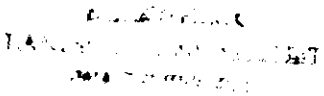
A genetic map of potato: construction and applications.

Een genetische kaart van de aardappel: constructie en toepassingen.



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A genetic map of potato: construction and applications

Jeanne M.E. Jacobs

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in de landbouw- en milieuwetenschappen
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Key-words: *Solanum tuberosum*, genetics, RFLP mapping, transposable elements, resistance, core map, potato cyst nematodes.

Bibliographic Abstract This thesis describes the construction of an integrated genetic map of potato (*Solanum tuberosum* L.) using molecular, morphological and isozyme markers. A general method for map construction, different from previous methods employed in non-inbred plants, is described using JoinMap 1.4. This basic map was used for various applications in potato genetics. For the purpose of targeted transposon tagging in potato, the integration sites of T-DNA's and transposable elements were determined and were found to cover the entire potato genome. Genetic maps, constructed from seven different potato populations, were compared and aligned. All data were combined and one common core map was constructed with the help of JoinMap 2.0. Furthermore, the genetic map was used to assist in the mapping of the resistance locus *GroV1* from *S. vernei*. This resistance locus, to the potato cyst nematode *Globodera rostochiensis* pathotype 1, was located on chromosome *5* with molecular (RFLP/PCR) markers.

PROPOSITIONS (STELLINGEN).

1. A genetic map, especially when combining data from distinct sources, is only as good as the most recent data, updated computer programmes and skills and effort of the mapper.

2. The concept of shorter maps from the male parent in Solanaceae, as revealed by classical genetic markers (Rick 1969), has been confirmed by recent mapping studies using molecular markers (DeVicente & Tanksley 1991; Van Ooijen *et al.* 1994; this thesis).

Rick, 1969 Genetics **62:** 753-768.
DeVicente & Tanksley, 1991 Theor. Appl. Genet. **83:** 173-178.
Van Ooijen *et al.*, 1994 Theor. Appl. Genet. **89:** 1007-1013.

3. It is surprising that the genetic map of potato, "described" by Tanksley *et al.* (1992), was accepted for publication, without any description of the mapping parent(s) used, the method of data collection and analysis, and significance of the resulting map.

Tanksley *et al.*, 1992 Genetics **132:** 1141-1160.

4. The term "double pseudo-backcross", used for non-inbred mapping populations to lure the vast majority of geneticists that are working with inbreeding plant species, is not entirely lucid.

Grattapaglia & Sederoff, 1994 Genetics **137:** 1121-1137.
Hemmat *et al.*, 1994 J. Hered. **85:** 4-11.
Van Eck, 1995 Ph.D. thesis. Wageningen Agricultural University.

5. "Single-parent" configurations are often indispensable but not always preferable.

6. The tendency of research groups to co-author everyone "who watered the plants", results in unnecessarily long citations.

7. In order to make waves, molecular biologists and scientists in other fields have to be surfing on the same wave length.

8. The trend in Dutch science to write a PhD thesis in the English language is in contrast with the fact that the propositions, historically the most important part of a PhD thesis, are generally published in Dutch.

9. Standing on the top of the world is not sufficient by itself to become a member of the Most Noble Order of the Garter.

Propositions belonging to the doctoral thesis 'A genetic map of potato: construction and applications', by Jeanne M.E. Jacobs, 30 October 1995.

Au fond, la recherche scientifique est une chose ludique

André Lwoff

Contents

General introduction

Genetic linkage maps.

A genetic linkage map represents the relative order of loci to each other, on the chromosomes. It is a valuable tool in basic genetical studies and applied breeding programmes, especially for the identification and selection of genotypes with specific combinations of traits. Traditionally, classical genetic markers required a large number of segregating populations to develop a linkage map, as only a limited number of loci segregated in each cross. The genetic loci studied, involved morphological traits and more recently biochemical (isozymes) markers. Tester lines were developed, mainly from wide crosses, in extensively studied species. These tester lines had the potential to contain many markers. However, it was never possible to develop one tester line with sufficient markers to cover the whole genome. Furthermore, in many species, including potato, it was difficult to develop appropriate tester lines. Consequently, the lack of genetic markers hindered the construction of linkage maps in many important plant species. The advent of molecular (RFLP) markers, has dramatically changed this situation. RFLP markers are available in unlimited amounts, well-spread over the genome, and are not subject to phenotypic variability. Their large numbers made it feasible to develop a linkage map in one single cross. Genetic linkage maps have now been developed for the most important crops (*eg.* maize, Gardiner *et al.* 1993; wheat, Liu & Tsunewaki 1991; rice, McCouch *et al.* 1988; tomato, Tanksley *et al.* 1992; soybean, Keim *et al.* 1990).

Inheritance studies in potato.

In cultivated potato (*Solanum tuberosum* L.), genetic analysis and mapping of loci via traditional approaches have been difficult for many years. Since this crop plant has a high degree of heterozygosity and an autotetraploid genome (2n=4x=48), genetical studies of specific loci is complex, with the inheritance of traits often being masked by multiple alleles and tetrasomic segregation (Howard 1970). Furthermore, genetic analysis of potatoes is also complicated by a high load of (sub)lethal alleles and severe inbreeding depression observed in this crop. The development of breeding methods to produce dihaploid clones of *S. tuberosum* (*eg.* Hermsen & Verdenius 1973), and the advent of molecular (RFLP) markers (*eg.* Van Eck *et al.* 1993; Van Eck *et al.* 1994a; Van Eck *et al.* 1994b) have simplified inheritance studies. This has made the study of potato genetics more feasible, although the development of specific genetic stocks and tester lines of diploid material containing interesting traits, is time-consuming and

hindered by self-incompatibility of *S. tuberosum* at the diploid level.

Linkage maps in non-inbred species.

In general terms, the development of genetic linkage maps for non-inbred species is more complex than in inbreeding species, such as *Arabidopsis* and tomato. Mapping populations in non-inbred species are usually derived from crosses between two heterozygous parents. Consequently, several modes of segregation are possible within the resulting mapping population. In diploid species it is possible for up to four different alleles to be segregating at a single locus. For the construction of genetic maps, various approaches have been used to solve the resulting complexities (Van Eck 1995). In the past these have ranged from disregarding a component of the data (*eg.* constructing a map using information from only one parent, or ignoring specific types of allele segregation), to combining the data obtained from both parents into one map.

The first genetic linkage map in a non-inbred species was developed for potato, using the segregation data from only one of the parents (Bonierbale *et al.* 1988). Essentially the same approach was subsequently followed for genetic maps of citrus (Durham *et al.* 1992; Jarrel *et al.* 1992) and loblolly pine (Devey *et al.* 1994). An alternative approach, also originally used in potato, involved the intercalation of three maps, separately constructed from the markers only segregating in either of the two parents, and for the markers segregating in both parents (Gebhardt *et al.* 1989b; Gebhardt *et al.* 1994). A similar approach, using allelic bridges to align the two parallel parental maps to varying extents has been utilised in alfalfa (Echt *et al.* 1994), loblolly pine (Groover *et al.* 1994), eucalyptus (Grattapaglia & Sederoff 1994), and apple (Hemmat *et al.* 1994). In some genetic mapping studies on gymnosperms, the availability of haploid tissue from megagametophytes has allowed the construction of linkage groups from a single parent, without the need of a mapping population (*eg.* white spruce, Tulsieram *et al.* 1992; maritime pine, Gerber *et al.* 1993; Norway spruce, Binelli & Bucci 1994).

Prior to this study there were two RFLP maps of potato, both based on diploid populations. One of these maps was based on the homology of the potato and tomato genome, using tomato RFLP markers in interspecific crosses of *S. tuberosum* and wild species. This map was initially constructed from the hybrid parent of the cross *S. phureja* x (*S. tuberosum* x *S. chacoense*)(Bonierbale *et al.* 1988), then later developed

using a population derived from the cross (*S. tuberosum* x *S. berthaultii*) x *S. berthaultii* (Tanksley *et al.* 1992; Bonierbale *et al.* 1994). The other map was based on potato RFLP markers in an intraspecific cross of *S. tuberosum* (Gebhardt *et al.* 1989b; Gebhardt *et al.* 1994; Leonards-Schippers *et al.* 1994). Both of these maps almost exclusively consist of molecular markers. However, to fully utilise genetic maps in basic research and applied breeding programmes, it is desirable that classical and molecular markers are integrated into the same genetic map (*eg.* Giraudat *et al.* 1992; Weide *et al.* 1993).

Value of comprehensive genetic maps.

Applications of molecular markers are anticipated to become increasingly important research tools for genetic studies in crop plants, including potato. Future applications of this technology will be greatly facilitated by the availability of comprehensive genetic maps in specific crops. The ability to construct combined genetic maps of a crop, that integrate the mapping data available from independent studies, is therefore valuable. However, with standard computer programmes this is difficult and inaccurate, especially when the data sets encompass information from different types of segregating populations, diverse sets of markers, and different closely related species. The computer programme for genetic mapping JoinMap (Stam 1993) has been developed to circumvent these problems. A new version, JoinMap 2.0, also includes an appropriate adaptation for non-inbred mapping populations (Stam 1995).

Transposon tagging of genes.

An important use of well established genetic maps include applications to basic research such as the tagging and cloning of genes controlling specific traits. Transposon tagging is a convenient approach for the isolation of genes which have an easy screenable mutant phenotype. Transposons are mobile segments of DNA that migrate through a genome, sometimes causing phenotypically visible mutations when they insert into or near a gene. The DNA sequence adjacent to the insertion site can be isolated using the known DNA sequence of the transposon tag. This sequence can then be used as a probe for the isolation of the complete wild type gene. This method has been particularly successful in plant species like maize and *Antirrhinum*, that contain well-characterised endogenous transposons (see review by Walbot 1992).

The maize *Ac-Ds* and *En-I* transposable element systems have been demonstrated to

be functional following their transformation into heterologous plant species (Baker *et al.* 1986; Pereira & Saedler 1989). This has resulted in the successful transposon tagging of a number of genes based on a random (non-targeted) approach for mutant selections in *Arabidopsis* (Aarts *et al.* 1993; Bancroft *et al.* 1993) and *Petunia* (Chuck *et al.* 1993). Non-random tagging of target genes with transposons, where selection is performed towards the inactivation of the target gene, has been performed for the *Cf-9* gene in tomato (Jones *et al.* 1994), the *N* gene in tobacco (Whitham *et al.* 1994) and the L^6 gene in flax (Ellis *et al.* 1995). In the case of the *Cf-9* gene, the finding that transposable elements preferentially jump to neighbouring positions in the genome was used for highly efficient tagging of the gene.

The maize transposon systems *Ac-Ds* and *En-I* are both known to be active in potato (Knapp *et al.* 1988; Pereira *et al.* 1991; Frey *et al.* 1989). This makes potato amenable for transposon tagging using these heterologous transposons. The availability of comprehensive genetic maps encompassing molecular and classical markers would therefore offer the potential for the targeted tagging and isolation of specific genes controlling important traits in potato.

Marker-assisted selection.

One of the first major applications of molecular markers in crop improvement has been marker-assisted selection. This is especially important for traits in which phenotypic screening is difficult or unreliable, such as pest and disease resistance. The potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida* are severe pests in potato crops throughout the world. The preferred way to avoid crop damage is to breed cultivars with resistance to the potato cyst nematodes. Since the cultivated potato does not contain resistance to both *Globodera* species, wild *Solanum* species have been used for the introgression of appropriate resistance genes into potato cultivars (Ross 1986).

The development of diploid potato populations, combined with molecular techniques for genetic mapping, has allowed the genetic mapping of several single loci or quantitative trait loci (QTL) for resistance to potato cyst nematodes that originate from *S. spegazzinii* (Barone *et al.* 1990; Kreike *et al.* 1993; Kreike *et al.* 1994) and *S. tuberosum* ssp. *andigena* (Pineda *et al.* 1993; Gebhardt *et al.* 1993). Other germplasm sources of resistance to *Globodera* species, such as *S. vernei*, are known (Plaisted *et*

al. 1962; Scurrah *et al.* 1973; Ross 1986). However, the accurate mapping of the loci involved is yet to be determined.

Scope of this thesis.

The aim of this thesis was to develop a basic genetic map for potato and to illustrate the use of this map in basic and applied research. To achieve this there were four separate components:

In Chapter 1, the construction of a genetic map of potato, that can serve as a basis for genetic studies as well as for breeding purposes, is described. This involved making two independent parental maps that integrate molecular markers with morphological and isozyme markers. These parental maps were subsequently combined into a common map, in a manner which fully exploits the segregation data present in a highly heterozygous crop like potato.

In Chapter 2, the development of a series of potato genotypes with transposable elements inserted into different genomic sites is described. The insertion sites of T-DNA constructs containing *Ac*, *Ds*, or *I* elements were mapped over the entire potato genome. Therefore, this set of transformants, with transposable elements mapped to a known position, can serve as a basis for targeted tagging of any locus in the potato genome.

In Chapter 3, the construction of a genetic map of potato, integrating the mapping data available from a number of previous studies is described. This encompasses information from different types of segregating populations and different potato species. Separate maps for all populations were (re)calculated, aligned, and subsequently joined into one basic core map with an updated version of the computer programme JoinMap.

In Chapter 4, the inheritance and mapping of *GroV1*, a resistance locus to *Globodera rostochiensis* pathotype Ro1, originating from *S. vernei*, is described. A diploid potato population was used, which was derived from a tetraploid clone commonly used for the introgression of potato cyst nematode resistance into potato cultivars. This included the use of bulked segregant analysis, coupled with PCR-based RAPD markers, and the subsequent development of SCARs for potential marker-assisted selection of this source of nematode resistance.

Chapter 1

A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons, and classical markers.

(with: Herman J. Van Eck, Paul Arens, Brigitte Verkerk-Bakker, Bas te Lintel Hekkert, Heleen J.M. Bastiaanssen, Ali El-Kharbotly, Andy Pereira, Evert Jacobsen, Willem J. Stiekema)

Abstract A genetic map of potato (*Solanum tuberosum* L.) integrating molecular markers with morphological and isozyme markers was constructed, using a backcross population of 67 diploid potato plants. A general method for map construction is described, different from previous methods employed in potato and other outbreeding plants. First, separate maps for the female and male parents were constructed. The female map contained 132 markers, whereas the male map contained 138 markers. Second, on basis of the markers in common the two integrated parental maps were combined into one with the computer programme JoinMap. This combined map consisted of 175 molecular markers, 10 morphological markers, and 8 isozyme markers. Ninety-two of the molecular markers were derived from DNA sequences flanking either T-DNA inserts in potato, or reintegrated maize transposable elements originating from these T-DNA constructs. Clusters of distorted segregation were found on chromosomes *1*, *2*, *8*, and *11* for the male parent and chromosome *5* for both parents. The total length of the combined map is 1120 cM.

Introduction

Genetic linkage maps are a valuable tool for the identification and selection of individuals with specific traits in basic genetical studies and applied breeding programmes. The accurate mapping of monogenic as well as polygenic traits, within an extensive map framework, allows their efficient introgression via marker-assisted selection. In cultivated potato (*Solanum tuberosum* L.) genetic analysis and mapping have been difficult for a long time. It is a highly heterozygous autotetraploid species (2n=4x=48), in which the segregation of traits is complex and often masked by this heterozygosity and tetrasomic inheritance (Howard 1970). Genetic analysis of potatoes is also complicated by severe inbreeding depression observed in this crop. The use of diploid wild species and especially the development of techniques to obtain dihaploid clones of *S. tuberosum* (*eg.* Hermsen & Verdenius 1973), have simplified inheritance studies and made the study of potato genetics more feasible. However, the development of specific stocks and tester lines of diploid material containing interesting traits, is time-consuming and hindered 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 in potato. These include tuber flesh colour (Bonierbale *et al.* 1988), tuber pigmentation loci (Gebhardt *et al.* 1989; Van Eck *et al.* 1994b), flower colour loci (Van Eck *et al.* 1993), and tuber shape (Van Eck *et al.* 1994a).

The development of molecular markers has dramatically increased the number of loci mapped in the potato genome. This resulted in two RFLP maps of potato to date, both based on diploid populations. One of these maps is based on potato RFLP markers in an intraspecific cross of *S. tuberosum* (Gebhardt *et al.* 1989b; Gebhardt *et al.* 1994; Leonards-Schippers *et al.* 1994). The other was based on the homology of the potato and tomato genome, using tomato RFLP markers in interspecific crosses of *S. tuberosum* and wild species (Bonierbale *et al.* 1988; Tanksley *et al.* 1992). Initially the latter map was mainly constructed from the hybrid parent of the cross *S. phureja* x (*S. tuberosum* x *S. chacoense*)(Bonierbale *et al.* 1988), then further developed using a population derived from the cross (*S. tuberosum* x *S. berthaultii*) x *S. berthaultii* (Tanksley *et al.* 1992). Both maps consist almost exclusively of molecular markers. However, to fully exploit genetic maps it is desirable that classical and molecular markers are incorporated into the same genetic maps (*eg.* Giraudat *et al.* 1992; Weide *et al.* 1993). The development of such an integrated genetic map can provide a

valuable tool for the screening and selection of desired genotypes.

In this paper we describe the construction of an integrated genetic map of potato that can serve as a basis for genetic studies as well as for breeding purposes. This initially involved making two independent parental maps that integrate molecular markers with morphological and isozyme markers. These parental maps were then combined into a common map. The resulting genetic map thus fully exploits the segregation data present in a highly heterozygous crop like potato.

Materials and methods

Plant material

Two diploid potato clones, coded C (USW5337.3; Hanneman & Peloquin 1967) and E (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. The offspring of these two non-inbred parental clones, C x E, consists of 67 genotypes. The clones C and E and their offspring, referred to as CE clones, have been used in previous genetic studies (Jongedijk & Ramanna 1989; Jongedijk *et al.* 1990; Van Eck *et al.* 1993; Van Eck *et al.* 1994a; Van Eck *et al.* 1994b).

Diploid potato clones were used for transformation with T-DNA constructs containing the maize transposable elements *Ac* as described before (Pereira *et al.* 1991). The diploid clone J92-6400-A16, harbouring the *R1* gene for resistance against *Phytophthora infestans*, was transformed with a T-DNA construct containing the maize *Ds* or *I* transposable elements (El-Kharbotly *et al.* 1995).

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 a polymorphic probe/enzyme combination giving segregation of alleles from both parental clones, was selected for analysis in the mapping population.

RFLP markers

Different sources of RFLP markers were used for segregation analysis and map construction. Random RFLP markers of potato were obtained from two sources. ST markers (*S. tuberosum*) originated from a potato leaf cDNA library (Nap *et al.* 1993) of the cultivar Bintje. Ssp markers originated from a genomic *Pst*I library of the wild species *Solanum spegazzinii* (Kreike *et al.* 1993). In addition, a series of cloned potato genes (BE and GBSS kindly provided by Dr. R. Visser, Wageningen Agricultural University) and a cDNA clone from *Petunia hybrida* (pVIP5043, kindly provided by Dr. R. Koes, Free University Amsterdam) were used as probes for mapping. Cloned genes mapped are BE (Branching enzyme); CHS*Ph* (Chalcone synthase *Petunia hybrida*); CHS*St* (Chalcone synthase *S. tuberosum*); GBSS and GBSSB (Granule bound starch synthase, major and minor locus); STF13 (*S. tuberosum* flower specific cDNA).

Potato genomic DNA sequences flanking the integration sites of either T-DNA constructs containing maize transposable elements, or reintegrations of the *Ac* element in the potato genome, were isolated by IPCR (Triglia *et al.* 1988). These were used for RFLP analysis as described previously (Pereira *et al.* 1992). The isolated IPCR fragments were cloned into the *Eco*RV site of pBluescript SK+ (Stratagene) and the identity of the clones was confirmed by hybridization to T-DNA/*Ac* border probes. For the T-DNA constructs containing a *Ds* element, the majority of flanking potato DNA sequences were obtained via plasmid rescue by electroporation (Dower *et al.* 1988). The nomenclature of these probes is as follows: TDs*X*, IPCR or plasmid rescue derived probe from T-DNA containing *Ds* transposable element, *X* = transformant number; TAc*X*, IPCR derived probe from T-DNA containing *Ac* transposable element, *X* = transformant number; TI*X*, plasmid rescue derived probe from T-DNA containing *I* transposable element, *X* = transformant number; Ac*X*-*Y*, IPCR derived probe from transposed *Ac* originating from TAc, *X* = TAc number, *Y* = transposed *Ac* number.

Forty-four markers from tomato, with known chromosomal positions in potato (TG's, Bonierbale *et al.* 1988) as well as two potato markers (GP's, Gebhardt *et al.* 1989) were also used for RFLP analysis in our mapping population. This enabled comparison with other potato maps (Gebhardt *et al.* 1991) and resulted in an analogous chromosome numbering of our map.

Different loci detected by a single probe were distinguished by the addition of a letter at the end of the locus name, *eg.* TAc13A and TAc13B. Whenever appropriate, the alphabetical order reflected the relative intensity of the RFLP band of the locus (major/minor bands).

Isozymes

Seven different enzyme systems were assayed in the mapping population (Table 1). Most isozyme patterns were determined using young leaf tissue. Tuber tissue was used in the case of ADH (*Adh-1* locus), and anthers for analysis of the *Adh-2* locus. For sample preparation, tissue was ground on ice with an equal volume of 0.05 M Tris-HCl extraction buffer (pH 6.9) containing 1 % β-mercapto-ethanol, followed by brief centrifugation to pellet debris. Immediately after centrifugation, the supernatant was applied to 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). Electrophoresis conditions, programmed on the control unit of the PhastSystem (Pharmacia), were as follows: for the 10-15 % and 8-25 % gradient gels (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; for the 12.5 % homogeneous gel (1) pre-run 400 V, 10 mA, 2.0 W, 10 Vh (2) sample application 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 % gel (1) pre-run 500 V, 10 mA, 3.0 W, 40 Vh (2) sample application run 500 V, 1 mA, 3.0 W, 10 Vh (3) separation 500 V, 10 mA, 3.0 W, 400 Vh. Temperature was maintained at 15°C. The gels were stained using standard procedures (Vallejos 1983).

Classical genetic markers

Classical genetic markers used in this study, along with their locus symbols, are summarised in Table 2. Analyses of classical genetic traits have been previously described in this mapping population for the flower colour loci *F*, *D* and *P* (Van Eck *et al.* 1993) and the tuber shape locus *Ro* (Van Eck *et al.* 1994a). 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 (Wageningen Agricultural University). Resistance to the

Table 1. Separation of isozymes and localisation of polymorphic loci on the potato genome.

Enzyme system	Gel*	Locus name	Chr.	Analogous Solanaceous locus
Alcohol dehydrogenase	(3)	*Adh-2*	4	*Adh-1*, chr.*4* tomato (Tanksley et al. 1992)
Diaphorase	(1)	*Dia-1*	5	*Dia-1*, chr.*5* potato (Bonierbale et al. 1988)
Malate dehydrogenase	(2)	*Mdh-2*	5	-
Alcohol dehydrogenase	(3)	*Adh-1*	6	*Adh-2*, chr.*6* tomato (Tanksley et al. 1992)
Glutamate oxaloacetate transaminase	(1)	*Got-2*	7	*Got-2*, chr.*7* potato (Bonierbale et al. 1988)
Glutamate oxaloacetate transaminase	(1)	*Got-1*	8	*Got-4*, chr.*8* potato (Bonierbale et al. 1988)
Acid phosphatase	(4)	*Aps-2*	8	*Aps-2*, chr.*8* tomato (Tanksley et al. 1992)
6-phosphogluconate dehydrogenase	(1)	*6-Pgdh-2*	12	*6-Pgdh-2*, chr.*12* tomato (Tanksley et al. 1992)
Triose phosphate isomerase	(2)	*Tpi-1*	-	-

* Gels types were: (1) 10-15%gradient gels; (2) 8-25% gradient gels; (3) 12.5% homogeneous gels; (4) 20% homogenoeous gels.

Table 2. Segregation of classical genetic markers and their localisation on the potato genome.

Locus	Parental genotypes C × E	Offspring genotypes	Segregation			Chromosome
			Observed	Expected	χ^2	
Self-incompatibility	$S_1S_2 \times S_2S_3$	$S_1S_3 : S_2S_3$	28:31	1:1 ♀	0.15	1
Metribuzin resistance	*Meme* × *meme*	*Meme* : *meme*	26:38	1:1 ♀	2.25	2
Red anthocyanins	*Dd* × *Dd*	*D-* : *dd*	58:9 *	3:1	11.95	2
Tuber flesh colour	*Yy* × *yy*	*Yy* : *yy*	37:30	1:1 ♀	0.73	3
Yellow margin	*YmYm* × *Ymym*	*YmYm* : *Ymym*	20:10 **	1:1 ♂	3.33	5
Desynapsis	*Ds1ds1* × *Ds1ds1*	*Ds1-* : *ds1ds1*	44:22	3:1	2.44	8
Crumpled	*Crcr* × *Crcr*	*CrCr*:*Crcr*:*crcr*	12:17:0 *	1:2:1	10.79	10
Tuber shape	*Roro* × *Roro*	*Ro-* : *roro*	49:17	3:1	0.02	10
Flower colour	*Ff* × *Ff*	*F-* : *ff*	44:19	3:1	0.89	10
Purple anthocyanins	*pp* × *Pp*	*Pp* : *pp*	10:57 *	1:1 ♂	32.97	11

* Segregation is significantly different from the expected ratio.

** Heterozygosity/homozygosity determined by backcrossing to clone E.

herbicide metribuzin (Sencor®) was screened using a nutrient solution test (De Jong 1983). Three replications, each with five cuttings, were assessed for the parental clones and all CE clones. Tuber flesh colour values were assigned according to the Dutch Descriptive List of Varieties (Anonymous 1988). In comparison to the flesh yellowness of standard varieties, the individuals of the mapping population could be classified on an ordinal scale ranging from 3 to 9. Although tuber flesh colour displays a continuous variation, clones were considered white (*yy*) when the mean trait value over three replications was $<5\frac{1}{2}$, and yellow (*Y-*) when the trait value was $>5\frac{1}{2}$ (Jongedijk *et al.* 1990). The recessive morphological leaf mutant "yellow margin" (*ymym*) (Simmonds 1965) did not segregate in the mapping population, because the parental clone C was homozygous dominant. Backcrosses of each CE clone to the parental clone E (*Ymym*) were performed to establish their genotype at the *Ym* locus. For each backcross, 25 seedlings were observed to detect segregants with yellow margins. The mutation desynapsis (*ds1*), affecting homologous chromosome pairing and thus recombination during the meiotic prophase I, was determined by cytogenetic observation, according to Jongedijk & Ramanna (1988). These results were confirmed by fertility observations in which fertile plants were scored *Ds1-*, and desynaptic individuals, lacking fertility, were scored homozygous recessive *ds1ds1*. Progeny plants displaying the recessive morphological mutation "crumpled" (*crcr*) were easily identified by their stunted phenotype. However, this trait is sublethal and these plants could not be used in RFLP analysis. Segregation at the *Cr* locus was therefore determined in that proportion of the CE clones that was non-mutant. Backcrosses to both parental clones were used to differentiate between the *CrCr* or *Crcr* individuals.

Linkage analysis and map construction

Data were collected in the mapping population for all segregating marker alleles, regardless whether segregation was from one parent or both. As a result of using non-inbred parents, five types of singe-locus segregation are found: 1:1♀, 1:1♂, 3:1, 1:2:1, and 1:1:1:1 (Table 3). Distorted segregation of markers was determined using the chi-square test. A single locus goodness-of-fit test (df=1) was used to examine gametic selection at 1:1♀, 1:1♂, and 1:1:1:1 segregating loci, for the gametic classes segregating from either clone C or clone E. Zygotic selection was determined using the chi-square contingency test (df=1) at 1:1:1:1 segregating loci. Two separate parental maps and the subsequent combined map were constructed with the computer programme JoinMap (Stam 1993). For parental map construction, the segregation data of markers segregating 1:1 from the relevant parent could be directly used for linkage analysis in JoinMap. Markers segregating from both parents in 1:2:1 and 3:1 ratios could be used as well. However, markers segregating from both parents showing a 1:1:1:1 ratio, have to be partitioned according to the alleles contributed by the relevant parent. For example, a marker displaying the parental genotypes *AB* x *AC*, will segregate into the classes *AA*, *AC*, *BA*, *BC*. The contribution of the female parent consists of the two classes *A-* (*AA* + *AC*) and *B-* (*BA* + *BC*), where the male alleles *A* and *C* do not interfere with the segregation from the female parent. Similarly, segregation of the two classes originating from the male parent in this example are *-A* (*AA* + *BA*) and *-C* (*AC* + *BC*). For the parental maps, allocation into linkage groups was based on a LOD threshold of 3.0. Marker orders within the linkage groups were initially determined using only the RFLP markers with a 1:1 segregation ratio. After the order of these markers was established for each linkage group, RFLP markers with 1:2:1 or 3:1 segregation ratios and the classical markers were allocated. This was done while maintaining the framework order initially established with 1:1 segregating markers (when appropriate, the option "fixed sequences" in JoinMap was used). Two separate genetic maps, one for each parental clone, were constructed in this manner. Markers segregating in both parental clones (1:1:1:1) were subsequently used as allelic bridges for the joining of the two maps into one combined map. The construction of the combined map was performed in a similar manner as the parental maps. When differences in marker orders were observed between the female and male maps, the two alternative marker orders were re-examined. Possible scoring errors in the data were corrected, and the "more

Table 3. Possible modes of segregation of single markers in the BC progeny from non-inbred parents, and numbers of markers examined in this study.

Parental genotypes	Offspring genotypes	Expected ratio's	Parental map	Marker type											
				Molecular									Isozyme	Classical	
				ST	Ssp	TG	GP	TDs	TAc	TI	Ac	genes			Total
AB × *AA*	*AB*:*AA*	1:1	♀ -	3	5	16	-	16	2	1	3	2	4	3	55
AA × *AB*	*AA*:*AB*	1:1	- ♂	5	3	8	-	29	3	1	6	0	4	2	61
AB × *AB*	*A*-:*BB*	3:1	♀ ♂	1	-	-	-	-	-	-	-	1	-	4	6
AB × *AB*	*AA*:*AB*:*BB*	1:2:1	♀ ♂	2	-	2	-	-	-	-	-	-	-	1	5
AB × *AC*	*AA*:*AC*:*BA*:*BC*	1:1:1:1	♀ ♂	6	4	18	2	18	1	4	10	3	-	-	66
Totals				17	12	44	2	63	6	6	19	6	8	10	193

likely marker order" was determined, based on a minimal number of double recombination events encountered. The more likely order was then imposed using the JoinMap option "fixed sequences", but only if these changes resulted in a minimal increase in the chi-square value for the overall goodness-of-fit of the map. When the alternative marker order contradicted the data, JoinMap could not be overruled with "fixed sequences". This might indicate the presence of chromosomal rearrangements of one parental clone relative to the other. Recombination frequencies were converted to map units (cM) with the Kosambi mapping function. The presented map reflects the chromosome numbering as proposed by Gebhardt *et al.* (1991) for genetic maps of potato. The computer programme Drawmap (Van Ooijen 1994) was used for graphic representation of the map.

Results

Segregation analyses

RFLP analysis. The mapping population of diploid potato plants used in this study for the construction of a genetic map, was obtained from a backcross involving two clones: *i.e.* clone C x clone E (= clone C x $VH^3$4211). These two parental clones contained mainly *S. tuberosum* germplasm, but also have the wild species *S. phureja* and *S. vernei* in their pedigree (Jongedijk & Ramanna 1988). Polymorphisms for molecular markers were easily found, reflecting the highly heterozygous state of potato. Most RFLP markers (60%) revealed polymorphism in only one parental clone (Table 3), with a similar frequency in clone C and clone E. The rest (40%) of these RFLP markers displayed polymorphism in both parental clones.

T-DNA's and transposable elements. The position in the genome of T-DNA's containing *Ac*, *Ds* or *I* elements, and of transposed *Ac* elements from a large set of independent diploid transformants, was determined. For this purpose potato genomic DNA fragments flanking T-DNA or reintegrated *Ac* transposable elements were isolated and used as probes for RFLP analysis in the mapping population. In total 92 markers consisting of flanking DNA were mapped (Table 3).

Isozyme loci. Seven enzyme systems tested revealed nine segregating isozyme loci in the population of CE clones (Table 1). Four of the nine segregating isozyme loci, *Dia-1*, *Mdh-2*, *Got-2* and *Adh-1*, were heterozygous in clone C, and five isozyme loci, *Got-1*, *6-Pgdh-2*, *Tpi-1*, *Adh-2* and *Aps-2*, were heterozygous in clone E.

Morphological markers. The observed segregation of the morphological traits and their map positions are shown in Table 2. The inheritance of the traits is well documented in literature. Some of the morphological markers were segregating in a 3:1 manner,

suggesting that both parental clones were heterozygous for that particular trait. Test crosses were performed in order to divide the phenotypic classes into their respective genotypic classes. In most cases the observed segregation was in agreement with the expected ratios. The segregations observed at locus *D* and locus *P* did not fit the genetic model (Table 2), but flanking RFLP loci showed a similar segregation distortion.

The *S* locus of gametophytic incompatibility, is known to cause distorted segregation of flanking markers in diploid potato. The genotypes of clone C and E are S_1S_2 and S_2S_3 respectively. In the population of CE clones the genotypes S_1S_3 and S_2S_3 were observed in the expected 1:1 ratio (Table 2). As anticipated, the absence of the S_1S_2 and S_2S_2 genotypes resulted in distorted segregation of markers surrounding this locus. An additional marker known to complicate linkage analysis in this population is *Cr*, resulting in the absence of the *crcr* class of phenotypes. Both parental clones are heterozygous for *Ds1*, a meiotic mutation preventing the development of normal gametes thereby hindering (back)crosses of mutant *ds1ds1* genotypes (Jongedijk *et al.* 1990). The *Ds1* mutation complicates the genetic analysis of all morphological markers that need to be (back)crossed to enable further analysis.

Construction of two independent parental maps

A genetic map was constructed for both parental clones (see Materials and Methods) with the computer programme Joinmap (Stam 1993). This programme, like all commonly available programmes for genetic mapping, was originally devised for inbreeding species. In these species all genetic markers in one population (either F_2 or BC) segregate in the same manner, and genotypes are specified with a single letter code. In non-inbred species like potato, different segregation patterns can be observed at different genetic loci within the mapping population. In addition to "F_2"(3:1 or 1:2:1) or "BC"(1:1) type segregations, 1:1:1:1 segregations are also found. These segregation ratios were redefined as a 1:1 ratio from the female parent and a 1:1 ratio from the male parent.

In our mapping population 116 marker loci with a 1:1 segregation ratio were mapped; 55 were polymorphic in the female parent C, while 61 marker loci were segregating 1:1 from the male parent E. Seventy-seven marker loci were segregating from both parental clones (Table 3). The female map (parental clone C) consists of 132 marker

loci (120 RFLP markers, 8 morphological marker loci, and 4 isozyme loci). The male map (parental clone E) consists of 138 marker loci (127 RFLP markers, 7 morphological marker loci, and 4 isozyme loci). In total 89% (193/218) of the markers that were polymorphic could be assigned to linkage groups.

Distorted segregation in one parental clone (gametic selection) was found in 15% (28/193) of the markers; 6% (8/132) in clone C, 14% (20/138) in clone E (Table 4). Zygotic selection, evident as absence or under-representation of a specific class of genotypes, was analysed in markers segregating from both parental clones and was found in 13% (10/77) of the markers. Loci with distorted segregation ratios mapped to clusters on chromosomes *1*, *2*, *8*, and *11* of the map of clone E, and to chromosome *5* of the map of both clone C and clone E.

Combination of the two parental maps into one genetic map

Once two independent parental maps were constructed, all markers were combined into one basic map. For this purpose, points in common to the two parental maps are essential. These "allelic bridges" (Ritter *et al.* 1990) are the markers polymorphic in both parental clones, mapped in both parental maps, and segregating in a 1:1:1:1 manner. Although markers segregating with 3:1 and 1:2:1 ratios are also polymorphic in both parents, they either mask the actual genotype (3:1) or are indecisive for the parental origin of marker alleles (1:2:1). Consequently they are less informative than the 1:1:1:1 segregating markers, and were therefore not used as allelic bridges.

The construction of the combined map was performed by merging the data sets created for the two parental clones into one input file for JoinMap. The female and the male meiosis leading to gamete formation are independent processes, and the combination of gametes into each offspring is in principal a random process. The data sets resulting from the analysis of the alleles segregating from each individual parent can therefore be regarded as data generated from two independent populations. In this manner three maps were constructed; one for the female parent C, one for the male parent E, and one that combines the data from C and E.

The combined map was made on the basis of markers shared by both parental maps. The parental maps share 77 marker loci, of which 66 marker loci were used as allelic bridges. The map presented in Figure 1 shows how the compilation of the two

Table 4. Loci with distorted segregation.

Chr.	Locus	Expected	Observed				χ^2-value	Selection
		1:1♀ 1:1♂ 1:1:1:1	*A-* *-A* *AA*	 *-C* *AC*	*B-* *BA*	 *BC*		
1	TDs109	1:1 ♂	4	53			22.04***	gametic
1	Ssp50	1:1 ♂	2	44			38.34***	gametic
1	TDs254	1:1 ♂	8	41			22.22***	gametic
2	STF13	1:1♀	32		18		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	10	15	20	10	3.91*	zygotic
4	TDs436	1:1:1:1	13	18	19	9	3.98*	zygotic
4	TDs69	1:1:1:1	11	3	8	14	6.12*	zygotic
5	Ssp72	1:1♀	12		42		16.66***	gametic
5	GP21	1:1♀	21		36		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	0	21	10	15	10.73**	zygotic
5	TDs416	1:1:1:1	9	19	20	14	4.39*	zygotic
6	TDs183	1:1:1:1	8	15	16	9	4.09*	zygotic
7	TG20A	1:2:1	AA:AB:BB = 5:30:8				7.14*	zygotic
7	*Got-2*	1:1♀	42		25		4.31*	gametic
8	GBSS	1:1♀	13		32		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♀	33		15		6.75**	gametic
10	*Cr*	1:2:1	*CrCr:Crcr:crcr* = 12:17:0				10.79**	zygotic
10	TAc13A	1:1♀	15		30		5.00*	gametic
10	TG63	1:1:1:1	6	14	14	8	4.75*	zygotic
11	TG44	1:1 ♂	11	39			15.68***	gametic
11	TG44	1:1:1:1	26	1	12	10	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

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

separate maps from the parental clones C and E was performed. Substantial differences in marker order between the female and male maps of some chromosomal regions were found which made the combining of the maps of clone C and E more complex for chromosomes *5* and *10*.

Discussion

RFLP maps of the potato genome have been constructed by two other groups to date. One of these maps is based on potato RFLP markers in an intraspecific cross of *S. tuberosum* (Gebhardt *et al.* 1989b), while the other used tomato RFLP markers in interspecific crosses of *S. tuberosum* and wild species (Bonierbale *et al.* 1988; Tanksley *et al.* 1992; Bonierbale *et al.* 1994). Following mutual exchange of a limited number of markers from each map, and their subsequent mapping using the other population, a consensus numbering of the linkage groups was devised (Gebhardt *et al.* 1991). In the construction of the map presented in this study, a set of these RFLP markers was used. As a consequence the chromosome numbering in our map is in accordance with the previously published maps.

Segregation analyses

The mapping population of diploid potato plants was obtained from a backcross of clone C x clone E (E = clone C x $VH^3$4211). A drawback of using a backcross population from non-inbred parents is the presence of (sub)lethal loci in both parental clones that are identical by descent, which can result in distorted segregation in the mapping population. Indeed Jongedijk *et al.* (1990) found that both clone C and E are heterozygous for the loci *Cr* (=crumpled), and *Ds1* (=desynapsis), complicating the interpretation of segregation analysis in the population. Plants homozygous recessive for *cr* have a poor growth, and only rarely set tubers. Consequently the homozygote *crcr* genotypes were lost from the mapping population over the course of repeated vegetative propagation via tuber production. Therefore, instead of the expected 1:2:1 segregation ratio for *CrCr*:*Crcr*:*crcr*, only *Cr*- phenotypes were observed. The genotypes *CrCr* and *Crcr* can not be distinguished phenotypically. The mapping of this trait therefore relied entirely on progeny testing of the 29 CE clones that could be backcrossed to both parents. The meiotic mutation desynapsis (*ds1*; Jongedijk & Ramanna 1988), was mapped to chromosome *8*. Although *ds1ds1* genotypes are viable plants in the population of CE clones, they do not produce viable gametes. Therefore,

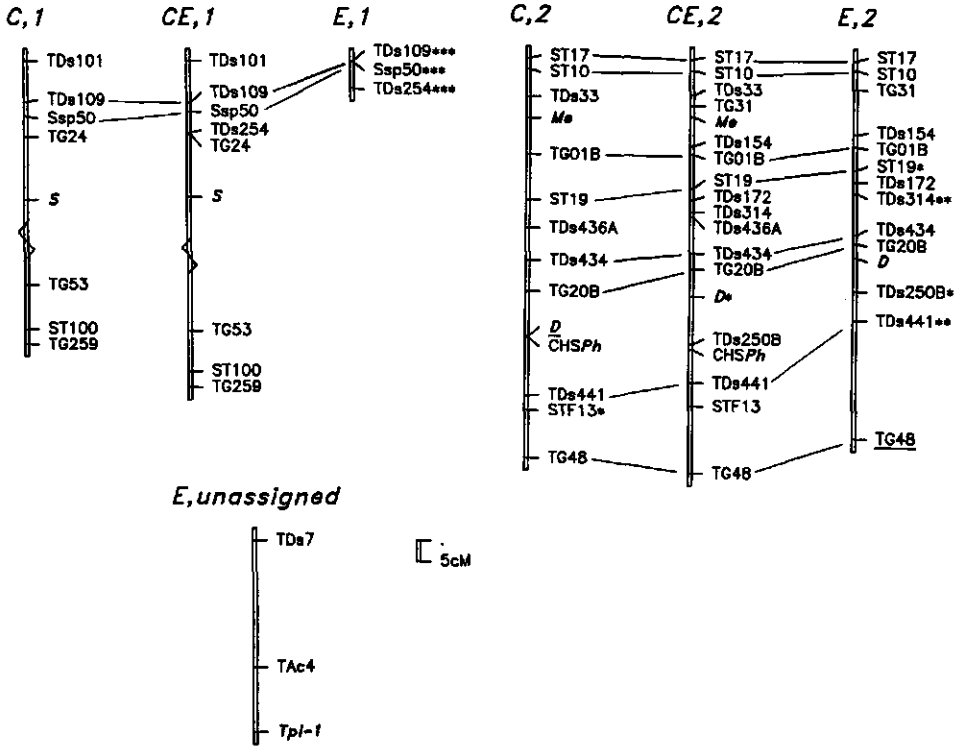
backcrosses for the distinction between homozygosity and heterozygosity of morphological traits with a dominant phenotype (*eg. Cr-*), could not be performed with these desynaptic plants. For the desynapsis mutant proportion of the mapping population, the actual genotypes of several dominant morphological traits therefore remained undetermined.

The position of the *S* locus on chromosome *1*, determined by analysis of stylar glycoproteins was in agreement with the mapping of an *S* locus cDNA (Gebhardt *et al.* 1991). The expression of gametophytic self-incompatibility in potato at the diploid level affects segregation of flanking markers. Therefore the observed distortion in the segregation of markers on chromosome *1* of clone E (Figure 1) is as expected and explains the poor development of the chromosome *1* map from clone E. Success or failure of backcrosses also corresponded with the expression of the *S* alleles.

Several other genomic regions of distorted segregation were observed on chromosomes *2* (clone C), *5* (clone C and E), and *11* (clone E). Given the high genetic load and marked inbreeding depression of potato (Howard 1970), we presume that other unidentified (sub)lethal loci are involved.

The occurrence of non-viable individuals homozygous for (sub)lethal loci that are identical by descent, would result in the absence of one class of genotypes (zygotic selection) and thereby produce segregation distortion. Distorted segregation can also involve only one parental clone (gametic selection). In clone E we observed that the loci with distorted segregation, with the exception of chromosome *1* markers surrounding the *S* locus, were inherited from the non-recurrent parent. This is for

Figure 1. Integrated genetic linkage map of potato from the backcross population C x E. For each chromosome the female (C), male (E) and combined (CE) map are given. Linkage groups are established with LOD > 3.0. Chromosomes were constructed with a LOD > 3.0, with the exception of chromosome *1* C (LOD= 0.41), chromosome *8* E (LOD= 1.44), and chromosome *12* C (LOD= 0.50). Individual markers mapped with a LOD < 3.0 are underlined. Marker names are described in Material and Methods. Isozymes and classical genetic markers are in italics, their abbreviations are described in Tables 1 and 2. Distorted segregation is indicated with asterisks following the marker name (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Markers used as allelic bridges are interconnected with solid lines between the parental and combined chromosome maps. Chromosome numbering and orientation is according to Gebhardt *et al.* (1991).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TG24
S
TG53
ST100
TG259
TDs109***
Ssp50***
TDs254***
C,2
CE,2
E,2
ST17
ST10
TDs33
Me
TG01B
ST19
TDs436A
TDs434
TG20B
D
CHSPh
TDs441
STF13*
TG48
TDs154
TDs172
TDs314**
TDs250B*
TDs441**
E,unassigned
TDs7
5cM
TAc4
Tpl-1
C,3
CE,3
E,3
TG08B
TG130
Y
TDs258
TI5-9
Ssp66
TG42
TDs83A
TDs383
TDs382
TG134
TI11
TAc13B
TDs369
TDs258*
TDs381
C,4
CE,4
E,4
TG123
Ac2-109B
TDsG60
TDs436
TDs168
Ac11-13
Ac8-22
Ac8-19
Ac8-99
Ac11-17
TDs491
TI31
TDs69
TI17
BE
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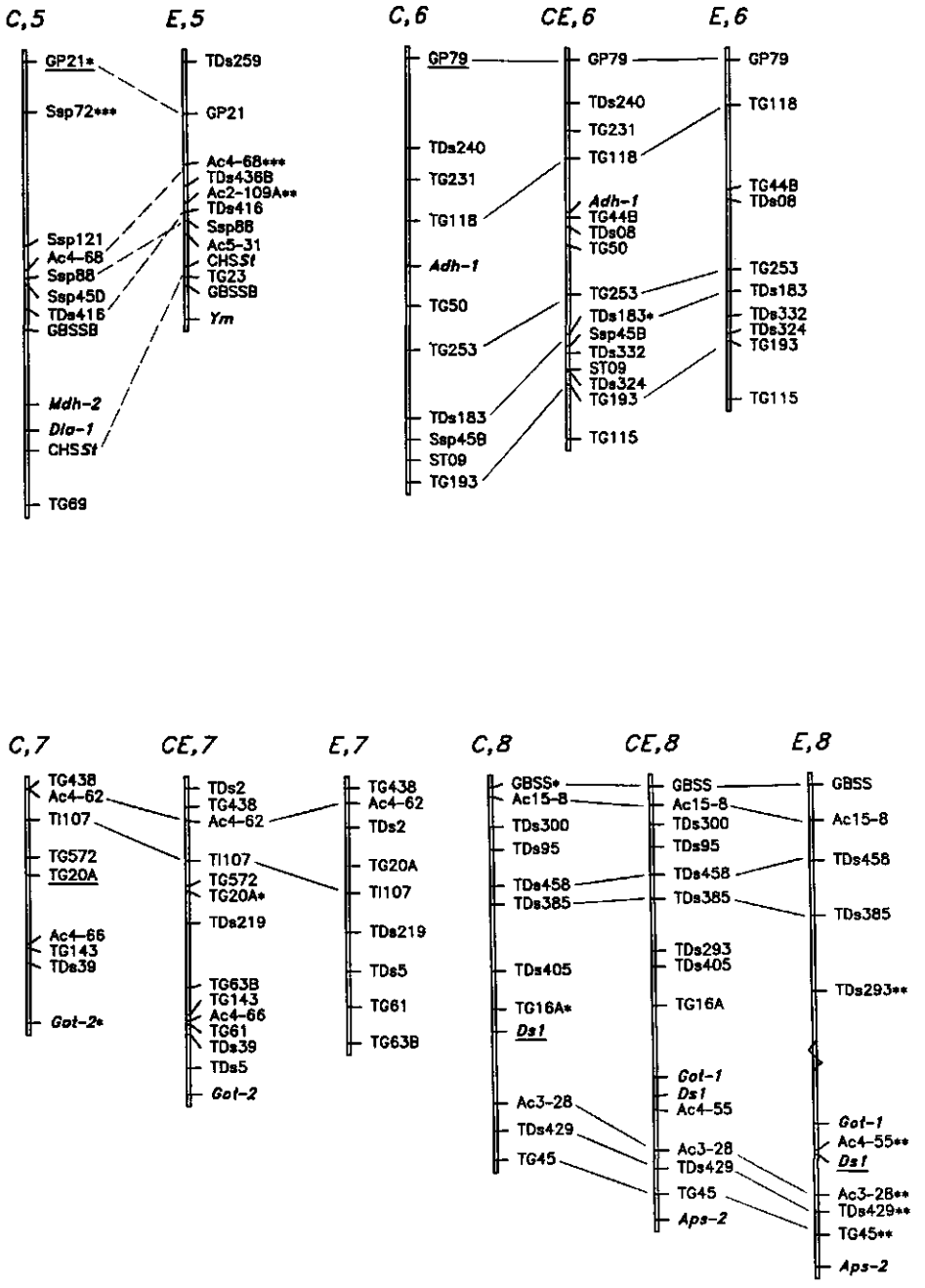
ST12
TAc11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GP21*
Ssp72***
Ssp121
Ac4-68
Ssp88
Ssp45D
TDs416
GBSSB
Mdh-2
Dia-1
CHSSt
TG69
TDs259
GP21
Ac4-68***
TDs436B
Ac2-109A**
TDs416
Ssp88
Ac5-31
CHSSt
TG23
GBSSB
Ym
C,6
CE,6
E,6
GP79
TDs240
TG231
TG118
Adh-1
TG50
TG253
TDs183
Ssp45B
ST09
TG193
GP79
TDs240
TG231
TG118
Adh-1
TG44B
TDs08
TG50
TG253
TDs183*
Ssp45B
TDs332
ST09
TDs324
TG193
TG115
GP79
TG118
TG44B
TDs08
TG253
TDs183
TDs332
TDs324
TG193
TG115
C,7
CE,7
E,7
TG438
Ac4-62
TI107
TG572
TG20A
Ac4-66
TG143
TDs39
Got-2*
TDs2
TG438
Ac4-62
TI107
TG572
TG20A*
TDs219
TG63B
TG143
Ac4-66
TG61
TDs39
TDs5
Got-2
TG438
Ac4-62
TDs2
TG20A
TI107
TDs219
TDs5
TG61
TG63B
C,8
CE,8
E,8
GBSS*
Ac15-8
TDs300
TDs95
TDs458
TDs385
TDs405
TG16A*
Ds1
Ac3-28
TDs429
TG45
GBSS
Ac15-8
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TDs293
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Got-1
Ds1
Ac4-55
Ac3-28
TDs429
TG45
Aps-2
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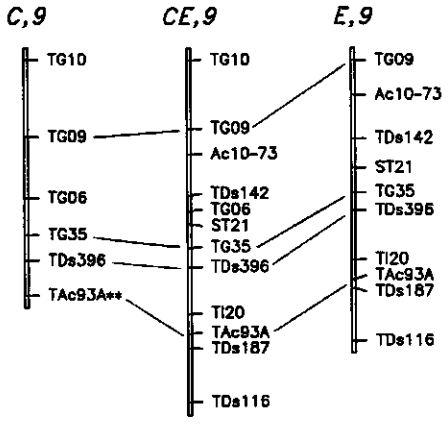
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TG09
TG06
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TAc93A**
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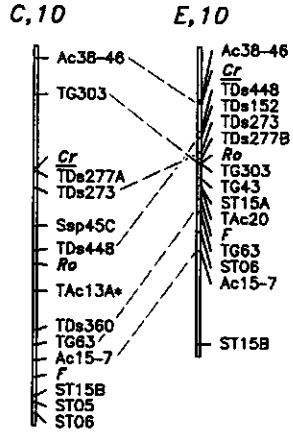
TDs187
TDs116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TG303
Cr
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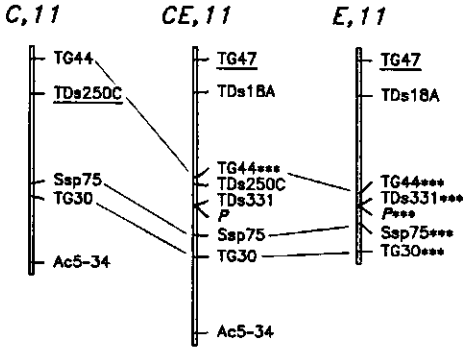
Ro
TAc13A*
TDs360
TG63
Ac15-7
F
ST15B
ST05
ST06
Ac38-46
Cr
TDs448
TDs152
TDs273
TDs277B
Ro
TG303
TG43
ST15A
TAc20
F
TG63
ST06
Ac15-7
ST15B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CE,11
E,11
TG44
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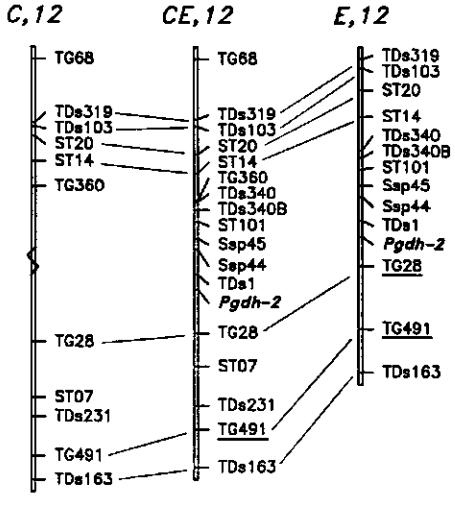
Ac5-34
TG47
TDs18A
TG44***
TDs250C
TDs331
P
Ssp75
TG30
Ac5-34
TG47
TDs18A
TG44***
TDs331***
P***
Ssp75***
TG30***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E,12
TG68
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ST14
TG360
TG28
ST07
TDs231
TG491
TDs163
TG68
TDs319
TDs103
ST20
ST14
TG360
TDs340
TDs340B
ST101
Ssp45
Ssp44
TDs1
Pgdh-2
TG28
ST07
TDs231
TG491
TDs163
TDs319
TDs103
ST20
ST14
TDs340
TDs340B
ST101
Ssp45
Ssp44
TDs1
Pgdh-2
TG28
TG491
TDs163

instance observed for chromosome *11* markers from clone E (Table 4). In the bottom distal region of the chromosome, alleles of the non-recurrent parent are under-represented (*eg. P*-locus; expected segregation 1:1, observed segregation 57:10).

Earlier genetic mapping work in potato has shown the same phenomenon. Bonierbale *et al.* (1988) found similar distorted segregation in several regions of the genome. Gebhardt *et al.* (1989b; 1991) also reported distorted segregation on several chromosomes, with chromosome *1*, displaying self-incompatibility, as an extreme example. Kreike (1995) detected distorted segregation as a result of gametic selection on all chromosomes, while zygotic selection against homozygous genotypes was found on chromosomes *2*, *3*, and *4*.

Mapping of molecular markers

The segregation analysis and mapping of RFLP markers was facilitated by the fact that the mapping population is a backcross. As a consequence, the parental clones C and E should have at least one shared allele at every locus, irrespective of the mode of segregation. Linkage analysis and map construction were straightforward in most cases, and turned out to be troublesome only in those regions of the parental genomes that show distorted segregation. A strong under-representation, or even absence, of some genotypic classes often suggested non-existent linkage to other markers with distorted segregation.

Mapping of (re)integrations of T-DNA and transposable elements

The positions of the loci representing T-DNA integration sites were evenly spread over the entire potato genome. No preference for particular chromosomes and/or chromosomal regions for the integration of T-DNA's was found. However, similar to observations in other Solanaceous species (Osborne *et al.* 1991), transposition of *Ac* in potato is mainly to closely linked sites, around the initial T-DNA integration site (Jacobs *et al.* 1994). An example is the lower region of chromosome *4*, showing Ac11-13 and Ac11-17 close to the initial TAc11 integration site, and Ac8-19, Ac8-22, Ac8-80, Ac8-99 clustering around the TAc8 integration site (the latter was not polymorphic in the mapping population, but mapped close to TAc11 in a different population). These results confirm the earlier observation that the maize *Ac-Ds* transposable element system is functional in potato (Knapp *et al.* 1988), and has the potential to be used for the tagging of genes of interest (Walbot 1992).

Mapping of isozyme markers

Most isozyme loci reported here have been mapped previously to similar genomic positions in either tomato (Tanksley & Loaiza-Figueroa 1985; Tanksley *et al.* 1992) or potato (Bonierbale *et al.* 1988; Tanksley *et al.* 1992). Mapping of isozyme loci in potato, was also performed by Douches & Quiros (1987; 1988a; 1988b), but no chromosome numbers could be assigned to the respective linkage groups found.

The numbering of the isozyme loci on our map is not always the same to that previously used in potato and/or tomato. Normally, 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}. The electrophoretic mobility however, is highly influenced by the method of separation used. This can affect the nomenclature and makes comparison with earlier work complicated. Second, locus names can be assigned on the basis of earlier studies in potato. This sometimes gives rise to contradictions with tomato literature, *eg.* the ADH loci (Table 1). The potato *Adh-2* locus and the tomato *Adh-1* (chromosome *4*) are both expressed in pollen, whereas potato *Adh-1* and tomato *Adh-2* (chromosome *6*) are expressed in roots and tubers (Martinez-Zapater & Olivier 1985; Douches & Quiros 1988b). Third, loci can also be named on basis of analogy to their map position on the tomato genome, as applied by Bonierbale *et al.* (1988) and Tanksley *et al.* (1992). We decided to be consistent with previous studies and wherever possible numbered the loci in analogy with the tomato map position. Until now, three *6-Pgdh* loci are mapped in tomato (Tanksley & Loaiza-Figueroa 1985) and one in potato (Bonierbale *et al.* 1988). The locus we mapped on chromosome *12* was indicated with *6-Pgdh-2*, in agreement with previous mapping studies, but disregarding its relative mobility. The same argument applied to the locus *Dia-1* mapped on chromosome *5*. The *Got-1* locus in this mapping population, previously studied by Jongedijk *et al.* (1990), mapped at about the same position as the potato locus *Got-4* in Bonierbale *et al.* (1988), which was named after the equivalent tomato locus. Allelism between potato *Got-1* and the tomato *Got-4* locus, is also suggested by recent studies using potato-tomato somatic hybrids (Jacobsen *et al.*, data not shown).

Mapping of classical markers

Morphological markers *F*, *D*, *P*, and *Ro*, analysed in this mapping population, were described before. In the case of the flower colour loci *F*, *D*, and *P*, crosses to tester

clones were performed to determine the exact genotype of the clones of the mapping population (Van Eck *et al.* 1993). For the tuber shape locus *Ro*, mapped on chromosome *10*, a qualitative and a quantitative approach was used. The latter revealed three different alleles, and enabled us to identify the *ro* allele that was identical by descent. The phenotypic classes could be sorted according to their flanking marker genotype, and multiple alleles could be assigned and analysed (Van Eck *et al.* 1994a). To date, gene mapping in potato has resulted in the localisation of only a few other morphological traits. The localisation of locus *Y*, involved in yellow tuber flesh colour, on chromosome *3* (Bonierbale *et al.* 1988) was confirmed by our results. The localisation of the purple skin colour (*PSC*) locus, involved in pigmentation of the tuber skin, on chromosome *10* (Gebhardt *et al.* 1991) was indirectly 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 & Long (1956), and linkage of tuber shape and skin colour was reported by De Jong & Rowe (1972). Recently the mapping of tuber skin colour loci on chromosome *10* was reported (Van Eck *et al.* 1994b).

Four additional morphological markers were localised on the genetic map of potato. Locus *Me* (metribuzin resistance) was mapped on chromosome *2*. The *Ym* locus was mapped on chromosome *5*. This is not in agreement with the location by means of trisomic analysis of this mutant phenotype on chromosome *12* (Wagenvoort 1982). This may be explained by assuming different chromosomal mutations causing the same mutant phenotype. A more likely explanation may be the highly similar morphology of potato chromosomes *5* and *12* in pachytene stage, which complicates trisomic identification (Ramanna & Wagenvoort 1976). Although we could only determine the *Ym* genotype for 30 CE clones, the mapping we report was accurately determined with the help of a large number of molecular markers. We therefore propose that the actual map position of *Ym* is on chromosome *5*. A linkage group consisting of the loci *Ds1*, *Got-1*, and *Cr* was earlier proposed by Jongedijk *et al.* (1990). They assumed that the segregation distortion they observed at the *Ds1* locus was the result of selection at the *Cr* locus. However, this study localised the *Ds1* and *Got-1* loci on chromosome *8*, whereas the *Cr* locus was mapped on chromosome *10*.

Integration of classical and molecular markers in one map

The genetic map of potato reported in this study has several features that clearly

distinguish it from previous genetic maps of potato and other outbreeding species. All segregation analyses, involving molecular, isozyme and morphological markers were performed in the same population. As a result, classical marker loci did not need to be mapped to approximate positions by using independent estimates from other populations. This allowed the map position of the classical markers to be determined more accurately. Due to the non-inbred nature of potato and the resulting high level of heterozygosity, segregation of markers from both parental clones was observed and could be exploited for linkage analysis. This maximised the mapping information obtained in one single population, and has led to the construction of a more extended basic map.

Construction of separate maps for the two parental clones

Initially two genetic maps, one for each parental clone, were constructed (see Materials and Methods). Most markers could be assigned to linkage groups with a LOD > 3.0 (Figure 1). In both clone C and clone E, 14 linkage groups were found. In clone C, these could be combined (LOD < 3.0, Figure 1) to 12 chromosomes by joining the two linkage groups found for the chromosomes *1* and *12*. In clone E chromosome *8* could be composed in a similar manner, leaving one unassigned linkage group. This linkage group remains unassigned since markers shared with other genetic maps of potato and tomato are lacking.

The basic map is composed from two maps

The genetic maps of potato published before were constructed in a different manner. Bonierbale *et al.* (1988) used a population originating from a cross of an interspecific hybrid to a third potato species. For a vast majority of the markers, the segregation data were collected from one parent, the interspecific hybrid. The use of such a hybrid to obtain mapping data, is most likely the reason why limited recombination was observed and the map had a small overall length (606 cM). The genetic map of potato by Tanksley *et al.* (1992) is based on the backcross of an interspecific hybrid (*S. tuberosum* x *S. berthaultii*) to *S. tuberosum*. The length of the map is also relatively short (684 cM). Unfortunately the actual manner in which the map was constructed, was not described. Gebhardt *cs.* (Gebhardt *et al.* 1991; Leonards-Schippers *et al.* 1994; Gebhardt *et al.* 1994) used an intraspecific mapping population, and analysed the segregation from both parents. As in this study, they used a backcross population. They divided their segregation data into three separate classes; alleles of markers

segregating from the female parent, alleles of markers segregating from the male parent, and alleles of markers segregating from both parents. Three separate maps were constructed, which were then aligned on basis of the markers present in all three maps. Their map is larger (1034 cM) than the Bonierbale *cs.* maps, most likely because they made use of an intraspecific cross. This is in accordance with the map length we found (1120 cM), using a cross that involved mainly *S. tuberosum* germplasm.

Markers showing polymorphism in both parental clones are essential in combining the two parental maps into one basic map. If two markers are segregating from both parents, two independent estimates for the marker pair can be determined. The computer programme JoinMap, especially devised for integration of mapping data from several sources, can combine both estimates for the construction of a combined map. An increase in the number of markers segregating from both parents, therefore increases the accuracy of the final combined map.

When using JoinMap to combine the two parental maps into one, marker orders sometimes differed from the original parental maps. The reason for this order difference results from the way JoinMap constructs the combined map. The recombination frequency between two loci, heterozygous in both parental clones, corresponds to the weighted average of the recombination frequencies between these loci in the separate clones. The shorter interval in one parent will be stretched, while the longer interval in the other parent will be compressed. However, parental differences in recombination frequencies are averaged only at the intervals between shared markers. In a few cases, this stretching/compressing effect caused different marker orders in the combined map. For example on the parental maps of chromosome *11*, the intervals between TG44 and Ssp75 are highly divergent. The resulting average, caused TDs250C to be mapped outside the TG44-Ssp75 interval in the combined map. However, from the data in clone C it was evident that the correct order was TG44-TDs250C-Ssp75. This type of artefact could be corrected using the JoinMap option "fixed sequences".

Marker orders between the female and male chromosomes were consistent, with the exception of chromosomes *4*, *5* and *10*. This made the combination of these chromosomes difficult. Clustering of markers in the lower region of chromosome *4*

complicated the construction of a combined map that was consistent with both parental maps. The differences in marker order are in this case due to minor random differences between the data sets of the two parental clones. Since the female and male marker orders in this cluster have a similar likelihood, we chose to represent chromosome *4* in the separate maps with slightly different orders. After re-examination of the data for chromosomes *5* and *10*, we concluded that the different marker orders were not due to scoring errors nor minor random differences between the data sets of the two parental clones. The most likely explanation for these marker order differences are chromosomal rearrangements. As a result, combined maps of chromosome *5* and *10* would be artificial and were therefore omitted from Figure 1. The combined map of chromosome *7* was less informative than the parental maps with regard to the relative marker order. This is because shared markers in the female and male map of chromosome *7* are limited to one end of the chromosome.

The use of a backcross population of non-inbred parents for mapping analyses, displayed an unexpected characteristic. The shared allele could not only be used as an internal check for accuracy of the segregation analysis, it also helped to order markers. In regions with high marker density, it is sometimes difficult to determine the correct marker order. However, when graphical genotypes (Young & Tanksley 1989) are made of the parental clones, on the basis of the marker order determined in the mapping population, additional information became evident. When comparing the shared alleles from clone C and clone E, we observed changes along the chromosomes, resulting in differences between clones C and E. Markers in coupling phase in clone E were not always in coupling phase in clone C. In other words, at some points a "jump" of shared alleles was observed, from one sister-chromatid of clone C to another sister-chromatid of clone C. Presumably this was due to a cross-over event that took place during the meiosis that gave rise to the gamete originating from clone C, incorporated in clone E. If, by chance such a cross-over had taken place amongst a cluster of markers, it made the ordering of the marker cluster easier. The order was determined by assuming a minimal amount of double-recombinants from clone C to clone E. It was often found that different orders within clusters only slightly differed in probability. When an order could be determined with the help of this "shared allele jump", it was regarded as more reliable, even if the probability of another marker order was slightly higher. In this way we could determine that approximately 25 cross-overs were contributed from clone C to the formation of the

gamete incorporated in clone E.

This basic genetic map of potato is being used as a reference framework for several goals. These include the mapping of resistance genes in populations other than the mapping population (Jacobs *et al.* 1995b), and the mapping of quantitative trait loci (Van Eck *et al.* in preparation). A basic genetic map, having the maximal possible content of mapping information integrated from distinct sources of markers and from several parental clones, is a major tool for further genetic analyses, whether it be the identification and selection of important agronomical traits, or resolving basic genetic questions.

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Chapter 2

Application of RFLP maps in heterologous transposon tagging in potato.

(with: A. El-Kharbotly, B. te Lintel Hekkert, E. Rutgers, M. Ramanna, E. Jacobsen, W.J. Stiekema, A. Pereira. Parts of this chapter have been described before in; Pereira *et al.* 1992; Jacobs *et al.* 1994; El-Kharbotly *et al.* 1995b.)

Abstract A strategy for targeted transposon tagging of disease and pest resistance genes from potato is described. This involves the use of maize transposable elements and consists of a two-step approach. In the first step, potato genotypes with transposable elements inserted near a gene of interest are identified. This is followed by the activation of the transposon and the screening of mutant phenotypes. The maize *Ac-Ds* transposable element system was shown to be functional in potato. Like in other Solanaceous species, *Ac* transposes mainly to closely linked sites. Therefore a transposable element closely-linked to a gene of interest is useful for subsequent tagging and isolation of that gene. Diploid potato clones were used for transformation with T-DNA constructs containing maize transposable elements (*Ac*, *Ds*, *I*). The position of the integration sites of these T-DNA constructs and transposons in the potato genome was determined. To enable this mapping, genomic DNA sequences flanking either the T-DNA inserts or reintegrated transposable *Ac* elements originating from these T-DNA constructs, were isolated with IPCR and plasmid rescue. These flanking sequences were used as probes for RFLP analysis in a mapping population of 67 diploid potato clones. The map positions of 94 flanking sequences were found to be spread over the entire potato genome.

Several transposon transformants were identified with an insertion located in a genomic region close to a resistance gene. The focus of our tagging research is chromosome *5*, because several disease resistance loci have been mapped to this chromosome. Transformants heterozygous for *R1* resistance, with T-DNA insertions on chromosome *5*, are being used for further crosses in order to recombine the transposon in coupling phase with the gene of interest. These recombinant genotypes will be retransformed with a transposase source to permit efficient transposon tagging of the *R1* gene.

Introduction

Transposons are mobile pieces of DNA that can cause a phenotypically visible mutation when inserted into or near a gene. With the help of the known DNA sequence of the transposon tag, the DNA sequence adjacent to the insertion site can be isolated. This sequence can then be used in the isolation of the complete wild type gene. This technique of gene isolation is called transposon tagging and is especially suited for the isolation of genes which have an easy screenable mutant phenotype. To select for mutations at a specific locus, a large number of individuals carrying mobilised transposons and heterozygous for the specific target locus (*A/a*) is screened. The mutant -transposon tagged- phenotype will become apparent as the rare individual in the population in which a transition to the mutant phenotype (conversion of *Aa* to *aa*) has taken place.

This method has been particularly successful in plant species like maize and *Antirrhinum*, that contain well-characterised endogenous transposons (see review by Walbot 1992). Some of the tagged mutants in maize were obtained by targeted tagging (*Ts2*, DeLong *et al.* 1993; *R-nj*, Dellaporta *et al.* 1988), using transposons closely linked to the locus. This transposon tagging approach, with the transposon targeted to the gene of interest, is more efficient because transposable elements are reported to jump preferentially to neighbouring positions in the genome. For example, in the well-characterised maize *Ac-Ds* transposable element system (McClintock 1951), it has been clearly established that *Ac* transposes to closely linked chromosomal positions (Greenblatt 1984). Consequently, transposon insertion frequencies as high as 10^{-3} to 10^{-4} have been obtained in maize with transposons linked to a target gene, whereas unlinked transposons resulted in lower insertion frequencies (10^{-5} to 10^{-6}; Döring 1989). In recent years the efficiency of obtaining mutants has been dramatically increased using these closely linked transposons. For example, the frequency for obtaining a *Ts2::Ac* tagged tasselseed mutant was 1/2,000 (DeLong *et al.* 1993), using an *Ac* located 2 cM from the *Ts2* locus (Walbot 1992). With such high frequencies it is also possible to efficiently tag and isolate genes which have more difficult phenotypes for screening, and may even be possible for quantitative traits.

The maize *Ac-Ds* and *En-I* transposable element systems also function in heterologous plant species, following their introduction by transformation (Baker *et al.* 1986; Pereira & Saedler 1989). This has resulted in the transposon tagging of a number of

genes based on a random (non-targeted) approach using selfed progeny for mutant selections in *Arabidopsis* (Aarts *et al.* 1993; Bancroft *et al.* 1993), and *Petunia* (Chuck *et al.* 1993). More recently, the *Ac* element has been used for targeted tagging of the *N* gene from tobacco (Whitham *et al.* 1994) and the L^6 gene from flax (Ellis *et al.* 1995). Very efficient tagging (1/4,000) has been documented for the *Cf-9* gene in tomato (Jones *et al.* 1994), using a line containing a *Ds* transposon at a genetic distance of 3 cM of the target gene.

In potato, both the maize transposon systems *Ac-Ds* (Knapp *et al.* 1988; Pereira *et al.* 1991) and *En-I* (Frey *et al.* 1989) have been shown to be active. This makes potato amenable for transposon tagging with these heterologous transposons, and offers an opportunity for the isolation of genes controlling important traits. With the application of DNA markers for RFLP analysis in potato diploids, detailed genetic maps have been obtained (Bonierbale *et al.* 1988; Gebhardt *et al.* 1989b; Jacobs *et al.* 1995a). In addition, a number of disease resistance loci have been localised on the potato genetic map (*e.g.* Barone *et al.* 1990; Ritter *et al.* 1991; Leonards-Schippers *et al.* 1992; Pineda *et al.* 1993; Gebhardt *et al.* 1993; El-Kharbotly *et al.* 1994; Kreike *et al.* 1994; Jacobs *et al.* 1995b). Such disease resistance loci without a characterised gene product, but giving rise to a phenotype that can be easily screened, are suitable targets for transposon tagging.

We describe here the development of a series of potato genotypes with transposable elements inserted at different sites in the genome. Mapping of the insertion sites of T-DNA constructs containing *Ac*, *Ds*, or *I* elements established that the entire potato genome is covered. This set of transformants, with transposable elements at a known position, can therefore serve as a start for targeted tagging of any locus in the potato genome.

Materials and Methods

Transposon constructs

A. The pHN::Ac construct (Figure 1A) is derived from the binary vector pGDW31 (Wing *et al.* 1989). The vector has a nos promoter-HPT gene conferring hygromycin resistance (20 μg/ml), as a selectable marker for plant transformation. pHN::Ac also contains the NPTII gene (kanamycin resistance, 100 μg/ml) as a phenotypic excision assay for transposition of *Ac* (Baker *et al.* 1987).
B. The pGBSS::Ac construct used is shown in Figure 1B. The NPTII gene was used as selectable marker for transformation (kanamycin resistance, 100μg/ml). Waxy variegation (GBSS gene) was

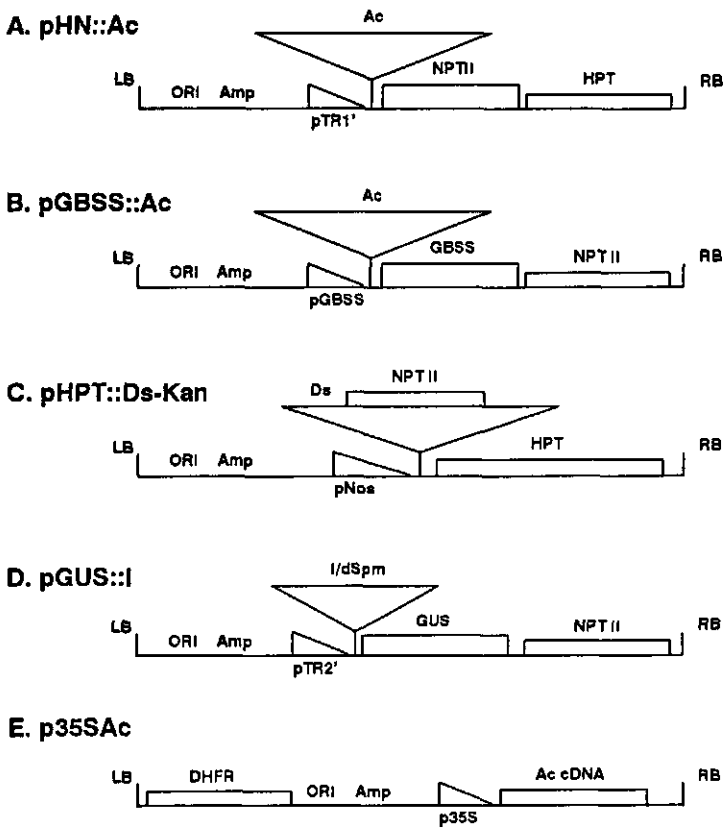


Figure 1. Schematic drawing of the T-DNA::transposon constructs used in transposon targeting. **A.** Construct pHN::Ac. The autonomous *Ac* element with 60bp of flanking DNA (Baker *et al.* 1987) is inserted between the TR1' promoter and the NPTII gene. The HPT gene is used for selection of transformants. **B.** Construct pGBSS::Ac. Excision of the *Ac* element from the GBSS gene provides a 'waxy' variegation phenotype (Pereira *et al.* 1991). The NPTII gene is used for selection of transformants. **C.** Construct pHPT::Ds-Kan. The Ds-Kan element is inserted between the nopaline synthase promoter and the HPT gene. The NPTII gene is used for selection of transformants. **D.** Construct pGUS::I. The *I* element is inserted between the TR2' promoter and the GUS gene. The NPTII gene is used for selection of transformants. **E.** Construct p35SAc, used for transactivation of non-autonomous *Ds*. The DHFR gene is used for selection of transformants. Abbreviations: LB / RB = Left and Right Borders of the T-DNA constructs; NPTII = neomycin phosphotransferase gene of transposon Tn5; HPT = hygromycin phosphotransferase gene; GBSS = granule-bound starch synthase gene; GUS = ß-glucuronidase gene; DHFR = dihydrofolate reductase gene; nos = nopaline synthase promoter.

used as phenotypic excision assay of *Ac* from the pGBSS::Ac construct. The waxy variegation was monitored by the blue staining of tuber tissue with iodine (Pereira *et al.* 1991).
C. The construction of the *Ds* plasmid pHPT::Ds-Kan (Figure 1C) is described by Pereira *et al.* (1992). The NPTII gene (kanamycin resistance, 100 μg/ml) was used as selectable marker for transformation. Construct pHPT::Ds-Kan contains the non-autonomous element *Ds*, that needs transactivation from *Ac* transposase in order to be mobilised. The HPT gene (hygromycin resistance, ≥20 μg/ml) can be used to monitor excision of *Ds*.
D. Construct pGUS::I (Figure 1D) contains a NPTII gene for selection of transformation (kanamycin resistance, 100 μg/ml). pGUS::I contains the non-autonomous element *I*, that needs transactivation from *En* transposase for excision. ß-glucuronidase activity (GUS gene) can be used to monitor excision of *I*.
E. The p35SAc construct harbours the *Ac* cDNA, for expression of transposase, under regulatory control of the 35S-CaMV promoter (Figure 1E). p35SAc was used to transactivate *Ds* elements. A DHFR (dihydrofolate reductase) gene was used as marker for (re)transformation (Eichholtz *et al.* 1987), by selecting for methotrexate resistance (0.15 μg/ml).

Plant material and transformation
The diploid potato clones 87.1017-5 and 87.1024-2 (Jacobsen *et al.* 1989) and J92-6400-A16 (El-Kharbotly *et al.* 1995a) were propagated in vitro using stem segments of 1-2 nodes. These potato clones were used in this study because of their high fertility, which was important to enable further crosses essential for transposon tagging of loci. In addition clone J92-6400-A16 harbours the *R1* locus for resistance to *Phytophthora infestans* in a heterozygous state. The *Agrobacterium tumefaciens* strain GV3101(pMP90RK) (Koncz & Schell 1986) was used for transformation as previously described (Visser *et al.* 1989). Potato clones 87.1024-2 and 87.1017-5 were transformed with the recombinant binary vectors pHN::Ac and pGBSS::Ac respectively. Potato clone J92-6400-A16 was used for transformation with either pHPT::Ds-Kan or pGUS::I. About 400 independent diploid potato transformants containing *Ac*, *Ds*, or *I* T-DNA inserts were obtained. A selection of J92-6400-A16 transformants with localised T-DNA::Ds position were retransformed with the *Ac* transposase construct p35SAc (El-Kharbotly *et al.* 1995b).

Isolation of T-DNA and transposable element flanking sequences
Potato genomic DNA sequences flanking the integration sites of either T-DNA constructs containing maize transposable elements, or reintegrations of the *Ac* element in the potato genome, were isolated by the inverse polymerase chain reaction (IPCR; Triglia *et al.* 1988). For IPCR, miniprep plant genomic DNA was isolated (Dellaporta *et al.* 1983) and primers near the T-DNA borders, or end of the *Ac* transposable element were used (Figure 2). The isolated IPCR fragments were cloned into the *Eco*RV site of pBluescript SK$^+$ (Stratagene) and the identity of the clones was confirmed by Southern hybridisation using T-DNA/*Ac* border probes.

For the T-DNA constructs containing a *Ds* or *I* element, the flanking potato DNA sequences were obtained via plasmid rescue by electroporation (Dower *et al.* 1988). For plasmid rescue, the pBR322 *ori*-sequences and the ampicillin resistance gene present in the left border end of the T-DNA construct were used. Restriction enzymes used were *Hin*DIII (*Ds*) as well as *Eco*RI (*Ds*, *I*).

RFLP mapping and linkage analysis
T-DNA/*Ac* flanking genomic DNA probes were used for RFLP analysis in a potato population of 67 diploid clones (Jacobs *et al.* 1995a). This mapping population was used for the construction of an integrated genetic map and contains molecular (RFLP) as well as classical (morphological and isozyme) markers. A set of tomato genomic DNA probes with known chromosomal positions in

potato (Bonierbale *et al.* 1988) was used to enable comparison with existing maps of potato (Gebhardt *et al.* 1991) and tomato (Tanksley *et al.* 1992). Segregation of markers in either one of both parental clones can be used, because independent maps of both parents are present. Linkage analysis, mapping, and subsequent integration of the two parental maps into one was performed with the computer programme JoinMap (Stam 1993) as described previously (Jacobs *et al.* 1995a). Map diagrams were made using the programme Drawmap (Van Ooijen 1994).

The nomenclature of the transposon markers is as follows: TDs*X*, plasmid rescue derived probe from T-DNA containing *Ds* transposable element; TAc*X*, IPCR derived probe from T-DNA containing *Ac* transposable element; TI*X*, plasmid rescue derived probe from T-DNA containing *I* transposable element; Ac*X*-*Y*, IPCR derived probe from transposed *Ac* originating from TAc. *X* = original transformant number, *Y* = transposed *Ac* number.

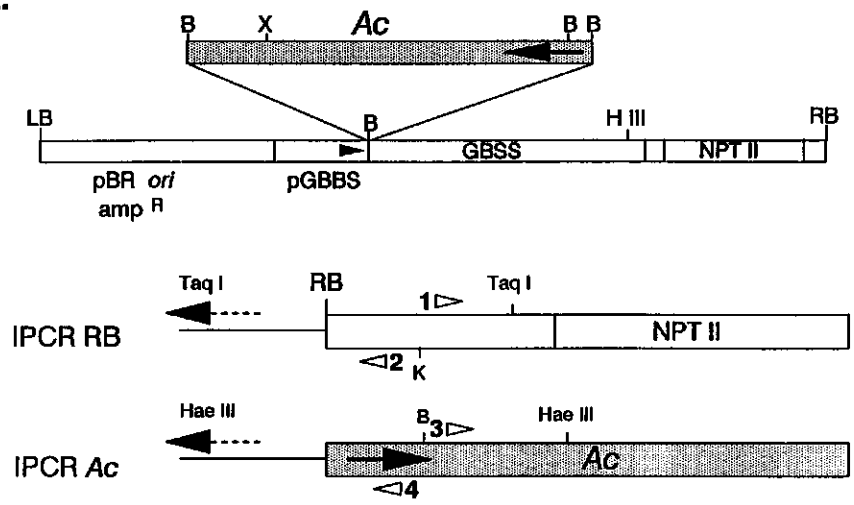
Results

Activity of maize transposable elements in the potato genome.

We have produced a large number of independent diploid potato transformants containing one of the various transposon constructs shown in Figure 1. To obtain data about the feasibility of transposon tagging in potato using maize transposable elements (*Ac*/*Ds* and *En*/*I*), the first series of transformants analysed contained T-DNA constructs with actively transposing *Ac* elements. Excision of *Ac* from the construct pHN::Ac (Figure 1A) in hygromycin resistant transformants was tested by screening on kanamycin containing media (Knapp *et al.* 1988). From the 42 hygromycin resistant transformants obtained, 27 (64%) were kanamycin resistant. All 27 gave rise to a PCR fragment specific for excision (Masterson *et al.* 1989), *i.e.* a PCR fragment of the expected size for an empty donor site. Outcross progenies were generated for five of these primary transformants in which excision occurred. From each of these progenies, 30-50 hygromycin resistant plants (*i.e.* containing the T-DNA) were screened for kanamycin resistance. The progenies displayed 95-100% resistance to kanamycin, suggesting that excision occurred in the majority of cells in primary transformants displaying kanamycin resistance.

Using a different construct, in which the *Ac* transposable element is present in the 5' leader of the GBSS gene (Figure 1B), we have developed an alternative assay for *Ac* excision in potato (Pereira *et al.* 1991). The assay is similar to the waxy variegation in maize and is based on the presence of amylose, produced by the GBSS gene after excision of *Ac*, in a mutant background of amylose-free starch (*amf*). The GBSS gene activity is monitored by the blue staining of tuber tissue with iodine, as compared to the red-brown staining of the *amf* background. The pattern of blue-staining revealed

A.



B. T-DNA primers

1. 5'CGGGATGATCCCGTTTCGTT3'
2. 5'GATAACGGTCGGTACGGGAT3'

***Ac* primers**

3. 5'GCTATCATTGCGGCCAAGCTC3'
4. 5'CATAACACGCACACTTACGATAG3'

Figure 2. **A.** Isolation of flanking DNA. IPCR was performed from the T-DNA integration sites and the reinsertions of *Ac* in the genome. The enzymes used for IPCR are indicated and the direction of primers used for synthesis shown as open arrowheads. The synthesized flanking DNAs used as probes are shown as dashed arrows. Plasmid rescue was performed using the pBR322 *ori* and ampicillin resistance (amp^R) in the case of the "TDs" and "TI" constructs (Figure 1C and 1D). **B.** Primers used for isolation of flanking sequences via IPCR. Abbreviations: LB / RB = Left and Right Borders of the T-DNA constructs; B = *Bam*HI, HIII = *Hin*DIII; K = *Kpn*I; NPTII = neomycin phosphotransferase gene of transposon Tn5; GBSS = granule-bound starch synthase gene; X = *Xba*I

a cell autonomous excision phenotype (waxy variegation) of the construct pGBSS::Ac in potato transformants, and displayed a high activity of *Ac* in potato (Pereira *et al.* 1991). Analogous to what was found in other plant species (Yoder 1990; Hehl & Baker 1990), a higher copy number of *Ac* resulted in an increased activity of *Ac*. In addition, the position of the integration site contributed to the activity of the transposable element (data not shown).

The genotype J92-6400-A16, known for its high efficiency of transformation (El-Kharbotly *et al.* 1995a), was used to obtain transformants harbouring a T-DNA with *Ds* element (pHPT::Ds-Kan, Figure 1C). In order to test mobilisation of the *Ds* element, a sample of four of these TDs clones were retransformed with p35SAc (Figure 1E) conferring *Ac* transposase activity. Most of the methotrexate-resistant 'retransformants' displayed hygromycin resistance, indicating the excision of *Ds* from the HPT gene. This confirmed that the introduced *Ac* transposase *in trans* could mobilise the *Ds* elements in our potato transformants. Since the *Ac* transposase gene is constitutively expressed under the control of the CaMV35S promoter, the *Ds* integrations are unstable. Therefore no attempts were undertaken to isolate *Ds* flanking sequences for mapping, and consequently no conclusions about the transpositional behaviour of trans-activated *Ds* can be made. For stable integrations of *Ds* an additional cross will be necessary in order to separate the *Ds* element from the *Ac* transposase source.

Numbers and map positions of transposon markers

In our strategy of targeted transposon tagging, based on preferential transposition to tightly linked sites in the genome, we screened for transposons that were randomly integrated close to genes of interest. Potato genomic DNA sequences flanking the insertions sites of T-DNA or transposable elements were isolated and used as probes for mapping. The position of 94 T-DNA and *Ac* flanking sequences have been located on the potato genetic map (Table 1), and are distributed as follows (Figure 3): 6 TAc on 4 chromosomes, 19 Ac on 7 chromosomes, 6 TI on 4 chromosomes, and 63 TDs on 12 chromosomes. All 12 chromosomes of the potato genome contain transposon markers. The insertion sites appear to be randomly distributed over the genome.

Clustered and scattered Ac *transposition*

For efficient tagging it is necessary to have a transposon source that is not only located close to the target locus but also produces transposition events which are tightly-linked, and therefore increasing the frequency of tagging the nearby target locus. In order to characterise the transpositional behaviour of *Ac* elements in potato we have used a set of transformants with actively transposing *Ac* elements. Independent transformants with a single-copy T-DNA::Ac insertion, were used to follow *Ac* transposition. In this approach, transposed *Ac* elements in one line will have originated from one and the same initial T-DNA insertion. Potato DNA sequences

TABLE 1. Marker loci from flanking sequences of T-DNA and transposable elements

Parental genotypes	Offspring genotypes	Expected ratio's	Marker types					
			TDs	TAc	TI	Ac	other[a]	Total
AB × *AA*	*AB:AA*	1:1♀	16	2	1	3	33	55
AA × *AB*	*AA:AB*	1:1♂	29	3	1	6	22	61
AB × *AB*	*A-:BB*	3:1	-	-	-	-	6	6
AB × *AB*	*AA:AB:BB*	1:2:1	-	-	-	-	5	5
AB × *AC*	*AA:AC:BA:BC*	1:1:1:1	18	1	4	10	33	66
Totals			63	6	6	19	99	193

[a] "other" includes RFLP markers from potato as well as tomato origin, isozyme loci, and morphological markers (see Jacobs *et al.* 1995a, for more extended description)

flanking transposed *Ac* elements as well as the original T-DNA insertion site were isolated and used as RFLP markers. As visualised in genomic Southerns of the transformants, the number of independent *Ac* reinsertions correlated in most cases with the number of IPCR bands obtained. The map positions of 19 transposed *Ac* elements is given in Figure 3. This revealed two different patterns of transposition; clustering around the initial integration site or scattering to sites all-over the genome.

The TAc11 T-DNA insert on chromosome *4* gives rise to two linked *Ac* transpositions (located within 35 cM of the T-DNA) labelled Ac11-13 and Ac11-17 (Figure 3). Similarly, Ac8-19, Ac8-22, Ac8-80, and Ac8-99 are clustered within 35 cM of TAc8 on chromosome *4*. These results suggest that *Ac* preferentially transposes to linked locations in potato, similar to that reported in maize (Greenblatt 1984) and other heterologous (Solanaceous) plant species (*eg.* Jones *et al.* 1990). However, some TAc locations do not give rise to linked *Ac* transpositions. Although we could not conclusively map the TAc4 flanking sequence, transpositions from this insertion site were located on chromosomes *5*, *7*, and *8*. This is consistent with earlier reports of *Ac* transposition in Solanaceae (Dooner *et al.* 1991; Osborne *et al.* 1991). Thus depending on the original location of the T-DNA (position effect) the reinsertion pattern of *Ac* may be clustered or scattered. This fact infers that not every trans-

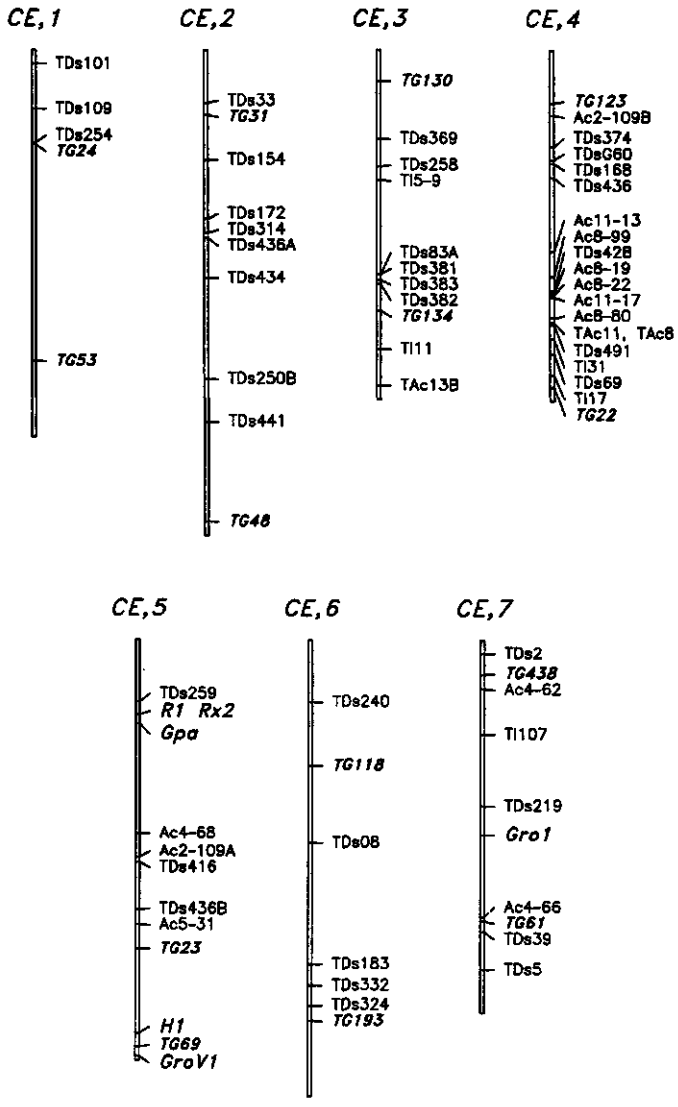
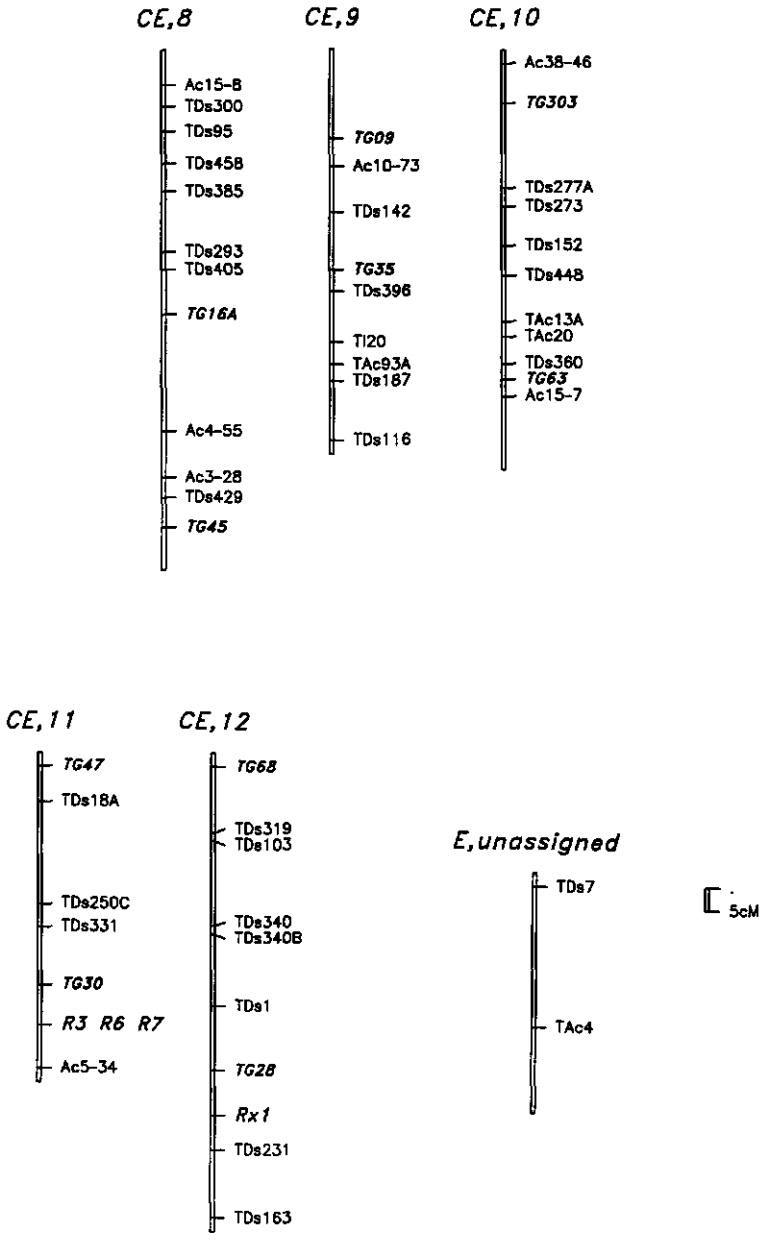


Figure 3. Diagram of the 12 chromosomes of potato, showing the TAc, Ac, TI, and TDs markers that were mapped. This is a compilation of the independent maps of both parental clones of the mapping population (Jacobs *et al.* 1995a). The markers are placed in a framework of 2 TG markers per chromosome (*italics*) to enable comparison with map positions in other potato maps. For clarity, all other markers are omitted from this diagram. The approximate positions of resistance loci for potato cyst nematodes, potato virus X and *Phytophthora infestans* identified so far in several different potato populations are also depicted (*large italics*).


CE,8
Ac15-8
TDs300
TDs95
TDs458
TDs385
TDs293
TDs405
TG16A
Ac4-55
Ac3-28
TDs429
TG45
CE,9
TG09
Ac10-73
TDs142
TG35
TDs396
TI20
TAc93A
TDs187
TDs116
CE,10
Ac38-46
TG303
TDs277A
TDs273
TDs152
TDs448
TAc13A
TAc20
TDs360
TG63
Ac15-7
CE,11
TG47
TDs18A
TDs250C
TDs331
TG30
R3 R6 R7
Ac5-34
CE,12
TG68
TDs319
TDs103
TDs340
TDs340B
TDs1
TG28
Rx1
TDs231
TDs163
E,unassigned
TDs7
TAc4
5cM

formant will give the same efficiency in tagging experiments. However, the number of TAc integration sites and subsequent reinsertion sites mapped in this study is too limited to be conclusive about the proportion of random T-DNA integrations that can be used for transposon tagging.

About 50% of the probes from T-DNA::Ac IPCR's were repetitive when used as probes on Southern blots and could therefore not be mapped. In contrast, most of the *Ac* probes (95%) were unique/low copy as judged by hybridisation to potato genomic DNA and could be used for mapping when polymorphic in the mapping population. This suggests that *Ac* elements transpose predominantly to low copy DNA, which makes the mapping of transposed *Ac* elements more efficient than that of the T-DNA's. The original T-DNA integrations from which the 'single copy' *Ac* elements excised, were both in unique/low copy and repetitive DNA sequences. Therefore we conclude that the sequences surrounding the initial T-DNA integration (low copy/repetitive), do not seem to influence the sequences in which the *Ac* elements reintegrate.

Localisation of integrations of T-DNA's containing Ds *and* I *transposons*

The diploid potato clone, J92-6400-A16, is heterozygous for the *R1* locus which confers race-specific resistance to the fungus *Phytophthora infestans*. It was transformed with the *Ds* construct pHPT::Ds-Kan (Figure 1C) or the *I* construct pGUS::I (Figure 1D), and over 400 independent transformants were selected. Potato genomic DNA flanking the T-DNA borders in the independent transformants was isolated and used as probes for mapping. The 63 mapped TDs markers (T-DNA::Ds flanking DNA) covered all chromosomes (Figure 3) and were randomly distributed over the potato genome (El-Kharbotly *et al.* 1995b). Similar to the TAc integrations, about 30-50% of the TDs/TI flanking sequences were repetitive. Based on the total number of flanking sequences for TDs/TI and TAc that we have isolated and analysed (>300), we deduce that a considerable fraction of T-DNA integrations (up to 50%) is into repetitive DNA. This complicates the mapping of these integration sites, and impedes the search for an appropriate clone for targeted transposon tagging.

Targeted transposon tagging of the R1 locus

The T-DNA integrations of the TDs lines TDs259 and TDs416 were located on chromosome 5 (Figure 3). Based on RFLP markers in common between this map

Figure 4. Southern blot of 24 genotypes of population J92-6400-A, segregating for the *R1* locus for resistance to *P. infestans*. Probe is TDs259, revealing linkage with the *R1* locus. P_R = resistant parental clone; P_S = susceptible parental clone; * = recombinant genotype.

(Jacobs *et al.* 1995a) and the map of the population used to locate the *R1* locus (Leonards-Schippers *et al.* 1992), these integrations were estimated to be within 25 cM from the *R1* locus. Therefore the two corresponding original transformants for these T-DNA insertions are suitable for tagging of the *R1* gene. In both transformants the T-DNA integrations were in repulsion to the *R1* locus, as determined by RFLP analysis in a population of 50 genotypes (J92-6400-A), segregating for *R1*, that included the original transformed potato clone (J92-6400-A16) and its siblings (Figure 4). Transformed line TDs416 (*R1r1*) was crossed to the *r1r1* clone J89-5040-2, in order to obtain recombinants between *R1* and the T-DNA integration. The genetic linkage between *R1* and the T-DNA was confirmed (18 cM; 6 recombinants in 39 progeny). Four recombinants were found in which the T-DNA TDs416 and the *R1* locus were in coupling phase. In order to obtain tagged mutants of the *R1* locus, retransformation of these recombinants with the *Ac* transposase construct p35SAc (Figure 1E) and other regulated *Ac* transposase constructs is currently in progress.

Discussion

Mapping of (re)integrations of T-DNA and transposable elements

In total 94 markers consisting of flanking DNA were mapped (Table 1, Figure 3). The loci were evenly spread over the entire potato genome. No preference for particular chromosomes and/or chromosomal regions for the integration of T-DNA's was found,

which is consistent with the mapping of T-DNA integration sites in other species (*eg.* tomato, Thomas *et al.* 1994). However, similar to observations in other (Solanaceous) species (Dooner *et al.* 1991; Osborne *et al.* 1991; Jones *et al.* 1990), transposition of *Ac* in potato is mainly to closely linked sites, clustered around the initial T-DNA integration site (Jacobs *et al.* 1994). These results confirm the earlier observation that the maize *Ac-Ds* transposable element system is functional in potato (Knapp *et al.* 1988), and has the potential to be used for the tagging of genes of interest.

The DNA sequences flanking the T-DNA/*Ac* integration sites were used as RFLP markers in a mapping population that is genetically different from the original transformants. The main advantage of this 'relative' mapping of the transformant's integration sites in an independent mapping population is that it circumvents the need to develop populations from every single transformant in order to map the integration site(s). However, since the mapping population is genetically different from the original transformant, we assume that the map position of the flanking sequences indicates a position equivalent to the integration site in the transformant itself.

Efficiency of tagging

Experimental work involving transposon tagging of disease resistance loci in maize (Bennetzen *et al.* 1988), tobacco (Whitham *et al.* 1994), and flax (Lawrence *et al.* 1993) revealed the high intrinsic instability of such loci. Therefore, for effective transposon tagging, the frequency of obtaining transposon insertion mutants should be high enough to resolve these mutations from the background level of mutations that may be expected by instability. For this reason targeted tagging of the gene of interest is generally used. With closer linkage of a transposable element to the gene of interest, the frequency of insertion is higher and the tagging of the gene is more efficient. With an unlinked transposable element we estimate that 500,000 plants will have to be screened to find a mutant (based on studies with maize kernels). This can be reduced to about 50,000 plants when the distance to the transposable element is 20 cM, and about 5,000 plants with a distance of 5 cM (Walbot 1992). Especially with a phenotype that only becomes evident after laborious (resistance) testing, this reduction in population size becomes very important. Therefore the additional effort needed for the search of a transformant containing a transposable element closely-linked to the gene of interest is more than compensated for at the stage of screening offspring for putative mutant (tagged) phenotypes in relatively small populations.

Tagging strategies

Many genes have been isolated in maize and *Antirrhinum* using transposon tagging, including some novel regulatory genes with low expression levels (Walbot 1992). Although only a few genes have been cloned to date by targeted transposon tagging in heterologous species (Jones *et al.* 1994; Whitham *et al.* 1994; Ellis *et al.* 1995), much information has been gathered to facilitate the development of tagging strategies (Haring *et al.* 1991).

The heterologous *Ac-Ds* two element system has the advantage of being able to manipulate the transposons *in vitro* before transformation into plants, thereby allowing the use of a transposon tagging system tailored for efficiency. The successful cloning of a gene following targeted tagging with closely-linked transposons is well illustrated by the cloning of the *Cf-9* gene from tomato using the *Ac/Ds* two element system (Jones *et al.* 1994). The high frequency of phenotypic mutants (37/160,000) found with targeted insertion mutagenesis demonstrates that high efficiency transposon tagging is possible when starting with a closely linked *Ds* element.

A promising strategy to increase the efficiency of targeted tagging is the use of different promoters to regulate *Ac* transposase expression to specific conditions (Scofield *et al.* 1992; Rommens *et al.* 1992). A stage specific source of transposase, *eg.* a pollen-specific promotor, limits (high) transposition to specific developmental stages and/or tissues of the plant. This way, the transposition events are more controlled and will cause stable mutant phenotypes rather than sectored mutant phenotypes.

The non-inbred potato is highly heterozygous, thereby making the development of transposon tagging strategies more complicated. However, targeted transposon tagging is feasible when well-developed genetic maps are combined with the use of: (1) the two-element system; (2) lines with transposons targeted to the gene of interest; (3) traits that are relatively easy to screen (Figure 5). The mapping of sequences flanking transposable elements has resulted in a series of potato clones containing transposable elements at different positions randomly spaced over the potato genome. This collection of clones has the potato genome saturated with transposable elements and makes targeted tagging of any locus possible. The non-autonomous TDs clones are especially useful for the tagging of loci in other potato genotypes, since the map

position of the *Ds* element remains stable while appropriate crosses are made. Several studies in tomato revealed different patterns of transposition of trans-activated *Ds*, to sites both linked and unlinked to the initial T-DNA integration site (Rommens *et al.* 1993; Healy *et al.* 1993; Knapp *et al.* 1994; Carroll *et al.* 1995). Nevertheless, the incidence of *Ds* elements transposing to closely linked sites is still considerably high. Therefore, for the tagging of a specific targeted gene, the first step involves the selection of a transformant with the TDs insertion closely linked in coupling phase with the dominant allele of the gene of interest. When the TDs and the target allele are in repulsion, it is more efficient to use a *Ds* linked loosely to a gene of interest (20-40cM) in order to first obtain recombinants in coupling phase. If the TDs is in a position too close to the target gene, a large population of offspring must be screened in order to find the rare cross-over events. Once the appropriate genotype, with the TDs and the gene in coupling phase is found, transposition of the *Ds* element is induced.

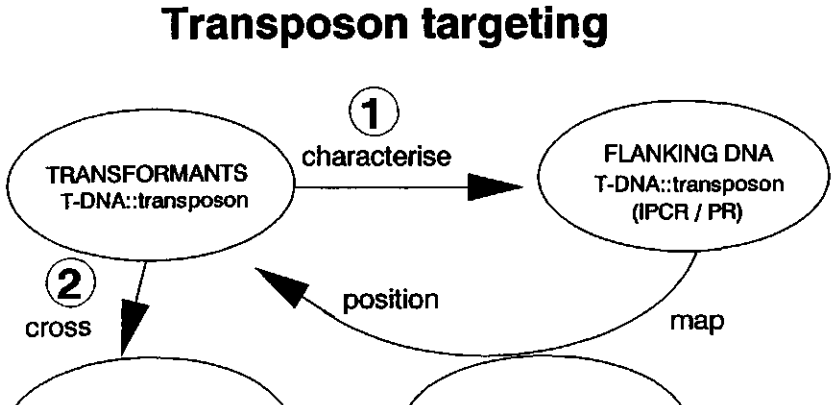


Figure 5. Strategy of transposon targeting showing the interrelationships between the various aspects discussed. (1) Transformants containing transposable elements are used for the generation of IPCR and plasmid rescue probes, that are analysed as molecular (RFLP) markers in a mapping population. The genomic positions of the target genes of interest (resistance genes) are determined. (2) Combination of all this information yields the appropriate transformant in which transposition is induced (retransformation or crosses) in order to efficiently tag the gene of interest.

Several approaches are possible to induce transposition (Figure 5). A crossing scheme to a potato clone containing transposase activity can be developed. It is important that crosses are planned so that the population being screened for transposition mutants is heterozygous for the target locus. Alternatively, retransformation with a T-DNA construct with transposase activity can be performed. The latter approach saves the time needed for additional crosses, but has the disadvantage that the plant material has to pass through tissue-culture. Once the *Ds* elements have transposed, putative mutants are identified by screening progeny for the desired phenotype. The reintegration sites in the target gene can then be identified using the *Ds* sequences as a probe.

Targeted tagging of disease resistance genes in potato

The potato genes we are targeting include those conferring resistance to pathogens, which are present in wild species or their introgressions into cultivated potato. By means of RFLP mapping, several resistance loci have been identified (Figure 3). Two loci for resistance against potato virus X (PVX) are mapped on chromosome *5* and *12* (Ritter *et al.* 1991). Monogenic resistances against the fungus *Phytophthora infestans* have been located on chromosomes *5* (*R1*, Leonards-Schippers *et al.* 1992) and *11* (*R3*, El-Kharbotly *et al.* 1994; *R6*, *R7*, C. Gebhardt pers. comm.). Several loci for resistance against potato cyst nematodes have been identified; *Gro1* from *Solanum spegazzinii* maps to chromosome *7* (Barone *et al.* 1990), whereas *H1* from *S. tuberosum* ssp. *andigena* (Gebhardt *et al.* 1993; Pineda *et al.* 1993) and *GroV1* from *S. vernei* (Jacobs *et al.* 1995b) are monogenic resistances directed against *Globodera rostochiensis* pathotype 1, both of which map to chromosome *5*. In addition a major locus for resistance against *Globodera pallida* pathotypes 2 and 3 from *S. spegazzinii* was located on chromosome *5* (*Gpa*, Kreike *et al.* 1994).

Appropriate crosses to introduce transposon constructs close to our target gene, *R1* on chromosome *5*, have been performed. Following the mobilisation of transposable elements targeted to the resistance gene, progeny populations will be screened for mutant phenotype(s). A suitable resistance test (easy to perform with little space, quick, and reproducible) is available and will contribute to a successful mutant screening (Whitham *et al.* 1994; Jones *et al.* 1994). Once a tagged mutant is identified, the corresponding resistance gene can be conveniently cloned.

Wild species have often been sources for resistance genes in potato breeding. Many

sources though are difficult to access because of the complications in quick introgression to suitable cultivars (*e.g.* reduced or no compatibility). In addition it will be necessary to remove undesired characters that are introgressed concomitantly with the resistance. Therefore the molecular isolation of resistance genes which are available in the repertoire of wild species would aid in their incorporation in potato cultivars. The general strategy we have initiated for transposon targeting in potato would therefore allow the tagging of useful resistance genes once they have been sufficiently characterised genetically.

Chapter 3

Genetic mapping data of potato combined into one core map with the computer programme JoinMap 2.0

(with: Nelleke C.M. Kreike, Herman J. van Eck, Evert Jacobsen, Willem J. Stiekema)

Abstract Molecular genetic maps of diploid potato have been constructed from several closely related *Solanum* species using different types of segregating populations. Due to the high level of heterozygosity in these *Solanum* species, many markers are polymorphic in both parental clones, giving up to four different alleles per locus in a single diploid mapping population. As a result, the segregation data in most mapping populations allow the construction of genetic maps derived from both parents. In order to compare and align maps obtained from several different *Solanum* species, we combined data from seven independent maps. These were available from our previous studies or extracted from the SolGenes database. Separate maps for all populations were (re)calculated, aligned, and subsequently joined into one basic core map with the help of a new version of the computer programme for genetic mapping; JoinMap 2.0. This new version allows a two digit input for each genotype/marker combination in a "cross-pollinating" mapping population. In view of the large diversity of markers and for convenience of use, the common map was constructed from a limited selection of markers. These 73 core markers can be used as landmarks on the independent genetic maps and can potentially bridge the gaps present in one genetic map by exchange of core markers present in another genetic map. The remaining markers are assigned to the intervals between these core markers, in the orders found in the original maps, without giving any significance to the intertwining orders of the different interval markers of the different maps.

Introduction

The construction of genetic linkage maps for non-inbred species is in general more complex compared to inbreeding species, such as tomato or *Arabidopsis*. Mapping populations are usually derived from crosses between two different, heterozygous parents. As a result, several modes of segregation are expected in a single mapping population, with up to four different alleles segregating at a single locus in diploid species. Various approaches have been used to solve the resulting complexities associated with map construction. These range from ignoring part of the data (*eg.* constructing a map from one parent only, or disregarding certain types of segregation), to combining the data obtained from both parents into one map.

A genetic linkage map for a non-inbred species was first constructed in potato, by using the segregation data from only one parent (Bonierbale *et al.* 1988). A similar approach was essentially followed for genetic maps of citrus (Durham *et al.* 1992; Jarrel *et al.* 1992) and loblolly pine (Devey *et al.* 1994). In several studies on gymnosperms, advantage has been taken of the availability of haploid tissue from megagametophytes allowing the construction of genetic maps from a single parent without the need of a mapping population (*eg.* white spruce, Tulsieram *et al.* 1992; maritime pine, Gerber *et al.* 1993; Norway spruce, Binelli & Bucci 1994). An alternative approach in potato has involved the intercalation of three separate maps, constructed for markers segregating in only one of the two parents and for the markers segregating in both parents (Gebhardt *et al.* 1989b, 1994). In a similar manner, two parallel parental maps, with various degrees of aligning the linkage groups with allelic bridges, have been constructed for alfalfa (Echt *et al.* 1994), loblolly pine (Groover *et al.* 1994), eucalyptus (Grattapaglia & Sederoff 1994), and apple (Hemmat *et al.* 1994). More recently, a novel method was introduced (Jacobs *et al.* 1995a; Van Eck 1995) for the construction of linkage maps for non-inbred species using JoinMap1.4 (Stam 1993). In this method two independent parental maps are constructed, followed by the construction of a joined map that is based on a weighted average of the recombination frequencies observed in the male and female meioses.

The mapping of genetic markers in cultivated potato (*S. tuberosum* L.) has been very difficult. It is tetraploid, suffers from inbreeding depression, and carries a load of (sub)lethal recessive alleles that interfere with segregation of linked markers. The introduction of diploid potato plants (either dihaploid genotypes obtained from

tetraploid potato, or diploid wild species) in genetic mapping studies, together with the development of molecular (RFLP) markers, have rapidly increased the number of markers which could be placed on the potato genome. This has resulted sofar in five RFLP maps of potato, all based on diploid populations. Two of these potato maps are based on the homology between the potato and tomato genomes, using tomato RFLP markers in interspecific crosses (*S. phureja* x [*S. tuberosum* x *S. chacoense*], Bonierbale *et al.* 1988, and [*S. tuberosum* x *S. berthaultii*] x *S. berthaultii*, Tanksley *et al.* 1992). The other three potato maps make use of potato RFLP markers in either an intraspecific cross of *S. tuberosum* (Gebhardt *et al.* 1989b; Gebhardt *et al.* 1991), a backcross of two *S. tuberosum* clones, with *S. phureja* and *S. vernei* in the pedigree (Jacobs *et al.* 1995a), or a combination of two F_1 and one backcross population (Kreike 1995). In these latter three maps, a series of tomato markers evenly spread over the genome (Bonierbale *et al.* 1988), were used to align linkage groups with previous potato maps.

Over recent years, molecular markers have been used for a wide range of purposes in potato genetics. These have included the fingerprinting of potato breeding lines and cultivars (*eg.* Gebhardt *et al.* 1989a; Debener *et al.* 1991; Görg *et al.* 1992), studies on phylogenetic relationships (Debener *et al.* 1990), verifying the nature of somatic hybrids (Williams *et al.* 1990; Baird *et al.* 1992) and products from anther culture (Rivard *et al.* 1989), as well as following homologous and homeologous recombination during introgression of genes from wild *Solanum* species (Williams *et al.* 1993). Molecular markers have also been used for the mapping of morphological markers (Van Eck *et al.* 1993; Van Eck *et al.* 1994a; Van Eck *et al.* 1994b; Jacobs *et al.* 1995a), quantitative trait loci (Van den Berg 1993; Freyre and Douches 1994; Bonierbale *et al.* 1994; Leonards-Schippers *et al.* 1994; Van Eck *et al.* 1994a; Kreike *et al.* 1994; Freyre *et al.* 1994), and pest and disease resistance loci (Barone *et al.* 1990; Ritter *et al.* 1991; Leonards-Schippers *et al.* 1992; Pineda *et al.* 1993; Gebhardt *et al.* 1993; Kreike *et al.* 1993; El-Kharbotly *et al.* 1994; Kreike *et al.* 1994; Jacobs *et al.* 1995b).

Applications of molecular markers are anticipated to become increasingly important research tools for genetic studies in crop plants, including potato. Future applications of this technology will be greatly facilitated by the availability of a basic reference map, since this would avoid the need to construct a complete genetic map in every

new population in which a trait of interest is segregating. Independent genetic maps constructed in different genetic backgrounds are not always easy to interchange, and often contain genomic regions not covered by the markers being used. In this paper we describe the construction of a genetic core map of potato, integrating the mapping data available from a number of previous studies. This encompasses information from different types of segregating populations and different potato species.

Materials and methods

Plant material

The data used in this study for the construction of a potato core map were derived from seven diploid populations (Table 1):

I. A BC population of *S. tuberosum*, with *S. phureja* and *S. vernei* in its pedigree, as described by Van Eck *et al.* (1993). A genetic map of this population based on 67 genotypes is described in Jacobs *et al.* (1995a).

II. A F_1 population *S. tuberosum* x *S. spegazzinni* consisting of 96 genotypes as described in Kreike *et al.* (1993).

III. One F_1 genotype (F_1-A-38) from population *II* was used as the female parent in a backcross to *S. tuberosum*. This BC population of 95 genotypes is described in Kreike *et al.* (1994).

IV. A F_1 population of *S. tuberosum* x *S. phureja* consisting of 58 genotypes as described in Kreike (1995).

V. The F_1 population *S. phureja* x [*S. tuberosum* x *S. chacoense*] consisting of 67 genotypes as described by Bonierbale *et al.* (1988).

VI. A BC population, BCB ([*S. tuberosum* x *S. berthaultii*] x *S. berthaultii*), consisting of 155 genotypes as described by Tanksley *et al.* (1992).

VII. A BC population, BCT ([*S. tuberosum* x *S. berthaultii*] x *S. tuberosum*), consisting of 158 genotypes as described by Bonierbale *et al.* (1994).

Data for populations *V*, *VI*, and *VII* were obtained from the USDA/NAL SolGenes database (curator C. Nelson, Cornell University, Ithaca NY; the SolGenes database is accessible via the WWW as well as Gopher at nightshade.cit.cornell.edu or probe.nalusda.gov).

Isozyme and classical genetic markers

Genetic markers involving classical traits and isozyme loci that were used in this study are summarised in Table 2.

RFLP markers

RFLP markers used are described previously in the original publications on the respective potato maps. In summary; TG, CD and CT markers originate from the work of Dr. S.D.Tanksley *cs.*, Cornell University, Ithaca NY, USA. Ssp markers are described in Kreike *et al.* (1993). ST, TAc, Ac, TDs and TI markers are described in Jacobs *et al.* (1995a). Genomic DNA and cDNA markers from potato (GP, CP) are described in Gebhardt *et al.* (1989b).

Linkage analysis and map construction

The programme JoinMap 2.0 was used for core map construction with the option CP for Cross Pollinators and the Kosambi function for converting recombination frequencies into map units (cM). Graphical representation of the maps was performed with the help of the computer programme

Table 1. Molecular genetic maps of potato, used in this study.

	Mapping population					Total map length (cM)		Reference
	P_1 (♀)	P_2 (♂)	type	size	map	a	b	
I	*S.tub* x *S.phu*	P_1 x *S.tub*	BC	67	♀♂	1120	855	Jacobs *et al.* (1995a)
II	*S.tub*	*S.spg*	F1	58	♀♂			Kreike *et al.* (1993)
III	*S.tub* x *S.spg*	*S.tub*	BC	95	♀♂	731[c]	626[c]	Kreike *et al.* (1994)
IV	*S.tub*	*S.phu*	F_1	58	♀♂			Kreike (1995)
V	*S.phu*	*S.tub* x *S.chc*	F_1	67	♂	606	726	Bonierbale *et al.* (1988)
VI	*S.tub* x *S.ber*	*S.ber*	BC	155	♀	546	660	Tanksley *et al.* (1992); Bonierbale *et al.* (1994)
VII	*S.tub* x *S.ber*	*S.tub*	BC	158	♀	644	639	Bonierbale *et al.* (1994)

a. Total length of map as described in the original study (see reference)
b. Total length of map as determined in this study using JoinMap 2.0
c. Segregation data of populations *II*, *III* and *IV* are combined for the construction of one genetic map (Kreike 1995)

Table 2. Localisation of classical genetic markers and isozyme loci on the potato genome.

A. Classical genetic markers

Locus		Chromosome	Reference
S	self-incompatibility	1	Gebhardt *et al.* 1991; Jacobs *et al.* 1995a
Me	metribuzin sensitivity	2	Jacobs *et al.* 1995a
D	red anthocyanins	2	Van Eck *et al.* 1993; Jacobs *et al.* 1995a
Y	tuber flesh colour	3	Bonierbale *et al.* 1988; Jacobs *et al.* 1995a
Ym	yellow margin	5	Jacobs *et al.* 1995a
Ds1	desynapsis	8	Jacobs *et al.* 1995a
Cr	crumpled	10	Jacobs *et al.* 1995a
Ro	tuber shape	10	Van Eck *et al.* 1994a; Jacobs *et al.* 1995a
F	flower colour	10	Van Eck *et al.* 1993; Jacobs *et al.* 1995a
PSC	Purple Skin Colour	10	Gebhardt *et al.* 1991
I_{ep}	tuber skin colour, epidermis	10	Van Eck *et al.* 1994b
I_{co}	tuber skin colour, cortex	10	Van Eck *et al.* 1994b
P	purple anthocyanins	11	Van Eck *et al.* 1993; Jacobs *et al.* 1995a

B. Isozyme loci

Locus		Chromosome	Reference
Idh-1	isocitrate dehydrogenase	1	Bonierbale *et al.* 1988*
Skdh-1	shikimic acid dehydrogenase	1	Bonierbale *et al.* 1988*
Prx-2	peroxidase	2	Bonierbale *et al.* 1988*
Pgm-1	phosphoglucomutase	3	Bonierbale *et al.* 1988*
Pgm-2	phosphoglucomutase	4	Bonierbale *et al.* 1988*
Adh-2	alcohol dehydrogenase	4	Jacobs *et al.* 1995a (tomato *Adh-1*)*
6-Pgdh-3	6-phosphogluconate dehydrogenase	5	Bonierbale *et al.* 1988*
Dia-1	diaphorase	5	Bonierbale *et al.* 1988; Jacobs *et al.* 1995a
Mdh-2	malate dehydrogenase	5	Jacobs *et al.* 1995a
Adh-1	alcohol dehydrogenase	6	Jacobs *et al.* 1995a (tomato *Adh-2*)*
Mdh-3	malate dehydrogenase	7	Bonierbale *et al.* 1988; Jacobs *et al.* 1995a*
Got-2	glutamate oxaloacetate transaminase	7	Bonierbale *et al.* 1988; Jacobs *et al.* 1995a*
Got-1	glutamate oxaloacetate transaminase	8	Bonierbale *et al.* 1988#; Jacobs *et al.* 1995a*
Aps-2	acid phosphatase	8	Jacobs *et al.* 1995a*
6-Pgdh-2	6-phosphogluconate dehydrogenase	12	Jacobs *et al.* 1995a*
Pgi-1	phosphoglucoisomerase	12	Bonierbale *et al.* 1988*
Tpi-1	triose phosphate isomerase	unassigned	Jacobs *et al.* 1995a

* = similar position was found in tomato.
# = locus is designated *Got-4*

Drawmap (Van Ooijen 1994). The separate maps of each mapping population were (re)calculated with JoinMap 2.0 (Stam 1995), taking the data of all markers into account. For populations *I-IV*, segregation data for both parental clones were available. These data were combined into a singlefile for map construction. Next, a limited set of core markers per chromosome were selected, evenly spaced over the chromosomes (optimal is 1 marker every 20 cM), well-fitting in the chromosome map (*i.e.* including high LOD scores), and present in as many of the 7 populations as possible. Only the pairwise estimates of combinations of these core markers are retained for the following steps. For each chromosome, core data from all 7 populations are combined into one file, to construct a new core map. Markers initially removed from the individual core maps are then "fitted" in the intervals between the core markers.

For the populations *V*, *VI* and *VII*, the segregation data used for map construction were mainly obtained from one parent. For population *V* this was the hybrid parent *S. tuberosum* x *S. chacoense*, with 3 additional markers segregating exclusively from the *S. phureja* parent (Bonierbale *et al.* 1988). The segregation data for the markers polymorphic in the *S. phureja* parent are not present in the SolGenes database, and are therefore not used in this study. For the backcross populations *VI* and *VII* the hybrid parent *S. tuberosum* x *S. berthaultii* was used for map construction. Although the maps from these two populations were made from the same parent, they involved a backcross using a different recurrent parent. Therefore differences in the resulting maps of populations *VI* and *VII* are based solely on a random (sampling) difference and could arise from (sub)lethal combinations of alleles present in one population as compared to the other.

Bonierbale *et al.* (1994) mention that in populations *VI* and *VII*, genetic maps were constructed in the recurrent parents as well. These maps contain 35 and 45 markers for the *S. tuberosum* and the *S. berthaultii* recurrent parents respectively. However, these data are not present in the SolGenes database, and were consequently not used in this study.

Results

Recalculation of the maps.

Separate genetic maps were (re)constructed for the diverse potato populations. The maps of populations *I*, *II*, *III* and *IV* recalculated with JoinMap 2.0 (Stam 1995) were shorter in length, as the original versions calculated with JoinMap 1.4 (Table 1, JoinMap 1.4; Stam 1993). Marker orders within clusters of closely-linked markers were found to be different in some instances. However, these differences were only minor and the average χ^2 of the two versions of the maps did not notably diverge. The recalculated maps of the populations *V* and *VI* were longer than the originally published maps constructed with MapMaker (Lander *et al.* 1987), while the maps of population *VII* constructed with MapMaker and JoinMap 2.0 had a similar length (Table 1).

In order to construct one basic reference map of potato, and to potentially fill gaps in the separate maps, a limited number of core markers was selected from the

(re)calculated maps. This selection was based on the following conditions, ranked in priority: (1) an optimal spacing of the core markers (approximately 20 cM), in order to create intervals long enough for determining an accurate order of the markers within the interval, and to enable an optimal number of markers to be used for quickly determining the position of a trait of interest; (2) the markers were chosen to be present in as many populations as possible, to make the intervals as uniform as possible. In addition, for reasons of easy interchange, presence of the marker on the genetic map of tomato was a consideration as well; (3) in those genomic regions where few markers were present in one or two (groups of) populations, concessions were made to the condition that a core marker should be present in all populations, in order to fill these gaps; (4) any marker that did not fit very well within one of the original (separate) maps (*i.e.* causing an increase in the χ^2 larger than the "jump" allowed in the settings of JoinMap2.0) was omitted whenever possible. It should be noted that distorted segregation caused by a single locus does not influence recombination fractions, and consequently does not affect genetic distances and map lengths (Van Ooijen *et al.* 1994). Therefore, in the selection of core markers, distorted segregation was not a main consideration. The integrated map was constructed from a core of markers from each mapping population with the computer programme JoinMap 2.0 (Figure 1). As expected, it was found that (re)calculating the core of the combined data from the seven populations did not make a difference in marker order, but in some instances did make a difference in the length of intervals between markers.

The markers in the intervals between the core markers are listed next to the chromosomes, in the order in which they map within the given intervals (Figure 1). The interval markers are presented in three different groups, for population *I*, populations *II*, *III* and *IV*, and populations *V*, *VI* and *VII*. This division was selected because it represents (groups of) populations used in separate studies, with a high number of markers in common. As a result, the genetic maps from the populations within such a group can be joined together with relative ease, and a reliable marker order within the interval can be given. Isozyme markers and classical genetic markers of which only the approximate position is known, are listed next to the map in the most likely interval. Whenever a core marker is not present in a population, the interval noted will expand beyond that core marker. *Eg.* the chromosome *1* interval 1.2-1.4 for populations *V*, *VI*, *VII* contains the seven markers listed for that interval in that particular order. Core marker 1.3, the *S* locus of gametophytic incompatibility,

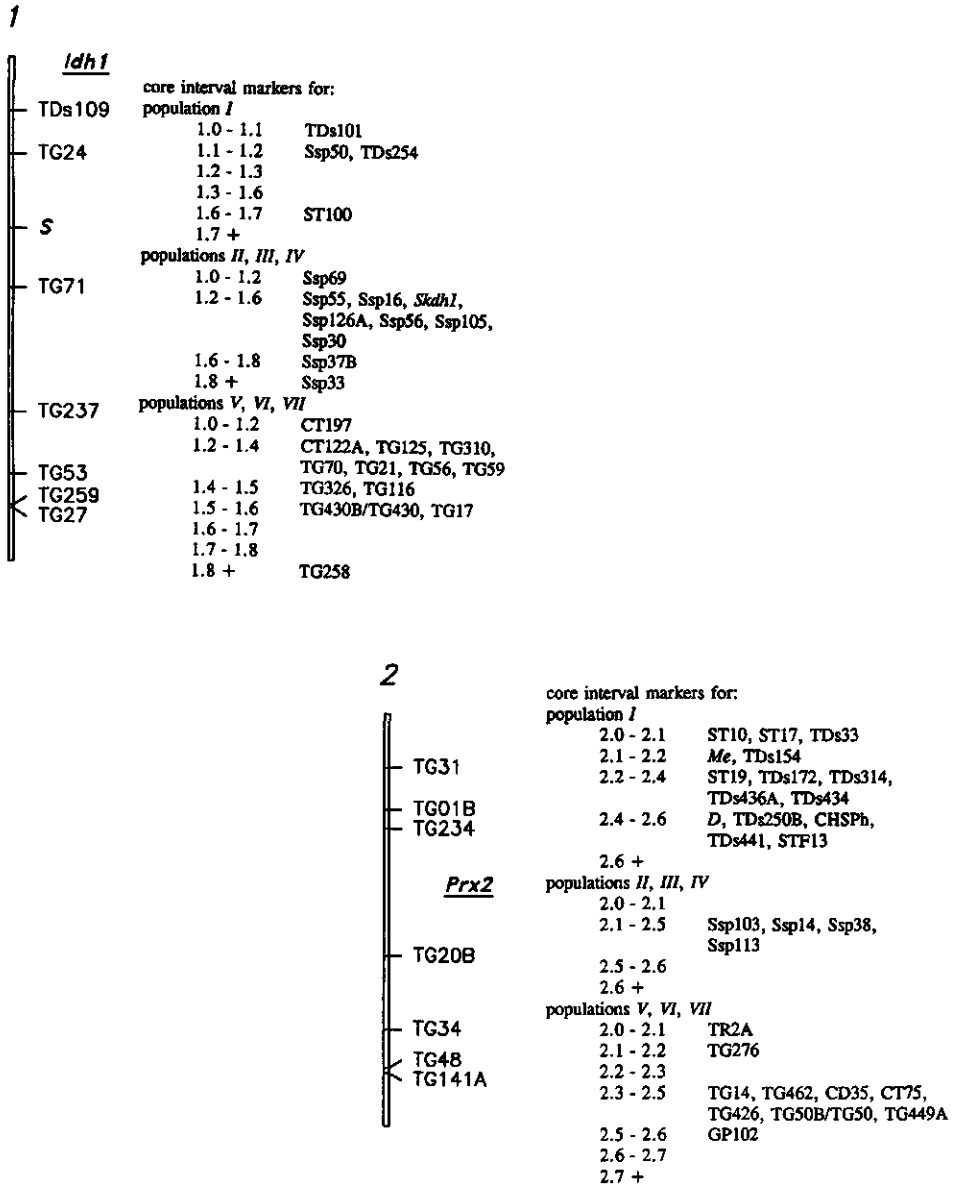
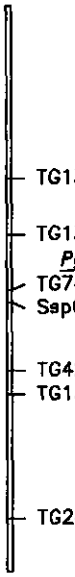


Figure 1. Core map of potato. Chromosomes are depicted with their core markers. Isozyme and classical markers, not present in the datasets used, are underlined and placed on the right-hand side of the map at their approximate position. Core interval markers are listed in the order determined in the separate (groups of) population(s). For chromosome *12* three separate maps are provided (see text).

3



core interval markers for:
population *I*

3.0 - 3.2	TG08B
3.2 - 3.4	TDs369, *Y*, TDs258, TI5-9
3.4 - 3.5	
3.5 - 3.6	TDs83A, TDs381, TDs383, TDs382
3.6 +	TI11, TAc13B

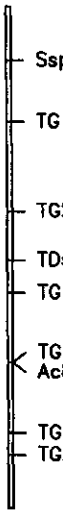
populations *II, III, IV*

3.0 - 3.2	TG56, Ac5-34, Ssp20A, Ssp08
3.2 - 3.4	Ssp80
3.4 - 3.5	PI4, PI2
3.5 - 3.6	
3.6 +	(Ssp26, Ssp66B, Ssp27B)

populations *V, VI, VII*

3.0 - 3.1	
3.1 - 3.2	TG131C, TG449B, CP6A, TG291B
3.2 - 3.3	TG364A, TG66, TG162
3.3 - 3.5	CP6B
3.5 - 3.6	TG364B
3.6 - 3.7	TG62, TG242, TG311, CD71, TG411
3.7 +	

4



core interval markers for:
population *I*

4.0 - 4.1	
4.1 - 4.2	Ac2-109B, TDs374, TDsG60
4.2 - 4.4	
4.4 - 4.7	TDs436, ST102, ST03, Ac11-13, Ac8-99, *Adh2*, TDs428
4.7 - 4.9	Ac8-22, Ac11-17, Ac8-80, ST12, TAc11, TDs491, TI31, TDs69, TI17, BE

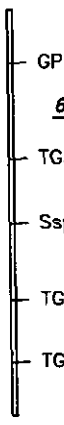
populations *II, III, IV*

4.0 - 4.1	
4.1 - 4.2	*Pgm2*
4.2 - 4.7	Ssp47, Ssp61, Ssp118B, Ac11-13
4.7 +	

populations *V, VI, VII*

4.0 - 4.2	CT122B, CT229, TG49, TG15
4.2 - 4.3	CD70, CT145, TR2B
4.3 - 4.5	TG652, TG272B/TG272, CT194
4.5 - 4.6	
4.6 - 4.8	TG345
4.8 - 4.9	
4.9 +	TG37, TG450, TG163, TG443

5



core interval markers for:
population *I*

5.0 - 5.1	TDs259
5.1 - 5.3	*El*, Ssp72, Ssp121, (Ac4-68, TDs436B, Ac2-109A, TDs416)
5.3 - 5.4	Ac5-31, Ssp45D, *Mdh2*, *Dia1*, CHSSt, GBSSB
5.4 - 5.5	
5.5 +	*Ym*

populations *II, III, IV*

5.0 - 5.1	Ssp124, Ssp112, Ssp37, Ssp118A
5.1 - 5.3	Ssp72, Ssp45A
5.5 +	GP265

populations *V, VI, VII*

5.0 - 5.2	TG475, CD31, CD64, TG441, CT101, TG569, TG568, CD127, TG619
5.2 - 5.4	TG291A, TG448, TG363, TG318, CD38B, TG358, CT172
5.4 - 5.5	
5.5 +	CD74A, CT51, CT130, TG185, GP22

6

core interval markers for:
population *I*

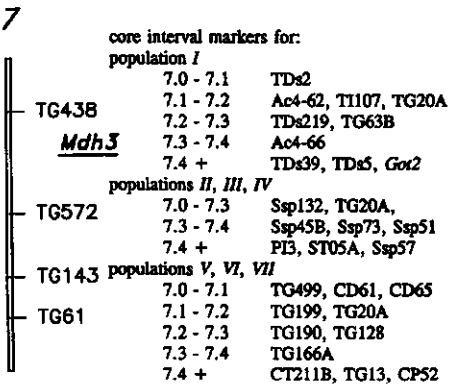
6.0 - 6.1	
6.1 - 6.2	TDs240
6.2 - 6.4	TG118, *Adh1*, TG44B, TDs08, TG50, TG253
6.4 - 6.5	Ssp45B, TDs332, ST09, TDs324, TG193
6.5 +	

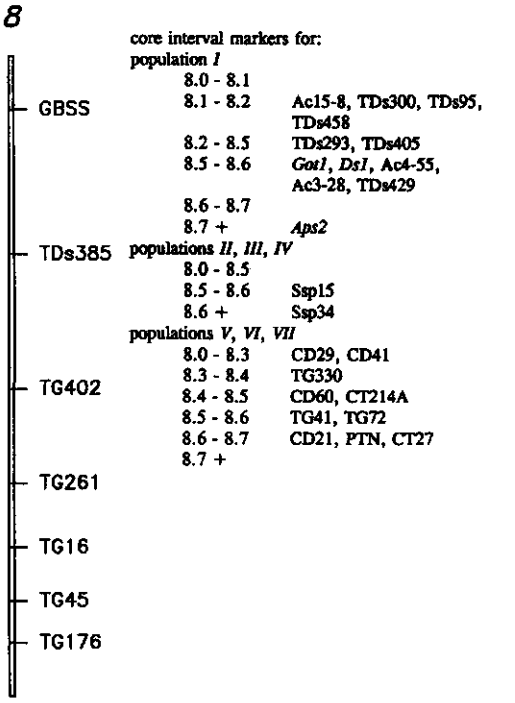
populations *II, III, IV*

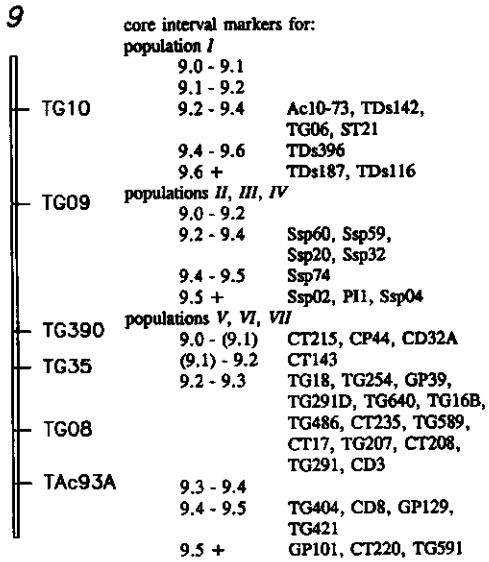
6.0 - 6.5	TG118
6.5 +	

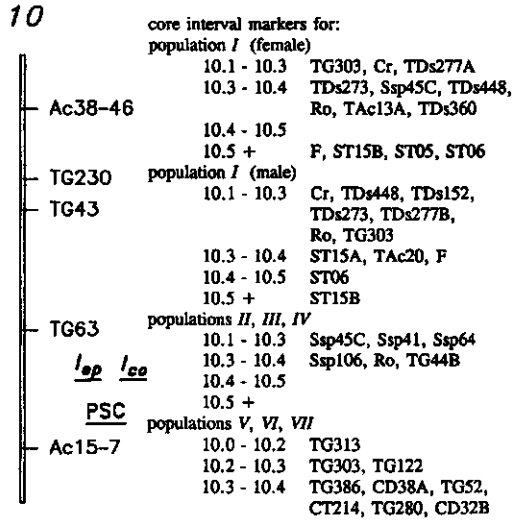
populations *V, VI, VII*

6.0 - 6.2	CT119, CD14
6.2 - 6.3	TG118, TG25, TG166B
6.3 - 6.5	TG62, TG233B, TG141B, TG364A, TG162, TG415
	TG193
6.5 +	CT107B

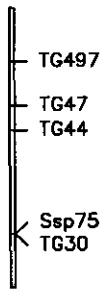

7
TG438
Mdh3
TG572
TG143
TG61
core interval markers for:
population I
7.0 - 7.1 TDs2
7.1 - 7.2 Ac4-62, TI107, TG20A
7.2 - 7.3 TDs219, TG63B
7.3 - 7.4 Ac4-66
7.4 + TDs39, TDs5, Got2
populations II, III, IV
7.0 - 7.3 Ssp132, TG20A,
7.3 - 7.4 Ssp45B, Ssp73, Ssp51
7.4 + PI3, ST05A, Ssp57
populations V, VI, VII
7.0 - 7.1 TG499, CD61, CD65
7.1 - 7.2 TG199, TG20A
7.2 - 7.3 TG190, TG128
7.3 - 7.4 TG166A
7.4 + CT211B, TG13, CP52


8
GBSS
TDs385
TG402
TG261
TG16
TG45
TG176
core interval markers for:
population I
8.0 - 8.1
8.1 - 8.2 Ac15-8, TDs300, TDs95, TDs458
8.2 - 8.5 TDs293, TDs405
8.5 - 8.6 Got1, Ds1, Ac4-55, Ac3-28, TDs429
8.6 - 8.7
8.7 + Aps2
populations II, III, IV
8.0 - 8.5
8.5 - 8.6 Ssp15
8.6 + Ssp34
populations V, VI, VII
8.0 - 8.3 CD29, CD41
8.3 - 8.4 TG330
8.4 - 8.5 CD60, CT214A
8.5 - 8.6 TG41, TG72
8.6 - 8.7 CD21, PTN, CT27
8.7 +


9
TG10
TG09
TG390
TG35
TG08
TAc93A
core interval markers for:
population I
9.0 - 9.1
9.1 - 9.2
9.2 - 9.4 Ac10-73, TDs142, TG06, ST21
9.4 - 9.6 TDs396
9.6 + TDs187, TDs116
populations II, III, IV
9.0 - 9.2
9.2 - 9.4 Ssp60, Ssp59, Ssp20, Ssp32
9.4 - 9.5 Ssp74
9.5 + Ssp02, PI1, Ssp04
populations V, VI, VII
9.0 - (9.1) CT215, CP44, CD32A
(9.1) - 9.2 CT143
9.2 - 9.3 TG18, TG254, GP39, TG291D, TG640, TG16B, TG486, CT235, TG589, CT17, TG207, CT208, TG291, CD3
9.3 - 9.4
9.4 - 9.5 TG404, CD8, GP129, TG421
9.5 + GP101, CT220, TG591


10
Ac38-46
TG230
TG43
TG63
Iep Ice
PSC
Ac15-7
core interval markers for:
population I (female)
10.1 - 10.3 TG303, Cr, TDs277A
10.3 - 10.4 TDs273, Ssp45C, TDs448, Ro, TAc13A, TDs360
10.4 - 10.5
10.5 + F, ST15B, ST05, ST06
population I (male)
10.1 - 10.3 Cr, TDs448, TDs152, TDs273, TDs277B, Ro, TG303
10.3 - 10.4 ST15A, TAc20, F
10.4 - 10.5 ST06
10.5 + ST15B
populations II, III, IV
10.1 - 10.3 Ssp45C, Ssp41, Ssp64
10.3 - 10.4 Ssp106, Ro, TG44B
10.4 - 10.5
10.5 +
populations V, VI, VII
10.0 - 10.2 TG313
10.2 - 10.3 TG303, TG122
10.3 - 10.4 TG386, CD38A, TG52, CT214, TG280, CD32B
10.4 + CD32C, TG403, CD72, TG420, TG408

11



core interval markers for:

population *I*

11.0 - 11.2	
11.2 - 11.3	TDs18A
11.3 - 11.4	TDs250C, TDs331, *P*
11.4 - 11.5	
11.5 +	Ac5-34

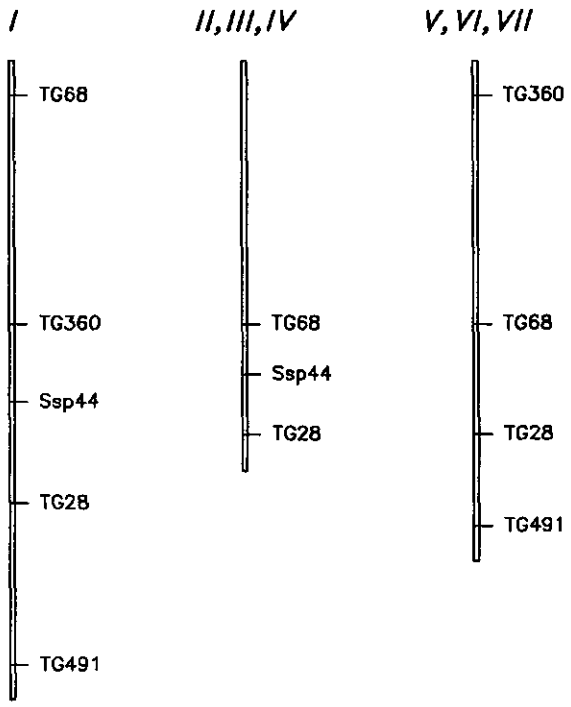
populations *II*, *III*, *IV*

11.0 - 11.3	Ssp85, GP21B
11.3 - 11.4	
11.4 - 11.5	Ssp29
11.5 +	

populations *V*, *VI*, *VII*

11.0 - 11.1	CP58A, CT182, TG523, TG508, CT168, TG194, CD74B, TG629
11.1 - 11.2	TG147, TG327, TG57, TG466, TG538
11.2 - 11.3	
11.3 - (11.5)	CT55, CT107A, TG46, CT107, TG384, TG546, CT107C, TG36
(11.5) +	TG26, TG393

12



core interval markers for:

population *I*

TG68	
12.2 - 12.1	TDs319, TDs103, ST20, ST14
TG360	
12.1 - 12.3	TDs340A, TDs340B, ST101, Ssp45
Ssp44	
12.3 - 12.4	TDs1, 6-Pgdh2
TG28	
12.4 - 12.5	ST07, TDs231
TG491	
12.5 +	TDs163

populations *II*, *III*, *IV*

12.0 - 12.2	Ssp126B, ST05B, Ssp67
TG68	
12.2 - 12.3	
Ssp44	
12.3 - 12.4	Ssp129, Ssp83
TG28	
12.4 +	

populations *V*, *VI*, *VII*

12.0 - 12.1	CT99
TG360	
12.1 - 12.2	CT219, CT211A, CT79, CT201
TG68	
12.2 - 12.4	TG263A, CD19, TG291C, TG387, TG263B, CD22, TG282, TG367
TG28	
12.4 - 12.5	TG296, CD2
TG491	
12.5 +	TG602

is not present in populations *V*, *VI* or *VII*, therefore the interval expands from core marker 1.2 to core marker 1.4.

In the calculation of the core map, data on 457 different markers were used of which 73 markers (16%) were designated as core markers (Table 3). In total 22 core markers were shared by all three groups of populations used, another 22 core markers were shared by two groups of populations, and 29 core markers were present in one (group of) population(s) only (Table 4). An additional 22 markers were shared by two groups of populations, but they were not chosen as core markers for the reasons explained above. The average number of markers per core interval in every group of populations is about the same (2.1 - 2.9), reflecting the effort to make a balanced choice of the number of core markers with respect to the total number of markers in each group of populations.

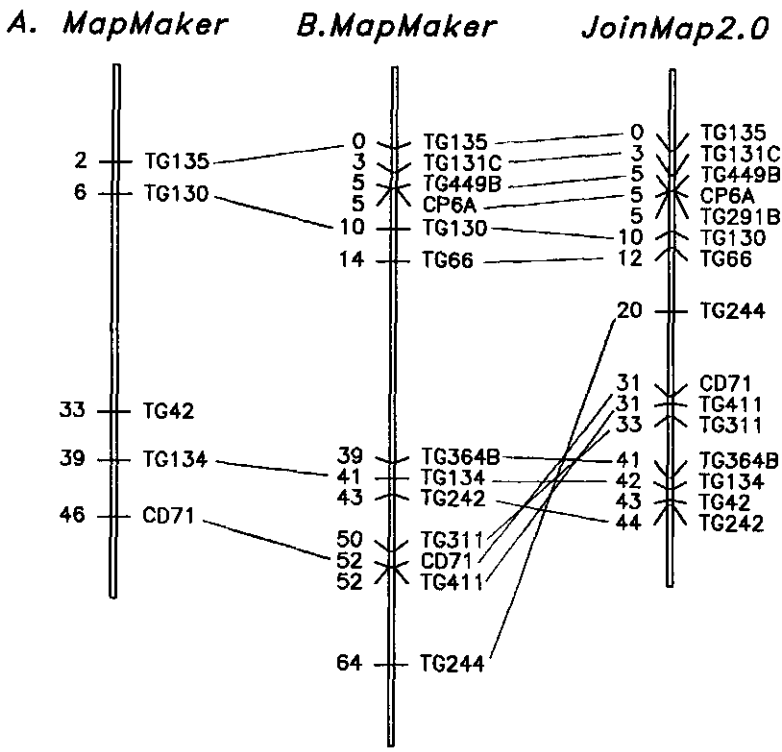


Figure 2. Map for chromosome *3* of population *V*. A. Map as presented in Bonierbale *et al.* (1988), B. Map as presented in Tanksley *et al.* (1992), C. Map as calculated in this study with JoinMap2.0.

The genetic map of chromosome *3* of population *V*, recalculated with JoinMap 2.0, showed an inversion, not observed before in the original map (Figure 2). The interval of TG244 to the tip of the chromosome (TG242) is inverted as compared to the maps of all other populations and the original map of population *V*. Inspection of the data of chromosome *3* of population *V* did not reveal any irregularities. However, in the initial grouping of the complete data set into separate linkage groups, the markers mapping to chromosomes *3* and *6*, were clustered together into one single linkage group. While all other linkage groups were separated with LOD=5.0, this cluster could only be split in the two linkage groups representing chromosome *3* and *6*, with LOD=9.0.

Table 3. Summary of the core map of potato

	Population *I*	Populations *II,III,IV*	Populations *V,VI,VII*	Total
Total number of markers	191	103	238	457
Number of core markers	51	35	53	73
Number of core intervals	53	33	63	85
Average number of markers per interval	2.6	2.1	2.9	4.5

Differences between maps of different mapping populations.

The core map of chromosome *6* was the most straightforward to construct. This can be explained by two reasons. First, in populations *II*, *III* and *IV*, only two segregating markers were found (TG115, TG118) that were expected to map on chromosome *6*. Therefore, data for chromosome *6* from this group of populations was lacking and a map of chromosome *6* could not be constructed in these populations. Second, in population *I* a relatively high number of TG-markers was used for the construction of the map of chromosome *6*.

The core map of chromosome *10* is presented with separate sets of interval markers for the male and female maps of population *I* (Figure 1). The chromosome *10* map of

population *I* is difficult to construct, because marker orders between the male and female parent show important differences. In addition, the male map length of chromosome *10* in population *I* is relatively short (59 cM). This suggests the presence of a chromosomal rearrangement between the two parental clones. Chromosomal rearrangements would result in irregular pairing of the homologous chromosomes, and recombination would give rise to incomplete or rearranged chromosomes. Therefore, selection will take place against gametes with recombinations on chromosomes *10*. As a result, reduced recombination will be observed, resulting in a shorter genetic map.

The marker order for chromosome *12* of population *I*, involving the region TG360-TG68, was different from the marker orders of the populations *V*, *VI* and *VII*. For this reason, separate maps for each group of populations are presented for chromosome *12* (Figure 1). Tanksley *et al.* (1992) found a paracentric inversion of the top half of chromosome *12* compared to the genetic map of tomato. However, the genetic map of population *I* indicated the same order of markers as the genetic map of tomato. It should be noted that in the construction of the genetic map of population *I*, it was not possible to join the two linkage groups that were expected to represent the two halves of chromosome *12* of the female parent with a significant LOD score. The female map of chromosome *12* was composed on basis of the order of markers in common with the male map of the same population. Since recalculations of chromosome *12* of the populations *V*, *VI* and *VII* confirmed the chromosome *12* map presented by Tanksley *et al.* (1992), this might indicate that this inversion is not present in all potato populations and/or species.

Discussion

Many applications of genetic markers in relation to traits of interest rely on some basic knowledge of map position of the trait of interest, or rely on the basic genetic maps to assign their associated markers to the appropriate linkage groups. In potato, maps constructed sofar have been derived from different (unrelated) populations, as well as from different tuberous *Solanum* species. The construction of a combined map is therefore a complicated and difficult task. However, aligning maps so that the position of markers is clearly in a given interval, common to as many maps as possible, will help to determine the approximate position of a marker/tait in other populations. It also increases the number of putative markers surrounding the marker/trait of interest,

Table 4. Presence of core markers in the separate populations and their type of segregation.

		I	*II,III,IV*	*V,VI,VII*			*I*	*II,III,IV*	*V,VI,VII*
1.1	TDs109	c			6.1	GP79	c		
1.2	TG24	a	*,*,a	b,b,b	6.2	TG231	a		b,b,b
1.3	S	a			6.3	TG240			b,b,b
1.4	TG71			b,b,b	6.4	TDs183	c		
1.5	TG237			b,b,b	6.5	TG115	b	b,a,*	b,b,b
1.6	TG53	a	a,*,b	b,*,*	7.1	TG438	c		b,b,b
1.7	TG259	a		b,b,b	7.2	TG572	a		b,b,b
1.8	TG27		*,*,a	b,*,*	7.3	TG143	a	c,c,*	b,b,b
2.1	TG31	b	a,*,c	b,*,*	7.4	TG61	b	a,c,a	b,b,b
2.2	TG01B	c		b,*,*	8.1	GBSS	c		
2.3	TG234			b,b,b	8.2	TDs385	c		
2.4	TG20B	c			8.3	TG402			b,b,b
2.5	TG34		b,a,c	b,b,b	8.4	TG261			b,b,b
2.6	TG48	c	*,a,a	b,*,*	8.5	TG16	a	a,c,*	b,b,*
2.7	TG141A			b,b,b	8.6	TG45	c	*,a,a	b,*,*
3.1	TG135			b,b,b	8.7	TG176			b,b,*
3.2	TG130	c	a,c,*	b,*,b	9.1	TG10	a		
3.3	TG74			b,b,b	9.2	TG09	c	b,a,a	b,b,b
3.4	Ssp66	c	*,a,a		9.3	TG390			b,b,b
3.5	TG42	a	*,a,a	b,*,b	9.4	TG35	c	*,*,c	b,*,*
3.6	TG134	a	b,a,a	b,b,*	9.5	TG08		b,a,*	b,*,*
3.7	TG244			b,b,b	9.6	TAc93A	a		
4.1	Ssp27	b	b,a,*		10.1	Ac38-46	c	*,a,*	
4.2	TG123	c	b,a,a	b,b,b	10.2	TG230			b,b,b
4.3	TG208			b,b,b	10.3	TG43	b	a,a,b	b,*,*
4.4	TDs168	c			10.4	TG63	c	b,c,b	b,b,b
4.5	TG65			b,b,b	10.5	Ac15-7	c	*,c,*	
4.6	TG155			b,b,b	11.1	TG497			b,b,b
4.7	Ac8-19	c	*,c,*		11.2	TG47	a		b,*,*
4.8	TG443			b,b,b	11.3	TG44	c	*,a,c	b,b,b
4.9	TG22	c		b,*,*	11.4	Ssp75	c	c,b,a	
5.1	GP21	c	*,a,*		11.5	TG30	c	b,c,*	
5.2	TG379			b,b,b	12.1	TG360	a		b,b,b
5.3	Ssp88	c	a,c,c		12.2	TG68	a	a,c,a	b,*,*
5.4	TG23	b	b,a,b	b,b,b	12.3	Ssp44	b	*,c,a	
5.5	TG69	a	a,c,c	b,b,*	12.4	TG28	c	*,*,a	b,*,*
					12.5	TG491	c		*,b,b

a = 1:1♀; b = 1:1♂; c = 1:1:1:1; * = not present in specified population.

which can be used for further fine-mapping. In addition, the high homology of the closely-related tomato and potato, reflected in the highly similar linear order of markers on their respective genetic maps (Bonierbale *et al.* 1988; Tanksley *et al.* 1992), gives a further value to the potato markers for use in genetic mapping studies in other Solanaceous species.

Construction of genetic maps of non-inbreds using JoinMap 1.4.

JoinMap 1.4 allows raw mapping data, from different (types of) populations and different independent estimates, to be combined into one single map. For non-inbreds, a map from a single mapping population can be established by regarding the mapping data obtained from one parent as an independent mapping population. In this context

the two parental maps are regarded as separate maps constructed in two different populations (Jacobs *et al.* 1995a). The segregation data obtained in a mapping population of non-inbreds, with two heterozygous parents, have to be recoded in order to "fit" in the format of commonly used mapping programmes, including JoinMap 1.4. Briefly, this is done as follows; 1:1♀, 1:1♂ and 1:1:1:1 segregation ratios are converted to "BC type" data, and 1:2:1 and 3:1 segregation ratios are converted to "F_2 type" data [see Jacobs *et al.* (1995a) and Van Eck (1995) for more extended description].

Genetic map construction in non-inbreds with JoinMap 2.0

For the construction of genetic maps of non-inbreds, JoinMap 2.0 includes the option "cross-pollinators" (CP), in which a two digit input per genotype/marker combination can be given. This enables the data scored from the segregating progenies to be directly used as input for the mapping programme, without the conversion or recoding necessary in the previous version of JoinMap. Up to four different alleles (two for each parent), and consequently up to four different allele combinations at one single locus, are therefore possible. This maximises the use of the available segregation data in genetic mapping calculations. Furthermore, the main improvement of JoinMap 2.0 in the application with "cross-pollinators" can be found in the calculation of the recombination frequencies between markers with different segregation ratios (1:1:1:1 vs. 1:1 vs. 3:1 vs. 1:2:1; see manual JoinMap 2.0)

One aspect of map construction with JoinMap 2.0 should be treated with caution; with segregation data originating from both parents, the CP option should preferentially be used solely for markers segregating in both parents. In some instances, an incorrect marker order can result when markers segregate from one parent only. This is especially the case when (1) recombination frequencies between the male and female map are highly divergent, so that marker intervals are significantly different between the male and female map, and (2) markers within the interval between two allelic bridges, segregate from one of the parents only. This is similar to what was reported for JoinMap 1.4 when combining data with highly divergent recombination frequencies (Wise & Schnable 1994; Jacobs *et al.* 1995a). In the direct construction of one combined map, these differences will not be observed. This is because an average is calculated, from which an incorrect marker order might result. This can be avoided by either not using the markers polymorphic in a single parent, or when these markers

are indispensable, calculate the separate parental maps as well. A large increase of the average χ^2 during map construction upon the addition of a marker, can be an indication of a poor fit of that marker, and inspection of the data is advised. Fixed sequences can be used to impose a correct marker order, deduced from the separate maps. The use of fixed sequences is a legitimate way of "guiding" JoinMap in map construction. In the construction of our core map this was an additional reason to choose core markers that were present in as many populations as possible, to avoid erroneous orders.

Differences between original and recalculated maps.

The maps of populations *I*, *II*, *III* and *IV* calculated with JoinMap 2.0 were shorter than the maps calculated with JoinMap 1.4. Recalculation of the maps for populations *V* and *VI* with JoinMap 2.0 resulted in longer maps than those originally published, calculated with MapMaker (Table 1). Such differences could not be explained by the mapping function, because in both cases the Kosambi mapping function was used in map construction. For the recalculation of the map for population *V*, only a limited number of markers of the original set is available in the SolGenes database. Therefore, the recalculation could only be accomplished with a subset of the data, which could have influenced the results. A contrasting result, the JoinMap map being shorter than the MapMaker map, has been reported in tomato. This was observed when the genetic map of tomato, originally constructed with the programme MapMaker (Tanksley *et al.* 1992) was recalculated with the programme JoinMap, using exactly the same mapping data (Van Ooijen *et al.* 1994). Furthermore, in this study the recalculated map of population *VII* using JoinMap 2.0 was similar in size as the original map constructed using MapMaker. Therefore, it would be an oversimplification to conclude that the differences are caused by the differences between the two computer programmes, and the result of the different algorithms used. Why these different results between different recalculations were found, is not clear.

The genetic maps of populations *V*, *VI* and *VII* are considerably shorter than the genetic map of population *I* (Table 1) and the genetic maps constructed by Gebhardt *et al.* (1991; 1994). In populations *V*, *VI* and *VII* the interspecific hybrids were used as the parent for map construction. Due to reduced recombination frequencies, caused by reduced homology between the chromosomes of the two parent species, interspecific hybrids often result in apparently shorter genetic maps. In addition, the

limited amount of markers used in populations *VI* and *VII* could have influenced the map length as well.

Concept of core markers and core intervals.
Several potato species were used for the construction of genetic maps. The comparison of these genetic maps revealed that chromosomal rearrangements exist between several mapping populations. Therefore, it is a delicate task to combine these genetic maps into one single consensus map. However, the combination of the available data into one map for future reference is desirable. Since it turned out to be impossible to combine all data into a useful map, we chose to use a limited number of markers from every mapping population, to construct a basic core map.

These core markers are used for the alignment of the different maps. A pair of adjacent core markers defines the borders of a core interval (similar to "bins", Gardiner *et al.* 1993). A limited set of core markers is selected, based on their position on the potato genome (evenly spaced) and their presence in as many different genetic maps of potato as possible. Such core markers can serve as starting points for genetic mapping of any trait of interest. Especially in a highly divergent crop species like potato, where an increasing number of wild species is used for introgression of genes of interest, the information stored in this way will be of more value than the construction of a "statistical average" map at all cost. The consensus core map is not a static map, and can be improved by adding additional markers to the intervals or by defining new subdivisions of the intervals by defining new core markers. This option of subdivision becomes increasingly important with the rapid expansion of PCR-based markers, that would make the number of markers per interval too high to be convenient. Core markers can also facilitate a quick assignment of linkage groups of markers like RAPDs and AFLPs, to chromosomes.

When initiating a new mapping study, the use of core map markers should allow quick determination of the core interval to which the new trait/marker maps. This can then be followed by more detailed mapping using as many markers as possible from within the core interval as well as (a limited number of) markers from the flanking intervals. In addition, pooling genotypes of the mapping population based on the alleles found with the core markers flanking the interval, should allow quick search for additional markers within the interval. Alternatively, core markers can be used on (pooled)

samples of a population with a trait of interest, to determine the appropriate genome interval. The reverse approach is applicable as well; markers found in a population with a trait of interest, can be quickly mapped in the basic population, relative to the core markers, by pooling the genotypes based on their phenotype for the trait of interest (eg. BSA-RAPD analysis, pooled AFLP).

With an increasing amount of mapping information that becomes available, especially with powerful techniques like AFLP, it is important to avoid unnecessary repetition of research effort. Therefore (one) basic population(s) with a well-developed reference core map will be a valuable resource for any species, and for non-inbred species in particular. This will allow efficient aligning of mapping information for any new trait to an existing basic map. In addition, in the process towards the construction of a reference core map, information can be gained on the importance of the potential differences, like chromosomal rearrangements, between different genetic maps. Widespread exchange of plant populations and/or of markers that have been placed on this reference core map is a prerequisite. The widespread use of tomato (TG) RFLP markers in potato, greatly assists the alignment of the maps, because they also define intervals that are common to many (Solanaceous) maps.

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Chapter 4

Mapping of resistance to the potato cyst nematode *Globodera rostochiensis* from the wild potato species *Solanum vernei*

(with: Herman J. van Eck, Karin Horsman, Paul Arens, Brigitte Verkerk-Bakker, Evert Jacobsen, Andy Pereira, Willem J. Stiekema)

Abstract A population of diploid potato (*Solanum tuberosum*) was used for the genetic analysis and mapping of a locus for resistance to the potato cyst nematode *Globodera rostochiensis*, introgressed from the wild potato species *Solanum vernei*. Resistance tests of 108 genotypes of a F_1 population revealed the presence of a single locus with a dominant allele for resistance to *G. rostochiensis* pathotype Ro1. This locus designated *GroV1*, was located on chromosome *5* with RFLP markers. Fine-mapping was performed with RAPD and SCAR markers. The *GroV1* locus was found in the same region of the potato genome as the *S. tuberosum* ssp. *andigena H1* nematode resistance locus. Both resistance loci could not excluded to be allelic. The identification of markers flanking the *GroV1* locus offers a valuable strategy for marker-assisted selection for introgression of this nematode resistance.

Introduction

The potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida* are severe pests in potato crops, causing a great loss in yield. To some extent, crop rotation and nematicides can reduce the soil-borne nematode population. However from a economical as well as an environmental point of view, the preferable way to avoid crop damage is to use cultivars resistant to the potato cyst nematodes. The cultivated potato, *Solanum tuberosum*, does not contain resistance to both *Globodera* species. Therefore wild *Solanum* species possessing resistance have been used for the introgression of this trait into potato cultivars (Ross 1986).

In the past, cultivars with resistance to some pathotypes of potato cyst nematodes have been developed using three different wild species for introgression: *S. tuberosum* ssp. *andigena*, *S. spegazzinii*, and *S. vernei* (Ross 1986). The monogenic *H1* locus from *S. tuberosum* ssp. *andigena* is used most widely in potato breeding and confers resistance to *G. rostochiensis* pathotypes Ro1 and Ro4. Inheritance of Ro1 resistance from *S. spegazzinii* and *S. vernei* is more complex and based on several loci as shown by classical genetic analysis (Plaisted *et al.* 1962; Scurrah *et al.* 1973; Ross 1986).

The development of diploid potato populations, combined with molecular techniques for genetic mapping, has allowed the genetic mapping of several loci for resistance to the potato cyst nematode *G. rostochiensis* pathotype Ro1. A major Ro1 resistance locus, *Gro1*, originating from *S. spegazzinii* was identified on chromosome *7* (Barone *et al.* 1990). This *Gro1* locus is most likely the *Fb* locus according to the nomenclature based on classical analyses (Ross 1986). The *H1* locus from *S. tuberosum* ssp. *andigena* was located on chromosome *5* (Pineda *et al.* 1993; Gebhardt *et al.* 1993). Quantitative loci from *S. spegazzinii* for resistance to *G. rostochiensis* Ro1 were mapped to chromosomes *10* (*Gro1.2*) and *11* (*Gro1.3*) (Kreike *et al.* 1993). In addition, a major locus from *S. spegazzinii* (*Gpa*) for resistance to the potato cyst nematode *G. pallida* was mapped on chromosome *5* (Kreike *et al.* 1994).

In this paper we describe the inheritance and mapping of a Ro1 resistance locus originating from *S. vernei* in a diploid potato population derived from a tetraploid clone commonly used for introgression of potato cyst nematode resistance into potato cultivars. Analyses involved phenotypic scoring of potato cyst nematode resistance, the use of RFLP markers for mapping of the resistance, and the development of PCR-

based markers such as RAPDs (Random Amplified Polymorphic DNA) and SCARs (Sequence Characterized Amplified Regions).

Materials and methods

Plant material

The diploid potato clone KW84-19-2471 (CPRO-DLO collection) was used as pollen donor in a cross to the diploid potato clone DH84-13-705 (CPRO-DLO collection) to obtain the F_1 population 89-24. KW84-19-2471 and DH84-13-705 were susceptible and resistant respectively to *G. rostochiensis* pathotypes Ro1 and Ro4 and *G. pallida* pathotype Pa2. From the F_1 population 89-24, 108 genotypes were used in this study. DH84-13-705 is a dihaploid, obtained from the tetraploid clone AM70-2115 via parthenogenesis after pollination with *S. phureja* (AM70-2115= $(VT^n)^2$62-33-3 x [$(VT^n)^2$62-33-3 x *S. tuberosum* clone B3769]). The tetraploid clone $(VT^n)^2$62-33-3, commonly used for introgression of potato cyst nematode resistance in potato cultivars (Ross 1986), was derived from a hybrid between *S. tuberosum* and *S. vernei*.

Resistance tests

The resistance tests were performed as described before (Kreike *et al.* 1993). Plants derived from at least two well-rooted cuttings (1990, 1991) or three tubers (1993) of each F_1 genotype were tested for resistance. In total 108 F_1 genotypes were tested for resistance to *Globodera rostochiensis* pathotype Ro1. From these 108 F_1 genotypes, 69 were tested for resistance to *G. rostochiensis* pathotype Ro4. A subset of 49 of these 69 F_1 genotypes were tested for resistance to *G. pallida* pathotype Pa2. Genotypes were designated resistant when the results of resistance tests consistently showed ≤ 30 newly formed cysts and susceptible when the results of the resistance tests consistently showed ≥ 100 newly formed cysts. The nematode populations used were Ro1 'Mierenbos A' (Arntzen & Van Eeuwijk 1992), Ro4 'F520' (CPRO-DLO collection), and Pa2 'P2-22' (Arntzen & Van Eeuwijk 1992). Both parents, as well as resistant and susceptible standards were included in each test. Resistant standards were the cultivars Saturna (Ro1, Ro4) and Astarte (Ro1), and the breeding clone $(VT^n)^2$62-33-3 for Pa2. Susceptible standards were the cultivars Maritta (Ro1, Ro4, Pa2) and Astarte (Ro4), and the breeding clone *S. vernei* hybrid 58-1642/4 (Pa2).

RFLP markers

DNA isolation, Southern blotting, and RFLP analyses were performed as previously described (Van Eck *et al.* 1993). RFLP markers mapped in a diploid potato population (Jacobs *et al.* 1995a) were used to determine the position of the *S. vernei* locus for potato cyst nematode resistance. TG markers were a kind gift of Dr. S.D. Tanksley, Cornell University, Ithaca NY (USA).

RAPD/BSA analyses

RAPD/BSA analyses were performed with random 10-mer primers from Operon Technologies Inc., Alameda (CA). The profile of the RAPD/BSA reaction using a Omnigene thermocycler (Hybaid) with tube-control for temperature was: 5 min. 92.5°C, 40 cycles of (5 sec. 92.5°C, 45 sec. 34.5°C, 45 sec. 72°C), 5 min. 72°C. The reaction was performed in a volume of 50 μl, with 0.1 U *Taq* polymerase. A total amount of 250 ng template DNA per reaction was used. Initial screening of primers in BSA reactions (Michelmore *et al.* 1991) was performed on samples of the resistant parent (P_R), the susceptible parent (P_S), a pool of DNA from 8 resistant F_1 genotypes (poolR), or 8 susceptible F_1 genotypes (poolS). To avoid possible incorrect scores from the resistance test, only genotypes at the extremes of the distribution were used; less than 10 cysts per plant for the resistant

pool and over 200 cysts per plant for the susceptible pool. Positive primers, which reproducibly amplified a fragment in P_R and poolR vs. no fragment in P_S and poolS, were tested on a second poolR and poolS, and when still positive, RAPD analyses of these primers with the individual genotypes from the F_1 population were performed.

SCAR development

RAPD fragments specific for the resistance were cloned in order to develop SCARs (Paran & Michelmore 1993). Three selected RAPD products amplified by the primers OpT08 (5'AACGGCGACA3'), OpU14 (5'TGGGTCCCTC3'), and OpX02 (5'TTCCGCCACC3') were cloned into the *Eco*RV site of pBluescript SK$^+$ (Stratagene). The DNA sequences of these clones were determined by the dideoxy chain termination method using an automated sequencer (Applied Biosystems Inc.). Longer primers (20- to 25-mers) were designed on the basis of these sequences. SCAR primer pairs were: T08, 5'AACGGCGACAGAAGAGTTGG3' and 5'GACATCGTCACATTATTGAAACAGC 3'; U14, 5'GGGCTTGTATAAGACCTCCGAGAGG 3' and 5'CCCTTCCTTGGGTAGTTTGAGCG 3'; X02, 5'CCACCAAACCCATAAAGCTGC 3' and 5'TGTGAATTGTGATGAATCTGCAACC 3'. The PCR conditions for the SCAR primer sets were: 7 min. 92°C, 25 cycles of (1 min. 92°C, 1 min. annealing, 2 min. 72°C), 5 min. 72°C. Annealing temperature was 57°C for SCAR T08 and SCAR U14, and 50°C for SCAR X02. The SCAR reaction mixtures were identical to the RAPD reaction mixtures.

Linkage analysis and map construction

Calculation of pairwise recombination frequencies between markers and the subsequent construction of the genetic map of chromosome 5 were performed with the computer programme JoinMap (Stam 1993). The Kosambi function for converting recombination frequencies into map units (cM) was used. The computer programme Drawmap (Van Ooijen 1994) was used for graphic representation of the map.

Results

Resistance testing

The resistant diploid potato clone DH84-13-705 was crossed to a fully susceptible diploid clone (KW84-19-2471). Initially 53 F_1 genotypes were screened for resistance to *G. rostochiensis* pathotype Ro1. A 1:1 ratio (26 resistant, 23 susceptible, $\chi^2_{1:1}$= 0.18, $P>0.3$, 4 indecisive genotypes) was found. This suggested that the resistant parent was heterozygous, and that the resistance was inherited as a monogenic and dominant trait. Consequently, the F_1 population was suitable for mapping the Ro1 resistance locus. Therefore, another 56 F_1 genotypes were tested for Ro1 resistance, which confirmed the 1:1 segregation ratio. The retesting of F_1 genotypes in successive years invariably resulted in consistent results. This often allowed individuals with indecisive response to be more definitively assigned to resistant or susceptible. Conflicting results were only apparent in rare instances. In total 108 F_1 genotypes were screened for resistance to *G. rostochiensis* Ro1, of which 102 could be classified into 46 resistant and 56 susceptible genotypes (46:56, $\chi^2_{1:1}$=0.98, $P>0.5$).

To determine the spectrum of potato cyst nematode resistances present in the parental clone DH84-13-705, a limited number of F_1 genotypes was also screened for resistance to the nematodes *G. rostochiensis* Ro4, and *G. pallida* Pa2. In the first Ro4 resistance test (1991), segregation of the response to Ro4 fitted a 1:1 ratio for resistance versus susceptibility (16:18, $\chi^2_{1:1}=0.12$, $P>0.25$) and showed correlation between resistance to Ro1 and Ro4. From the genotypes of which Ro1 and Ro4 resistance could be compared, two genotypes were found that appeared to be recombinant; resistant to Ro1 and susceptible to Ro4 in two consecutive years of testing (Table 1). Combined results over several years of testing the Ro4 resistance, revealed a significantly higher frequency of susceptible genotypes than expected (19:45, $\chi^2_{1:1}=10.56$, $P<0.005$). This overrepresentation of susceptible genotypes probably reflects a selective loss of progeny during successive years of clonal propagation. Resistance to Pa2 did not show a clear segregation into separate classes, indicating that Pa2 resistance might be determined by more than one locus. However, a correlation between Pa2 resistance and Ro1/Ro4 resistance was observed (R=0.69, based on log[average cyst number + 1], n=49).

Table 1. Resistance of population 89-24 to *Globodera rostochiensis*, pathotypes Ro1 and Ro4.

	G. rostochiensis Ro1	
	resistant[1]	susceptible[1]
G. rostochiensis Ro4		
resistant[1]	14	0
susceptible[1]	2	38

[1]resistant < 30 cysts, susceptible > 100 cysts.

RFLP mapping of the GroV1 locus

In order to map the resistance locus from *S. vernei*, a limited set of RFLP markers was initially used that mapped to regions in the genome previously correlated with monogenic resistances to potato cyst nematodes. Segregation of markers on chromosome *7*, in the region of the *Gro1* locus from *S. spegazzinii* (Barone *et al.* 1990), did not show any correlation to resistance from *S. vernei*. However, RFLP marker TG69, that mapped to chromosome *5* in the region of the *H1* locus (Pineda *et al.* 1993), did show linkage to the *S. vernei* resistance (Figure 1). Two additional chromosome *5* RFLP markers, polymorphic in population 89-24, confirmed that the *S. vernei* resistance locus is located on this chromosome.

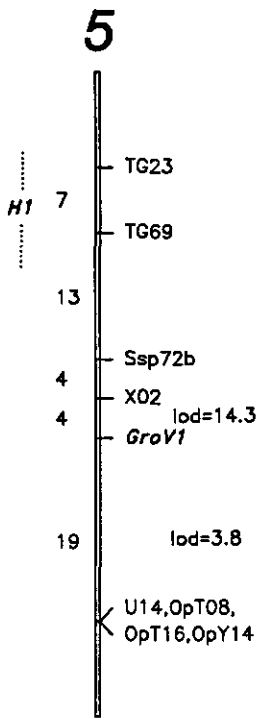

Figure 1. Genetic map of the lower part of chromosome *5*. TG23, TG69, and Ssp72b are RFLP markers; OpT08, OpT16, and OpY14 are RAPD markers; X02 and U14 are SCAR markers. Genetic distances are given in cM. Linkage of *GroV1* with flanking markers; X02 4 cM (lod=14.3) and U14 19 cM (lod=3.8). The approximate position of the *H1* locus for resistance to *G. rostochiensis* pathotype Ro1 (Pineda *et al.* 1993; Gebhardt *et al.* 1993) is depicted with a dashed line.

RFLP markers were primarily chosen for polymorphism in the resistant parent, but whenever the RFLP markers were polymorphic in the susceptible parent these segregating alleles were scored in the F_1 population as well. In this approach, independent genetic maps of chromosome *5* from both parents could be constructed, analogous to that previously described for potato (Jacobs *et al.* 1995a). The orders of the RFLP markers in the resulting chromosome *5* maps of each parent were the same. Although the *S. vernei* resistance locus and the *H1* locus map to the same region on the potato genome, the resistance locus we describe here is, based on its pedigree and resistance spectrum, clearly from a different origin. Therefore this resistance locus was named *GroV1* (V1 from *S. vernei* Ro1) to distinguish it from the *H1* locus.

Fine-mapping with RAPD markers by Bulked Segregant Analysis (BSA)

Based on the Ro1 resistance tests, the F_1 genotypes could be divided into two classes: resistant and susceptible. This allowed BSA analyses (Michelmore *et al.* 1991) on pooled DNA from individuals of each class. In total, 312 RAPD primers were tested

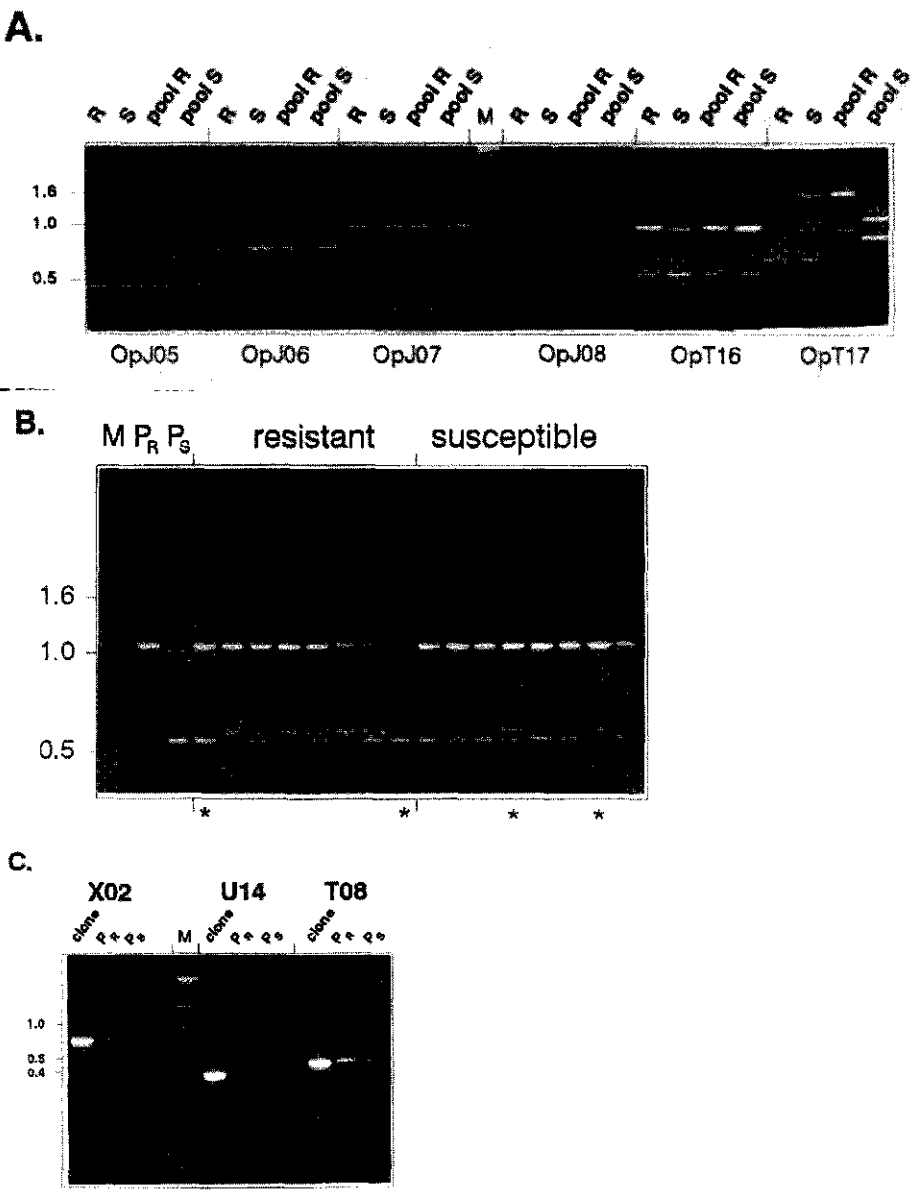

Figure 2. Markers linked to the *GroV1* locus. **A.** BSA reactions; primer OpT16 amplifies a band correlated with resistance. R = resistant parent, S = susceptible parent, poolR = pooled DNA from 8 resistant genotypes, poolS = pooled DNA from 8 susceptible genotypes. **B.** RAPD profiles of the individual genotypes of the pools, shown in panel a, with RAPD primer OpT16. Recombinant genotypes are marked with an asterisk. M = marker lane, P_R = resistant parent, P_S = susceptible parent. **C.** SCAR profiles of 3 cloned RAPD fragments. Template DNA was of the cloned fragment, resistant parent (P_R), and susceptible parent (P_S).

with DNA from the resistant parent (P_R), the susceptible parent (P_S), a pool of 8 resistant genotypes (poolR), and a pool of 8 susceptible genotypes (poolS) (Figure 2). The pool size of 8 genotypes for the BSA reactions was experimentally determined on the basis of detection level and desired interval of the genome to be covered (Giovannoni *et al.* 1991; Michelmore *et al.* 1991).

Six RAPD primers reproducibly gave an amplification product in P_R and 2 independent poolR, whereas no product was found in P_S and poolS. Therefore, the fragments amplified by these RAPD primers seemed correlated to the resistance locus. When these 6 positive RAPD primers were tested with DNA of 35 individual F_1 genotypes, 5 RAPDs were linked to the resistance locus (Figure 1), while one RAPD (OpV07) could be located on the same linkage group but at a distance of more than 40 cM. Four of the 5 RAPD products linked to the resistance (OpT08, OpT16, OpU14, and OpY14) were clustered, *i.e.* no recombinants between them were found in the 35 F_1 plants tested. This cluster of RAPD markers was located 19 cM distal to the resistance. The closest linkage (4 cM) was found with RAPD marker OpX02, located proximal to the resistance locus. OpU14 and OpT08 gave the clearest results in terms of presence or absence of a fragment. Both of these primers amplified several DNA fragments, each with only one fragment specific for the resistance (649 bp for OpU14, 517 bp for OpT08). Primer OpX02 also amplified several fragments, with a 854 bp fragment present in both resistant and susceptible genotypes, that showed a clear difference in intensity between the two classes.

Development of SCAR markers

RAPD products linked to *GroV1*, amplified by the primers OpT08, OpU14, and OpX02 were cloned and sequenced. On the basis of these nucleotide sequences, longer primers (20 to 25 nucleotides) were designed. The pairs of primers designed for OpU14 and OpX02 were chosen to be located within the sequence of the cloned fragment, not containing the sequences of the RAPD primers. As a result, the fragments produced with the longer OpU14 and OpX02 primers are expected to be shorter than the original RAPD products. In all cases, PCR fragments of the predicted length were produced with template DNA from both the plasmid containing the cloned fragment and DNA of the resistant parent. SCAR fragment sizes were T08 511 bp, U14 366 bp, and X02 798 bp.

The SCAR-X02 primers amplified a fragment of the correct size in the P_R and poolR. However, SCAR-X02 also showed a fragment of the same length in the susceptible parent and susceptible F_1 genotypes. This 'susceptible' fragment, like the 'susceptible' fragment amplified by RAPD primer OpX02, clearly showed a lower intensity on ethidium bromide stained agarose gels. Increase of the annealing temperature in the PCR reaction or digestion of the PCR products with restriction enzymes prior to separation on agarose gels did not result in a more specific profile for resistance.

In contrast to the RAPD OpT08, the SCAR-T08 primers produced a fragment of similar size in the resistant as well as the susceptible genotypes. Since the primers designed for this SCAR included (part of) the RAPD primer-sequence, the difference found in the RAPD reaction between the susceptible and resistant genotypes might be based on a difference within the 10 nucleotides of the RAPD primer. Changing the reaction conditions of the PCR (raising the annealing temperature, varying the $MgCl_2$ concentration in the PCR reaction mixture, or digestion of the DNA products with restriction enzymes prior to gel-electrophoresis) failed to alter the pattern.

The SCAR-U14 primers (Figure 3) amplified a fragment of the correct size correlated to the resistance. These primers also produced additional DNA fragments that were not related to the resistance. Changing the PCR conditions did not result in a more specific PCR amplification profile. This means that fluorometric screening of the results from the PCR reactions, desirable for large-scale screenings in marker-assisted selection, can not be performed. Gel-electrophoresis and ethidium bromide staining was therefore compulsory to distinguish genotypes producing the fragment (resistant) from those not producing the fragment (susceptible).

Discussion

Resistance testing

The clear segregation found between resistant and susceptible genotypes in simple Mendelian ratios, had not previously been described for *S. vernei* sources of Rol resistance. Therefore, a part of the F_1 population 89-24 was screened for resistance to the Ro4 pathotype of *G. rostochiensis*. This allowed an assessment of possible similarity to the *H1* locus from *S. tuberosum* ssp *andigena* CPC1673 (Ross 1986), which confers resistance to both Rol and Ro4. A correlation between the Rol and

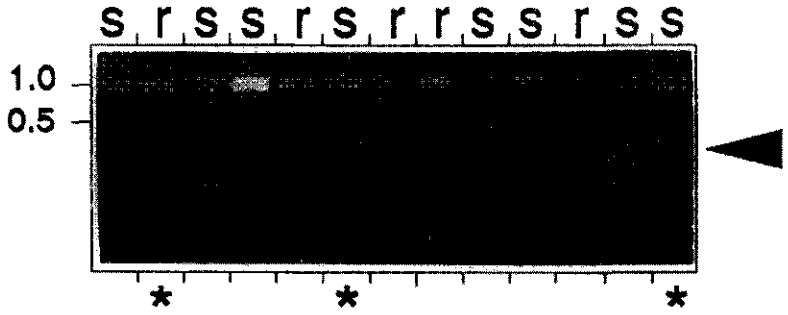

Figure 3. SCAR profiles of primer pair U14 with individual genotypes. r = resistant genotype, s = susceptible genotype. Recombinant genotypes are marked with an asterisk.

Ro4 resistances as observed for the *H1* locus was also found for the *GroV1* locus in F_1 population 89-24. However, two Ro1/Ro4 recombinants suggest a different spectrum of resistance for *GroV1*. Additional resistance tests will confirm this recombinant nature.

To further investigate potential similarities of the *GroV1* resistance locus from *S. vernei* with the *H1* locus, a resistance test with the potato cyst nematode *Globodera pallida* pathotype Pa2, population P2-22, was performed. Resistance derived from *S. tuberosum* ssp. *andigena* CPC1673, the source of the *H1* locus, to a single specific Pa2 population (D236) was recently described (Arntzen *et al.* 1993). In the same study, several other Pa2 populations, including P2-22, were tested but only resistance to the D236 Pa2 population was found. However, we observed a correlation between Ro1/Ro4 resistance and resistance to Pa2 population P2-22, indicating that the *GroV1* locus under study, based on its spectrum of resistance, is phenotypically different from the *H1* locus.

Mapping of the GroV1 locus

Classical genetic studies on Ro1 resistance from *S. vernei* in tetraploid potato populations indicated that this resistance could be based on two independent major loci (Plaisted *et al.* 1962). In contrast, our population segregated for only one locus conferring Ro1 resistance. One explanation for this difference could be that the second

resistance locus is present in the resistant parent, but in a homozygous state and therefore not segregating. The difference could also be due to loss of the second locus from the original resistant line during repeated (back)crosses to *S. tuberosum*, or to the reduction from tetraploid to diploid level. Furthermore, from the pedigree data available it can be concluded that the *S. vernei* background used in this research is derived from a different accession to that previously studied (Plaisted *et al.* 1962; Scurrah *et al.* 1973).

By screening progenies from different intra- and interspecific crosses at the tetraploid level, it was found that one of the two proposed loci for Ro1 resistance from *S. vernei* seemed to be segregating at the same locus as the *H1* locus from *S. tuberosum* ssp. *andigena* CPC1673 (Scurrah *et al.* 1973). RFLP mapping of the *GroV1* locus as reported in this paper on chromosome *5* distal to TG69, compared to the mapping of *H1* closely-linked proximal to TG69 (Pineda *et al.* 1993), established that *GroV1* is indeed located in the same region of the potato genome. RFLP markers on chromosome *5* used by Gebhardt *et al.* (1993) for the mapping of the *H1* locus, were not polymorphic in our population. Therefore, their *H1* mapping results could not be further compared with our mapping data of the *GroV1* locus. In addition to the lack of markers in common, the three mapping studies are based on relatively limited numbers of genotypes. Therefore, we can neither confirm conclusively that the *GroV1* locus resides at a locus different from *H1*, nor exclude that *H1* and *GroV1* are different alleles of the same resistance locus.

Fine-mapping with RAPD and SCAR markers

Testing 312 RAPD primers with pools of DNA from 8 resistant or 8 susceptible genotypes initially gave 11 RAPD fragments correlated with the resistance. With an average of 4 to 5 fragments per reaction, about one percent of the amplified fragments were therefore correlated with nematode resistance. This is the expected frequency of positive associations given the pool size of 8 genotypes and an estimated size of the potato genome of about 1000cM (Gebhardt *et al.* 1991; Jacobs *et al.* 1995a).

The primer pairs based on the 3 cloned and sequenced RAPD products were effective at amplifying SCAR fragments. SCAR-U14 could be used directly for screening for the presence of the *GroV1* resistance locus. The RAPD primer OpT08 showed a dominant phenotype linked to resistance, whereas the corresponding longer SCAR

primers gave rise to a product in both resistant and susceptible genotypes. The third primer pair (SCAR X02) gave the same intensity difference between resistant and susceptible genotypes as the original RAPD primer X02. This result for the conversion of RAPD markers into SCAR markers, is similar to what was described before (Paran & Michelmore 1993). They also found instances where fragments, specifically amplified in one class of genotypes by 10-mer RAPD primers, became visible in all genotypes when using longer primers based on the sequence of the cloned fragment.

Although the SCAR T08 and SCAR X02 primer pairs were initially not fit for use in (pre)screenings for resistance, with further research they can be developed for marker-assisted selection. Since the complete sequences of the cloned RAPD fragments have been determined, restriction enzyme sites within the fragments are known. Restriction enzymes can therefore be used to produce fragments of known length from the PCR product, which may further differentiate resistant and susceptible genotypes. Unfortunately, none of the restriction enzymes we used, known to have a restriction site in the 'resistant' fragment, revealed a difference between resistant and susceptible genotypes. Cloning and sequencing of the 'susceptible' amplified fragment could assist in the search for appropriate restriction enzymes. Alternatively different primers could be designed for the SCAR reaction, or a combination of different primer pairs, with variable stringencies during their respective reaction cycles could be used.

Marker-assisted selection

Seventeen primers produced a fragment in P_R and poolS, with no fragment in poolR. These fragments were in repulsion phase with the resistance and thus were amplified from the 'susceptible allele' of the resistant parent. Although these RAPD products can be used to distinguish resistant from susceptible genotypes, they were not considered in further experiments. Amplification of a 'susceptible' fragment is less appropriate for marker-assisted selection, since selection for resistance is based on absence of a PCR product. Consequently, false-positives resulting from a failed PCR reaction, may be wrongly interpreted as presence of resistance.

This study has identified markers flanking both sides of the *GroV1* locus conferring nematode resistance in potato. The genetic distance of these SCAR markers to the resistance locus will determine the success of marker-assisted selection. The SCAR-U14 primer pair amplifies a 366 bp fragment that mapped 19 cM distal to *GroV1*,

whereas the SCAR-X02 primer pair amplifies a 854 bp fragment 4 cM proximal to *GroV1*. Since these markers flank both sides of the resistance locus, the total number of seedlings that have to be screened can be substantially reduced while maintaining a high confidence of avoiding false positives and recombinants.

Clustering of resistance loci

The positions of different resistance loci in potato, even to different types of pathogens, are found to be clustered together in the genome (Gebhardt *et al.* 1993). Loci mapped to chromosome *5* of potato include resistance against potato virus X, *Rx2*, and resistance to *Phytophthora infestans* race 1, *R1*, at approximately the same locus (Ritter *et al.* 1991; Leonards-Schippers *et al.* 1992). Chromosome *5* also contains the earlier mentioned resistance to the potato cyst nematode *G. rostochiensis* pathotype Ro1, *H1* (Pineda *et al.* 1993; Gebhardt *et al.* 1993), and a major locus for quantitatively inherited resistance against the potato cyst nematode *G. pallida* pathotype 2, *Gpa* (Kreike *et al.* 1994). This paper describes the mapping of another resistance to potato cyst nematodes, *GroV1*, on chromosome *5*.

With the increasing number of disease resistance loci that have been mapped in several plant species, clustering of resistances against different pathogens at particular regions in the genome is commonly found (*eg.* Dickinson *et al.* 1993; Kesseli *et al.* 1994; Kaloshian *et al.* 1995). The question can be raised whether the equivalent genomic positions (*eg.* in potato the *R1* and *Rx2* loci, or the *H1* and *GroV1* loci) are purely coincidental, or are due to a general mechanism giving rise to resistance to a variable set of pathogens, present at similar genomic positions in different genetic backgrounds. To investigate such relationships, the search for markers specific for the different (alleles of) resistance loci becomes more important (Niewöhner *et al.* 1995).

Genetic mapping of potato cyst nematode resistance from different genetic backgrounds will enable more extensive studies on the identity of the resistance found in different germplasm sources, their effect, and their mode of action. Cloning of the different resistance genes will be essential for these studies. In anticipation of cloning these genes, we have chosen the approach of transposon tagging. This involved the development of potato lines containing the maize transposable elements *Ac/Ds* and *En/I*. The genomic positions of the transposable elements in these lines have been determined (Jacobs *et al.* 1994). Clones containing *Ac/Ds* elements on chromosome

5, close to the position of the nematode resistance loci are now being used for crosses in order to obtain transposon tagged mutants.

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General discussion.

An extensive genetic map of potato (*Solanum tuberosum* L.) based on morphological, isozyme and DNA markers, is a useful tool for a wide range of breeding applications as well as for basic science. The aim of this thesis was to develop such a basic genetic map for potato as a framework for other potato populations and *Solanum* species, and to illustrate the use of this map in basic and applied research. The project "Construction of a genetic map of potato", of which the results are described in this thesis and in Van Eck (1995), was granted at a time that no genetic maps of potato were available. A limited number of morphological and isozyme markers were described. However, none of these markers were located on specific linkage groups. Furthermore, recent genetic analyses of potato had been simplified by using diploid populations and closely related diploid wild species, rather than tetraploid cultivated potato.

Similar genetic mapping studies were previously performed in tomato (*Lycopersicon esculentum*), a diploid, inbreeding species, that is related to but not cross-hybridising with potato. Just as the research for this project started, the first molecular genetic map of potato was published (Bonierbale *et al.* 1988), that made use of RFLP markers, originating from tomato, and mapped on the molecular genetic map of tomato. It was found that there was high colinearity between the potato and tomato genome. This colinearity, and the absence of an adequate numbering of the potato chromosomes, led to the numbering of the potato linkage groups in accordance with the numbering for the tomato chromosomes. This numbering, based on homoeology with tomato, is now commonly used in potato genetics (*eg.* Gebhardt *et al.* 1991; Van Eck *et al.* 1993; Jacobs *et al.* 1995a). An advantage of potato over tomato is the high level of heterozygosity in potato, and the ease with which polymorphism is found. Therefore, genetic maps can be constructed from closely related parental lines. In contrast to inbreeding species, like tomato, with low intraspecific variability, potato as a non-inbred species carries a large potential for the use of genetic maps in breeding via the introgression of traits with marker-assisted selection. The high level of homology and colinearity between the tomato and potato genomes, makes exchange of mapping information, whether it be (molecular) markers, the position of monogenic traits of interest or even QTL, a very attractive feature. However, the genetic map of pepper (Prince *et al.* 1993), based partly on tomato RFLP markers, showed only a limited number of intervals conserved between pepper and tomato. Therefore, it

remains to be seen whether the orthologous counterpart of a trait mapped in one species is always found in the other one. However, especially for traits that are easier to assess in one species rather than in the other, mapping in the "easier" species is an option that should be taken into account.

A genetic map of potato.

Chapter one of this thesis describes the construction of a genetic map of potato, in a diploid backcross population. Separate maps for the female and male parents of the mapping population were constructed, that included molecular markers as well as morphological and isozyme markers. With the help of JoinMap 1.4, a computer programme for genetic mapping, these maps were combined together on basis of the markers in common (allelic bridges; Ritter *et al.* 1990). This method of map construction fully utilises the segregation data that can be obtained in a mapping population of non-inbreds (Jacobs *et al.* 1995a; Van Eck 1995), and is essentially different from the methods described sofar for non-inbreds.

Only a limited number of classical markers were previously described for potato, the majority of which could be mapped in this population. The morphological traits mapped, involve the flower colour markers *D*, *F* and *P* (Van Eck *et al.* 1993), the tuber shape locus *Ro* (Van Eck *et al.* 1994a), and loci for self-incompatibility (*S*), metribuzine resistance (*Me*), tuber flesh colour (*Y*), yellow margin (*Ym*), desynapsis (*Ds1*), and crumpled (*Cr*) (Jacobs *et al.* 1995a). In addition, a major QTL involved in earliness was mapped on chromosome *5* (Van Eck 1995). Data on more quantitative trait loci were collected, and analysis and mapping is currently being performed (Van Eck *et al.* in preparation).

In addition to the mapping population, derived from the backcross of clone C x clone E (Jacobs *et al.* 1995a), we also anticipated using genotypes of the reciprocal cross (ExC) for the construction of the genetic map. This would have been expected to improve the map with regard to marker orders, especially in those regions where the backcross in one direction, but not the other, resulted in distorted segregation. It could also be used as a means to study possible differences in the two parental clones, with regard to differences in recombination frequencies during female and male meiosis, a phenomenon commonly found in Solanaceae (*eg.* Rick 1969; DeVicente & Tanksley 1991). However, we encountered problems in obtaining a reasonably-sized population

of ExC genotypes. The rare berries that were obtained from this reciprocal cross often contained only a few seeds. Therefore it was necessary to pool seeds from several crosses in order to obtain sufficient individuals to serve as a mapping population. In RFLP analysis of this EC population, unexpected progeny genotypes were encountered for many progeny clones. The segregation of molecular markers in these unexpected genotypes either suggested tetraploidy, caused by the union of unreduced 2n gametes from both parents, or diploid genotypes arising parthenogenically from unreduced 2n female gametes. The tetraploid nature of genotypes was subsequently confirmed by flow cytometry analysis of the EC population. The size of the EC population after discarding the tetraploid genotypes and the "parthenogenic" diploid genotypes was only 23. This would be a statistically insufficient number for map construction and/or analysis of the possible differences between male and female meiosis. The reciprocal backcross was therefore not used in RFLP analysis and map construction.

The mapping population used in this study consisted of 67 genotypes. This population size appears limited, in comparison with backcross mapping populations used for inbreeding species. However, for the same number of progeny genotypes, the true population size of sampled gametes in non-inbreds can be twice as large as that for inbred species. Cross-over events in the recurrent parent of a mapping population of inbreds, will not be visible, due to the homozygosity of the inbred line. A population size of 67 backcrossed individuals in an inbreeding species will result in the information from 67 meioses. In contrast, in a non-inbred crop like potato, both parents will be heterozygous, therefore a large proportion of the markers will show polymorphism in both parents. Consequently, the effective number of meioses that can be studied in a non-inbred mapping population of 67 will be 2 x 67 = 134, assuming that alleles are segregating from both parents. Nevertheless, in many studies concerning non-inbred species, the information contained in one of the parents has been neglected in the analysis (*eg.* Bonierbale *et al.* 1988; Durham *et al.* 1992). This reduced the effective population size to the actual number of individuals analysed.

The first genetic map of potato (Bonierbale *et al.* 1988) had a total length of 606 cM, which was much smaller than expected on basis of similarity with tomato regarding its genome size. There are several reasons that may account for this "short" map. First, the male parent was used as the mapping parent. In Solanaceous species it is routinely found that maps derived from the male parent are shorter than the maps

derived from female parents (tomato, DeVicente & Tanksley 1991; Van Ooijen *et al.* 1994; potato, Jacobs *et al.* 1995a). Second, an interspecific hybrid was used as the mapping parent. Interspecific hybrids are well known to have reduced pairing of chromosomes, due to reduced homology between two distinct species, which results in less recombination and therefore shorter genetic maps. The second genetic map of potato (Gebhardt *et al.* 1989b), constructed from an intraspecific cross was already slightly longer (690 cM), and the latest version of this map (Gebhardt *et al.* 1994) is of a similar size as the tomato map (Tanksley *et al.* 1992). The genetic map of potato presented in this study (Jacobs *et al.* 1995a) also has approximately the same length as the genetic map of tomato.

Integration of classical and molecular markers in one map.

In numerous plant species segregation data for classical as well as molecular markers have been obtained and linkage maps subsequently constructed. However, in general classical and molecular maps have been developed separately. Integrated maps containing both classes of markers were published only recently (*eg.* Tanksley *et al.* 1992; Hauge *et al.* 1993; Kiss *et al.* 1993; Kesseli *et al.* 1994). Whenever integration has been performed, it is often limited to either small regions of the genome or only a few classical markers. Most of the time only the approximate map position of the classical markers on the molecular genetic map is known. Accurate map integration is primarily impeded by: the limited number of classical markers that can be scored in a particular cross; the difficulties in many species to obtain tester lines for classical markers; and the lack of marker exchange between mapping populations (allelic bridges) necessary for accurate map integration. Due to the large effort involved to align the classical and molecular mapping information in an appropriate manner, highly accurate integration of classical and molecular maps is mostly restricted to those regions of the genome containing genes of interest. Existing examples of such restricted, but well-documented map integrations, can be found in tomato for chromosome *1*, surrounding the *Cf-2/Cf-5* resistance genes (Balint-Kurti *et al.* 1995), chromosome *3*, surrounding a locus for regeneration capacity (Koornneef *et al.* 1993), and chromosome *6*, surrounding the M_i resistance gene (Weide *et al.* 1993). Since populations of non-inbred species, including potato, are highly heterozygous and difficult to repeatedly backcross due to inbreeding depression, the presence of morphological genetic markers is masked most of the time. Therefore, extensive classical genetic maps of non-inbred species are generally not available, and often only

single genetic markers are recorded. However, we have been able to construct a genetic map of potato that contains the majority of the classical genetic markers known in potato (Jacobs *et al.* 1995a).

Advantages versus disadvantages of mapping in non-inbred species.
The optimal cross for map construction should be a balance between maximum polymorphism, and a high level of homology for chromosome pairing and recombination to take place. In general, a high level of polymorphism can be obtained by using distantly related parents for BC or F_2 populations. However, the genetic distance between the parents used, can affect the recombination frequencies in the cross. The more closely related the parents in the cross, both for parents within the same species as well as for parents from different species, the greater the homology. This results in increased chromosome pairing, and higher recombination frequencies based on crossing-over. Therefore, longer genetic maps are expected. However, a high level of homology between the parents is countered by a decrease in polymorphism, and the recombination events might go unnoticed. An advantage of using a non-inbred species for map construction is the high level of polymorphism, even within species. This often allows the use of an intraspecific F_1 population for map construction, thereby avoiding the chance of reduced recombination, due to a lack of homology, encountered in interspecific crosses. A clear disadvantage of working with a cross-fertilising, highly heterozygous crop like potato, is the general phenomenon of inbreeding depression. Consequently, many uncharacterised (sub)lethal loci are expected to be present in the potato genome. These become visible as distorted segregation of alleles in one of the parents, due to gametic selection, or as (partial) absence of genotypic classes in the progeny, due to zygotic selection. The latter case is especially found in a backcross population, where both parents can be heterozygous for the same (sub)lethal allele, resulting in these loci segregating with some individuals in a homozygous state. The mapping of loci linked to such a (sub)lethal locus will also be affected. As a consequence, clusters of markers with distorted segregation are expected to arise. However, the regions where they might occur can not be predicted. They may vary for each cross and will mainly depend on the parents used. Linkage to a (sub)lethal locus could account for the observed results of distorted segregation in regions such as chromosome *11* in the male parent of our mapping population (Jacobs *et al.* 1995a). Distorted segregation originating from one locus per linkage group, will not influence recombination frequencies and the correct

construction of genetic maps will not be impeded (Van Ooijen *et al.* 1994).

Relative mapping.

The use of the mapping population (Jacobs *et al.* 1995a) is anticipated to be continued, either for the mapping of additional traits segregating directly in the mapping population, or for the "relative" mapping of other traits. The latter involves loci in other populations, which can be mapped on the potato genome by linkage to markers that were already located in the mapping population used in this study. An example of "relative" mapping is given in Chapter 2. This involved the isolation of DNA sequences flanking T-DNA/transposable elements from genotypes that are genetically different from the mapping population. The genomic location of the integration sites in the transformants was then determined in the mapping population. This circumvents the need for developing separate mapping populations of every transformant in order to determine the integration site. This approach also has another positive effect; these flanking markers contribute to the value of the genetic map, by increasing the total number of markers mapped, thereby improving the quality of the map. In addition, the flanking markers are not anonymous random markers, but represent valuable information on processes like integration sites of T-DNA's and transpositional behaviour (clustering) of introduced heterologous transposable elements.

(Targeted) transposon tagging

The technique of targeted tagging with heterologous transposable elements has now been used for the cloning of several resistance genes in plants. The approaches used were via targeted tagging, either starting with a transposon closely-linked to the gene of interest (*Cf-9*, Jones *et al.* 1994), or with a transposon in any position in the genome (*N* gene, Whitham *et al.* 1994; L^6 gene, Ellis *et al.* 1995). As expected, the tagging frequencies with a transposon in a closely-linked position were much higher. Sofar, transposon tagging with heterologous transposons has been successfully used for self-fertilising plant species only. This most likely reflects the fact that the genetics of self-fertilising species is generally far better developed than in cross-fertilising species. Therefore, transposon tagging is anticipated to be much more complicated in non-inbred species. Nevertheless, we believe that targeted transposon tagging is feasible in potato, when a transformant with a transposable element closely linked to a trait of interest is used in conjunction with the appropriate assessment of the plant material.

By determining the position of the integration sites in a series of independent single-copy transformants, we were able to develop a collection of potato transformants with transposable elements at different positions, evenly spread over the potato genome. This collection has the potential for targeted tagging of any locus of interest in potato. Our major target for transposon tagging is the *R1* locus on chromosome *5*, a monogenic resistance against *Phytophthora infestans* (Leonards-Schippers *et al*, 1992), although other important resistance loci are also clustered on this chromosome (see below).

Implications for mapping QTL.

Potato was not only the first non-inbred species in which (molecular) genetic linkage maps were obtained (Bonierbale *et al.* 1988; Gebhardt *et al.* 1989b), it was also the first non-inbred in which QTL were determined (Leonards-Schippers *et al.* 1994; Van Eck *et al.* 1994a; Freyre & Douches 1994a; Freyre *et al.* 1994b). In the past, maps of non-inbred parents were composed from segregating markers from one parent only (*eg.* potato, Bonierbale *et al.* 1988; citrus, Durham *et al.* 1992; alfalfa, Kiss *et al.* 1993; apple, Hemmat *et al.* 1994). Alternatively, separate maps for the two parents were produced (*eg.* Bonierbale *et al.* 1994; Grattapaglia & Sedoroff 1994). However, this approach is questionable, especially when evaluating QTL. Neglecting the contribution of alleles from one of the parents of the (QTL) mapping population can lead to loss of information with regard to the QTL under study. Multiple (quantitative) alleles are described for tuber shape in potato (Van Eck *et al.* 1994a). Such multiple alleles are very likely to be involved in many quantitative traits, resulting in phenotypes with a more gradual effect for the quantitative trait under study, with less extreme phenotypes. In addition to the interaction of multiple alleles at a specific locus (Van Eck *et al.* 1994a), the interaction with alleles at other loci (from the other parent) should be taken into account. For example, transgressive variation for a range of quantitative traits in a F_2 population of the tomato species *L. esculentum* and *L. pennellii* was found (Eshed & Zamir 1994)

Not using the mapping information contained in one of the parents has the danger of misjudging the effect of quantitative traits, possibly resulting in QTL not being recognised. The importance of using the allelic contributions from both parents is shown in an example of QTL mapping in the inbreeding crop, tomato. A F_2 population of *Lycopersicon esculentum* and *L. pimpinellifolium* was studied for several

QTL, including fruit size (Tanksley 1995). The wild species *L. pimpinellifolium*, having very small fruits, showed an unexpected major QTL with a large positive effect on the fruit size in the population.

A genetic core map for potato.

Genetic maps in potato have been constructed with variable purposes, from a number of different populations (Bonierbale *et al.* 1988; Gebhardt *et al.* 1989b; Gebhardt *et al.* 1991; Tanksley *et al.* 1992; Bonierbale *et al.* 1994; Jacobs *et al.* 1995a; Kreike 1995). The various maps differ in the type of cross studied, the population size, the *Solanum* species used, and in the way the data are analysed (one, two or three parental maps or one combined map). In order to derive a consensus map of potato, that can be used as a reference for different purposes, it is important that the available information is combined. This includes data on molecular markers, isozymes and classical genetic markers. The demand for a good reference map, which is generally applicable within a species, is becoming increasingly important, especially with the rapid manner in which PCR-based markers, such as RAPD and AFLP (amplified fragment length polymorphism) markers, can be used to find linkage to a trait of interest. In addition, in the process towards the construction of a reference core map, information can be gained on the importance of the potential differences, such as chromosomal rearrangements, between different genetic maps. Combining the data from different mapping populations will also be very useful in generating a more comprehensive picture of the potato genome.

The diversity of species that is used, or will be used in the future in genetic mapping studies of potato, makes the construction of one consensus map complicated. To avoid the construction of a combined map that is not more than a "statistical average" of the independent maps, with no real biological value, we constructed a consensus map composed of core markers. This map can be used for future reference for the allocation of newly mapped markers or traits of interest to the core intervals, provided some of the markers used are core markers or markers located to a specific core interval.

Application of a basic reference map

The importance of a well-defined reference map is illustrated by the following example. The recently developed AFLP technique (Zabeau & Vos 1992) has been successfully used for the rapid search of markers linked to potato cyst nematode

resistance in a diploid potato population (J. Rouppe van de Voort, pers.comm.). An AFLP map was developed in a diploid population containing nematode resistance. Linkage groups were obtained, but because no loci other than AFLP markers were mapped in this population, it was not known to which chromosomes these linkage groups were referring. The same set of AFLP primers was then used in a mapping population with a well-defined RFLP map (Jacobs *et al.* 1995a). Many of the AFLP bands were found to be equal in size in both populations and became grouped in similar linkage groups (Van Eck *et al.* in preparation). In this manner, the AFLP linkage groups of the population segregating for nematode resistance could be assigned to chromosome numbers in accordance with earlier potato maps, by using a reference (RFLP) map of potato.

Mapping of nematode resistance.

The genetic map developed in our mapping population was used to assist in the mapping of a resistance locus in another potato population. A locus for resistance to the potato cyst nematode *Globodera rostochiensis*, pathotype Ro1, was located to chromosome *5*. This *GroV1* locus, originating from the wild species *S. vernei*, was mapped using RFLP and PCR markers. In the same region of the potato genome, another resistance to *G. rostochiensis* Ro1 was mapped; the *H1* locus from *S. tuberosum* ssp. *andigena* (Pineda *et al.* 1993; Gebhardt *et al.* 1993).

RFLP mapping located the *GroV1* locus on chromosome *5*, distal to the marker TG69. In contrast, Pineda *et al* (1993) mapped the *H1* locus proximal to TG69. This implied that *GroV1* is located in the same region of the potato genome, but did not seem to be in the same position as the *H1* locus. However, as was found in the construction of the potato core map (Chapter 3), chromosomal rearrangements are not uncommon in potato, and are especially encountered when different species are compared. In this example of nematode resistance, two different potato species (*S. vernei* and *S. tuberosum* ssp. *andigena*) are involved in the comparison between the *H1* and the *GroV1* locus. In addition, the low copy RFLP marker Ssp72, was shown to be linked not only to the *GroV1* locus (Jacobs *et al.* 1995b), but also to the *Gpa* locus originating from *S. spegazzinii*, conferring resistance to the potato cyst nematode *G. pallida* pathotype Pa2 (Kreike *et al.* 1994). However, the *Gpa* resistance locus was mapped to the other distal end of chromosome *5*. Based on additional markers linked to both resistances, and the genetic maps of chromosome *5* in both populations, we

conclude that the *GroV1* and *H1* resistance loci may be truly dissimilar loci at different genomic positions. However, it is striking that two resistance loci (*GroV1* and *Gpa*) to different nematode species originating from different *Solanum* species, are linked to distinct loci of the same RFLP marker. This result, together with the presence of chromosomal rearrangements between potato species (Chapter 3) lead to the impression that either rearrangements or duplicated regions of the genome could be involved in their evolution.

Clustering of resistance loci.

Clustered resistance loci against a certain pathogen, with duplications of these clusters in the genome, have been reported in for example flax (Ellis *et al.* 1988), lettuce (Kesseli *et al.* 1994), maize (Hulbert *et al.* 1993), and tomato (Jones *et al.* 1993). A further example in potato, are the loci *R3*, *R6*, and *R7* for resistance against the fungal pathogen *Phytophthora infestans*, located in the same distal region of chromosome *11* (El-Kharbotly *et al.* 1994; C. Gebhardt pers. comm.). In addition to clustering of resistance loci to one pathogen, an increasing number of examples of clustering of resistance loci to different pathogens is found (*eg.* Dickinson *et al.* 1993; Kesseli *et al.* 1994; Kaloshian *et al.* 1995). For potato, clustering of resistance loci to distinct pathogens was found on chromosome *5*, and involves loci for resistance to potato virus X, *Rx2* (Ritter *et al.* 1991), resistance to *Phytophthora infestans* race 1, *R1* (Leonards-Schippers *et al.* 1992) and a major locus for resistance against the potato cyst nematode *Globodera pallida*, *Gpa* (Kreike *et al.* 1994).

In view of the high level of genome homology between potato and tomato, the clusters of resistance loci can be further extended. Several resistance loci in potato are located in homeologous genomic positions to resistance loci in tomato. The equivalent position of the *Rx2* and *R1* loci on chromosome *5* of potato, contains the *Pto* gene for resistance against *Pseudomonas solanacearum* in tomato (Martin *et al.* 1991). The same holds for the potato cyst nematode resistance locus *Gro1* (Barone *et al.* 1990) and the *Fusarium oxysporum* resistance locus *I1* (tomato, Sarfatti *et al.* 1991) on chromosome *7*. It is anticipated that the detection of clustered loci will be further extended with more resistance loci being mapped. The close genetic relationship between potato and tomato, will no doubt disclose more clustering of analogous traits of interest over the species boundaries.

Marker-assisted selection.

Introgression of qualitative, as well as quantitative loci, into desirable genetic backgrounds, is facilitated by linkage of these traits to molecular markers. In addition, marker-assisted selection with closely-linked markers allows the identification of recombinants with minimal amounts of "foreign" DNA flanking the trait being introgressed (Young & Tanksley 1988). The identification of markers flanking the *GroV1* locus therefore offers a valuable strategy for marker-assisted selection for introgression of this nematode resistance. The development of SCAR markers in this population was aimed at the application of PCR-based markers in potato breeding programmes. The genetic distance between RAPD/SCAR markers and the *GroV1* resistance locus will determine the success of marker-assisted selection. Tight linkage to the trait of interest is important. Although these distances are 4 cM for SCAR-X02 and 19 cM for SCAR-U14, these markers flank both sides of the resistance locus. Initial screening using flanking markers, allows the elimination of genotypes susceptible to nematodes. In this manner the total number of lines for phenotypic resistance testing can be substantially reduced. Identification of recombinant lines between the two flanking markers are valuable for fine-mapping, and can assist in the eventual map-based cloning of the gene(s).

Conclusion

The research in this thesis developed a comprehensive reference genetic map for potato. The value of this map as a framework for basic research on the mapping of integration sites of T-DNA's and transposing *Ac* elements was demonstrated. This reference map was also successfully used for the mapping of a new locus for nematode resistance, and the subsequent development of SCAR markers with potential use in marker-assisted selection in applied breeding programmes.

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Summary

The research in this thesis involved the construction of an integrated genetic map of the non-inbred, heterozygous potato (*Solanum tuberosum* L.). This genetic map incorporates molecular, morphological, and isozyme markers, and can therefore serve as a basis for genetic studies as well as for breeding purposes. Using a backcross population of 67 diploid potato plants, a general method for map construction is described, different from previous methods employed in potato and other outbreeding plants. This initially involved making two independent parental maps; a female map containing 132 markers, and a male map containing 138 markers. On the basis of markers shared by these two parental maps, a combined genetic map was constructed with the computer progamme JoinMap 1.4. The resulting map integrates both molecular and classical genetic markers into a single map, and fully exploits the segregation data present in a highly heterozygous crop like potato. It consists of 175 molecular markers, 10 morphological markers, and 8 isozyme markers, and has a total length of 1120 cM. Clusters of distorted segregation were found on several chromosomes, especially in the map from the male parent.

Included in the map are 94 RFLP markers, consisting of cloned DNA flanking insertions of T-DNA's or transposable elements in the potato genome. These were isolated via inverse polymerase chain reaction (IPCR) or plasmid rescue from diploid clones of potato transformed with T-DNA constructs containing maize transposable elements (*Ac*, *Ds*, *I*). In this manner, the integration sites of T-DNA constructs in potato were found to cover the entire potato genome. Furthermore the *Ac-Ds* transposable element system was established to be functional in potato, with *Ac* transposing mainly to closely linked loci in the potato genome. This set of transformants, with transposable elements at a known position, can therefore serve as starting material for the targeted transposon tagging and subsequent cloning of any locus in the potato genome. A strategy for targeted transposon tagging of disease and pest resistance genes from potato is described.

Several transposon transformants were identified with their insertion site linked to the loci for resistance genes. A majority of the transformants is heterozygous for the *R1* gene for resistance to *Phytophthora infestans*, located on chromosome *5*. Transformants with a transposon linked in repulsion phase to the *R1* locus have been identified. In order to efficiently tag the *R1* gene, appropriate crosses will be made.

The availability of comprehensive genetic maps for potato is important for genetic studies and future applications to plant breeding. To compare and align maps obtained from several different *Solanum* species, the data from seven independent mapping populations were combined into one common map. Separate maps for all populations were (re)calculated, aligned, and subsequently joined into one basic core map with a new version of the computer programme for genetic mapping, JoinMap 2.0. This new version contains an option for use with mapping populations of non-inbred parents, by using a two digit input for each genotype-marker combination. In this manner the input data for every marker takes both alleles from each genotype into account, and can be composed from up to four different parental alleles. Consequently, the segregation data available in a mapping population of a non-inbred species is fully utilised.

The segregation data from the seven mapping populations of potato used, represent a data set from different types of segregating populations, different closely related species, and a diverse set of molecular markers. The integration of the maps was therefore performed on the basis of 73 core markers, common to as many of the maps as possible. The remaining 384 markers were assigned to the intervals between these core markers. The order of the interval markers, as determined in each original map, was retained. No attempt was undertaken to determine the intertwining orders of the interval markers in the integrated map, since this would not have resulted in an outcome with practical use. A number of chromosomal inversions between the existing genetic maps of potato, and between the potato and tomato maps were recognised.

The value and application of a comprehensive reference map of potato was demonstrated, using a diploid potato population segregating for resistance against the potato cyst nematode *Globodera rostochiensis* (pathotype Ro1). Extensive resistance screening of 108 genotypes of a F_1 population, repeated over three years, established the presence of a single locus with a dominant allele for resistance to this nematode. This locus, originating from the wild species *S. vernei*, was designated *GroV1*. After the initial allocation with RFLP markers of the resistance locus to chromosome *5*, bulked segregant analysis with random amplified polymorphic DNA (RAPD) primers was performed. Additional markers were identified in the region surrounding the resistance locus. The cloning and sequencing of RAPD products, closely linked to the *GroV1* locus, allowed the subsequent development of more specific SCAR (sequence-

characterised amplified region) markers. The location of the resistance locus *GroV1*, on the distal part of the long arm of chromosome 5, was in the same genomic region as the *H1* locus, another Ro1 resistance locus that originates from the species *S. tuberosum* ssp. *andigena*. The identification of SCAR markers flanking the *GroV1* locus offers a valuable strategy for marker-assisted selection for introgression of this nematode resistance into new potato cultivars.

Samenvatting

Het onderzoek dat wordt beschreven in dit proefschrift heeft betrekking op de constructie en toepassing van een genetische kaart van de aardappel (*Solanum tuberosum* L.), die kruisbevruchtend en heterozygoot is. Deze kaart bevat moleculaire, morfologische en isozym merkers en kan gebruikt worden als basis voor verdere genetische studies en voor veredelingsdoeleinden. Een algemene methode voor kaartconstructie is beschreven, gebruikmakend van een terugkruisingspopulatie van 67 diploide aardappelplanten. Deze methode wijkt af van de methoden die eerder zijn gebruikt voor aardappel en andere kruisbevruchters. De eerste stap in de constructie van de kaart bestond uit het maken van twee onafhankelijke ouderkaarten; een vrouwelijke kaart van 132 merkers en een mannelijke kaart van 138 merkers. Een gecombineerde kaart, gebaseerd op de merkers die in beide kaarten uitsplitsen, werd samengesteld met behulp van het computerprogramma JoinMap 1.4. Het eindresultaat was één kaart die zowel moleculaire als klassieke merkers integreert en die de waarnemingen van splitsende merkers in een heterozygoot gewas als aardappel ten volle benut. De kaart is samengesteld uit 175 moleculaire merkers, 10 morfologische merkers en 8 isozym merkers. De totale lengte bedraagt 1120 cM. Clusters van merkers met scheve uitsplitsingsverhoudingen werden aangetroffen op verscheidene chromosomen, vooral in de mannelijke kaart.

Vierennegentig RFLP merkers, bestaande uit gekloneerd DNA, flankerend aan inserties van T-DNA of transposons in het aardappelgenoom, zijn opgenomen in de kaart. Deze merkers werden geïsoleerd door middel van "inverse polymerase chain reaction" (IPCR) of "plasmid rescue" van diploide aardappelklonen, die waren getransformeerd met T-DNA constructen die transposons uit maïs (*Ac*, *Ds*, *I*) bevatten. De integratieplaatsen van T-DNA constructen in aardappel bleken over het volledige aardappelgenoom verdeeld te zijn. De serie van transformanten die op deze manier werd gekarakteriseerd, met transposons op een bekende plek in het genoom, kan dienen als uitgangsmateriaal voor "doelgerichte transposon tagging" en vervolgens clonering van ieder willekeurig locus in het aardappelgenoom. Een strategie om te komen tot doelgerichte transposon tagging in aardappel van resistentiegenen tegen ziekten en plagen, met behulp van transposons wordt beschreven.

Diverse transposon transformanten waarvan de plaats van insertie gekoppeld is aan resistentieloci werden geïdentificeerd. Een groot deel van de transformanten is

heterozygoot voor het *R1* locus voor resistentie tegen *Phytophthora infestans*, dat op chromosoom *5* is gelokaliseerd. Enkele transformanten bezitten een T-DNA integratie die gekoppeld is aan het *R1* locus. Deze integraties waren echter alle in afstotingsfase met de resistentie. Aanvullende kruisingen om een efficiënte tagging van het *R1* gen te verkrijgen, zullen worden uitgevoerd.

Een uitgebreide genetische kaart van de aardappel is van belang voor genetisch onderzoek en voor toepassing in de plantenveredeling. Gegevens van 7 verschillende plantpopulaties, gebruikt voor kaartconstructie, werden gecombineerd om zo te komen tot een vergelijking van de kaarten die in de verschillende *Solanum* soorten zijn verkregen. Aparte kaarten van alle populaties werden (opnieuw) berekend, naast elkaar gelegd en samengevoegd in één basiskaart. Dit alles werd uitgevoerd met een nieuwe versie van het computer programma voor het maken van genetische kaarten; JoinMap 2.0. Deze nieuwe versie bevat een mogelijkheid voor gebruik met karteringspopulaties van kruisbevruchtende planten. Bij iedere genotype-merker combinatie kunnen beide allelen worden ingevoerd en daardoor kunnen de gegevens voor elke merker de vier verschillende ouderallelen bevatten. Dit leidt ertoe dat de uitsplitsingsgegevens die aanwezig zijn in een populatie van kruisbevruchters ten volle worden benut.

De zeven aardappelpopulaties die werden gebruikt, vertegenwoordigen diverse typen uitsplitsende populaties, diverse nauw verwante soorten en een uiteenlopende collectie van moleculaire merkers. Het samenvoegen van de kaarten werd daarom gebaseerd op 73 kernmerkers, die in zoveel mogelijk populaties aanwezig waren. De overige 384 merkers werden in de intervallen tussen deze kernmerkers geplaatst. De volgorde van de merkers in de intervallen die was bepaald in de oorspronkelijke kaarten, werd gehandhaafd. Een bepaling van de samengevoegde volgorde van interval merkers, aanwezig in de verschillende kaarten, werd niet uitgevoerd, omdat dit niet tot een resultaat zou hebben geleid dat praktisch bruikbaar is. Enkele chromosoom inversies tussen de bestaande genetische kaarten van aardappel onderling en tussen de kaarten van aardappel en tomaat werden gevonden cq. bevestigd.

De waarde van een uitgebreide basiskaart van aardappel wordt gedemonstreerd, door middel van een toepassing in een diploide aardappelpopulatie die uitsplitst voor resistentie tegen het aardappelcyste-aaltje *Globodera rostochiensis* (pathotype Ro1). Uitgebreide resistentietoetsen, herhaald over 3 jaar, van een F_1 populatie van 108

individuen, wees op de aanwezigheid van een enkel locus met een dominant allel voor resistentie tegen deze nematode. Dit locus, afkomstig van de wilde soort *S. vernei*, werd *GroV1* genoemd. Na een grove kartering van het resistentielocus met behulp van RFLP merkers op chromosoom *5*, werd een zgn. "bulked segregant analysis" met "random amplified polymorphic DNA" (RAPD) primers toegepast. Aanvullende merkers in het gebied rondom het resistentielocus werden geïdentificeerd. Het kloneren en sequencen van de RAPD produkten die nauwe koppeling met het *GroV1* locus aangaven, leidde vervolgens tot de ontwikkeling van meer specifieke "sequence-characterised amplified regions" (SCAR) merkers. De locatie van het resistentielocus *GroV1*, op het distale deel van de lange arm van chromosoom *5*, valt in hetzelfde stuk van het chromosoom als het *H1* locus, een andere monogene Rol resistentie uit de soort *S. tuberosum* ssp. *andigena*. SCAR merkers die het *GroV1* resistentielocus flankeren, kunnen worden toegepast bij indirecte selektie met behulp van merkers om de introgressie van deze resistentie in nieuwe aardappelcultivars te bewerkstelligen.

Account

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Curriculum vitae

Jeanne Jacobs werd geboren in Kessel op 12 november 1960. Na het behalen van het Atheneum-B diploma aan het Blariacum College in Venlo-Blerick (mei 1979), begon zij in augustus 1979 aan de toenmalige Landbouwhogeschool in Wageningen aan de studie "Moleculaire Wetenschappen". Het doctoraalexamen werd behaald in maart 1988 met als hoofdvakken Moleculaire Biologie en Celbiologie en een stage Moleculaire Biologie, die werd doorgebracht aan het INRA in Toulouse (Frankrijk). Per januari 1989 trad zij als OIO (Onderzoeker In Opleiding) in dienst bij de Nederlandse organisatie voor Wetenschappelijk Onderzoek, NWO, in het kader van het BION/STW projekt "Constructie van een genetische kaart van de aardappel". Zij werd aangesteld voor het moleculair-biologische onderzoek binnen dit projekt en gedetacheerd bij het ITAL, later het CPRO-DLO, in Wageningen. Deze aanstelling, die per 1 december 1993 eindigde, was vanaf 1 januari 1991 in deeltijd (0.8). Daarnaast had zij van mei 1991 tot mei 1993 zitting in de Dienstcommissie voor Projectmedewerkers van NWO, met als taak het ombudswerk. Vanaf 15 augustus 1995 is zij als post-doc werkzaam in de groep van prof. Michelmore bij de vakgroep "Vegetable Crops" van de University of California in Davis (USA). Het onderzoek aldaar heeft betrekking op valse meeldauw resistentie in sla.

Nawoord

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Nothing venture, nothing win

Sir Edmund Hillary