* and *F* descend from the different parents.

When the progenies of 88-402-12 x I_1G254-77 and 88-402-12 x I_1G254-83 are compared it is possible to characterise the effect of the dominant *D* allele contributed by I_1G254-77. Locus *D* is involved in the ability to produce red anthocyanins throughout the plant, but tuber skin colour and flower colour is only observed in the presence of a complementary allele at locus *I* or *F*. Without complementary gene action red pigments produced by locus *D* can be observed in the tip of the sprouts, but not in the tuber skin or flowers.

Observations on skin and flower colour in the offspring of 88-402-12 demonstrate epistasic dominance of locus *P* over locus *D*. This epistasis is complete in the tuber, but incomplete in the flower. This was also described for the interaction between locus *P* and R/R^{pw} (Dodds and Long, 1955).

The conclusion of allelism between locus R/R^{pw} and locus *D* as drawn from the results shown in the Tables 2 and 3 is also confirmed by the results from Table 4. The presence of a dominant allele at locus *D* in clone 88-402-25 was inferred from the presence of red pigmented flesh and red pigmentation of the tip of the spouts. Heterozygosity at locus *D* was inferred from the 1:1 segregation for pigmented sprout tips in its offspring.

RFLP mapping of the locus involved in skin colour in the cortex

The offspring of 88-402-12 x I_1G254-77 comprised of 91 descendants was used to map the locus causing pigmentation of the cortex. Cosegregation was found between skin colour and RFLP marker CP105 with three recombinants out of 74 observations (4.1% recombination; $\chi^2_{(1)}$= 62.5; LOD = 16.8). Cosegregation was also observed with marker GP218 with only one recombinant out of 62 observations (1.6 % recombination; $\chi^2_{(1)}$= 58.1; LOD = 16.4). These RFLP markers encompass the skin colour locus as the largest recombination fraction was found between CP105 and GP218 (3 out of 59, 5.1 % recombination; $\chi^2_{(1)}$= 47.7; LOD = 12.6). The locus involved in pigmentation of the cortex, probably locus *R*, appeared to be located on

chromosome 10 at approximately the same position as the skin colour locus involved in pigmentation of the epidermis. Because allelism is not demonstrated between the locus segregating in this material derived from diploid cultivated species and locus *R* involved in pigmentation of the cortex in tetraploid Group Tuberosum (Lunden, 1937) the position of this locus is indicated with symbol I_{co} on the map shown in Fig. 1B. The suffix indicates that this allele is involved in pigmentation of the cortex.

Construction of an integrated map

The two separate maps based on 88-405-5 and 88-402-12 were integrated with data from previous publications (Van Eck *et al.*, 1993, 1994) referring to the map position of flower colour locus *F* (Lunden, 1937) and the tuber shape locus *Ro* (Masson, 1985) (Fig. 1C). The order of loci as described in Gebhardt (1991) were also taken into account by adding a fixed sequence file (GP218 - TG63 - CP105) to the computer program JOINMAP. This integrated map of chromosome 10 (Fig. 1D) is showing a short distance between the skin colour gene(s) at locus *I* and the flower colour locus *F* (Lunden, 1937). This provides further evidence that the linkage group *B-I-F* (Dodds and Long, 1956) is allelic to the linkage reported by Lunden (1960) between flower locus *F* and skin colour locus *R* in tetraploid Group Tuberosum germplasm.

Discussion

Evidence for allelism

In this report the relations are examined between two genetic models, proposed by different investigators for different germplasm. The loci and/or phenotypes were compared to find evidence for allelism between loci. Allelism between loci can be studied with three different methods. The first approach is based on the phenotype of the trait. The similarity between two phenotypes is a strong indication that these traits are controlled by the same locus, but this is no evidence for allelism. A second and much stronger procedure to test allelism is on the basis of the position of the locus on the genetic map. Loci with distinct map positions are not allelic. However, when two traits can be located on approximately the same locus, it is difficult to distinguish between allelism

and close linkage of different loci. The third and reliable strategy to test for allelism is genetic complementation. This method requires crosses with well defined tester genotypes.

Allelism between locus *D* (Lunden, 1937) and locus *R/R^pw* (Dodds and Long, 1955) was demonstrated with the third method using the diploid tester clones derived from cv. Gineke. Also on the basis of phenotypic observations the effects of loci *D* and *R/R^pw* are identical. Allelism between locus *D* (Lunden, 1937) located on chromosome 2 (Van Eck, 1993) and *I* (Dodds and Long, 1956) located on chromosome 10 (this report) as suggested by De Jong (1991) is rejected.

The tuber skin colour loci present in 88-405-5 and 88-402-12 and the *PSC* locus (Gebhardt *et al.*, 1989) were mapped on chromosome 10. The phenotype of the *PSC* locus is similar to the phenotype observed in clone 88-405-5 (Gebhardt, personal communication). The small difference between the map order reported in Gebhardt *et al.* (1989, 1991) and that in this study can be explained as an artefact due to integration of loci which are polymorphic in only one parent. Integration of the female and male meiosis is based on loci which are polymorphic in both parents. These loci serve as 'allelic bridges' between separate parental maps. Even when all loci are at the same chromosomal position, the different phenotypes caused by locus *E* and locus *R* can be due to different but closely linked genes.

The hypothesis of two closely linked skin colour genes is supported by many other closely linked loci involved in tissue specific expression of anthocyanin pigmentation (Kessel and Rowe, 1974; De Jong, 1987), including flower colour (Fig. 1) in this specific region of potato chromosome 10.

Evaluation of the genetic model as proposed by Lunden (1937, 1960, 1974)

The extensive studies by Lunden have been a major achievement for potato genetics, particularly because the genetic studies were carried out with tetraploid varieties. Major drawbacks of the tetrasomic inheritance are the complex segregation ratios and the difficulties involved in establishing linkage relations.

The weak aspect of the genetic model proposed by Lunden has been the

contradictory information about correlation between flower and skin colour. To detect linkage between flower and skin colour loci it is necessary that the loci are simplex and the dominant alleles are in linkage phase. When they are in repulsion phase or not in the simplex condition a large progeny size is required to detect linkage (Wu *et al.*, 1991). From the approx. one hundred crosses investigated by Lunden (1937), only in six cases the parent had a simplex genotype at the flower and skin colour loci. Additionally, the segregation of the anthocyanin producing loci *D* or *P* was interfering with the segregation of flower and skin colour. It is, therefore, not unlikely that genetic linkage between separate flower and skin colour loci has been overlooked.
Lunden (1937) proposed a model incorporating loci involved in the production of red or blue anthocyanins which are active throughout the plant, as well as loci controlling tissue specific expression of these anthocyanins. This complementary inheritance of pigmentation is a very important aspect of this genetic model. The presence of complementary loci was confirmed by studies at the diploid level (Van Eck *et al.*, 1993).

Evaluation of the genetic model as proposed by Dodds and Long (1955, 1956)
The genetic model for diploid cultivated species has recognised the linkage between genetic loci for flower and skin colour. This linkage is confirmed by RFLP mapping of a flower colour locus (Van Eck *et al.*, 1993) and a skin colour locus (Gebhardt *et al.*, 1989; this study) on chromosome 10. The different skin colour phenotypes were not described for diploid cultivated species. The evidence for maintaining two different skin colour genes at one genetic locus *I* is based on differences in phenotype. Many different genetic loci involved in tissue specific expression of anthocyanins are located at this small region on chromosome 10 (Dodds and Long, 1956; De Jong and Rowe, 1972; Kessel and Rowe, 1974; De Jong, 1987). This clustering of loci involved in pigmentation is another argument in support of the presence of two different skin colour genes at locus *I*. The detection of genetic linkage between flower and skin colour loci is most likely due to the greater genetic resolution at the diploid level rather than to differences between the loci involved in anthocyanin pigmentation.

A major difference between the models seems to be the monogenic inheritance of colour traits in diploid cultivated species and the complementary inheritance of pigmentation as described by Lunden. However, from the description by Dodds and Long (1956) of the inheritance of white, flecked and self-coloured flowers it can be deduced, that also in this material flower colour is the result of complementary action of the *F* and R/R^{pw} locus. According to Dodds and Long (1956) the *F* locus is involved in the contrast between self-coloured and flecked flowers. The tester genotype I_1G254-77 with genotype *DDff* can produce pigments throughout the plant. Pigmentation can be observed in the sprouts, stem and calyx but also some pigmentation is expressed in the tips of the petals, in a pattern similar to that shown by Dodds and Long (1956) for flecked flowers. Our interpretation is as follows: *R.F.* = self-coloured flowers; *R.ff* = flecked flowers, which are white flowers with some pigmentation due to leaky alleles of *ff*; $R^{pw}R^{pw}F.$ and $R^{pw}R^{pw}ff$ = white flowers. Complementary inheritance is also present for tuber skin colour but again it is not described as such. Locus *I* is described as a recessive inhibitor rather than as a dominant locus for skin colour. Locus *I* is dependent on locus R/R^{pw} for its full expression.

Nomenclature

To avoid further confusion between the nomenclature used in the different genetic models it is proposed to revoke the symbol R/R^{pw} and to indicate the locus involved in the biosynthesis of red anthocyanins with the symbol *D*. Firstly, because locus *D* was already proposed by Salaman (1910), long before the introduction of locus R/R^{pw}. Secondly, because the description of this locus as given by Dodds and Long (1955) is not correct. Pink tuber colour is not the phenotypic expression of the recessive R^{pw} allele, but that of the dominant *I* allele in the absence of a dominant *D* allele.

Four different names, *R*, *E*, *I* and *PSC* have been used for a locus on chromosome 10 involved in tuber skin colour. It is justified to revoke the symbol *PSC* (Gebhardt *et al.*, 1989). This symbol was only used because it was unknown whether the segregation for skin colour was due to segregation of a locus involved in anthocyanin production or a locus for tissue specific expression. Because as yet no evidence is presented in favour of two different

genetic loci, it is not possible to conclude whether the older names *R* and *E* or the more often used, but relatively new symbol *I* is to be preferred. Because genetic stocks used in this study were derived from diploid cultivated species, the symbol *I* was used in Fig. 1 to indicate the skin colour locus. The differences between the skin colour alleles tracing back to the clones 88-405-5 and 88-402-12 are indicated by the suffices I_{ep} and I_{co}.

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CHAPTER 5

MULTIPLE ALLELES FOR TUBER SHAPE IN DIPLOID POTATO DETECTED BY QUALITATIVE AND QUANTITATIVE GENETIC ANALYSIS USING RFLPs.

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Summary

Tuber shape in potato is commonly regarded as displaying continuous variation, yet at the diploid level phenotypes can be discerned visually, having round or long tubers. Inheritance of qualitative tuber shape can be explained by a single locus *Ro*, round being dominant to long. With RFLPs the *Ro* locus was mapped on chromosome 10. Tuber shape was also studied as a quantitative trait, using the length/width ratio as trait value. The estimated broad sense heritability was $h2$=0.80. The morphologically mapped *Ro* locus explained 75 % of the genetic variation, indicating the presence of a major QTL at the *Ro* locus and minor genetic factors. RFLP alleles linked with *Ro*-alleles were used to divided the progeny into four genotypic classes: $Ro^{♀}Ro^{♂}$: $Ro^{♀}ro$: $roRo^{♂}$: *roro* = 1:1:1:1. The recessive *ro* allele is identical by descent in both parents. The significantly different effects (p=0.0157) of the non-identical alleles $Ro^{♀}$ and $Ro^{♂}$ provided evidence for multiallelism at the *Ro* locus. Linkage mapping of the *Ro* locus was compared with QTL-mapping. Only those markers which are polymorphic in both parents allow accurate QTL-mapping when genetic factors segregate from both parents. This finding applies to QTL-mapping in all outbreeders without homozygous inbred strains.

Introduction

Since the introduction of RFLP markers many applications of this technique have proven to be very useful for the identification of genetic variability in practical breeding and scientific research. Initially attention has focused on the construction of genetic linkage maps. The convenient availability of saturated RFLP maps has greatly stimulated quantitative genetic investigations. Individual

loci of quantitative traits (QTLs) have been recognized and mapped on the genome (Paterson *et al.* 1988). Recently, RFLP markers have also been used to investigate inter- and intra-locus interactions of loci involved in quantitative traits. Fatokun *et al.* (1992) reported epistasis between QTLs: an inter-locus interaction. Stuber *et al.* (1992) detected QTLs playing a significant role in heterosis: an intra-locus interaction. Another example of an intra-locus interaction is the interaction between multiple alleles. Multiallelism is a common and frequently reported phenomenon for qualitative traits. The existence of multiple alleles for quantitative trait loci has only been considered in a theoretical model (Forkmann and Seyffert 1977); experimental evidence is not available.

The experimental model used in this paper is tuber shape in diploid potato. Cultivated potato is a highly heterozygous tetraploid outbreeder. The shape of the tuber is one of the most eye-catching traits of the potato crop. The tuber shape selected by breeders is determined by the preference of consumers and the processing industry. The consumer preference may vary with country. To minimize waste, varieties with long tubers are preferred for french fries, but varieties with round tubers are used for crisps. Although tuber shape is an easily selectable trait for breeders, the continuous variation complicates classification into Mendelian ratios. The range from round to oval or long suggests a polygenic inheritance. Studies on tuber shape carried out at the tetraploid level did not give any clear genetic model (DeJong and Burns 1993; DeMaine and Fleming 1991). Genetic studies are complicated by the heterozygosity, the high genetic load and the tetrasomic inheritance. However, at the diploid level, DeJong and Rowe (1972), Taylor (1978), Okwuagwu (1981) and Masson (1985) concluded that the inheritance of tuber shape is monogenic. A single dominant gene *Ro* was postulated by Masson (1985); round being dominant to long.

Our objectives in this research were: (1) to describe inheritance of tuber shape both in a qualitative and in a quantitative way, (2) to map the *Ro* locus on the potato genome, (3) to compare the results of linkage analysis using qualitative data with QTL-mapping, and (4) to elucidate the phenotypic effect of the different alleles at the *Ro* locus.

Materials and methods

Plant material

The experimental material was developed by crossing two diploid (2n=2x=24) potato clones. The female parent, clone US-W5337.3 (Hanneman and Peloquin 1967) was a hybrid between *S. phureja* PI225696.1 and the dihaploid US-W42 extracted from cv. Chippewa. This clone is widely used in many types of research. The male parent, clone 77.2102.37 (Jacobsen 1980), was a hybrid between VH^34211 (a *S. vernei* - *S. tuberosum* backcross) and the US-W5337.3 clone. Both parental clones have round tubers. Descendants derived from this cross are maintained as a population for mapping studies. Morphological observations on tuber shape were done when the population size was 102 clones. RFLP analysis was performed in a smaller randomly chosen subset. From fifty clones (virus free, with good tuber formation), three plants were grown in a screenhouse during the summer in 10 litre pots. The tubers were harvested when mature and bulked per clone.

Data collection

Qualitative data on tuber shape were obtained by dividing the clones of progeny US-W5337.3 x 77.2102.37 visually into two phenotypic classes: round and long. This classification was based not only on the visual perception of tuber shape, but also on tapering. Long tubers often taper at the apical and stolon ends (rose and heel), while round tubers may have a longitudinal axis which is shorter than the transversal axis.

Quantitative data were obtained by measuring the length/width ratio of the tubers. The length of a tuber is defined as the distance between the apex (rose) and the place of stolon attachment (heel). The width of the tuber is the length of the transversal axis perpendicular to the longitudinal axis. This method of measuring the transversal axis is very sensitive for irregular tuber shape and deviant types like 'kidney' and 'pebble'. Therefore, the width was measured twice, in such a way that the two directions were representative for the irregularity of the tuber. In most cases we took the largest transversal axis (if it could be found unambiguously), and the width perpendicular to the previous direction. The length/width ratio is a numerical measure describing the

phenotypic value of tuber shape, the width being the mean of the two measurements in both directions perpendicular to the longitudinal axis.

RFLP markers and linkage analysis

The progeny used in this experiment was also used to construct a genetic map of potato in our laboratories. This map is comprised of morphological, isozyme, and RFLP markers (Eck, van *et al.* 1993). Additional RFLP markers were obtained from Dr. S.D. Tanksley (Cornell, Ithaca, NY) to align the linkage groups with the published genetic map of potato (Tanksley *et al.* 1992). Linkage analysis was performed using JOINMAP (Stam 1993) and Linkage-1 (Suiter *et al.* 1983). The significance of pairwise linkage between loci is expressed in LOD scores, the logarithm of the Odds ratio of the likelihood of the data assuming that two loci are linked with a given recombination value over that assuming the two are not linked. A LOD value of three means that the chances are greater than thousand to one that the loci are linked for a given recombination estimate.

Statistical analysis and QTL mapping

The quantitative data on tuber shape were analyzed with the statistical computer programme SAS (SAS Institute Inc. 1990). The variation in $\underline{L/W}$ ratio between 'long' (*roro*) clones and between tubers within a 'long' clone is much larger than the variation between and within 'round' clones (*Ro.*). Therefore, log transformation was performed on the $\underline{L/W}$ ratio to produce a normally distributed error term.

With the procedure GLM (Generalized Linear Models) the log transformed data on the $\underline{L/W}$ ratio were analyzed using standard analysis of variance (ANOVA) methods. Variance was partitioned according to the experimental factors: (i) Phenotypic classes for the morphological types round = *Ro.* or long = *roro*), (j) Genotypic classes within morphological classes($Ro^{♀}Ro^{♂}$: $Ro^{♀}ro$: $roRo^{♂}$: *roro*), (k) Clones within genotypic classes and (l) Tubers within clones. This unbalanced nested design is represented by the mixed model:

$$\underline{\log(L/W)}_{ijkl} = \mu + Mo_i + Ge_{ij} + \underline{Cl}_{ijk} + \underline{Tu}_{ijkl}.$$

The terms Cl and Tu for clones and tubers are considered random. The other terms are considered fixed. The total variance explained by the model was partitioned into components of variance belonging to the terms of the model. Since the terms for morphological and genotypic classes (Mo_i and Ge_{ij}) represent fixed terms, the variation explained by these terms is not expressed as a variance component (σ^2) but is indicated by Q (SAS Institute Inc. 1990). The *F*-value was calculated using the Type I Mean Square (SAS Institute Inc. 1990) and the appropriate error term. (Type 1 sums of squares are the incremental improvement in error sum of squares as each effect is added to the nested model. They can be computed by fitting the model in steps and recording the difference in error sum of squares at each step.)

Results

The inheritance and mapping of tuber shape as a qualitative trait

The differences in tuber shape between descendants of the cross US-W5337.3 x 77.2102.37 allowed a preliminary visual separation into two morphological classes. Nevertheless, considerable and typical differences could be observed between clones ordered within the same morphological group. Based on this preliminary classification, a segregation in tuber shape of 68:29 = 'round' : 'long' was observed. As both parents have 'round' tubers this segregation fits a 3:1 monogenic ratio ($\chi^2_{(3:1)} = 1.24$; $p=0.26$); round being dominant to long. The genetic model proposed for this cross (*Roro* x *Roro* → *Ro.* + *roro*) was confirmed by intercrossing several 'round' and 'long' descendants and by backcrossing with the parents. (data not shown). The gene symbol *Ro* is used according to Masson (1985), who also reported a single dominant gene for tuber shape, as did Taylor (1978), Okwuagwu (1981) and DeJong and Burns (1993).

Cosegregation between RFLP markers and tuber shape (visually classified) was observed for several markers located on chromosome 10 (Fig.1, Table 3). The RFLP markers Ac38-46, TG303, ST06, TG63, Ac15-7 and ST15a detected polymorphism between alleles segregating from the female parent as well as between alleles segregating from the male parent. Markers TAc20 and TG43

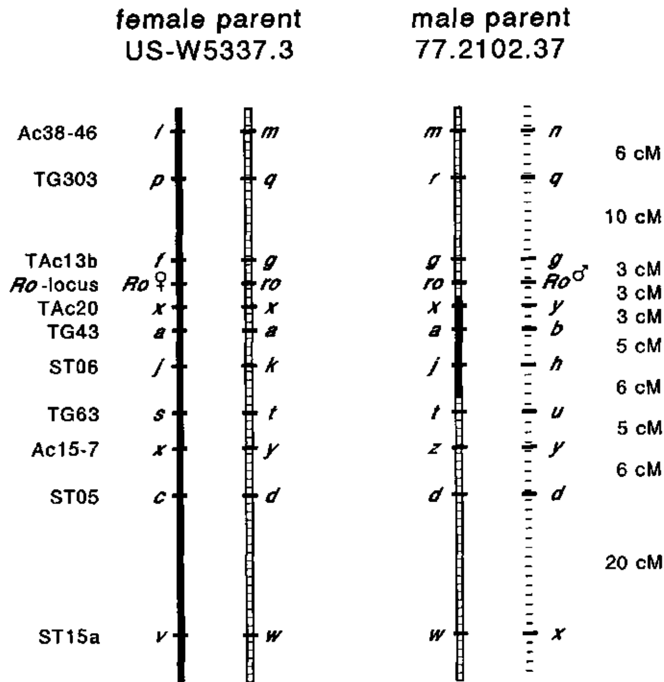

Fig. 1. Map of potato chromosome *10* showing the position of the alleles on the chromatids of both partens. Different RFLP alleles are indicated by different letters.

were polymorphic in the male parent, but were homozygous in the female parent. Markers TAc13b and ST05 showed polymorphism only for the female alleles. For the construction of the map a strategy was chosen not to evaluate the data on the genotypes of the descendants of this cross, but to evaluate the data on the genotypes of the male and female gametes which produced the descendants. The resulting linkage maps represent the independent maps of the male and female parent. (The separate linkage maps of the male and female parent are not shown.) The map based on the female meiosis in US-W5337.3 revealed a much higher amount of crossing-over relative to the map based on the male parent 77.2101.37. For example, linkage of RFLP markers Ac38-46 and TG303 with the *Ro* locus is very close in the male parent, while linkage

Table 1. Overview of estimated amounts of explained variation per term per model.

Term	Estimated component	Estimate	
		L/W[a]	log(L/W)[b]
Model: $\underline{\log(L/W)}_l = \mu + \underline{Tu}_l$			
Total	σ^2_{total}	0.12536	0.10404
Model: $\underline{\log(L/W)}_{kl} = \mu + \underline{Cl}_{ijk} + \underline{Tu}_{ijkl}$			
Clones	$\sigma^2_{genet.}$	0.09864	0.08337
Environment	$\sigma^2_{environment}$	0.02672	0.02068
Model: $\underline{\log(L/W)}_{ikl} = \mu + Mo_i + \underline{Cl}_{ik} + \underline{Tu}_{ikl}$			
Morphological classes	$Q_{gen_major(3:1)}$[c]	0.07883	0.06225
Clones (within morphol.classes)	$\sigma^2_{gen_minor(3:1)}$	0.01981	0.02112
Environment	$\sigma^2_{environment}$	0.02672	0.02068
Model: $\underline{\log(L/W)}_{jkl} = \mu + Ge_j + \underline{Cl}_{jk} + \underline{Tu}_{jkl}$			
Genotypic classes	$Q_{gen_major(1:1:1:1)}$[c]	± 0.07954	± 0.06643
Clones (within genotypic classes)	$\sigma^2_{gen_minor(1:1:1:1)}$	0.01910	0.01694
Environment	$\sigma^2_{environment}$	0.02987	0.02121

[a] Untransformed data [b] log transformed data [c] not a variance component

was not significant in the female parent. With the computer package JOINMAP (Stam 1993) the female and male maps were integrated into the map presented in Figure 1. This joined linkage map of chromosome 10 shows the position of the *Ro* locus between RFLP loci. The significance of the pairwise linkage of the tuber shape locus with the RFLP loci is expressed in LOD scores, which are included in table 3.

Quantitative genetic analysis of tuber shape

From fifty clones the tubers were harvested and measured. The number of tubers per clone ranged from 3 to 42, with an average of 19.8 ± 9.2 tubers per clone. To calculate the broad sense heritability (h^2) of tuber shape, the genetic variance between clones ($\sigma^2_{gen.}$) and the environmental variance between tubers within clones ($\sigma^2_{env.}$) were estimated according to the model

$$\underline{\log(L/W)}_{kl} = \mu + \underline{Cl}_k + \underline{Tu}_{kl},$$

assuming the clone effect was random. The calculated heritability h^2 (see Table 1) of 0.80 indicated that tuber shape is hardly affected by environmental factors.

Nevertheless, tuber shape is not completely determined by the *Ro* locus and environmental factors, because differences can be observed between clones belonging to the same morphological class (eg. 'round' and 'long'). These differences have to be associated with minor genetic factors. To calculate the amount of the genetic variance explained by minor genetic factors ($\sigma^2_{gen_min.}$) and the *Ro* locus ($\sigma^2_{gen_maj.}$) the following model was used:

$$\underline{\log(L/W)}_{ikl} = \mu + Mo_i + \underline{Cl}_{ik} + \underline{Tu}_{ikl}.$$

Table 1 shows the estimates of the variance components. From the total genetic variation estimated by this model 75 % was due to the Mo_i term representing the qualitative classification and 25 % was due to genetic variation between clones within the same morphological class.

Establishing the underlying genotypes of the phenotype 'round'

The pedigree of the material shows that the male parent 77.2102.37 of this cross is a descendant of the female parent US-W5337.3. As a consequence of the relatedness of the parents, this cross can be regarded as a backcross. The segregation of RFLPs which are polymorphic in both parents can be described by the general genetic model **ab x bc → ¼ ab + ¼ ac + ¼ bb + ¼ bc.** At every locus both parents have at least one allele in common (allele **b**). This common allele is the allele which was transmitted from clone US-W5337.3 to clone 77.2102.37. Fig. 1 shows the distribution of alleles at the RFLP loci flanking the *Ro* locus on the chromatids. Coupling and repulsion phases of alleles within a parent were deduced from the cosegregation of alleles in the offspring. The recessive allele *ro* in clone 77.2102.37 is identical by descent to the recessive allele *ro* in clone US-W5337.3, while the dominant alleles are indicated with the symbols $Ro^{♀}$ and $Ro^{♂}$ to distinguish between their origin. Fig. 1 also shows that two crossing-over events occurred in clone US-W5337.3 when the gamete was formed which contributed to clone 77.2102.37. The configuration of the alleles of markers TG303 and Ac15-7 in the male parent is unexpected, because the alleles from these loci that are transmitted from the

parent US-W5337.3 are in repulsion phase with alleles at other loci.
For the clones descending from the cross US-W5337.3 x 77.2102.37 it is possible to deduce the genotype at the *Ro* locus using the linkage with unique alleles at RFLP loci closest flanking the *Ro* locus. The initial 3:1 segregation into two morphological classes ('round' : 'long' = *Ro.:roro* = 3:1) can be separated into a 1:1:1:1 segregation of the four genotypic classes $Ro^{♀}Ro^{♂}$: $Ro^{♀}ro$: $roRo^{♂}$: *roro*. The prediction of the phenotypes based on this method was in agreement with the initially used visual classification.

For the fifty clones used in this experiment their underlying *Ro* genotypes were determined. This resulted in eight homozygous dominant clones $Ro^{♀}Ro^{♂}$, nine heterozygous clones $Ro^{♀}ro$, ten heterozygous clones $roRo^{♂}$ and eleven homozygous recessive clones *roro*. For the twelve remaining clones, the genotypes could not be determined unambiguously. Eight clones could not be identified because of crossing-over between flanking markers, the cross-over events occurring in five cases in the female meiosis and in three cases on the male side. Missing RFLP data prevented the determination of the *Ro* genotype of four clones.
To estimate again the amount of the genetic variation explained by the major gene at the *Ro* locus, but now using the 1:1:1:1 classification instead of the 3:1 classification, the following model was used:

$$\underline{\log(L/W)}_{jkl} = \mu + Ge_j + Cl_{jk} + Tu_{jkl}.$$

From the total genetic variance estimated by this model 80 % was due to the Ge_i term representing the 1:1:1:1 classification (Table 1). The higher value of the variation explained by this classification compared with the value of 75 % for the 3:1 segregation indicates the presence of genetic variation within the 'round' class and a smaller influence of minor loci.

Detection of multi allelism

Genetic variance due to the major QTL for tuber shape at the *Ro* locus between the three genotypic classes $Ro^{♀}Ro^{♂}$, $Ro^{♀}ro$ and $roRo^{♂}$ was expected. Typical differences were already observed between clones belonging to the same morphological class. The mean length/width ratios of the four genotypic classes,

Table 2. Mean L/W-ratios for tubershape

Class	Mean L/W-Class ratios ± SE	LSD (α=0.05)
All tubers	0.9782 ± 0.0112	
Morphological class 'round'	0.8133 ± 0.0270	
Morphological class 'long'	1.5112 ± 0.0517	
Genotype $Ro^{♀}/Ro^{♂}$	0.8057 ± 0.0665	ab
Genotype $Ro^{♀}/ro$	0.7530 ± 0.0472	a
Genotype $ro/Ro^{♂}$	0.8898 ± 0.0499	b
Genotype ro/ro	1.5112 ± 0.0517	c

listed in Table 2, show that heterozygous clones which are 'round' due to the presence of the $Ro^{♀}$ allele have a much lower length/width ratio than 'round' clones having the $Ro^{♂}$ allele. The difference in tuber shape between the four genotypic classes was investigated using mixed model analysis of variance and was significant (F = 44.72, p < 0.0001). The high F-value was mainly due to the difference between round and long clones. With the subset including only data from the two heterozygous genotypic classes, $Ro^{♀}ro$ and $roRo^{♂}$, the F-value for the mixed model analysis of variance was still 12.24 (p = 0.0028). The significance of the difference between the heterozygous genotypes analyzed by a protected least significant difference test between the Least Significant Means of the classes $Ro^{♀}ro$ and $roRo^{♂}$ was t = -2.5437 (p = 0.0157). With the subset including only data from the two heterozygous genotypic classes $Ro^{♀}ro$ and $roRo^{♂}$ the significance improved slightly: t = -3.0803 (p = 0.0068). The homozygous dominant genotype $Ro^{♀}Ro^{♂}$ gave an intermediate round tuber shape and did not differ significantly from both the heterozygous genotypes. The finding of a significant difference in the mean tuber shape varying with the parental origin of the dominant *Ro* allele provides evidence for the presence of three alleles at the *Ro* locus.

QTL-mapping of the Ro locus

The position already given of the *Ro* locus on chromosome 10 of the potato genome was determined by a qualitative approach. A QTL-mapping procedure is expected to give a similar result. QTL-mapping was performed by analysis

of variance using log(L/W) value as the dependent variable and the genotypic classes of the RFLP loci as the treatment source of variation. The results of this analysis presented in table 3 reveal that proximal RFLP markers ST06, TG63 and Ac15-7 are associated with tuber shape, whereas the distal markers Ac38-46, TG303 and ST15 lack significant association. Markers TAc20 and TG43 failed to show a highly significant association with tuber shape represented by the log(L/W) value. This lack of significance is caused by the type of polymorphism displayed by these RFLP markers. Five types of polymorphisms can be found at RFLP loci in the offspring of outbreeders: (♀1:1), (♂1:1), (3:1), (1:2:1) and (1:1:1:1). The first and second type of polymorphism is found at loci where either the female or male parent is segregating (heterozygous), while the other parent is homozygous at that locus. The third and fourth type of polymorphism is found at loci where both parents, with the same genotype, are heterozygous for a dominant (e.g. RAPD markers) and codominant marker, respectively. When both parents have different heterozygous genotypes the fifth type is found (multiple alleles, **ab x bc**).
When clones are classified according to a polymorphic marker of the second type (♂1:1), the clones are classified with respect to the paternal alleles, but lumped together with respect to the maternal alleles ($Ro^{♀}Ro^{♂}$ + $roRo^{♂}$ versus $Ro^{♀}ro$ + *roro*). The contrast in tuber shape (Length/Width) between these groups is expected to be small, because the genotypic class $Ro^{♀}ro$, with the smallest L/W value, and the genotypic class *roro*, with the highest L/W value (shown in table 2), are in this situation lumped together into the same class. Figure 2 illustrates that allele *a* of marker TG43 indicates both genotypic classes $Ro^{♀}ro$ and *roro*. The distribution of genotypic classes $Ro^{♀}Ro^{♂}$ and $roRo^{♂}$ indicated by allele *b* coincides with the minimum in the bimodal distribution indicated by allele *a*. This explains why markers TAc20 and TG43 cannot detect significant association with tuber shape despite their close linkage. On the other hand, when the clones are lumped together in groups according to the maternal RFLP alleles and the paternal alleles are ignored, the contrast between the groups is expected to be much larger. The highly significant association between RFLP marker TAc13b and tuber shape can be explained by this lumping together of clones with different paternal alleles and classifying

Table 3. LOD scores of genetic linkage between RFLP markers along chromosome 10 and the qualitative *Ro* locus, and significance of association between RFLP markers and log(L/W)-ratio.

	Linkage with *Ro* locus			ANOVA (QTL-mapping)					
	♀ map	joined map	♂ map	Only female alleles (1:1)		Complete classification (1:1:1:1)		Only male alleles (1:1)	
RFLP marker	LOD	LOD	LOD	*F*	*P*	*F*	*P*	*F*	*P*
Ac38-46	0.09	1.16	1.56	0.120	0.7314	1.058	0.3821	1.029	0.3182
TG303	0.15	3.42	5.50	4.119	0.0488	2.158	0.1082	3.397	0.0724
TAc13b	1.67	1.67		23.912	<0.0001				
TAc20		3.16	3.16					3.821	0.0308
TG43		4.17	4.17					2.701	0.1083
ST06	1.19	2.08	0.92	13.782	0.0008	5.891	0.0030	0.775	0.3857
TG63	0.05	1.79	3.92	2.155	0.1519	6.189	0.0021	5.871	0.0213
Ac15-7	0.59	1.99	2.04	6.760	0.0132	3.905	0.0164	0.370	0.5468
ST05	0.11	0.11		6.129	0.0194				
ST15		3.16	3.16	0.370	0.547	0.6519	0.5279	1.265	0.2689

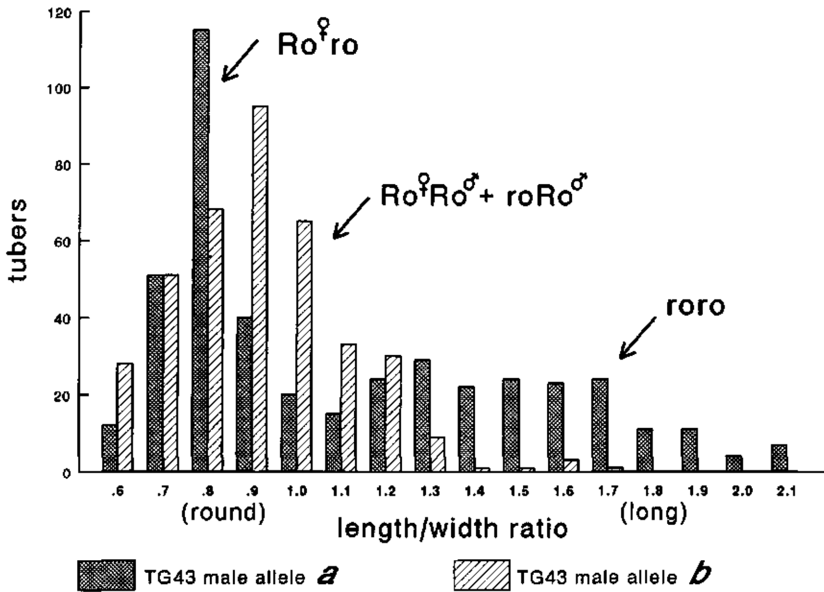

Figure 2: Distribution of tuber shape lenght/width ratio within the TG43 offspring class *aa* and class *ab*.

according to maternal alleles. This is also illustrated by the still significant *F*-value for marker ST05 although it is located at 30 cM map distance from *Ro*.

To verify the point that different types of polymorphism have different powers to detect a QTL, the 1:1:1:1 segregating RFLP loci were converted into ♀1:1 and ♂1:1 segregating loci. Table 3 shows the resulting pattern of *F*-values. These *F*-values which display the effect of lumping are in good accordance with the different effect of the $Ro^{♀}$ and $Ro^{♂}$ allele reported in Table 2. The higher recombination frequency in the female meiosis is disturbing the effect for the markers Ac38-46 and TG303 which are not linked to *Ro* in a map based on the female meiosis alone.

Discussion

The inheritance and qualitative mapping of tuber shape

The visual classification of tuber shape is not unambiguous, but it was demonstrated to be a suitable approach not only in this study, but also in previous studies using diploid material (Taylor 1978, Okwuagwu 1981, Masson 1985, DeJong and Burns 1993). In this study the visual classification appeared to be completely in agreement with the predictions based on the flanking RFLP markers. The localization of the *Ro* locus on potato chromosome 10 described in this paper should help in revising the classical genetics of the potato. Many contradictory genetic models were postulated to explain the inheritance of tuber shape (DeJong and Burns 1993). A genetic model with a major QTL with multiple alleles will explain the data of many of the earlier papers.

Localization of the *Ro* locus on chromosome 10 is in agreement with the earlier observation of linkage between tuber shape and skin colour (DeJong and Rowe 1972, Masson 1985, DeJong and Burns 1993), for skin colour is now also localized on chromosome 10 (Gebhardt *et al.* 1989). Probably, the locus *Ro* is the only major QTL for tuber shape in Group Tuberosum germplasm, since the linkage between tuber shape and skin colour was reported several times. In several other crosses which were studied in our lab, tuber shape always mapped on chromosome 10. This justifies the use of the symbol *Ro* after Masson (1985). Moreover, clone US-W5337.3 was also used by Masson.

Detection of multiple alleles

Multiple alleles were detected at the *Ro* locus after making a distinction among the descendants between the four possible genotypes which can be obtained with three different alleles ($Ro^{♀}Ro^{♂}$, $Ro^{♀}ro$, $roRo^{♂}$ and *roro*). The correct genotype of each clone of the progeny was uncovered by using the information of the flanking RFLP alleles. The disadvantage of this method is the absence of the truly homozygous genotypes $Ro^{♀}Ro^{♀}$ and $Ro^{♂}Ro^{♂}$. However, it is not possible to design one single cross from which $Ro^{♀}Ro^{♀}$, $Ro^{♂}Ro^{♂}$ and *roro* can segregate, and therefore, these genotypes cannot be selected within (on average) the same genetic background. Without RFLP techniques there is an alternative method

to compare the effect of multiple alleles in a common genetic background, i.e. the development of near isogenic lines by repeated backcrossing. Unfortunately, this technique is limited to species which can be selfed indefinitely. Furthermore, the diploid potato is self-incompatible, and only one generation per year can be obtained. A common genetic background is required to study the effects of a single locus. A minimum number of eight clones per (marker) genotypic class would be adequate to cancel the effects of other chromosomal regions on tuber shape, outside chromosome 10.

Two distinct dominant alleles and one recessive allele could be distinguished in this cross; the presence of other alleles in potato germplasm is very likely, especially in cultivated species where other types of tuber shape can be observed (See pictures in DeJong and Burns 1993). Further research may reveal to what extent this variation is due to other minor loci or to other alleles of the *Ro* locus. Studies on tuber shape at the tetraploid level have never resulted in a clarification of the inheritance of this trait (DeMaine and Fleming 1991, DeJong and Burns 1993). An explanation as to why these investigations have been so difficult may be the large amount of intra-locus interaction between the four possibly different alleles in the tetraploid genome.

Although experimental evidence for the presence of multiple alleles for a QTL is only now being reported, this study in potato can be compared with a case reported in maize. Beavis *et al.* (1991) showed associations between the chromosomal localization of QTLs for plant height and qualitative genetic loci like the GA dwarf locus, d3, on chromosome 9. Their results provide circumstantial evidence in support of Robertson's hypothesis on the relationship of qualitative mutants to quantitative traits. Robertson (1985) proposed that major mutants are actually null or near-null alleles at a QTL. According to this approach the recessive *ro*-allele for tuber shape can be regarded as the null or near-null allele which is recognized qualitatively. The variation among dominant *Ro*-alleles is of a quantitative nature.

The view that the (most) recessive allele can be regarded as a null or near-null allele is in accordance with the model suggested by Forkmann and Seyffert (1977) to describe the quantitative effects at a multiallelic locus. In the

parameterization of Forkmann and Seyffert the most recessive homozygote is the reference point and all contributions of the other alleles are unidirectional and positive, whereas the interactions between alleles are unidirectional and negative. This parameterization may, to some extent, reflect the biological and/or biochemical basis of gene action. However, its usefulness in terms of operational and observable parameters on the population level, which are necessarily allele frequency dependent, is questionable.

When more metric traits are resolved into Mendelian factors, in experimental designs which use heterozygous parents, statements can then be made concerning the importance of multiple alleles relative to multiple loci in explaining quantitative genetic variation. In this mapping population multiple alleles were detected at more than one third of the RFLP loci. This abundance of multiple alleles at the DNA sequence level may be indicative for a resembling profusion of multiallelism for quantitative trait loci.

QTL-mapping of the Ro locus

By carrying out linkage analysis of tuber shape as a qualitative trait prior to the QTL analysis we were able to demonstrate the possible weakness of a QTL analysis in a heterozygous outbreeding crop. The qualitative analysis resulted in location of the *Ro* locus on the map and also revealed the presence of multiple alleles. Had we started with the QTL analysis by treating tuber shape as a true quantitative character we would not have been able to map the major QTL at the *Ro* locus properly. Most QTL mapping procedures are designed for autogamous species, in many cases assuming a typical F2 or BC type of segregation. Standard QTL mapping software cannot properly deal with multiallelism resulting in a 1:1:1:1 ratio for markers. One way to get around this is to carry out two analyses, one for the paternally segregating alleles (disregarding the maternal segregation) and one for the maternally segregating alleles (disregarding the paternal segregation). In doing so we demonstrated that this grouping of alleles may lead to false conclusions: in our case the effect of the paternally segregating alleles is almost completely masked by the joint effect of the maternal alleles. Had all of the markers been segregating in a 1:1 ratio,

either from the paternal or the maternal side, only the maternal segregation would have indicated a QTL for tuber shape. Moreover, had we not known (from the pedigree and the qualitative analysis) the heterozygous state at the *Ro* locus in the male parent, we would not have detected it by a QTL analysis based on 1:1 ratio's only.

Where dominant markers are used, such as RAPDs, to analyse the offspring in an outbreeding crop, these markers will segregate in a 1:1 ratio either from the male or female side (a dominant marker which is heterozygous in both parents is not likely to be used, because it detects no polymorphism between the parents in the first place). Our present analysis clearly demonstrates the limitations of a straightforward QTL analysis in such a situation; a QTL effect may go unnoticed due to heterozygosity of the other parent. Therefore we recommend performing QTL-analysis with markers which detect different polymorphisms in both parents (1:1:1:1) when quantitative genetic factors are also segregating from both parents.

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Chapter 6

General discussion and concluding remarks

Differences between potato and other crop species

Molecular markers have been used for the construction of genetic linkage maps for several agriculturally important species including barley (Heun *et al.*, 1991), *Brassica oleracea* (Slocum *et al.*, 1990), lettuce (Landry *et al.*, 1987), maize (Helentjaris *et al.*, 1987), potato (Bonierbale *et al.*, 1988; Gebhardt *et al.* 1989), rice (McCouch *et al.*, 1988), soybean (Keim *et al.*, 1990), tomato (Bernatzky and Tanksley, 1986) and *Triticum tauschii* (Gill *et al.*, 1991). These maps have been used for the localisation of genes for morphological and agronomically important traits, and for the molecular isolation of resistance genes (Martin *et al.*, 1993; Jones *et al.*, 1994). This thesis describes the construction of a genetic map of the potato genome with morphological, biochemical and molecular markers using a cross between diploid heterozygous *Solanum tuberosum* genotypes. This cross and the resulting structure of the offspring population has shown to be useful in various ways. In the first part of this chapter the exceptional properties of potato genetics will be evaluated. Firstly, potato is highly heterozygous. Therefore, it was possible to construct a genetic map of potato integrating molecular, biochemical and morphological markers. Within the same mapping population morphological traits segregated and sufficient polymorphisms for isozyme loci as well as for RFLP loci were found. This abundance of molecular polymorphisms within *S. tuberosum* must be due to its autotetraploid nature and its mode of sexual and vegetative reproduction. The situation in potato is clearly different from the situation in for example tomato. Here, progenies from interspecific hybridisations are required to obtain enough molecular polymorphisms to allow the construction of RFLP maps. However, these interspecific molecular maps and the intraspecific classical maps of tomato are difficult to integrate (Weide *et al.*, 1994) because of the limited amount of DNA polymorphisms found within *L. esculentum* germplasm (Van der Beek *et al.*, 1992).

Secondly, as potato is a vegetatively propagated crop, the parental and offspring

plants involved in this study could be maintained and propagated by means of tubers. Therefore, field trials can be designed in four-hill plots in three replications to study agronomically important traits (Kreike *et al.* 1993, 1994; Van Eck, unpublished data). Replicated phenotypic assessment of a trait of an individual genotype allows more accurate estimation of the genotypic value for that trait and the total variance can be dissected into the genotypic and environmental variance to estimate the heritability of the trait under investigation (chapter 5, Van Eck *et al.*, 1994). Maintenance and propagation of mapping populations of sexually reproduced annual crops is usually troublesome. For some crops, the genetic material can be used for one generation only. To circumvent such problems methods have been designed to produce an 'immortalised' mapping population. Examples are recombinant inbred lines (RILs) (Burr and Burr, 1991), or bulked F3 seed lots (Gardiner *et al.*, 1993), which have been successfully applied in species that can be selfed. Thirdly, in potato, features occur which are characteristic to those of obligatory outbreeders, such as a high degree of heterozygosity, inbreeding depression, and mechanisms preventing selfing. In most diploid species gametophytic self-incompatibility precludes selfing or inbreeding (Cipar *et al.*, 1964). In a few diploid *Solanum* species and in all polyploids (except *S. tuquerrense*) self-compatibility is the rule, but because of sterility or severe inbreeding depression the production of homozygous inbred lines remains troublesome (De Jong, 1972). Heterozygosity of the parents has important implications for the genetic structure of the mapping population and requires different methods for genetic analysis, map construction (chapter 2), and QTL-mapping (chapter 5, Van Eck *et al.*, 1994). The following consequences of heterozygosity of the parents are relevant for RFLP analysis: **(1)** The F_1 and BC_1 of heterozygous parents is a segregating generation, whereas the F_1 generation of homozygous inbred lines is uniform and segregation is expected not before the F_2 or BC_1 generations. **(2)** Heterozygous parents will result into an F_1 or BC_1 population where different marker loci may segregate in a mixture of up to five different types of single locus segregations. At loci where both parents are heterozygous (*ab* x *ab*; *A.* x *A.*), the 1:2:1 or 3:1 ratio is expected in case of co-dominance or dominance respectively, and a 1:1:1:1 ratio is frequently observed at an RFLP locus with

multiple alleles (*ab* x *bc*). However, if one parent is homozygous (*aa* x *ab*; *ab* x *bb*) the resulting ratio is 1:1♀ or 1:1♂, depending on which parent is segregating. Linkage analysis, given such a mixture of ratios, demands an appropriate computer package such as Linkage-1 (Suiter, 1983). After adaptation of the data input it is possible to use JOINMAP (Stam, 1993). This adaptation involves the splitting of the data set having two alleles per locus into two separate datasets with only the single alleles which descended from either the female or the male parent. This type of mapping population is presently indicated as a 'double pseudo-testcross' (Hemmet *et al.*, 1994, Grattapaglia and Sederoff, 1994). Currently, JOINMAP is being revised to make this computer package suitable for linkage analysis and for QTL-mapping in outbreeding species (Stam, pers. comm.). **(3)** The number of alleles at segregating loci can range from two up to four in the F_1 offspring from heterozygous parents, or three in case of the BC_1, whereas in the F_1 from inbred lines invariably two alleles are found at all loci where the parental inbreds carry different alleles. **(4)** In the offspring of inbred parents it is known in advance that at two adjacent loci alleles from the same parent are in linkage phase. In the F_1 offspring from non-inbred parents linkage configurations are unpredictable. A special advantage of a BC_1 from non-inbreds is the presence of an allele which is shared by both parents. This common allel is helpful in several aspects (see chapter 2), such as the discrimination between alleles from the same locus or from duplicate loci, when an RFLP probe detects multiple bands. **(5)** Two genetic maps can be constructed from one mapping population derived from non-inbred parents. The first map represents the recombination events in the female meiosis and the second map reflects the male meiotic recombinations. This allows the detection of parental differences in frequency of crossing-over in female and male meiosis (Hemmet *et al.*, 1994). **(6)** When the size of the mapping population is N plants, the F_1 or BC_1 from a cross between non-inbred parents is more informative than the BC_1 or F_2 from inbred parents, because in the mapping population of non-inbreds those N plants trace back to 2N gametes which are informative with respect to recombination in the female and the male meiosis. The 2N gametes resulting in an F_2 from inbred parents are less informative because in the 1:2:1 segregation the parental origin of the alleles cannot be

inferred. (7) Merging of the female and male map is only possible when at least two loci per linkage group are polymorphic in both parents. These so called 'allelic bridges' should preferably be well separated and located at the distal ends of a linkage group, but even then erroneous ordering of loci may occur. (8) Quantitative genetic variation may segregate from both parents. As explained in chapter 5, multiallelic marker loci, segregating in a 1:1:1:1 ratio, are required for the proper detection and estimation of the location of the QTL and the magnitude of QTL effects.

Up to now there are only a few publications where the above mentioned differences between mapping populations derived from inbred or non-inbred parents are fully acknowledged (Echt *et al.*, 1994). Nevertheless there are many genome mapping projects in progress where allogamous species are involved that cannot be selfed indefinitely. The work described in this thesis may therefore serve as a model for most of the forest and fruit tree species; for dioecious species such as asparagus, hop, date-palm, as well as for the outbreeding crops such as carrot, onion, leek, tulip, narcissus, sugar-cane, cassava and many grasses. RFLP studies on the outbreeding crops with homozygous inbred strains such as maize, turnip, cabbage (*Brassica spp.*) or beets (*Beta spp.*) are similar to those on autogamous crops.

RFLPs and the inheritance of morphological traits

Relative to the importance of the potato as a crop species, the genetics of potato is poorly developed. Only two out of the few reported morphological markers had a known map location: the *Y* locus, involved in yellow flesh colour on potato chromosome *3* (Bonierbale *et al.*, 1988), and the *PSC* locus involved in purple skin colour on chromosome *10* (Gebhardt *et al.* 1989; See table 1 in chapter 1). Besides, the *S* locus involved in gametophytic self-incompatibility on chromosome *1* was mapped before using a cDNA clone (Gebhardt *et al.*, 1991).

An alternative approach to the mapping of morphological traits is the combination of half-tetrad analysis (Mendiburu and Peloquin, 1979) determining the distance of the locus relative to the centromere, and trisomic analysis associating the trait loci with trisomic chromosomes. Trisomic analysis was

applied to four recessive mutants. Topiary (*tp* locus) is associated with chromosome *3* and another three mutants (locus *a* for albinism; locus *v* for virescence; locus *Ym* for yellow margin) with chromosome *12* (references: see Wagenvoort *et al.*, 1988). The localisation of *Ym* was not confirmed by our results, described in chapter 2, where *Ym* was mapped on chromosome *5*. Gene-centromere distances were estimated for several morphological trait loci. Mendiburu and Peloquin (1979) mapped locus *P* involved in purple anthocyanins at 13.0 map units (mu) from the centromere. The distance to the centromere of the dominant locus *Y* involved in yellow flesh colour was determined by Mok *et al.* (1976): 13 mu, Singsit *et al.* (1989): 15.6 mu, Stelly and Peloquin (1986): 18.5 mu, and Douches and Quiros (1987): 16.8 mu. The gene-centromere distance of the *Ro* locus involved in tuber shape was determined at 12.2 mu by Masson (1985). The distance between the centromere and the *Ym* locus is estimated 33.3 mu (Jongedijk *et al.*, 1991) and 31.7 mu (Wagenvoort, 1993).

The research described in this thesis has added several other classical markers to the map. The eleven classical genetic traits localized on this map are: The *S* locus, involved in gametophytic self-incompatibility on chromosome *1*; The *Me* and *D* locus, involved in metribuzin sensitivity and the production of red anthocyanins, respectively, mapped on chromosome *2*. On chromosome *3* the yellow flesh colour locus *Y* was mapped. Earliness and yellow margin, at the loci *El* and *Ym*, mapped on chromosome *5*. Desynapsis (*Ds*) mapped on chromosome *8*. Three loci could be placed on chromosome *10*: tuber shape (*Ro* locus), pigmented flowers (*F* locus) and crumpled (*Cr*). Locus *P*, involved in the production of purple anthocyanins was assigned to chromosome *11*. The mapping of the loci *F* and *I* on chromosome *10*, implies the localisation of the earlier reported linkage group comprising the loci *B-I-F* (seed spot - skin colour - flower colour; Dodds and Long, 1956), *Pf* (Pigmented flesh; De Jong, 1987), *Pw* and *Ul* (Pigmented whorl and Under leaf pigmentation; Kessel and Rowe, 1974) in a cluster on chromosome *10*.

Since many of the morphological traits described in potato have now been localised on the potato genome, further research is required to elucidate the hereditary basis of more complex morphological traits, such as eye depth (deep

- shallow), shape of light sprouts (spherical - oval - conical - cylindrical), habit of the tip of lightsprout (closed - open), pubescence of the stem and sprouts, number and shape of root tips on light sprouts, protrusion of lenticells on light sprouts, and leaf shape. These morphological characters are employed by the UPOV for the description of potato cultivars and for testing of distinctness (UPOV, 1986). The traits mentioned are expected to have stable phenotypes hardly affected by environmental conditions, which facilitates genetic analysis. Several aspects which hamper genetic studies of morphological traits, are described in this thesis, and it was clearly shown that RFLP markers facilitated this type of research. These various aspects are summarised below.

(1) Skewed segregations

Skewed segregations are frequently encountered in potato. The most predominant cause of skewed segregations is a high genetic load, since many deleterious alleles accumulated in the tetraploid genome are exposed in primary diploids. In chapter 2 it was shown that 35 RFLP loci (16 %), clustered in regions on chromosomes *1*, *2*, *4*, *5*, *8* and *11* did not fit Mendelian ratios. Consequently, the inheritance of purple anthocyanins, described in chapter 3, did not fit a single gene model. A skewed segregation of RFLP markers closely linked to locus *P* with a similar skewed segregation confirmed that purple was under control of one single locus *P* on chromosome *11*. Skewed segregations were also described for *R* loci involved in late blight resistance (El-Kharbothly *et al.*, 1994) and for the *Gpa* locus on chromosome 5 involved in nematode resistance (Kreike *et al.*, 1994), where cosegregation with RFLP marker loci confirmed the genetic model and allowed their localisation.

(2) Epistasis

The complex inheritance of e.g. flower colour, which is based on the complementary action of loci involved in anthocyanin production and expression, could be unravelled with backcrosses to tester clones. The genetic model proposed was confirmed and the relevant loci were unequivocally mapped with the aid of RFLP analysis (chapter 3). Many other examples of non-monogenic qualitative traits waiting for their dissection into individual genetic loci by RFLP analysis are available, such as tuber skin colour, seed spot, and russet tuber skin. The russet phenotype is determined by three

complementary loci (DeJong, 1981) of unknown position.

(3) Multiple alleles

In potato, multiple alleles are described for qualitative traits, for instance the orange flesh allele *Or* at the *Y* locus (Brown *et al.*, 1993) and the complex *B* locus involved in pigmention of the floral abscission layer, tuber eyebrows, embryo spot, and nodal bands (Dodds, 1955). In this thesis multiple alleles were reported for tuber shape (chapter 5). At the diploid level the presence of long, oval, round and flat tuber types due to multiple alleles obscured the classification into Mendelian ratios. However, by using RFLPs the quantitative and qualitative inheritance of tuber shape could be investigated profoundly. At the tetraploid level multiple alleles may cause a continuous distribution of length/width ratios of tubers, impeding qualitative genetic analysis. Similarly, multiple alleles may be involved in other morphological traits like eye depth or leaf shape, and fade away discrete Mendelian classes.

(4) Allelism

Allelism between the loci *I*, *E*, *R* and *PSC* was conferred from their map position. Although evidence for allelism should be based on genetic complementation, a map based approach is very useful to genetic studies in potato. The development of well-defined genetic stocks of potato genotypes is time-consuming and reference stocks are not readily available. Allelism can be excluded when loci are on different map positions. Especially for the identification and characterization of loci involved in resistance, information on the map position is useful. On the one hand to distinguish between different sources of resistance genes, and on the other hand to investigate the putative clustering of resistance genes.

RFLP mapping of quantitative traits

The two most significant results of the research on quantitative traits as described in chapter 5 are **(1)** the detection of multiple alleles at a quantitative trait locus and **(2)** the recognition that quantitative genetic variation in a progeny from non-inbred parents should be studied with codominant multiallelic marker loci.

Detection of multiple alleles

The existence of multiple alleles has been described for many qualitative traits in plants (hybrid necrosis in wheat (Hermsen, 1963), chevron patterns on white clover, self-incompatibility), animals (coat colour in rabbits and horses) and humans (ABO bloodgroup system). In early genetics (Nilsson-Ehle, 1908) and at present (Thoday, 1961; Thompson, 1975; Paterson *et al.*, 1988; Tanksley, 1993) the common explanation for the inheritance of traits which exhibit a continuous distribution for the trait value, is based on polygenes (polymery). However, for quantitative traits the presence of multiple alleles has also been considered. Sirks used data for seed weight genes in garden beans (Sirks, 1925) and put forward the general question whether multiple allelomorphs or multiple factors could explain the inheritance of quantitative traits and certain qualitative traits which did not segregate in simple Mendelian ratios (Sirks, 1929).

This question should be reconsidered in the perspective of the recent achievements of quantitative genetic studies using extensive linkage maps of RFLPs (see review: Tanksley, 1993), and the results described in chapter 5. Probably the true genetic nature of quantitative traits is a combination of both multiple loci and multiple alleles. A quantitative trait which is solely based on either a number of di-allelic loci or on one single multiallelic locus may be regarded as an extreme situation. Further investigations will reveal whether or not multiple alleles are an underestimated source of quantitative genetic variation.

Molecular markers and saturated linkage maps are indispensable tools to investigate these fundamental questions on the nature of quantitative traits. In chapter 2 it was shown that in potato the number of alleles per locus revealed with RFLPs is high. The structure of the mapping population used in this study, which actually is a backcross, allows the detection of up to three alleles per locus. In this mapping population multiple alleles were detected at more than one third of the RFLP loci, when using five probe-enzyme (6-cutter) combinations. Using three probe-enzyme (4-cutter) combinations Leonards-Schippers (1994) detected in 34 of the 111 cases (31 %) four alleles per locus. This abundance of multiple alleles at the DNA sequence level may be indicative for a similar frequency of multiallelic genes, including quantitative trait loci.

Localisation of QTLs in outbreeders.

According to Paterson *et al.* (1988) QTLs can be located on the genome using a complete linkage map. However, when the offspring from non-inbred parents is used as mapping population, the map which is created is a composition of two independent maps, one reflecting the recombination in the female meiosis and one in the male meiosis. In chapter 5 it was demonstrated that only those RFLP loci, which showed a 1:1:1:1 segregation, could accurately monitor the segregation of quantitative genetic variation for tuber shape in a progeny from non-inbred parents. When those multiallelic loci segregating 1:1:1:1 were converted into a female and a male locus each showing a 1:1 segregation, the detection of the *Ro* locus was not allways possible, despite close linkage.
Using only the paternal alleles of the markers loci it was hardly possible to detect the QTL at the *Ro* locus. When using the markers which segregated from the female parent it was possible to discover the locus involved in tuber shape, but the size of its effect would be estimated wrongly.
The localisation of QTLs for horizontal resistance to *Phytophthora infestans* on the potato genome as performed by Leonards-Schippers *et al.* (1994) is an example of the alternative method. Analysis of QTLs was performed using a separate female and male map, which does not affect the position of a QTL. However, the size of the effect of a QTL locus, and therefore the possibility to detect that locus is influenced by this method. The detection of QTLs using separate female and male maps as applied by Leonards-Schippers (1994) depends on the within-parent variation of quantitative trait alleles, although the trait value was measured in the progeny where between-parent allele interactions occur. After the detection of the position of the QTLs on the separate parental maps, the size of the effects of the QTLs were reexamined using single point analysis of variance with the four progeny classes.
Several statistical procedures have been proposed for QTL analysis such as single point analysis (Tanksley *et al.*, 1982; Edwards *et al.*, 1987), interval analysis (Lander and Botstein, 1989), multiple regression (Haley and Knott, 1992) and improved combinations thereof (Jansen, 1993; Jansen and Stam, 1994). The single point analysis of variance as employed by Kreike *et al.* (1993), Van Eck *et al.* (1994; chapter 5) and Leonards-Schippers *et al.* (1994)

is a simple method for QTL analysis in the offspring from non-inbred parents allowing the use of markers segregating 1:1:1:1. The current procedures for interval mapping do not allow the use of such markers, and using the oversimplified pseudo-backcross data will result in biased estimates. The development of computer software for interval mapping fully employing the information of multiallelic (RFLP) markers for the accurate detection and characterisation of multiallelic QTLs, is presently carried out at the DLO-Centre for Plant Breeding and Reproduction Research. A procedure for mapping QTLs in crosses between non-inbred parents assuming multiallelic (RFLP) markers, but ignoring multiallelism at the quantitative trait locus is described by Haley *et al.* (1994), and was applied in mapping of QTLs for growth and fatness in pigs (Andersson *et al.* 1994). The procedure should allow complete classification of the descendants with respect to the origin of the quantitative trait alleles. However, intervals flanked by di-allelic RFLP loci, which segregate in a 1:2:1, 1:1♀ or 1:1♂ ratio cannot completely classify the descendants. Therefore the demarcation of the intervals depends on more marker loci adjacent to the interval, until the identity of the four possible QTL genotypes in the offspring can be established.

Inter- and intralocus interactions between QTL alleles.

As a result of the structure of a mapping population derived from diploid non-inbred parents, it is possible to study intralocus interactions between individual alleles of a quantitative trait locus. Using this experimental design the effects of up to four alleles and four combinations of alleles can be assessed, in a common genetic background. In chapter 5 the effects caused by four combinations of three alleles of the *Ro* locus on tuber shape are analysed. A clear intra-locus interaction was observed at this locus. Other examples of investigations of allele effects and allele interactions are provided in the publication by Fatokun *et al.* (1992) on epistasis between quantitative trait loci and by Stuber *et al.* (1992) on heterosis on the basis of (pseudo-) overdominance at QTLs. When specific inter- and intralocus interactions between alleles of QTLs with a beneficial performance have been discovered with the use of molecular markers, the same markers can be used for marker

assisted selection to retain these effects during the breeding program.

Interactions between QTL alleles and the genetic background of the breeding material.

Lander and Botstein (1989) suggested that the use of contrasting parents for QTL mapping experiments would be advantageous to obtain clear results. However, the estimated effect of the beneficial allele in such a donor population may not have any predictive value for the effect of the selected allele in the genetic background of the recurrent parent. As indicated by Rasmusson (1934) the effect of each allele on the genotypic value is not constant, but dependent upon all the other factors present. The effect of the allele is smaller as the number of other genetic factors acting in the same direction is larger. Therefore, it is important to plant and animal breeders to realise that the value of a beneficial allele is dependent on the inter- and intralocus interactions, as well as on the number of positive factors already present in the genetic background of the breeding material.

Applicability of molecular markers in potato breeding

In the Netherlands the role of the governmental institutions and private breeding companies is strictly divided. The institutions have restricted themselves to scientific research, the development of genetic tools and breeding methods and the production of advanced parental material, whereas the private sector mainly produces new varieties, and takes care of pure and healthy maintenance and propagation of seed and plant material. The discussions on the applicability of marker technology in potato breeding has besides technical also economical aspects. Therefore, governmental research institutes and breeding companies may have a different opinion about the use of molecular markers.

At present there seems to be no limitations to the application of molecular markers in genetic and plant breeding research. Many questions, which previously could not be tackled, are presently investigated thoroughly, and the genetics of the potato is developing with unprecedented speed. The hereditary basis of many agronomically important and probably polygenic traits is being investigated. Molecular marker techniques play also a role in the development

of genetics, new selection and introgression methods, identification and evaluation of valuable genetic variation in wild species, taxonomy and variety identification, and the investigation of the effects of transgenes in breeding material.

Costs and complexity of molecular marker techniques are limiting factors in their application in commercial breeding, especially when revenues from potatoes for seed, processing and consumption are under pressure. Markers are mostly being used as diagnostic tools and have to compete economically with classical diagnostics, such as field trails, resistance and quality tests. However, there are traits of which the evaluation is highly complicated, time-consuming and expensive or requiring much plant material. Evaluation of such traits is logically postponed to later stages of the selection proces. There are even traits for which no adequate test is available, for instance sweetening after cold storage, resistance to black leg and tolerance to black spot bruising. Other tests can be performed only in regions with specific pathogens, pests or environmental conditions which are not present in the Netherlands. Unfortunately, the ability to develop molecular markers for indirect selection is relying on proper evaluation of trait values as well.

The costs of molecular diagnostics for plant breeding were calculated by Ragot and Hoisington (1993). Cost per data point ranged from $0.14 to $0.55 for chemiluminiscent RFLPs, $0.13 to $0.76 for radioactive RFLPs, and $0.18 to $1.30 for RAPDs. The costs of RAPDs is highly dependent on the costs of *Taq* polymerase, which may range from $0.05 to $0.50 per amplification. Re-use of membranes has a large effect on the costs per data point. When membranes can be used for more than three determinations per sample, RAPD analysis is more expensive than RFLPs.

The costs of resistance and quality tests presently employed by potato breeders range from $1.00 for a closed container test for nematode resistance, or ELISA tests for virus resistance, up to $15.00 measuring the total glycoalkaloid contents. However, only if the relation between phenotype and genotype of an agronomically important trait has been established the costs of phenotypic and molecular-genetic tests can be compared and an unbiased choice made.

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Summary

In proportion to the importance of the potato as a world food crop, the hereditary basis of many important agronomical and quality traits is poorly understood. The reasons for this scanty genetic knowledge of this crop are found in tetraploidy, the high degree of heterozygosity, the absence of homozygous inbred lines or a collection of genetically well defined marker stocks. Besides of that, the long generation time, the frequently observed distorted segregation ratios probably due to a high genetic load, discourage geneticist to choose potato as a model species for genetic research. But still, a profound understanding of its genetic composition is the basic requirement for developing more efficient breeding methods, including techniques of gene cloning and genetic modification. By using diploid plants in combination with molecular markers, it has been shown that most of the above mentioned drawbacks can be overcome.

The aims of the research described in this thesis were **(1)** to construct a genetic linkage map of the potato genome, comprising molecular, isozyme and morphological markers, **(2)** to apply this map for the analysis and localisation of morphological and agronomically important traits, and **(3)** to gain a better insight in how to detect and to map quantitative trait loci in the offspring from obligatory outbreeders using non-inbred parents.

The linkage map of potato reported here was constructed using a 67 backcross progeny generated from non-inbred parents. The high degree of heterozygosity of the parents resulted in the observation of a mixture of five types of single locus segregations. These are the 1:1 segregation ratio from loci which were heterozygous in either the female or the male parent. Other types were the 1:2:1, 3:1 and 1:1:1:1 segregation ratio. A novel method was described for map construction. This method was developed because the currently available mapping software, designed for inbred parents, could not handle this type of segregation data. The method involves the construction of a separate maternal and paternal map, using only the segregation data of the relevant parent, disregarding the alleles segregating from the other parent. This mapping strategy is known as the "double-pseudo-test-cross". However, the computer

program JOINMAP allows the construction of linkage maps that combine segregation data from independant crosses. Merging the maternal and paternal segregation data allowed the construction of a combined map. Alignment of the maternal and paternal linkage groups is based on marker loci which are heterozygous in both parent. At least two of these 'allelic bridges' are required to find the correct orientation. In the discussion in Chapter 2 our mapping strategy is judged on its merits, and compared with other methods found in literature, to overcome the obstacles encouterd caused by the non-inbred nature of the parental clones. The segregation distortion, observed at 16 % of the loci, was predominantly caused by gametic selection. Using the 1:1:1:1 segregating markers it was possible to recognize zygotic selection a loci clustered on chromosomes *4* and *5*.

The resulting map consists of 197 RFLP, 9 isozyme and 11 classical genetic markers. The majority of the RFLP markers were derived from potato genomic DNA sequences flanking the intregration sites of a transposable element, or T-DNA constructs containing a transposable element. These RFLP markes not only represent loci on this map, they als reflect genomic positions of T-DNAs and transposable elements in an qually large population of independant potato genotypes. The eleven classical genetic traits localized on this map are:
The *S* locus, involved in gametophytic self-incompatibility on chromosome *1*; The *Me* locus, involved in metribuzin sensitivity and locus *D*, involved in the production of red anthocyanins, both mapped on chromosome *2*. On chromosome *3* the yellow flesh colour locus *Y* was mapped. Earliness and yellow margin, at the loci *El* and *Ym*, mapped on chromosome *5*. Desynapsis (*Ds*) mapped on chromosome *8*. Three loci could be placed on chromosome *10*: tuber shape (*Ro* locus), pigmented flowers (*F* locus) and crumpled (*Cr*). Locus *P*, involved in the production of purple anthocyanins was assigned to chromosome *11*.

As the small amount of morphological markers in potato is mainly comprising loci involved in anthocyanin pigmentation, and as furthermore the present genetic models on anthocyanin pigmentation do not agree with one another, the

needful attention was spend to this matter. To establish allelism between the originally loci described in literature and those which segregate in the mapping population, three diploid tester clones were used. The alleles in these testers descended from cv. Gineke; a tetraploid potato variety investigated by Lunden. The genotypes of these testers with white flowers and white skin colour are: *DDff*, *ddFF* and the double recessive *ddff*. By back-crossing individuals of the CE-population with these tester lines is was possible to recover the flower colour genotype of practically every CE-individual. In potato the flower colour phenotype of is determined by three unlinked loci *D*, *F* en *P*. A dominant allele of locus *D* and/or *P* together with a dominant allele of locus *F* is required for coloured flowers. Because of genetic complementation with these testers it was also possible to establish the genotype in the white flower colour phenotypes. Since it is was possible to expose the monogenic inheritance for every locus, the positions could be determined of these three loci on the potato genome by linkage analysis using RFLP markers. Locus *D* is involved in the biosynthesis of red anthocyanins and is placed on chromosome *2*, while locus *P*, involved in the biosynthesis of blue anthocyanins was localised on chromosome *11*. Locus *F* is situated on chromosome *10* and is involved in the flower specific expression of the gene(s) accommodated by loci *D* en *P*. Furthermore, this study demonstrated that usage of well defined tester clones was of importance to identify loci, to establish allelism, and to simplify flower colour genetics with complementary acting loci, in spite of distorted segregations which did not fit the expected ratios.

Potato clones representing germplasm of diploid cultivated *Solanum* species were used to study the inheritance of tuber skin colour. The genotypes of this material was assigned according to the nomenclature used by Dodds and Long. By hybridizing this diploid material with the above-mentioned tester lines allelism could be established between locus *D* from the model by Lunden and locus R/R^{pw} from the model by Dodds and Long. In the genetic model by Dodds and Long the function of locus R/R^{pw}, accommodating a basic gene for anthocyanin pigmentation, is defined in a completely different way. Due to this different definition skin colour seemd to inherit like a monogenic dominant

character, as opposite to the model by Lunden proposing complementary inheritance, which is similar to the situation described for flower colour. Another difference between these genetic models relates to the number of skin colour phenotypes. Lunden made a distinction between locus *E* which is involved in pigmentation of the epidermis and a second locus *R* which plays a role in the pigmentation of the outer layers of the cortex. The model by Dodds en Long recognized only the locus *I* to explain the inheritance of skin colour without discerning the different phenotypes.

In diploid cultivated *Solanum spp.*, with skin colour localized in either the epidermis or the cortex, complementary inheritance was observed between the skin colour locus and the anthocyanin producing locus *D*. The skin colour locus, involved in tissue specific expression of pigments in the epidermis, could be mapped on chromosome *10*, using RFLPs in offspring from 88-405-5 crossed with the tester clones. In the offspring descending from clone 88-402-12, with skin colour localized in the cortex, the second skin colour phenotype mapping approximately at the same position. On this very position on chromosome *10* Gebhardt mapped the purple skin colour locus (*PSC*). On the basis of their position on the genetic map allelism cannot be proven, nor excluded between all those loci *E*, *R*, *I* and *PSC*. In the close proximity of these skin colour loci the flower colour locus *F* was mapped in the CE-population. This shows that the previously published linkage group *B-I-F* is located on potato chromosome *10*. With this result, all presently known loci involved in tissue specific expression of anthocyanin pigmentation, are situated in a narrow cluster on this chromosome. This keeps the possibility open that locus *I* accommodates two closely linked genes involved in the pigmentation of the epidermis and the cortex of the tubers.

Finally, is this thesis attention is focused on the localisation of quantitative traits loci (QTLs) in the progeny from non-inbred parents. Tuber shape is commonly regarded as a quantitative character because of the continuous variation ranging from round, via oval to long tubers. However, it was possible to discern visually among clones of the CE-population between two distinct phenotypic

classes: round en long. On the basis of this visual classification the inheritance of tuber shape was explained by presuming a monogenic dominant locus *Ro*, round being dominant to long. With RFLPs the *Ro* locus was mapped on chromosome *10* using normal linkage analysis. Tuber shape was also studied as a quantitative trait, using the length/width ratio as phenotypic value. The broad sense heritability, was estimated: $h^2 = 0{,}80$. The morphologically mapped *Ro* locus could explain 75 % of the genetic variation, indicating the presence of a major QTL at the *Ro* locus and minor quantitative genetic factors outside it.

On the basis of cosegregation linkage or repulsion phase can be determined between alleles from adjacent loci. By using this type of information about linkage between unique alleles of flanking RFLPs in coupling phase with *Ro* alleles, it was possible to identify the origin of the alleles at the *Ro* locus. The 3:1 (round:long) segregating CE-progeny was divided into four genotypic classes specified by their allelic composition: $Ro^{♀}Ro^{♂}$: $Ro^{♀}ro$: $roRo^{♂}$: $roro$ = 1:1:1:1. The recessive *ro* allele was identical by descent in both parents. The effect on tuber shape of the non-identical alleles $Ro^{♀}$, $Ro^{♂}$ en *ro* was evaluated by comparing the mean length/width ratio of the four genotypic classes. The heterozygous genotypic classes $Ro^{♀}ro$ en $roRo^{♂}$ differed significantly in their length\width ratio ($p=0.0157$). This difference in length\width ratio was explained by postulating multiple alleles at the *Ro* locus. From this it is conceivable why at the tetraploid level never a monogenic inheritance for tuber shape was described. Complex intralocus interactions between multiple *Ro* alleles cause at the tetraploid level a continuous variation for tuber shape. In fact, in diploid potato multiple alleles were observed at approx. one third of the RFLP loci. Therefore, multiallelism for QTLs may be an underestimated source of quantitative genetic variation. Accordingly, it would be reasonable to assume that also for other quantitative traits the hereditary basis is determined by multiple loci in combination with multiple alleles.

A procedure to map quantitative trait loci in the offspring of heterozygous parents ought to take into account the above explained situations. The difference

between the classes which are indicated by the molecular markers can be annulled by intralocus interactions of QTL-alleles. Usage of dominant markers like Random Amplified Polymorphic DNA markers (RAPDs) as well as the use of codominant markers which are heterozygous in only one of the parents should be avoided in QTL-mapping experiments. These type of markers identify only two out of the four possible classes. Multiallelic RFLPs are the most appropriate type of markers since they can discriminate between all four allele combinations found in the offspring of non-inbred parents, allowing unbiased estimation of the presence and the magnitude of QTLs.

Samenvatting

In verhouding tot het belang van de aardappel als een wereldwijd geteeld voedselgewas, is slechts weinig bekend van de erfelijke basis van de belangrijke landbouwkundige en kwaliteitskenmerken. De oorzaak van deze gebrekkige genetische kennis kan in verband gebracht worden met de sterke mate van heterozygotie, tetraploïdie en het ontbreken van homozygote inteeltlijnen of een collectie van genetisch gekarakteriseerde testerlijnen. Voorts is er de lange generatieduur en treden er vaak scheve splitsingsverhoudingen op, veroorzaakt door een sterke *'genetic load'*, die ertoe hebben bijgedragen dat aardappel niet bepaald een modelgewas is voor genetisch onderzoek. Desalniettemin is een grondige kennis van de overerving van belangrijke eigenschappen een eerste vereiste voor de ontwikkeling van meer efficiënte veredelingsprogramma's, waaronder ook technieken als gen-isolatie en genetische modificatie begrepen worden. Door gebruik te maken van diploïde planten in combinatie met moleculaire merkers, is het mogelijk gebleken om veel van de eerder genoemde nadelen te overwinnen.

Het doel van het onderzoek dat in deze dissertatie wordt beschreven is (1) de constructie van een genetische kaart van het aardappelgenoom, bestaande uit moleculaire, isozym en morfologische merkers, (2) het benutten van deze kaart voor de analyse en het karteren van morfologische en/of landbouwkundig belangrijke eigenschappen en (3) het verwerven van beter inzicht in de wijze waarop een locus betrokken bij een kwantitatieve eigenschap ontdekt en gekarteerd kan worden, in de nakomelingschap van sterk heterozygote ouders, zoals die voorhanden zijn bij obligate kruisbevruchters.

De hier beschreven genetische kaart van het aardappelgenoom is gebaseerd op de splitsingsgegevens van een terugkruisingspopulatie van 67 planten, verkregen uit niet-ingeteelde ouders. Door de hoge mate van heterozygotie van de ouders kunnen merker loci op vijf verschillende manieren uitsplitsen. Dit zijn de 1:1 splitsingsverhouding voor loci die heterozygoot zijn in óf de vader, óf de moeder. Verder zijn dit de 1:2:1, de 3:1 en de 1:1:1:1 splitsingsverhouding. Een nieuwe methode wordt beschreven voor het construeren van een kaart. Deze methode moest ontwikkeld worden, omdat de

thans beschikbare computer programma's, die ontworpen zijn voor kartering in kruisbevruchters, zich niet lenen voor de analyse van dit type splitsingsgegevens. The methode bestaat uit het berekenen van aparte genetische kaarten voor de vader en de moeder. Hierbij worden alleen de splitsingsgegevens gebruikt die betrekking hebben op de ene ouder, terwijl de allelen genegeerd worden, die afkomstig zijn van de andere ouder. Deze wijze van genoom kartering staat bekend als de "dubbele pseudotestkruising". Echter, met het computer programma JOINMAP kunnen koppelingskaarten geconstrueerd worden, op basis van splitsingsgegevens van onafhankelijke kruisingen. Door het samenvoegen van de afzonderlijke gegevens van de vader en de moeder kan dan een gecombineerde genetische kaart vervaardigd worden. De identiteit en de orientatie van maternale en paternale koppelingsgroepen kan vastgesteld worden met merkers die in beide ouders uitsplitsten. Tenminste twee van deze allelische bruggen zijn vereist om de koppelingsgroepen in de juiste orientatie samen te voegen. In de discussie van Hoofdstuk 2 wordt deze methode op zijn waarde beoordeeld en vergeleken met andere in de literatuur gevonden methoden voor het omzeilen van de problemen die veroorzaakt worden door de heterozygotie van de ouders. Zestien procent van de loci vertoonden afwijkende spitsingsverhoudingen, die voornamelijk veroorzaakt waren door selectie van gameten. Door de 1:1:1:1 splitsende merkers te gebruiken kon herkend worden dat scheve uitsplitsing van loci op chromosomen 4 en 5 ontstaan was door selectie van zygoten.

De resulterende kaart bestaat uit *197* RFLP, *9* isozyme en *11* klassiek genetische merkers. The meerderheid van de RFLP merkers zijn afgeleid van genomische DNA sequenties die gelegen zijn naast de integratie punten van transposons of van T-DNA constructen die een transposon bevatten. Deze RFLPs representeren niet alleen loci op deze genetisch kaart, maar geven tevens de positie in het genoom weer van de T-DNA's en de transposons in evenzovele onafhankelijke aardappel genotypen. De elf gekarteerde klassiek genetische eigenschappen zijn: de *S* locus, betrokken bij gametofytische zelfincompatibiliteit, ligt op chromosoom *1*. De *Me* en de *D* locus, respectivelijk betrokken bij metribuzine gevoeligheid en de productie van rode

anthocyanen, zijn geplaats op chromosoom *2*. Op chromosoom *3* ligt de *Y* locus voor gele vleeskleur. Vroegrijpheid en gele bladrand, op de loci *El* en *Ym* zijn gekarteerd op chromosoom *5*. Desynapsis (*Ds*) vond zijn plek op chromosoom *8*. Op chromosoom *10* liggen die loci: knolvorm op de *Ro* locus, gekleurde bloemen op de *F* locus en een schrompelige mutant op de *Cr* locus. Tenslotte ligt locus *P*, betrokken bij de produktie van blauwe anthocyanen op chromosoom *11*.

Omdat in aardappel het geringe aantal morfologische merkers voornamelijk bestaat uit loci die betrokken zijn bij anthocyaan pigmentatie, en omdat bovendien de huidige genetisch modellen van anthocyaan pigmentatie niet met elkaar overeenstemmen, is aan dit aspect de nodige aandacht besteed.
Voor diploïde gecultiveerde *Solanum* soorten is door Dodds en Long een genetisch model voorgesteld voor bloem- en schilkleur, dat niet teruggrijpt op een model gepostuleerd voor de tetraploïde cultuur aardappel (*Solanum tuberosum* L.) dat eerder door Salaman en Lunden is beschreven. Om allelisme vast te stellen tussen de in de literatuur beschreven loci en de loci die uitsplitsten in de CE-populatie waarop de bovengenoemde genetische kaart is gebaseerd, werd van drie diploide testerklonen gebruik gemaakt. De allelen in deze testers stammen af van cv. Gineke; een door Lunden onderzocht tetraploïd aardappelras. De genotypen van deze testers met witte bloemen en blanke schilkleur zijn: *DDff*, *ddFF* en de basisrecessief *ddff*. Door de individuen van de CE-populatie terug te kruisen met deze testerlijnen was het mogelijk om van vrijwel elk CE-individu het genotype voor bloemkleur te stellen. Het bloemkleur fenotype in aardappel wordt bepaald door de drie ongekoppelde loci *D*, *F* en *P*, waarbij een dominant allel van locus *D* en/of *P* samen met een dominant allel van locus *F* vereist is voor gekleurde bloemen. Door complementatie met de testers kon ook in witte bloemen het genotype voor deze loci bepaald worden. Nadat voor elke locus de monogene vererving vastgesteld kon worden, was het mogelijk om door middel van koppelingsanalyse met RFLP merkers deze drie loci op het aardappelgenoom te karteren. Locus *D* is betrokken bij de biosynthese van rode anthocyanen en is geplaatst op chromosoom *2*, terwijl locus *P*, betrokken bij de biosynthese van blauwe

anthocyanen gelocaliseerd is op chromosoom *11*. Locus *F* ligt op chromosoom *10* en is betrokken bij de bloem-specifieke expressie van de genen die ondergebracht zijn op de loci *D* en *P*.

Het onderzoek laat verder zien dat het gebruik van goed gedefinieerde testers van groot belang is voor de identificatie van loci, het vaststellen van allelisme, en het vereenvoudigen van de bloemkleurgenetica met complementair overervende loci, ondanks scheve uitsplitsingen die niet overeenkomen met de verwachte splitsingsverhoudingen.

Voor de overeving van schilkleur werd gebruik gemaakt van aardappelklonen die wat herkomst en nomenclatuur betreft representatief zijn voor diploïde gecultiveerde *Solanum* soorten. Door dit materiaal te kruisen met de bovengenoemde testerlijnen kon allelisme vastgesteld worden tussen locus *D* uit het model van Lunden en locus R/R^{pw} uit het model van Dodds and Long. In het genetische model van Dodds en Long is de rol van dit basisgen voor anthocyaanpigmentatie volstrekt anders gedefinieerd. Hierdoor lijkt schilkleur een monogene dominante eigenschap, terwijl in het model van Lunden sprake is van complementaire overerving voor schilkleur, vergelijkbaar met die voor bloemkleur. Een tweede verschil tussen beide genetisch modellen, betreft het aantal schilkleur fenotypen. Lunden maakt onderscheid tussen locus *E* welke in aanwezigheid van een basisgen voor anthocyaan biosynthese, betrokken is bij pigmentatie van de epidermis en locus *R* welke op vergelijkbare wijze een rol speelt bij pigmentatie van de buitenste lagen van de cortex. Het model van Dodds en Long kent slechts een onafhankelijke locus *I* voor schilkleur zonder deze fenotypen te onderscheiden.

In nakomelingschappen van klonen afgeleid van de diploide gecultiveerde *Solanum spp.*, waarin schilkleur gelocaliseerd kan zijn in de epidermis, dan wel in de cortex, kon complementaire overeving vastgesteld worden tussen een locus voor schilkleur en locus *D*, die verantwoordelijk is voor de productie van anthocyanen. De schilkleur locus die betrokken was bij weefselspecifieke expressie van pigmenten in de epidermis kon in een kruising tussen kloon 88-

405-5 en de testerklonen met RFLPs op chromosoom *10* gekarteerd worden. In nakomelingen van kloon 88-402-12, waarin de cortex gepigmenteerd is, werd de locus die dit tweede schilkleur fenotype bepaald, bij benadering op dezelfde positie op chromosoom *10* gelocaliseerd. Eerder werd door Gebhardt de *purple skin colour* locus (*PSC*) op deze positie op chromosoom *10* geplaatst. Op grond van hun positie op de genetische kaart kan allelisme tussen de loci *E*, *R*, *I* en *PSC*. niet uitgesloten, noch bewezen worden. Deze schilkleurloci liggen in de nabijheid van de bloemkleurlocus *F*, waaruit blijkt dat de eerder gepubliceerde koppelingsgroep *B-I-F* op aardappelchromosoom *10* ligt. Hiermee zijn alle thans bekende loci betrokken bij weefselspecieke expressie van anthocyaanpigmentatie geplaatst in een zeer klein gebied op dit chromosoom. Dit houdt de mogelijkheid open dat locus *I* twee nauw gekoppelde genen voor pigmentatie van de epidermis en de cortex van de schil bevat.

In deze dissertatie wordt tenslotte aandacht besteed aan de localisatie van kwantitatieve eigenschappen (*QTL-mapping*) in de F_1 nakomelingschap van niet ingeteelde kruisingsouders. Knolvorm kan beschouwd worden als een kwantitatieve eigenschap vanwege de continue variatie van ronde, via ovale naar lange knollen. Het is echter ook mogelijk om de klonen van de CE-populatie visueel te classificeren in twee discrete fenotypische klassen: rond en lang. Op grond van deze visuele indeling kan de overerving van knolvorm verklaard worden door een monogene dominante locus *Ro* voor knolvorm te veronderstellen, waarbij rond dominant is over lang. Met RFLP's kon deze *Ro* locus via normale koppelingsanalyse gekarteerd worden op chromosoom *10*. Door de lengte/breedte verhouding van de knollen te gebruiken als maat voor knolvorm, kon deze eigenschap bestudeerd worden als een kwantitatieve eigenschap. Op grond van de variatie tussen klonen en de variatie binnen klonen kon de overervingsgraad geschat worden: $h^2 = 0{,}80$. Wanneer de als morfologische eigenschap gekarteerde *Ro* locus gebruikt werd als verklarende variabele, dan kon daarmee 75 % van de genetische variatie verklaard worden. Dit duidt op een hoofdgen voor knolvorm op de *Ro* locus (*major QTL*) en kleinere kwantitatief genetische factoren daarbuiten (*minor QTLs*).

Op grond van cosegregatie kan van allelen van aangrenzende loci vastgesteld worden of deze gekoppeld zijn in koppelings- of afstotingsfase. Door op deze wijze gebruik te maken van informatie over koppeling met unieke allelen van RFLP loci aan weerszijden van de *Ro* locus, was het mogelijk om informatie te verkrijgen over de herkomst van de allelen van de *Ro* locus. De CE-populatie die in een verhouding van 3 ronde (*Ro.*) op 1 lange (*roro*) uitsplitst kon zodoende volledig geklassificeerd worden in een 1:1:1:1 verhouding. Omdat beide ouders het recessieve *ro* allel gemeenschappelijk hebben leidt dit tot de volgende klassen: $Ro^{♀}Ro^{♂}$, $Ro^{♀}ro$, $roRo^{♂}$ en *roro*. Het effect van de niet-identieke allelen $Ro^{♀}$, $Ro^{♂}$ en *ro* op knolvorm kon nu bepaald worden door vergelijking van de gemiddelde vorm van de knollen in de vier aldus vastgestelde genotypische klassen. De beide heterozygote klassen $Ro^{♀}ro$ en $roRo^{♂}$ bleken significant verschillend in hun lengte\breedte verhouding (p=0.0157). Dit verschil in lengte\breedte verhouding kan verklaard worden door multiple allelie op de *Ro* locus te veronderstellen. Hieruit kan verklaard worden waarom op tetraploïd niveau nooit een monogene overerving voor knolvorm beschreven is. Complexe intralocus interacties tussen multiple *Ro* allelen kunnen in tetraploïden een continue variatie voor knolvorm veroorzaken. Overigens komt in diploide aardappel op ± eenderde van de RFLP loci multiple allelie voor, zodat multiple allelie voor QTL's misschien een onderschatte bron is van kwantitatief genetische variatie. Dan zou ook voor andere kwantitieve eigenschappen de genetisch basis gevormd kunnen worden door polygenen in combinatie met multiallelisme.

Een schattingsprocedure voor het localiseren van loci van kwantitatieve eigenschappen (QTL's) in de nakomelingschap van heterozygote ouders dient rekening te houden met situaties zoals hierboven beschreven. Door intralocus interacties van QTL-allelen kunnen verschillen tussen de klassen, die met moleculaire merkers aangetoond worden, te niet gedaan worden. Het gebruik van dominante merkers zoals *Random Amplified Polymorphic DNA* merkers (RAPDs) en het gebruik van codominante merkers die slechts in één ouder heterozygoot zijn dienen voor QTL-mapping vermeden te worden. Deze merkers kunnen slechts twee van de mogelijk vier klassen identificeren.

Multiallele RFLP's zijn daarom het meest informatief. Deze merkers kunnen in de nakomelingschap de vier mogelijke allel combinaties volledig classificeren, waardoor de aanwezigheid en de grootte van een QTL zuiver geschat kan worden.

Curriculum Vitae

Herman Johannes van Eck werd op 9 april 1961 te Rotterdam geboren als tweede zoon van E.J. van Eck en C.H. van Eck-Gijsbers. Hij behaalde in juni 1978 het HAVO- en in juni 1980 het VWO-diploma aan de Chr. scholengemeenschap Melanchthon te Rotterdam. In 1980 werd begonnen met de studie plantenveredeling (N-13) aan de Landbouwhogeschool in Wageningen. Na het behalen van het kandidaatsexamen in september 1984 is de studie gedurende het cursusjaar 1984-1985 onderbroken geweest vanwege het bekleden van de functie van secretaris van de Senaat van de W.S.V. Ceres. Na een stage bij het veredelingsbedrijf De Ruiterseeds te Bleiswijk, werd in 1986 begonnen aan de ingenieursstudie bestaande uit een verzwaard hoofdvak plantenveredeling en een hoofdvak moleculaire biologie. Na het behalen van het doctoraaldiploma op 24 juni 1988 begon een periode met wisselende werkzaamheden o.a. voor het computerbedrijf Pink Elephant bv te Voorburg. In het kader van het BION/STW project "Constructie van een genetische kaart van aardappel d.m.v. moleculair biologische, celgenetische en klassiek genetische methoden" werd hij op 1 april 1989 door de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) aangesteld als onderzoeker in opleiding bij de Vakgroep Plantenveredeling, onder begeleiding van de hoogleraar Dr.Ir. E. Jacobsen. In januari 1993 nam hij ontslag bij NWO voor een aansluitende aanstelling tot 1 februari 1995 als toegevoegd onderzoeker in dienst van de Landbouwuniversiteit bij de Vakgroep Plantenveredeling. Het onderzoek resulteerde in enkele wetenschappelijke publicaties welke gebundeld zijn in dit proefschrift.

Nawoord

Na een universitaire studie, of als schoolverlater, begint (als je geluk hebt) een periode waarin je betaald wordt voor hetgeen je doet. Het vinden van een werkkring is overigens steeds minder vanzelfsprekend, maar dit terzijde. Na enkele jaren heb je in die betaalde uren het nodige verricht. Niet iedereen slaat dan een paar nietjes door het resultaat en plakt er een kaftje om. Het is ook moeilijk als je in de wegenbouw zit, om de A12 met nietjes te bundelen. En als je *pro deo* kinderen opgevoed hebt, dan zou je ze soms achter het behang willen plakken, maar nooit in een kaftje. Toen ik in april 1989 bij de Vakgroep Plantenveredeling als OIO een baan kreeg, was het van het begin af aan de bedoeling om de resultaten op te schrijven. Het resultaat is de dissertatie die je thans in handen hebt, die trouwens zonder de hulp van velen niet tot stand gekomen was.
Eigenlijk was ik in het begin niet bijster enthousiast over dit onderzoek naar de genetische kaart van aardappel. RFLP-werk staat bekend als saai en zeer routinematig. De doelstelling van het project was weinig origineel. Nadat voor gewassen als tomaat en maïs al genetische kaarten gemaakt waren, moest aardappel er ook maar aan geloven. Zo kunnen we voortgaan met de genetisch kaart van prei, worteltjes of peterselie.

Achteraf kijk ik terug op een leuke en leerzame periode. Aardappel bleek één van de allerleukste gewassen! Het gewas vertoont een grote rijkdom aan genetische variatie, waarover je ook met de buurvrouw van je achterneef van gedachten kunt wisselen. Echter, de genetische basis van blauwe plekken, afkokers en diepe ogen is nog steeds niet onderzocht. Wat dat betreft lijkt de genetica van de aardappel nog op een onontgonnen gebied. Geheel onverwacht leverde een experiment naar knolvorm het leuke resultaat van multipele allelie op. Het bleek mogelijk om de conclusies te generaliseren en tot je verbazing ben je opeens bezig met origineel genetisch onderzoek. Verder is het maken van een kaart van een kruisbevruchter zo simpel nog niet. Over onze methode van kartering is het laatste woord nog niet gesproken en de methode is nog steeds

voor verbetering vatbaar. Tenslotte lijken moleculaire merkertechnieken, door hun toepasbaarheid in indirecte selectie, een goede toekomst te hebben. Ik had geen leuker werk kunnen bedenken!

In dit nawoord wil ik allereerst mijn dankbaarheid en complimenten uiten naar mijn promotor Evert Jacobsen voor zijn wijze van begeleiding. Evert kent zijn mensen goed, en hij weet wat hij moet doen en laten om alles en iedereen tot zijn recht te laten komen. Je hebt me veel vrijheid gegeven, zonder me aan mijn lot over te laten. Je maakte duidelijk wat je wilde, zonder te domineren. Vervolgens gaat mijn dank uit naar drs. Paul Beckers van BION / NWO voor zijn uitstekende financiële en administratieve begeleiding van dit STW-project. De heren ir. C.D. van Loon, ir. Joost Vermeulen en ir. P. Hijma waren leden van de vanwege de STW ingestelde gebruikerscommissie. Zij worden bedankt voor hun belangstelling en bijdrage aan de halfjaarlijkse voortgangsrapportages. Ook de inbreng vanuit de Groningse tak van het STW-project van Laas Pijnacker en Gert Baarda aan deze halfjaarlijkse bijeenkomsten mag niet onvermeld blijven. Binnen het STW-project is Groningen altijd op 200 km afstand blijven liggen, maar met het CPRO-DLO kon een nauwe samenwerking gerealiseerd worden. Daarom bedank ik co-promotor Dr. Willem Stiekema voor de samen-uit, samen-thuis samenwerking en zijn commentaren op de manuscripten. De intensieve samenwerking met Jeanne Jacobs en onze wederzijdse afhankelijkheid heeft in ons beider voordeel gewerkt. Zonder jouw RFLP-gegevens zouden de morfologische en isozyme loci niet gemept zijn.

Prof. J.G.Th. Hermsen komt de eer toe, dat hij de aanvraag voor dit STW-project geschreven heeft. Daarnaast was u beschikbaar als aardappel-encyclopedie en corrector van de Engelse taal. Erik Jongedijk en Coco van der Wolk: jullie hadden al een begin gemaakt met de karakterisering van de CE-populatie. Een grote hoeveelheid werk is verzet door de analisten Anneke Thea Hertog-van 't Oever, Brigit Jansman en Petra van den Berg. Hartelijk bedankt voor jullie inzet en incasseringsvermogen om het soms grillige verloop van het onderzoek en mijn nukkigheden te verdragen. De verzorging van de planten was in goede handen bij Jan en Teus. Jullie zijn niet alleen vakmensen, maar

ook jullie betrokkenheid bij de mensen en het onderzoek van de aardappelafdeling is te prijzen. Zonder Dirk-Jan zou de gehele coördinatie van de aardappelafdeling wegvallen. Isolde, Jan en Mart worden bedankt voor de *in vitro* instandhouding. Met grote voortvarendheid heeft Jan van Dijk bloemkleur en Jacqueline Ton knolvorm vastgelegd in eindeloze getallenreeksen. Het tempo waarmee René Luyten luminogrammen het licht deed zien was zo mogelijk nog groter. Bedankt voor jullie geleverde bijdrage. De mannen van de overkant: Herman, Barto, Frans, Anton en Arie worden bedankt voor het verzorgen van de veldproeven, chipsbakken, onderwaterwegen enz. Herman, Arnold en Johan: zonder jullie zou geen computer z'n werk kunnen doen. Alle mensen van het lab boven en beneden: bedankt voor de hulp, praktische tips, gezelligheid en taart. Vaste en tijdelijke onderzoekers, met name kamergenoten Ronald, Ronald, Charlotte, Anja, Ellen, Heleen en Karin: bedankt voor alle nuttige wetenschappelijke besprekingen en gezellige gesprekken.

En de collega's van buiten de vakgroep: Hans Sandbrink, Sjaak van Heusden, Nelleke Kreike, Rob Weide, Ruud Verkerk, Monique van Wordragen, Paul Arens, Brigit Verkerk-Bakker, Wendy Oude-Breuil en Jacq de Koning. Toen RAPD's, PCR, QTL's en JoinMap nog nauwelijks bekend waren in Wageningen hielden we RFLP-lunch, die in de beginjaren goed functioneerde als een "AIO-netwerk", waar domme vragen gesteld konden worden en veel kennis en ongepubliceerde informatie uitgewisseld werd.

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Herman van Eck