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ORIGINAL PAPER

Y. Bai · R. van der Hulst · C. C. Huang · L. Wei · P. Stam · P. Lindhout

Mapping *Ol-4*, a gene conferring resistance to *Oidium neolycopersici* and originating from *Lycopersicon peruvianum* LA2172, requires multi-allelic, single-locus markers

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Abstract *Lycopersicon peruvianum* LA2172 is completely resistant to *Oidium neolycopersici*, the causal agent of tomato powdery mildew. Despite the large genetic distance between the cultivated tomato and *L. peruvianum*, fertile F_1 hybrids of *L. esculentum* cv. Moneymaker × *L. peruvianum* LA2172 were produced, and a pseudo-F_2 population was generated by mating F_1 half-sibs. The disease tests on the pseudo-F_2 population and two BC_1 families showed that the resistance in LA2172 is governed by one dominant gene, designated as *Ol-4*. In the pseudo-F_2 population, distorted segregation was observed, and multi-allelic, single-locus markers were used to display different marker-allele configurations per locus. Parameters for both distortion and linkage between genetic loci were determined by maximum likelihood estimation, and the necessity of using multi-allelic, single-locus markers was illustrated. Finally, a genetic linkage map of chromosome 6 around the *Ol-4* locus was constructed by using the pseudo-F_2 population.

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Y. Bai (✉) · R. van der Hulst · P. Stam · P. Lindhout (✉)
Graduate School of Experimental Plant Sciences, Laboratory of Plant Breeding, Wageningen University,
The Netherlands
e-mail: Pim.Lindhout@wur.nl
Tel.: +31-317-483454
Fax: +31-317-483457

C. C. Huang
Syngenta Seeds BV,
Postbus 2,
1600 AA Enkhuizen, The Netherlands

L. Wei
Henan Agricultural University,
Wenhua Road No. 95,
450002 Zhengzhou, China

Introduction

Lycopersicon peruvianum is one of the wild relatives of tomato and provides a vast reservoir of valuable traits for crop improvement, such as disease, pest and virus resistances (e.g. Brüggemann et al. 1996; Ammiraju et al. 2003). *L. peruvianum* LA2172 is almost immune to *Oidium neolycopersici*, which was previously named *O. lycopersici* (Kiss et al. 2001), the causal agent of powdery mildew in tomato (Lindhout et al. 1994). The resistance in *L. peruvianum* LA2172 is considered as complete, compared to the incomplete resistance conferred by the *Ol-1* and *Ol-3* genes that map on chromosome 6 and originate from *L. hirsutum* G1.1560 and G1.1290, respectively (Huang et al. 2000; Van der Beek et al. 1994).

L. peruvianum is reproductively isolated from the *esculentum* complex by severe crossing barriers (Taylor 1986). These barriers can be partially overcome by using either in vitro techniques or bridge accessions like *L. peruvianum* LA1708 and LA2172 (Poysa 1990; Rick 1982; Van Heusden et al. 1999; Veremis and Roberts 1996).

L. peruvianum is an out-crossing species, with accessions and individuals within accessions differing in marker alleles at the same locus (Baudry et al. 2001; Ganal and Tanksley 1996; van Ooijen et al. 1994). In several studies, where intra-specific crosses were used for mapping, multi-alleles at a single locus were encountered, and a mapping strategy was used to deal with multi-allelic loci. One example is the study of van Ooijen et al. (1994), in which a restriction fragment length polymorphism (RFLP) linkage map of *L. peruvianum* was constructed using three reciprocal backcross populations from an intra-specific cross between *L. peruvianum* LA2157 and LA2172. Multiple alleles were observed within the LA2172 accession, but only RFLP markers that yielded polymorphism between, but not within, the two parents were used. In this way, the multi-allelic loci were excluded. In the study of Ganal and Tanksley (1996), an F_1 mapping population was generated by crossing two *L. peruvianum* plants. For some RFLP probes, more than two alleles were

identified between the two parents. Only RFLP markers, which simultaneously segregated in both male and female gametes (Ritter et al. 1990), were included and scored dominantly. Via this strategy, the number of useful RFLP markers was limited, and the co-dominant information usually provided by RFLP markers was neglected.

Distorted marker segregation has been reported frequently in mapping studies of *L. peruvianum*, as is also reported in other inter- and intra-specific crosses within *Lycopersicon* (Haanstra et al. 1999; Kaloshian et al. 1998; Sandbrink et al. 1995; Van Heusden et al. 1999; Zamir and Tadmor 1986). In *L. peruvianum*, this may be caused by both self-incompatibility and unilateral incompatibility, resulting in preferential transmission of certain alleles. One gametophytic self-incompatibility locus in *L. peruvianum* has been mapped on chromosome 1 (Tanksley and Loaiza-Figueroa 1985). Three loci for the unilateral incompatibility in *L. pennellii* were identified on chromosomes 1, 6 and 10 (Chetelat and De Verna 1991).

All of these phenomena, crossing barriers, multi-alleles per locus and distorted segregation hamper genetic studies of *L. peruvianum*. In this study, we characterised the resistance to *O. neolycopersici* in *L. peruvianum* LA2172 by using different populations from an interspecific cross between *L. esculentum* cv. Moneymaker and *L. peruvianum* LA2172. Multi-allelic, single-locus markers were exploited to unfold the complexities such as multi-allelism and distorted segregation in a pseudo-F_2 population. We illustrated the risk of exclusive use of bi-allelic markers.

Materials and methods

Plant and fungal materials

Hundreds of pollinations were made on plants of *L. esculentum* cv. Moneymaker (MM) with pollen from two plants of *L. peruvianum* LA2172 (LA2172), and only 11 F_1 plants could be raised (Table 1). These F_1 plants were self-incompatible, so pseudo-F_2 populations were obtained by cross-pollinations between individual F_1 plants. Finally, a pseudo-F_2 population (hereafter referred to as the F_2 population) of 194 plants, derived from a cross between two F_1 plants (F_{1a} and F_{1b}) that originated from different individual LA2172 pollen parents, was produced for the present study (Fig. 1). Different backcross populations were generated by using MM as a recurrent parent. Two BC_1 families (in total, 80 plants) of the two F_1 plants (F_{1a} and F_{1b}) were used in the present study (Fig. 1).

Table 1 Generating of F_1 hybrids from crosses between *Lycopersicon esculentum* cv. Moneymaker (*MM*) and *L. peruvianum* LA2172 (*LA2172*)

	MM × LA2172 (plant no. 1)	MM × LA2172 (plant no. 2)
Crosses	>100	>100
Fruits	17	20
Fruits with seeds	6	9
Number of seeds in total	22	29
Number of F_1 plants in total	9	2

Twenty informative F_2 plants of the *Ol-1* reference mapping population, which was derived from an interspecific cross of MM × *L. hirsutum* G1.1560 (Fig. 3b; Huang et al. 2000), were used to position molecular markers linked to the *O. neolycopersici* resistance in LA2172.

The pathogenic fungus *O. neolycopersici*, which originated from infected tomato plants (Lindhout et al. 1994), was maintained on MM plants in a growth chamber at a temperature of 21°C with 70% relative humidity (RH).

Disease test

The inoculum preparation and the inoculation were performed as described by Bai et al. (2003). The experimental setup was according to a randomized block design. For the disease test on the F_2 population, six blocks were used, and each contained 32–33 F_2 plants, three LA2172 plants as resistant control and five MM plants as susceptible control. The inoculated plants were grown in a greenhouse at 20±3°C with 30–70% RH. The plant was scored as resistant (no visible fungal sporulation) or susceptible (with fungal sporulation) at 14, 17 and 21 days post inoculation (dpi). For the disease tests on the BC_1, one block contained 80 BC_1 plants of the two BC_1 families, 20 plants of each MM and LA2172 as susceptible and resistant control, respectively. The inoculated plants grew in a greenhouse at 22°C with 70% RH, and were evaluated as described above at 9 dpi and 12 dpi.

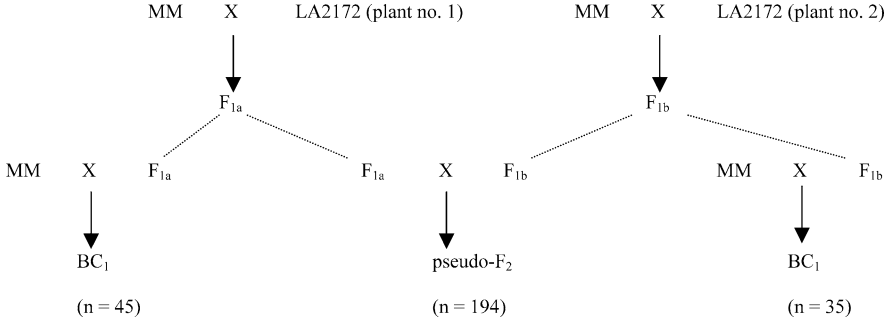

Fig. 1 Cross-pollinating scheme of BC_1 populations and a pseudo-F_2 (F_2) population from a cross of *Lycopersicon esculentum* cv. Moneymaker (*MM*) × *L. peruvianum* LA2172 (*LA2172*)

Molecular markers and linkage analysis

As described by Bai et al. (2003), total DNA was extracted from leaves of the F_2 and BC_1 plants, and the AFLP fingerprints were generated. Bulked segregant analysis (BSA) was performed for AFLP analysis on the resistant and susceptible pools, which were composed of equal volumes of AFLP pre-amplification products of eight resistant and eight susceptible F_2 plants, respectively. AFLP markers were converted into cleaved amplified polymorphic sequence (CAPS) markers according to Brugmans et al. (2003). The CAPS marker *Aps*1 was generated as described by Bai et al. (2003).

The genetic linkage maps were constructed using the software package JoinMap, version 3.0 (van Ooijen and Voorrips 2001). A full account of linkage analysis, using marker data of the F_2 population is given in the Appendix.

Results

The resistance to *O. neolycopersici* in LA2172

Fertile F_1 hybrids of MM × LA2172 were produced despite the large genetic distance between these two species. The F_1 progeny was not tested for resistance to *O. neolycopersici*, since it was very difficult to generate the F_1 plants and the development of these F_1 plants was very irregular. Instead, two BC_1 families from two F_1 plants (F_{1a} and F_{1b}) that were used for generating the F_2 population (Fig. 1) were analyzed for resistance to *O. neolycopersici*. As shown in Table 2, all plants of the susceptible control MM showed fungal sporulation, and all LA2172 plants were immune (no symptoms). The BC_1 plants could unambiguously be classified as either resistant or susceptible. One BC_1 family (MM × F_{1a}) was segregating for resistance (12 resistant:33 susceptible, Table 2), suggesting that the ancestor F_{1a} plant was heterozygously resistant. Thus, resistance in LA2172 should be dominant, although the segregation in this BC_1 family did not follow the 1:1 ratio of a monogenic model (χ^2=8.889, P=0.003). All BC_1 plants (n=35) of MM × F_{1b} were susceptible, indicating that the F_{1b} plant was homozygously susceptible. In the last 10 years, not a single LA2172 plant has been found to be susceptible to *O. neolycopersici*, despite extensive testing of this accession (data not shown). No susceptible LA2172 plants have been found so far; however, a susceptibility allele was transferred to the F_{1b} plant, indicating that the resistance gene is heterozygously present in the LA2172 parent. To verify whether the resistance in LA2172 is monogenic, a large-scale disease test was performed on the F_2 population derived from the cross of F_{1a} × F_{1b}. As shown in Table 2, 100 F_2 plants were resistant and 94 were susceptible, which is in agreement with a segregation ratio of 1:1 for a monogenic model (χ^2=0.1289, P=0.72). We therefore concluded that the resistance in LA2172 is governed by one dominant gene, designated as *Ol-4*.

Table 2 Results of different disease tests on two BC_1 families and one pseudo-F_2 (F_2) population from a cross of MM × LA2172

Test	Plant	Number of plants		
		Total	Resistant	Susceptible
BC_1 families	MM	20	0	20
	LA2172	20	20	0
	BC_1 (MM × F_{1a})	45	12	33
	BC_1 (MM × F_{1b})	35	0	35
F_2 population	MM	30	0	30
	LA2172	18	18	0
	F_2	194	100	94

Identification of markers linked to the *Ol-4* locus

Since the F_2 population was derived from a cross between two F_1 plants (F_{1a} × F_{1b}) that originated from different LA2172 plants (Fig. 1), it might harbour different marker alleles at one locus. Assuming this can also be true for markers linked to the *Ol-4* locus, the heterozygous resistant plant F_{1a} has one marker allele from LA2172 (coded as 'p', linked to the resistance allele of the *Ol-4* locus) and one marker allele from MM (coded as 'e'). The homozygous susceptible plant F_{1b} has one marker allele from LA2172 (coded as 'p*', linked to the susceptibility allele of the *Ol-4* locus) and one marker allele 'e' from MM. Therefore, four marker genotypes (p/p*, e/p, e/p* and e/e) segregating at a 1:1:1:1 ratio were expected in the F_2 population. To test for this hypothesis, multi-allelic, single-locus markers were required to differentiate the four marker genotypes in the F_2 population.

In order to efficiently identify the marker allele p linked in coupling phase to the *Ol-4* locus, BSA was performed by using AFLP on the resistant and susceptible pools of the F_2 plants (see Materials and methods). By using a total of 256 *Pst*/*Mse* primer combinations, 16 AFLP markers were identified that were present only in the resistant pool. Ten of these AFLP markers showed close co-segregation with *Ol-4* (Fig. 3a) by testing them on 56 F_2 individuals (20 resistant and 36 susceptible). To assign the AFLP markers to a particular chromosome, one AFLP marker (P18 M51–450) was successfully converted into a CAPS marker, designated By-4 (Table 3). By using the *Ol-1* reference mapping population (see Materials and methods), By-4 was positioned on chromosome 6 between RFLP markers *Aps*1 and TG153 (Fig. 3b).

Map position of the *Ol-4* locus

In order to verify whether *Aps*1 is linked to the *Ol-4* locus, the RFLP marker *Aps*1 on chromosome 6 was converted into a CAPS marker (Table 3) that co-segregated with the original RFLP marker in the *Ol-1* reference mapping population (see Materials and methods). The CAPS marker *Aps*1/*Taq*I revealed the expected multiple alleles in the F_2 population. As shown in Fig. 2a, the e allele was from MM, two other alleles (p and p*) were from LA2172.

Table 3 Primer sequences, lengths of PCR products and enzymes revealing a polymorphism for cleaved amplified polymorphic sequence (CAPS) markers

CAPS marker	Primer sequence (5′-3′)	Annealing temperature (°C)	PCR products (bp)	Enzymes detecting polymorphism between	
				LA2172 and MM	*L. hirsutum* G1.1560 and MM
*Aps*1	F: atggtgggtccaggttataag R: cagaatgagcttctgccaatc	56	1300	*Sau*96I (p=p*≠e) *Taq*I (p≠p*≠e)	*Dde*I
By-4	F: catagtgtagctttgattcttgta R: ccaattgccgggaaggaa	46	300	*Apo*I (p≠p*=e) *Hyp*CH4IV (p=e≠p*)	*Mse*I

By testing this marker on the F_2 population, four marker phenotypes were observed (p/p*, e/p, e/p* and e/e, Table 4). Only the marker allele p was linked to the resistance allele of the *Ol-4* locus. In addition, for the CAPS marker By-4 (Fig. 2b; Table 4), restriction enzyme *Hyp*CH4IV was specific for the p* allele (in equation p*≠p=e), and restriction enzyme *Apo*I was specific for the p allele (in equation p≠p*=e). Thus, when the results from these two restriction enzymes were combined, marker By-4 uncovered the four distinct marker genotypes of the F_2 plants. The three marker alleles for CAPS markers *Aps*1 and By-4 were confirmed in the two BC_1 families. The expected ratio for the four marker genotypes (p/p*, e/p, e/p* and e/e) in the F_2 population was 1:1:1:1, while the observed frequencies were 74:15:80:17 for marker *Aps*1 and 73:16:80:17 for marker By-4, respectively (Table 4). For both CAPS markers By-4 and *Aps*1, distorted marker segregation was caused by preferential transmission of the *L. peruvianum* allele by the male parent (genotype e/p*, Appendix 3), with estimated transmission rates of about 18% and 82% for alleles e and p*, respectively.

All possible marker-allele configurations in the F_2 population are shown in Table 4 and Fig. 2, and marker data for cross pollinator (CP) population type were prepared for JoinMap to construct a linkage map around the *Ol-4* locus. As the JoinMap program assumes no distortion, parameters for both distortion and linkage between the *Ol-4* and CAPS markers were also estimated using maximum likelihood estimations. A full account of the linkage analysis for various types of the CAPS markers is given in the Appendices. A linkage map was constructed for a part of tomato chromosome 6, where the *Ol-4* locus was positioned above CAPS marker *Aps*1 (Fig. 3a). A comparison of the *Ol-4* maps with the *Ol-1* map showed that the order of the marker loci on the maps was identical. Therefore, we concluded that *Ol-4* is on chromosome 6, but at a position different from the *Ol-1* locus.

Discussion

L. peruvianum is an out-crossing species and can harbour multi-alleles per locus. as illustrated in the present study and by others (Ganal and Tanksley 1996; Kaloshian et al. 1998; Miller and Tanksley 1990; van Ooijen et al. 1994). In several mapping studies, mapping strategies have been applied in order to deal with multi-allelic loci (Ganal and Tanksley 1996; van Ooijen et al. 1994). In the present study, it was illustrated that the information of multi-alleles at one locus can be fully exploited. Taking into account the high degree of heterogeneity in the donor parent *L. peruvianum* LA2172, we anticipated the possibility of more than two marker alleles per locus in the F_2 population that was generated by mating F_1 half-sibs. To test this hypothesis, multi-allelic CAPS markers for single loci were developed, which uncovered all marker-allele configurations (four marker genotypes, Fig. 2) in the F_2 population. Consequently, markers could not be processed in the same way as a normal

Table 4 Marker genotypes in a F_2 population derived from a cross of MM × LA2172

Marker genotype	Expected ratio	Number of the F_2 plants per marker phenotypes[a]				
		*Aps*1/*Taq*I (p≠p*≠e)	*Aps*1/*Sau*96I (p=p*≠e)	By-4 (*Apo*I + *Hyp*CH4IV) (p≠p*≠e)	By-4/*Apo*I (p≠p*=e)	By-4/*Hyp*CH4IV (p=e≠p*)
p/p*	1:4	74 (71R + 3S)	74 (71R + 3S)	73 (70R + 3S)	89 (85R + 4S) (e/p=p/p*)	153 (78R + 75S) (p/p*=e/p*)
e/p	1:4	15 (15R)	95 (22R + 73S) (e/p=e/p*)	16 (15R + 1S)	See p/p*	33 (15R + 18S) (e/p=e/e)
e/p*	1:4	80 (7R + 73S)	See e/p	80 (8R + 72S)	97 (8R + 89S) (e/p*=e/e)	See p/p*
e/e	1:4	17 (17S)	17 (17S)	17 (17S)	See e/p*	See e/p

[a] *R* resistance; *S* susceptible

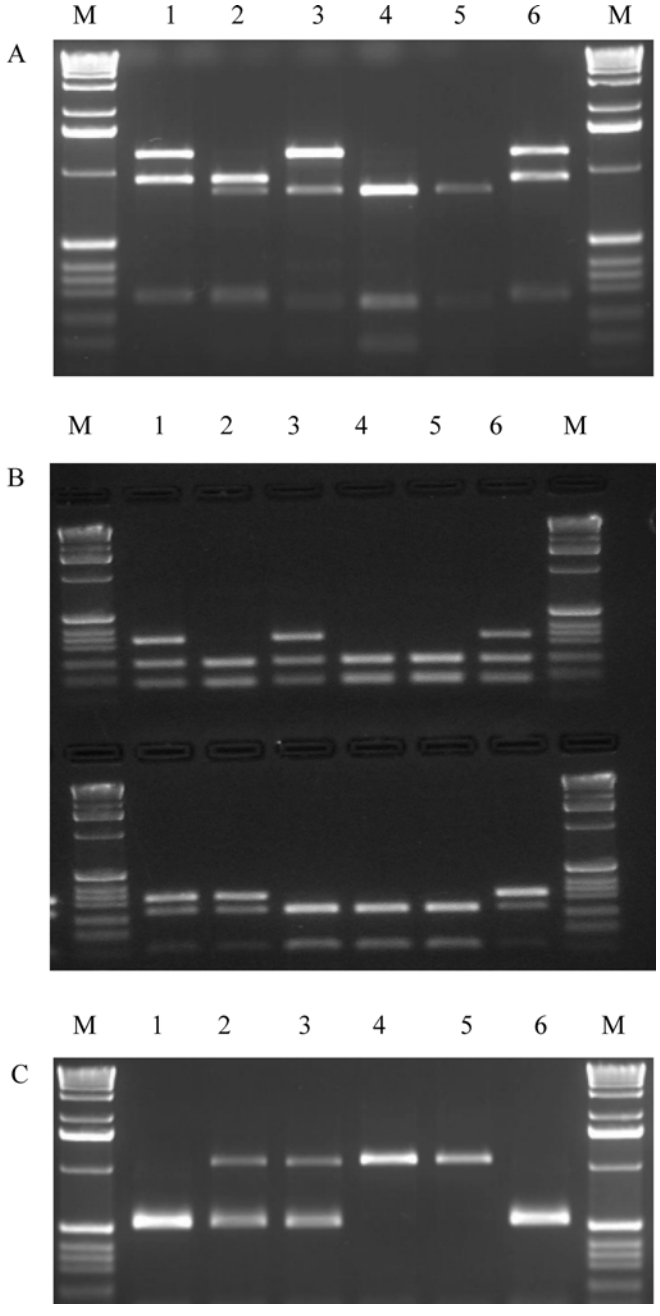

Fig. 2a–c Electrophoretic patterns of cleaved amplified polymorphic sequence (CAPS) markers (2% agarose gel) to show marker phenotypes in a F_2 population from a cross of MM × LA2172. *M* DNA size marker of 1-kb ladder, *lanes 1* and *2* resistant F_2 plants, *lanes 3* and *4* susceptible F_2 plants. *Lanes 1–4* show marker phenotypes of p/p*, e/p, e/p* and e/e, respectively; *lane 5* MM with marker phenotype of e/e; *lane 6* LA2172 (DNA is pooled from several plants) and shows marker phenotype of p/p*. **a** CAPS marker *Aps*1/*Taq*I (p≠p*≠e). The upper fragment in *lane 6* (LA2172) is the p* allele and the lower fragment is the p allele; the single fragment in *lane 5* (MM) is the e allele. **b** CAPS marker By-4. In *lane 6* (LA2172) of the *upper panel*, *Hpy*CH41V (p=e≠p*) reveals the upper fragment for p* allele; in *lane 6* (LA2172) of the *lower panel*, *Apo*I (p≠p*=e) yields the upper fragment for p allele. **c** CAPS marker *Aps*1/*Sau96*I (p=p*≠e). The fragment in *lane 6* (LA2172) is diagnostic for both p and p* alleles

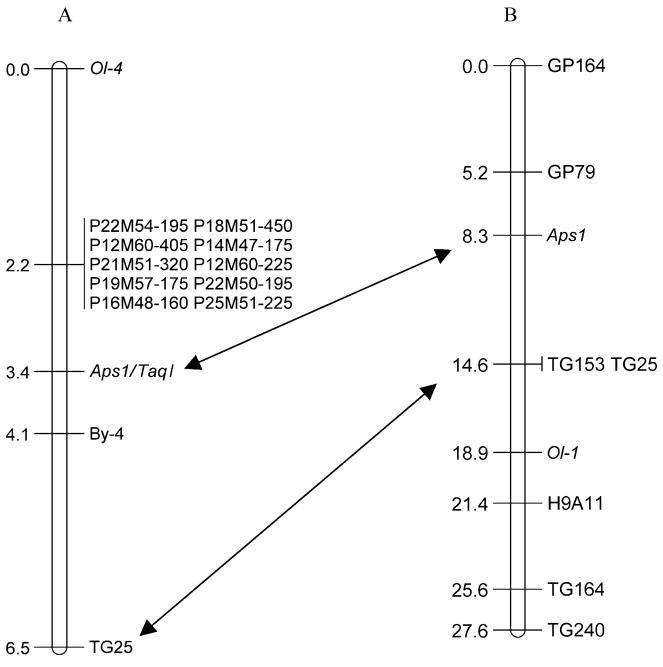

Fig. 3a, b Genetic maps of part of chromosome 6 showing map positions of *Ol-4* and *Ol-1* loci, based on two different populations. **a** The *Ol-4* locus map position in a F_2 population derived from the cross of MM × LA2172, respectively. AFLP and CAPS markers were used. **b** Skeleton map showing positions of the *Ol-1* locus and some restriction fragment length polymorphism markers on chromosome 6, which was retrieved from Huang et al. (2000)

backcross or F_2 population from inbreeding species, but rather as a population resulting from a cross between two heterogenously heterozygous diploid parents (coded as CP in JoinMap program).

The recognition of multi-alleles at a single locus in the F_2 population by the multi-allelic markers has been of crucial importance. As shown in Table 4, CAPS markers uncovered two, three or four genotypes in the F_2 population, depending on the restriction enzymes used (Fig. 2). For example, the CAPS marker *Aps*1/*Sau96*I could not differentiate between the alternative *L. peruvianum* alleles p and p* (Fig. 2c). Thus, this marker revealed only three marker phenotypes in the F_2 population: homozygous as LA2172 (p/p*), homozygous as MM (e/e) and heterozygous (marker genotype of e/p and e/p*). F_2 plants carrying the heterozygous marker phenotype segregated into classes: resistant (n=22) and susceptible (n=73) (Table 4). As explained in Appendix 4, incorrect estimates of recombination frequency between the *Ol-4* locus and this marker could be obtained, if the distortion cannot unambiguously be attributed to either of the parents. Thus, the usage of bi-allelic markers of this type may complicate the mapping study because of the joint effect of linkage and preferential transmission of certain alleles. Similarly, bi-allelic markers like CAPS marker By-4/*Hyp*CH4IV (where p=e≠p*) were uninformative for mapping in this F_2 population (Appendix 1), as this marker type uncovers two marker phenotypes (Fig. 2b; Table 4). One is heterozygous as LA2172 (marker genotypes of p/p* and e/p*) with 153 F_2 plants (78

resistant and 75 susceptible), while the other is homozygous as MM (marker genotypes of e/p and e/e) with 33 F_2 plants (15 resistant and 18 susceptible). Without discrimination of the p and e alleles, one would conclude that By-4/*Hyp*CH41V segregates independently from the *Ol-4* gene. For a reliable genetic analysis, it is crucial to have markers that reveal different marker-allele configurations, like the multi-allelic, single-locus marker *Aps*1/*Taq*I that detected the three marker alleles (p, p* and e) and distinguished the four marker genotypes in the F_2 population (Fig. 2a; Table 4).

By recognition of multi-alleles at a single locus in the F_2 population, distorted segregation in the F_2 population became uncovered that was caused by preferential transmission of *L. peruvianum* alleles by the male parent only. In this case, it was demonstrated that the selection acted against male gametes having *L. esculentum* alleles at the *Ol-4* locus, which hardly influences the estimated recombination frequencies between the genetic loci (Appendix 3). In agreement with this, other authors (Ritter et al. 1990; van Ooijen et al. 1994) suggested that the estimated recombination frequency is unbiased when the segregation distortion is caused by selection at one locus per chromosome, but is biased when the selection acts on two (or more) loci on one chromosome.

In summary, we mapped the *Ol-4* gene on the tomato chromosome 6, which governs complete resistance to *O. neolycopersici* and originates from *L. peruvianum* LA2172. It was illustrated that the hypothesis of close linkage between genetic markers can be erroneously rejected in case bi-allelic marker data are used for multi-allelic loci. The recognition of multiple alleles allowed accurate mapping of *Ol-4* and revealed the large segregation distortion in the F_2 population of MM × LA2172.

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Appendix

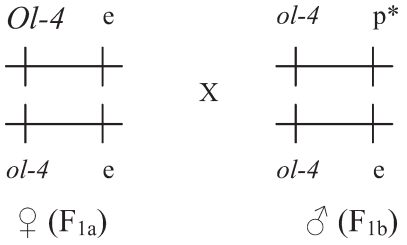
Estimation of recombination frequency between *Ol-4* and (multi-allelic) markers from the F_2 population

Altogether, we used four types of markers (all possible marker-allele configurations in the F_2 population), for each of which we discuss the linkage analysis below.

Appendix 1

Markers that distinguish between the alternative *L. peruvianum* alleles (p, p*), but only one of these alleles is distinct from the *L. esculentum* allele (p*≠p=e)

Marker By-4/*Hyp*CH4IV is of this type (Table 4; Fig. 2). The configuration of the cross between the two F_1 genotypes (F_{1a} and F_{1b}, see Fig. 1) looks as follows:



Since neither of the parents is heterozygous at both loci, markers of this type are uninformative for linkage. In fact, the loci behave as if they segregate independently (see below). Not being aware of this non-informative nature, the joint segregation can be misinterpreted as 'unlinked loci'.

Later on, we will have to introduce a parameter for preferential transmission of marker alleles. Let this parameter be β for allele transmission rate in the male parent (β=0.5 corresponds to a 1:1 Mendelian ratio). This leads to the following table of gamete combinations and their frequencies:

♀	♂	
	β (*ol-4* p*)	1−β (*ol-4* e)
(1/2) *Ol-4* e	1/2β	1/2(1−β)
(1/2) *ol-4* e	1/2β	1/2(1−β)

The following two-way table shows genotypes for the progeny (numbers in parentheses are the observed numbers for the marker By-4/*Hyp*CH4IV from Table 4).

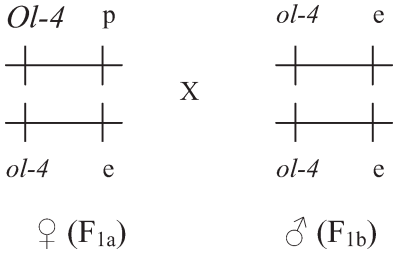
	Ol-4/ol-4	*ol-4/ol-4*	Sum
e/p*	1/2β (78)	1/2β (75)	β (153)
e/e	1/2(1−β) (15)	1/2(1−β) (18)	(1−β) (33)
Sum	1/2 (93)	1/2 (93)	1 (186)

Despite the distorted segregation, we indeed observe independent segregation between this marker and the *Ol-4* locus. The distortion parameter β is estimated as $\hat{\beta} = 153/186 = 0.8226$.

Appendix 2

Markers that distinguish between the two alternative *L. peruvianum* alleles (p, p*), but only one of these is distinct from the *L. esculentum* allele (p≠p*=e)

This is the 'mirror' situation of (p*≠p=e), discussed above. Marker By-4/*Apo*I is of this type. The configuration of the cross looks like:



This represents the classical test cross configuration, from which recombination frequency (r), is readily estimated by counting the recombinant genotypes among the offspring. However, for the sake of completeness we introduce a parameter, α, for preferential transmission of marker alleles by the female parent (α=0.5 corresponds to a 1:1 ratio). This leads to the two-way table of genotype frequencies below (observed numbers are in parentheses, which are taken from Table 4 for marker By-4/*Apo*I).

	Ol-4/ol-4	*ol-4/ol-4*	Sum
e/p	α(1−r) (85)	**αr** (4)	α (89)
e/e	(**1**−**α**)**r** (8)	(1−α)(1−r) (89)	(1−α) (97)
Sum	(93)	(93)	1 (186)

Notice that the boldfaced genotypes together occur with frequency r. So we estimate

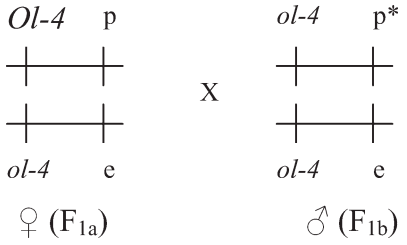
$$\hat{r} = \frac{12}{186} = 0.0645, \text{ and}$$
$$\hat{\alpha} = \frac{89}{186} = 0.4785.$$

We note that α is close to 0.5, indicating that no significant preferential transmission of alleles by the female parent occurs at this marker locus.

Appendix 3

Markers that distinguish between the two *L. peruvianum* alleles, as well as the *L. esculentum* allele (p≠p*≠e)

This represents the most informative class of markers that allow straightforward estimation of the two distortion parameters (α and β) as well as r. Marker By-4 and *Aps*1/*Taq*I are of this category. The configuration of the cross reads like:



Using the same parameter notation as above, we have the following table of gamete combinations

♀	♂	
	β (*ol-4* p*)	1−β (*ol-4* e)
α(1−r) (*Ol-4* p)	α β(1−r)	α(1−β)(1−r)
(1−α)r (*Ol-4* e)	(1−α)βr	(1−α)(1−β)r
αr (*ol-4* p)	α**β**r	α(1−β)r
(1−α)(1−r) (*ol-4* e)	(1−α)β(1−r)	(1−α)(1−β)(1−r)

Thus, the two-way table of genotypes reads:

	Ol-4/ol-4	*ol-4/ol-4*	Sum
p/p*	αβ(1−r)	**αβr**	αβ
e/p*	(**1**−**α**)**βr**	(1−α)β(1−r)	(1−α)β
e/p	α(1−β)(1−r)	**α(1−β)r**	α(1−β)
e/e	(**1**−**α**)(**1**−**β**)**r**	(1−α)(1−β)(1−r)	(1−α)(1−β)

Notice that the boldfaced genotypes together represent a proportion, r (independent of α, β). So r is estimated by counting these genotypes. Likewise, α and β are estimated by adding the appropriate classes in the right margin of the above table (pp*+ep for α, pp*+ep* for β). Using the numbers given in Table 4 we obtain for

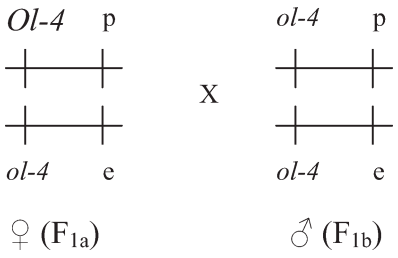
$$\text{By}-4: \quad \hat{\alpha} = 0.479,\ \hat{\beta} = 0.823;\ \hat{r} = 0.0645,$$
and for
$$\textit{Aps}1/\textit{Taq}\text{I}: \quad \hat{\alpha} = 0.479,\ \hat{\beta} = 0.828;\ \hat{r} = 0.0538.$$

We observe that the estimates for α and β are the same as the ones obtained for markers By-4/*Hyp*CH4IV and By-4/*Apo*I, which is not surprising, since they represented the same locus (By-4).

Appendix 4

Markers that distinguish between the *L. peruvianum* and *L. esculentum* alleles, but not between the two *L. peruvianum* alleles (p*=p≠e)

Marker *Aps*1/*Sau96*I is of this type. The configuration of the cross reads like:



From this we see that, considering the marker locus only, any segregation distortion at the marker cannot be ascribed to either parent or to both parents. In other words, estimation of the distortion parameters must go along with estimation of r, and vice versa. In this sense, this marker category represents the most 'difficult' one: it requires simultaneous estimation of α, β and r.

Proceeding as before, using α and β for female and male transmission frequencies at the marker and r for recombination frequency, we obtain the following two-way table of genotypes (numbers in parentheses are observed numbers for marker *Aps*1/*Sau96*I in Table 4).

	Ol-4/ol-4	*ol-4/ol-4*	Sum
p/p	$\alpha\beta(1-r)$ (71)	$\alpha\beta r$ (3)	$\alpha\beta$ (74)
e/p	$\alpha(1-\beta)(1-r)+(1-\alpha)\beta r$ (22)	$\alpha(1-\beta)r+(1-\alpha)\beta(1-r)$ (73)	$\alpha(1-\beta)+(1-\alpha)\beta$ (95)
e/e	$(1-\alpha)(1-\beta)r$ (0)	$(1-\alpha)(1-\beta)(1-r)$ (17)	$(1-\alpha)(1-\beta)$ (17)
Sum	(93)	(93)	1 (186)

For the simultaneous estimation of α, β, and r, we proceed as follows. As can be seen from the table above, α and β can be estimated from the observed frequencies at the marker locus (probabilities in the right margin). We also see that for the marker genotype frequencies, α and β are interchangeable, which means that, in case α and β are not equal, we cannot ascribe the estimate to either parent. However, we observe a 1:1 ratio at the *Ol-4* locus, which means that for a marker closely linked to *Ol-4*, the value of α must be close to 0.5 (α is the female transmission rate: a clear deviation from 0.5 would 'drag' along the alleles at *Ol-4*). Estimates of α and β are obtained by solving the appropriate likelihood equations, using probabilities and observed frequencies at the marker locus. The resulting quadratic equation yields two equivalent solutions, i.e.

$$\left(\hat{\alpha}, \hat{\beta}\right) = (0.483,\ 0.823) \text{ and}$$
$$\left(\hat{\alpha}, \hat{\beta}\right) = (0.823,\ 0.483).$$

We accept the first one, since α should be close to 0.5; it also is in close agreement with the estimates obtained for the other markers. Next, we substitute $(\alpha,\ \beta)$= (0.483,0.823) in the expressions for the probabilities in the body of the table and (numerically) solve the resulting likelihood equation for r. This yields $\hat{r} = 0.0394$. However, should we have used the 'mirror' estimates of$(\hat{\alpha}, \hat{\beta})$, i.e.$(\hat{\alpha}, \hat{\beta}) = (0.823, 0.483)$, the incorrect estimate of r would have been$\hat{r} = 0.287$. This, again, shows the necessity of carefully interpreting the joint segregation data in order to avoid wrong conclusions.

Using the obtained estimates, we have calculated the corresponding LOD values for linkage. The table below summarizes the results.

Marker	$\hat{\alpha}$	$\hat{\beta}$	$\hat{r}$	LOD
*Aps*1/*Taq*I	0.479	0.828	0.054	39.1
*Aps*1/*Sau*96I	0.483	0.823	0.039	27.7
By-4	0.479	0.823	0.065	36.7
By-4/*Apo*I	0.479	–	0.065	36.7
By-4/*Hyp*CH4IV	–	0.823	–	–

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