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

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**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 that differs 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 the 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.

**Key words** Morphological markers · Isozymes · Non-inbred species · Combined map · JoinMap

## 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 by tetrasomic inheritance (Howard 1970). Genetic analysis of potatoes is also complicated by the severe inbreeding depression that occurs in this crop. The use of diploid wild species and especially the development of techniques to obtain dihaploid clones of *S. tuberosum* (e.g. Hermsen and 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 the 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 and has 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. 1989; Gebhardt et al. 1994; Leonards-Schippers et al. 1994). The other is 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). The latter map was initially mainly constructed from the hybrid parent of the cross *S. phureja* × (*S. tuberosum* × *S. chacoense*) (Bonierbale et al. 1988), then further developed using a population derived from the cross (*S. tuberosum* × *S. berthaultii*) × *S. berthaultii* (Tanksley et al. 1992). Both maps consist almost exclusively of molecular mark-

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ers. However, to fully exploit genetic maps it is desirable that classical and molecular markers are incorporated into the same genetic maps (e.g. 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 a 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 and 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$4211 (Jacobsen 1978). The diploid mapping population [C×(C×$VH^3$4211)] is therefore a backcross population. The offspring of these two non-inbred parental clones, C×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 and Ramanna 1989; Jongedijk et al. 1990; Van Eck et al. 1993; Van Eck et al. 1994a,b).

Diploid potato clones were used for transformation with T-DNA constructs containing the maize transposable elements *Ac* as described previously (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, in preparation).

### Molecular techniques

DNA isolation, Southern blotting, hybridisation and autoradiography were performed as previously described by Van Eck et al. (1993). Survey blots with DNA from both parental clones C and E, digested with *Dra*I, *Eco*RI, *Eco*RV, *Hin*DIII and *Xba*I, were used for finding polymorphisms. Whenever possible, a polymorphic probe/enzyme combination giving the 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. The 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: (1) TDs*X*, IPCR or plasmid rescue-derived probe from T-DNA containing *Ds* transposable element, *X*=transformant number; (2) TAc*X*, IPCR-derived probe from T-DNA containing *Ac* transposable element, *X*=transformant number; (3) TI*X*, plasmid rescue-derived probe from T-DNA containing *I* transposable element, *X*=transformant number; (4) 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 (TGs, Bonierbale et al. 1988), as well as 2 potato markers (GPs, 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, e.g. 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 anther tissue was used 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%

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

| Enzyme system | Gel[a] | Locus name | Chromosome | 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* | – | – |

[a] Gels types were: (1) 10–15% gradient gels; (2) 8–25% gradient gels; (3) 12.5% homogeneous gels; (4) 20% homogeneous 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 | $\chi^2$ | |
| 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[a] | 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[b] | 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[a] | 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[a] | 1:1 ♂ | 32.97 | 11 |

[a] Segregation is significantly different from the expected ratio
[b] Heterozygosity/homozygosity determined by backcrossing to clone E

b-mercapto-ethanol, followed by a brief centrifugation to pellet debris. Immediately after centrifugation, the supernatant was applied to the gel in 1-$\mu$l wells. Electrophoresis was carried out on precast polyacrylamide PhastGels (Pharmacia). The gels were buffered with 0.112 *M* TRIS-acetate (pH 6.4), and 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. The temperature was maintained at 15°C. The gels were stained using standard procedures (Vallejos 1983).

## Classical genetic markers

The 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 using standard clones provided by R. Eijlander (Wageningen Agricultural University). Resistance to the 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 less than 5½, and yellow (*Y-*) when the trait value was greater than 5½ (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*), which affects homologous chromosome pairing and thus recombination during the meiotic prophase I, was determined by cytogenetic observation (Jongedijk and 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* and *Crcr* individuals.

## Linkage analysis and map construction

Data were collected in the mapping population for all segregating marker alleles, regardless of whether segregation was from one parent or both. As a result of using non-inbred parents, five types of singe-locus segregation were 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*×*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 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 manner similar

**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 ratios | Parental map | Marker type | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Molecular | | | | | | | | | Isozyme | Classical | |
| | | | | St | Ssp | TG | GP | TDs | TAc | TI | Ac | Genes | | | |
| *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 |

to that of 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 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 the 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×clone E (=clone C×VH$^3$4211). These two parental clones contained mainly *S. tuberosum* germ plasm, but they also have the wild species *S. phureja* and *S. vernei* in their pedigree (Jongedijk and 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 frequency that was similar 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-DNAs 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*

The 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 these traits is well-documented in the 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 the distorted segregation of markers surrounding this locus. An additional marker known to complicate linkage analysis in this population is *Cr*, which results 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 female parent C, while 61 marker loci segregated 1:1 from the male parent E. Seventy-seven marker loci segregated 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 1 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 the 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 datasets 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 datasets 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 female parent C, one for male parent E, and one that combines the data from C and E.

The combined map was constructed 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 Fig. 1 shows how the compilation of the two 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 that 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. 1989), 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, Gebhardt et al. (1991) devised a consensus numbering of the linkage groups. In the construction of the map presented in this study, a set of these RFLP markers was used. Consequently, 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×clone E (E=clone C×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), thereby 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 segrega-

**Table 4** Loci with distorted segregation

| Chromosome | Locus | Expected | Observed | | | | $\chi^2$-values | Selection |
|---|---|---|---|---|---|---|---|---|
| | | 1:1 ♀<br>1:1 ♂<br>1:1:1:1 | *A-*<br>*-A*<br>*AA* | <br>*-C*<br>*AC* | *B-*<br><br>*BA* | <br><br>*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 |

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

tion ratio for *CrCr*:*Crcr*:*crcr*, only *Cr-* phenotypes were observed. The genotypes *CrCr* and *Crcr* cannot 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 and 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 (e.g. *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 the segregation of flanking markers. Therefore, the observed distortion in the segregation of markers on chromosome *1* of clone E (Fig. 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 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 (e.g. *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. (1989, 1991) also reported distorted segregation on several chromosomes, with chromosome *1* displaying self-incompatibility, as an extreme example. C. M. Kreike (personal communciation) 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. Consequently 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-DNAs was found. However, similar to observations in other Solanaceous species (Osborne et al. 1991), the 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 of the isozyme loci reported here have been mapped previously to similar genomic positions in either tomato (Tanksley and Loaiza-Figueroa 1985; Tanksley et al. 1992) or potato (Bonierbale et al. 1988; Tanksley et al. 1992). The mapping of isozyme loci in potato has also been performed by Douches and Quiros (1987, 1988a,b), 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 as that previously used in potato and/or tomato. The nomenclature used to indicate loci and alleles is normally 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*$^1$. Electrophoretic mobility, however, is highly influenced by the method of separation used, thus affecting the nomenclature and making comparison with earlier work complicated. Second, locus names can also be assigned on the basis of earlier studies in potato. This sometimes gives rise to contradictions with tomato literature, e.g. 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 and Olivier 1985; Douches and Quiros 1988b). Third, loci may be named on the 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 have been mapped in tomato (Tanksley and Loaiza-Figueroa 1985) and one in potato (Bonierbale et al. 1988). The locus we mapped on chromosome *12* was indicated with *6-Pgdh-2*, which is in agreement with previous mapping studies but in disregard of 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 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 has also been suggested by recent studies using potato-tomato somatic hybrids (Jacobsen et al., data not shown).

## Mapping of classical markers

The morphological markers *F*, *D*, *P* and *Ro*, analysed in this mapping population have already been described. In

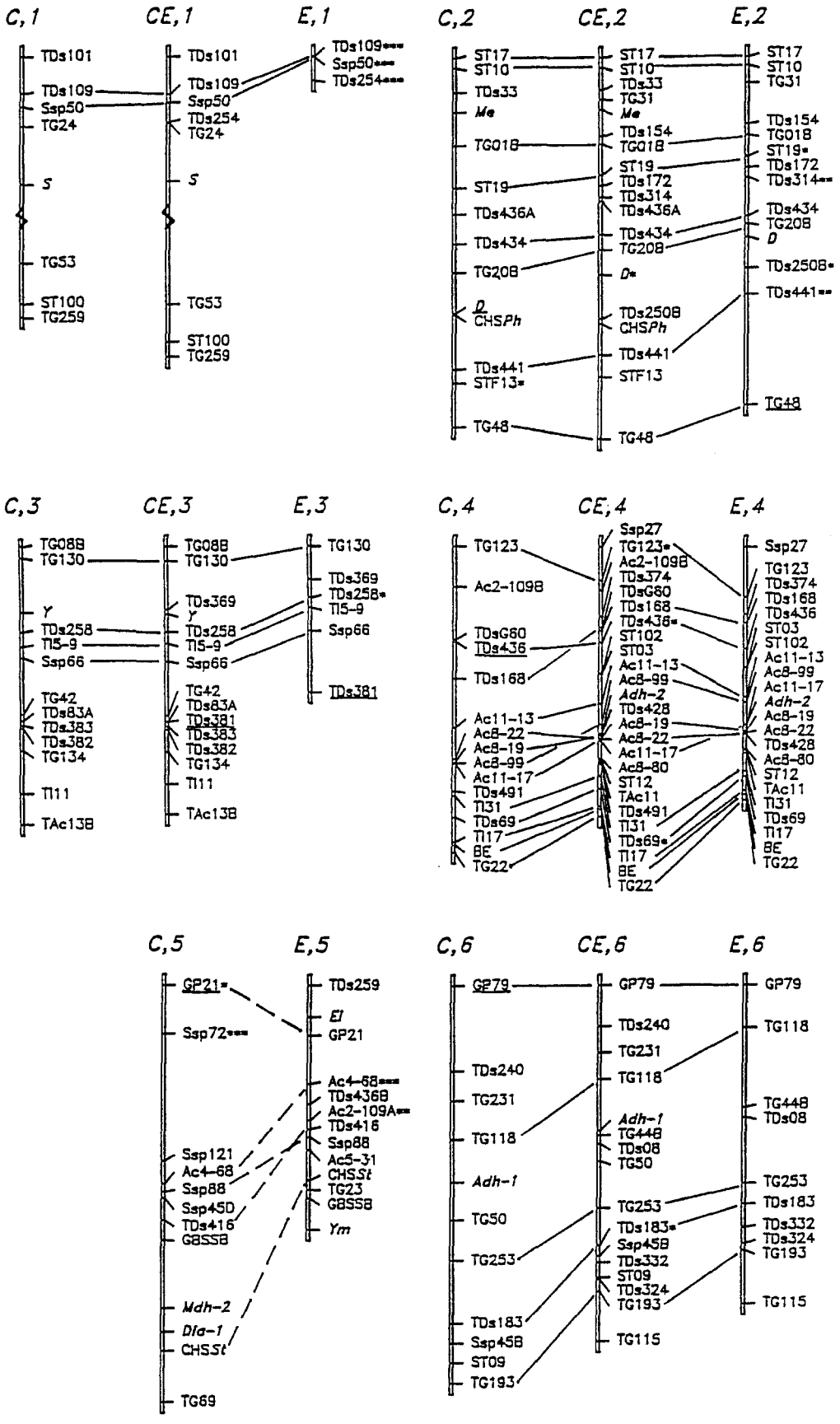


**Fig. 1** Integrated genetic linkage map of potato from the backcross population C×E. For each chromosome the female (*C*), male (*E*) and combined (*CE*) map are given. Linkage groups are established with LOD>3.0. Chromosomes were constructed with a LOD>3.0, with the exception of chromosome *1* C (LOD=0.41), 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 the Materials and methods. Isozymes and classical genetic markers are in *italics*, and 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)

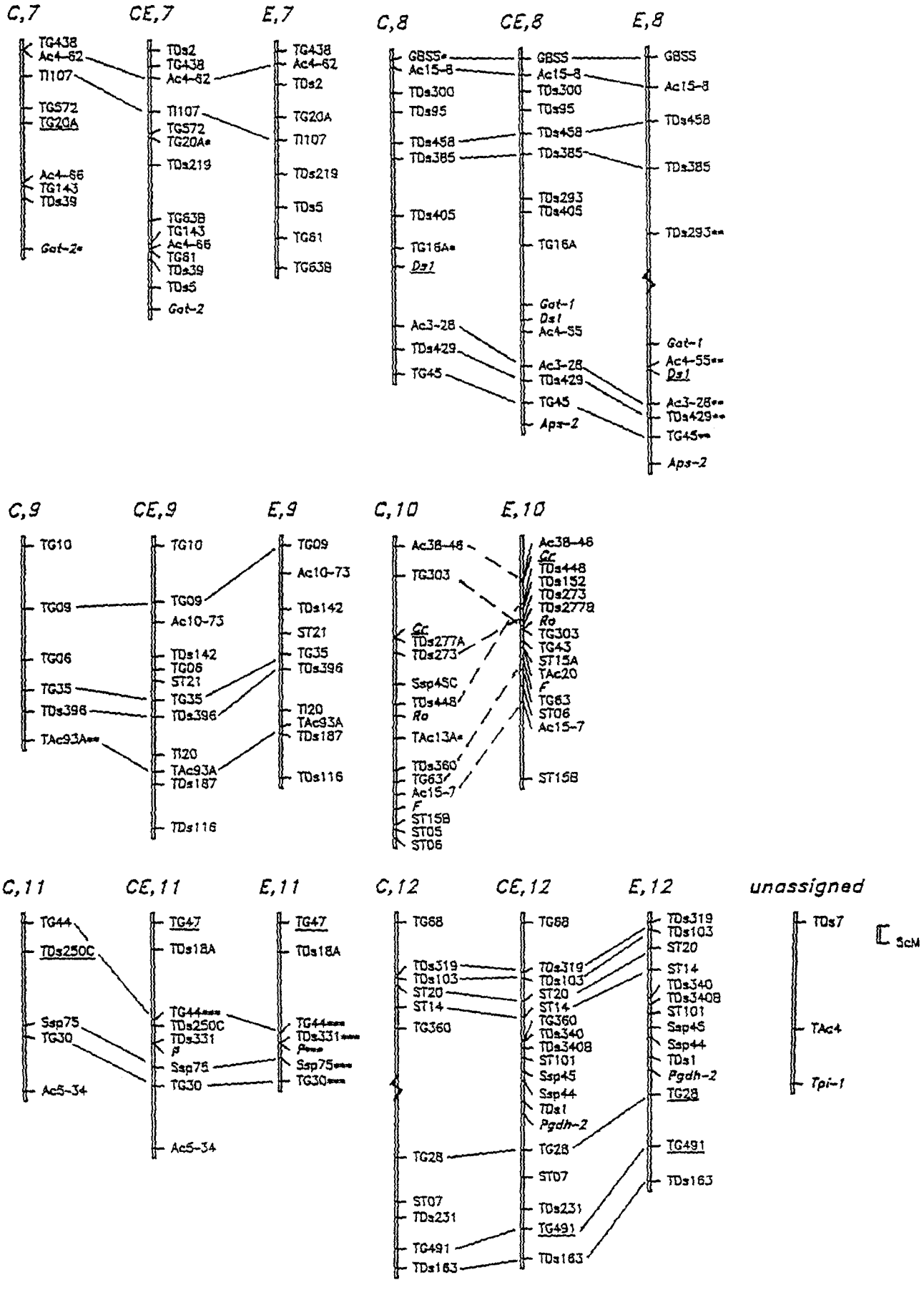
C,7
CE,7
E,7
TG438
Ac4-62
TI107
TG572
TG20A
Ac4-66
TG143
TDs39
Got-2*
TDs2
TG438
Ac4-62
TI107
TG572
TG20A*
TDs219
TG63B
TG143
Ac4-66
TG61
TDs39
TDs5
Got-2
TG438
Ac4-62
TDs2
TG20A
TI107
TDs219
TDs5
TG61
TG63B
C,8
CE,8
E,8
GBSS*
Ac15-8
TDs300
TDs95
TDs458
TDs385
TDs405
TG16A*
Ds1
Ac3-28
TDs429
TG45
GBSS
Ac15-8
TDs300
TDs95
TDs458
TDs385
TDs293
TDs405
TG16A
Got-1
Ds1
Ac4-55
Ac3-28
TDs429
TG45
Aps-2
GBSS
Ac15-8
TDs458
TDs385
TDs293**
Got-1
Ac4-55**
Ds1
Ac3-28**
TDs429**
TG45**
Aps-2
C,9
CE,9
E,9
TG10
TG09
TG06
TG35
TDs396
TAc93A**
TG10
TG09
Ac10-73
TDs142
TG06
ST21
TG35
TDs396
TI20
TAc93A
TDs187
TDs116
TG09
Ac10-73
TDs142
ST21
TG35
TDs396
TI20
TAc93A
TDs187
TDs116
C,10
E,10
Ac38-48
TG303
Cc
TDs277A
TDs273
Ssp4SC
TDs448
Ro
TAc13A*
TDs360
TG63
Ac15-7
F
ST15B
ST05
ST06
Ac38-48
Cc
TDs448
TDs152
TDs273
TDs277B
Ro
TG303
TG43
ST15A
TAc20
F
TG63
ST06
Ac15-7
ST15B
C,11
CE,11
E,11
TG44
TDs250C
Ssp75
TG30
Ac5-34
TG47
TDs18A
TG44**
TDs250C
TDs331
β
Ssp75
TG30
Ac5-34
TG47
TDs18A
TG44**
TDs331**
β*
Ssp75**
TG30**
C,12
CE,12
E,12
TG68
TDs319
TDs103
ST20
ST14
TG360
TG28
ST07
TDs231
TG491
TDs163
TG68
TDs319
TDs103
ST20
ST14
TG360
TDs340
TDs3408
ST101
Ssp45
Ssp44
TDs1
Pgdh-2
TG28
ST07
TDs231
TG491
TDs163
TDs319
TDs103
ST20
ST14
TDs340
TDs3408
ST101
Ssp45
Ssp44
TDs1
Pgdh-2
TG28
TG491
TDs163
unassigned
TDs7
TAc4
Tpi-1
5cM

Fig. 1

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 loci *Ro* and *F* on chromosome *10*, since linkage of a flower and skin colour locus has been reported by Dodds and Long (1956), and linkage of tuber shape and skin colour by De Jong and 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*, which is not in agreement with the location determined by means of trisomic analysis of this mutant phenotype on chromosome *12* (Wagenvoort 1982). This may be explained by assuming that different chromosomal mutations can cause the same mutant phenotype. A more likely explanation may be the highly similar morphology of potato chromosomes *5* and *12* in the pachytene stage, which complicates trisomic identification (Ramanna and 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* has been 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, our 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 of the segregation analyses involving the 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 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, the 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 (Fig. 1). In both clone C and clone E, 14 linkage groups were found. In clone C, these could be combined (LOD<3.0, Fig. 1) to 12 chromosomes by joining the 2 linkage groups found for the chromosomes *1* and *12*. In clone E chromosome *8* could be composed in a similar manner, leaving 1 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 earlier 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* × *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 the 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* germ plasm.

Markers showing polymorphism in both parental clones are essential in combining the two parental maps into one basic map. If 2 markers are segregating from both parents, two independent estimates for the marker pair can be de-

termined. The computer programme JoinMap, especially devised for the 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 2 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. The 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 datasets 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 to minor random differences between the datasets 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 Fig. 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 the 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 and Tanksley 1989) were 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 and 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 achieving several goals. These include the mapping of resistance genes in populations other than the mapping population (Jacobs et al. in preparation), and the mapping of quantitative trait loci (Van Eck et al. in preparation). A basic genetic map that has 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 the resolving of basic genetic questions.

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