Jurnal Agro*Biogen* 11(1):1–6

Segregation Analysis of SSR, SNP, and AFLP Markers in F_2 Population of *Solanum lycopersicum* × *S. arcanum*

(Analisis Segregasi Marka SSR, SNP, dan AFLP pada Populasi F_2 Persilangan *Solanum lycopersicum* × *S. arcanum*)

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Submitted: 2 December 2014; Revised: 22 December 2014; Accepted: 27 February 2015

ABSTRAK

Penyimpangan segregasi marka biasa terjadi pada persilangan antarspesies pada berbagai komoditas tanaman. Penelitian terdahulu mengenai pemetaan lokus ketahanan terhadap jamur hawar dini (*Alternaria solani*) menunjukkan penyimpangan segregasi dari 52% marka yang terpetakan pada peta pautan 176 progeni F_2 dari persilangan *Solanum lycopersicum* cv. Solentos × *S. arcanum* LA2157. Tujuan penelitian ini adalah menganalisis lebih detil segregasi marka pada peta pautan tersebut dan menentukan penyebab penyimpangan segregasi dengan menghitung frekuensi alel dan genotipe F_2 dari tiap marka. Dari 371 marka yang terpetakan, 192 di antaranya menyimpang dari rasio Mendel 1 : 2 : 1. Penyimpangan marka terjadi pada semua kromosom, sebesar 1% sampai 9%. Surplus alel homozigot *S. arcanum* (40%) menjadi penyumbang terbesar penyimpangan segregasi, diikuti oleh heterozigot (18%) dan homozigot *S. lycopersicum* (5%). Frekuensi alel dari 152 marka menyimpang dari frekuensi homogenitas alel yang diharapkan, yang mungkin disebabkan oleh seleksi gametofitik. Enam puluh satu marka menyimpang dari distribusi frekuensi genotipe F_2 yang diharapkan, yang dapat diakibatkan oleh segregasi zigotik. Segregasi 37 marka menyimpang dari frekuensi homogenitas alel dan distribusi genotipe F_2 yang diharapkan. Marka dengan segregasi menyimpang tetap dapat digunakan dalam analisis pautan karena lokus ketahanan terhadap jamur hawar dini juga teridentifikasi pada daerah kromosom yang mengandung marka-marka terdistorsi. Identifikasi lebih lanjut mengenai mekanisme penyimpangan segregasi memerlukan kajian pemetaan yang ekstensif dan mendalam.

Kata kunci: Tomat, *Solanum arcanum*, distorsi segregasi marka.

ABSTRACT

Distorted marker segregation is a common phenomenon in interspecific cross of various crops. Previous mapping study of early blight fungus (*Alternaria solani*) resistance loci showed 52% marker distortion in the genetic linkage map of 176 F_2 progenies derived from *Solanum lycopersicum* cv. Solentos × *S. arcanum* LA2157. The objectives of this study were to analyze in detail the marker segregation in the map and to determine the cause of segregation distortion by calculating the allele and genotype frequencies of each marker. Out of 371 mapped markers, 192 markers deviated from the expected Mendelian ratio of 1 : 2 : 1. Distorted markers occurred in all chromosomes, ranging from 1% to 92%. Surplus of *S. arcanum* homozygotes contributed most to the skewness (40%), followed by heterozygotes (18%), and *S. lycopersicum* homozygotes (5%). The allele frequencies of 152 markers deviated from the expected allele homogeneity frequency, indicating that their segregation might be affected by gamethophytic selection. Sixty-one markers deviated from the expected F_2 genotype frequency distribution, indicating that their segregation might be influenced by zygotic selection. Thirty-seven of the distorted markers showed deviation from expected frequencies of allele homogeneity and F_2 genotype frequency distribution. Distorted markers can be retained in linkage analysis since chromosomal regions containing distorted markers showed linkage with early blight fungus resistance loci. Further identification of the mechanism contributing segregation distortion requires detailed and extensive mapping studies.

Keywords: Tomato, *Solanum arcanum*, marker segregation distortion.

INTRODUCTION

Segregation distortion (SD) or deviation of the observed genotypic frequencies from the expected Mendelian ratio within a segregating population is commonly observed in tomato interspecific crosses. The extent of distortion was higher in wider crosses; less skewed segregation rate (8–10%) was observed in crosses of tomato with *Solanum pimpinellifolium* (syn. *Lycopersicon pimpinellifolium*), a closely related species of the cultivated tomato (Chen and Foolad, 1999; Grandillo and Tanksley, 1996); whereas 50% distortion rate was reported in a *S. lycopersicum* × *S. cheesmaniae* F_2 population (Paterson *et al.*, 1991), 52% to 55% in a *S. lycopersicum* × *S. arcanum* F_2 population (Chaerani *et al*., 2007; van Heusden *et al*., 1999), and up to 80% in a *S. lycopersicum* × *S. pennellii* F_2 population (de Vicente and Tanksley, 1993).

Distorted segregation can occur because of statistical bias (genotyping and scoring errors) or biological factors, such as chromosome loss, gametophytic competition which lead to preferential transmission of certain alleles; zygotic selection which is observed in differential survival ability to mature; incompatibility genes; unilateral incongruity or non homologous recombination; viability selection of segregating plants; gene transfer; transposable element; and environmental agents (Christiansen, 1980; Liu *et al*., 2011; Saliba-Colombani *et al*., 2000; Semagn *et al*., 2006; Zhao *et al*., 2006). Zhao *et al.* (2006) inferred the potential factors involved in marker segregation distortion of an F_2 population of rice by calculating the allele frequency (p and q) and the distribution of F_2 genotype frequency (p^2:$2pq$:q^2). According to the Hardy-Weinberg principle for a gene with two alleles, homogeneity of allele frequency ($p = q$) and F_2 genotype frequency distribution is expected in the absence of disturbing forces such as mate choice, mutation, selection, genetic drift, gene flow, and meiotic drive (Hartl, 1987). Deviation of allele frequency from the expected ratio as determined by Chi-squared test indicates the occurrence of gametophytic selection, whereas deviation from the expected genotype frequency indicates zygotic selection (Zhao *et al*., 2006).

Previously, Chaerani *et al.* (2007) published a framework map of SSR, SNP, and AFLP markers in an F_2 population derived from *S. lycopersicum* cv. Solentos × *S. arcanum* LA2157 showing positions of quantitative trait loci (QTLs) effect on early blight fungus without addressing marker segregation analysis in detail. In this paper, we present the details of the map and extend the marker segregation analysis to determine the potential factors of segregation distortion by calculating the proportions of allele and F_2 genotype distribution.

MATERIALS AND METHODS

Plant Material, Molecular Markers, and Linkage Mapping

The mapping population, molecular markers, and the construction of marker linkage map were described earlier in Chaerani *et al.* (2007). Marker maps were drawn using the program MapChart version 2.0 (Voorrips, 2001).

Analysis of Marker Segregation

The null hypothesis of a 1 : 2 : 1 marker segregation in F_2 progenies (*ll*, *la*, and *aa*, i.e. homozygous *S. lycopersicum*, heterozygous *S. lycopersicum*/*S. arcanum*, and homozygous *S. arcanum*, respectively) was tested for each marker by performing a χ^2 test (df = 2) using the module available in JoinMap® 3.0 (van Ooijen and Voorrips, 2001).

Analysis of Distorted Marker

The allele and genotype frequencies of distorted markers and their significance as determined by χ^2 test (df = 1) were calculated with the aid of Powermarker V3.25 (Liu and Muse, 2005). Deviation from allele frequency homogeneity ($p = q$) indicates that segregation is influenced by gametic selection, whereas deviation of F_2 genotype frequency (p^2:$2pq$:q^2) indicates that segregation is caused by zygotic selection (Zhang *et al*., 2003).

RESULTS AND DISCUSSION

Analysis of Marker Segregation

A total of 371 markers were mapped to 12 tomato chromosomes (Table 1, Figure 1). The number of markers mapped per chromosome ranged from 17 (chromosome 5) to 53 (chromosome 1) and the length of linkage group ranged from 70.2 cM (chromosome 9) to 142.5 cM (chromosome 1), resulting in a total of 1178.4 cM map length. The average marker density is one marker per 3.2 cM. When considering that the haploid DNA content of tomato is estimated to be approximately 950 Mbp, 1 cM in our map equals to about 800 kb (Arumuganathan and Earle, 1991).

The allele frequency at 192 marker loci (52%) deviated significantly from the expected 1 : 2 : 1 segregation ratio at 5% to 0.01% significance level. For each marker type, 11 (65%) SNPs, 15 (48%) SSRs, and 158 (51%) AFLPs showed skewed segregation

Table 1. Map length, number of mapped loci, segregation distortion, and distribution of molecular markers among 12 chromosomes in *Solanum lycopersicum* cv. Solentos × *S. arcanum* LA2157 F_2 population.

	Chr 1	Chr 2	Chr 3	Chr 4	Chr 5	Chr 6	Chr 7	Chr 8	Chr 9	Chr 10	Chr 11	Chr 12	Total
Number of SSR mapped	3	3	6	3	1	2	2	1	2	2	3	3	31
Number of SNP mapped	3	2	0	3	2	2	1	2	1	0	1	3	20
Number of AFLP mapped	47	32	30	30	14	25	31	22	23	19	29	19	320
Total number of markers	53	37	36	36	17	29	33	25	26	21	33	25	371
Number of distorted segregation marker (%)	25 (47.2)*	33 (89.2)	1 (2.8)	33 (91.7)	7 (41.2)	5 (17.2)	26 (76.5)	23 (92.0)	20 (76.9)	2 (9.5)	12 (36.4)	5 (20.0)	192 (51.6)**
Number of excess "*Il*" (%)	7 (13.2)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.8)	1 (4.8)	0 (0.0)	8 (32.0)	19 (5.1)
Number of excess "*Ia*" (%)	9 (17.0)	4 (10.8)	0 (0.0)	7 (19.4)	11 (64.7)	7 (24.1)	6 (17.5)	1 (4.0)	14 (53.8)	0 (0.0)	5 (15.2)	1 (4.0)	65 (17.5)
Number of excess "*aa*" (%)	12 (22.6)	29 (78.4)	6 (16.7)	28 (77.8)	13 (76.5)	0 (0.0)	24 (80.0)	22 (88.0)	8 (30.8)	2 (9.5)	11 (33.3)	0 (0.0)	150 (40.3)
Map length (cM)	142.5	89.0	131.5	106.5	83.9	95.0	94.0	88.3	70.2	79.0	94.2	104.3	1178.4
Average distance between markers (cM)	2.7	2.4	3.7	2.9	4.9	3.3	2.8	3.5	2.7	3.8	2.9	4.2	
Largest gap between markers (cM)	16.2	16.9	13.9	13.3	24.8	24.3	12.2	12.8	13.4	19.8	14.0	25.7	

Chr = chromosome, "*Il*" = homozygous *S. lycopersicum,* "*Ia*" = heterozygous *S. lycopersicum*/*S. arcanum,* "*aa*" = homozygous *S. arcanum.*

*Based on number of marker per chromosome. **Based on total number of markers.

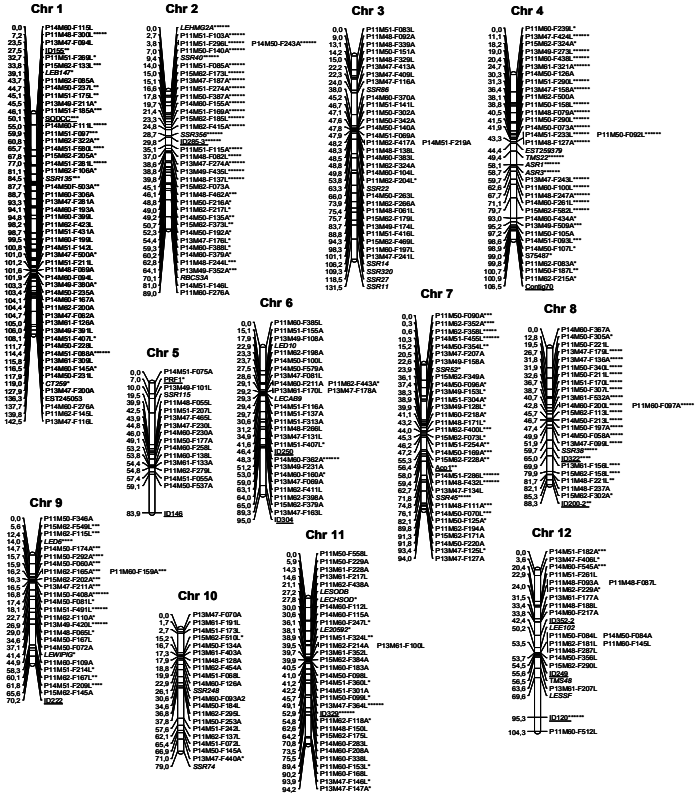

Figure 1. SSR, SNP, and AFLP marker maps based on 176 F_2 plants derived from *Solanum lycopersicum* cv. Solentos × *S. arcanum* LA2157 cross. Chromosome numbers are above the linkage groups. Italicized markers are SSRs, whereas underlined markers are SNPs, and the remaining markers are AFLPs. Map distances are in cM (left). The orientation of chromosome 4 is unknown. Asterisks indicated significance level of *P* value χ^2 test. *$P < 0.05$. **$P < 0.01$. ***$P < 0.005$. ****$P < 0.001$. *****$P < 0.0005$. ******$P < 0.0001$.

(Table 1). The aberrant segregation was observed on all chromosomes, ranging from 1% for chromosome 3, to 92% for chromosome 8. The direction of the skewness was mostly caused by excess of *S. arcanum* homozygotes (40%), suggesting that the *S. arcanum* alleles were transmitted at higher frequencies. Surplus by heterozygotes and *S. lycopersicum* homozygotes occurred at lower rates, which was 18% and 5%, respectively.

Strong marker segregation distortion can hamper the linkage analysis since biased markers can overestimate the recombination frequency and therefore map distances between markers with skewed segregation ratios may be inaccurate (Cervera

et al., 2001; Saliba-Colombani *et al.*, 2000; Sim *et al*., 2012). However, distorted markers often showed linkage with resistance loci. For example, the death of *Melampsora*-susceptible plant in poplar were caused by distorted markers, but they were retained in linkage map since they cosegregated with the resistance gene (Cervera *et al*., 2001). In our previous QTL study, resistance loci to early blight fungus were mapped in regions both with skewed segregations (chromosomes 2, 6, 7, and 9) and in regions without skewed segregation (chromosomes 1 and 5) (Chaerani *et al*., 2007). Zhang *et al*. (2003) also reported that the presence of early blight QTLs in tomato was consistent with the extent of skewed marker segregation.

Table 2. SSR, SNP, and AFLP markers showing deviation from expected homogeneity allele frequency in *Solanum lycopersicum* cv. Solentos × *S. arcanum* LA2157 F_2 population.

Chr	Marker	Allele frequency		χ^2 value	Chr	Marker	Allele frequency		χ^2 value	Chr	Marker	Allele frequency		χ^2 value
		a	b				a	b				a	b	
1	LEB147	0.42	0.58	8.81 ***	4	ASR1	0.30	0.70	55.68 *	7	P14/M51-F-286-P1	0.37	0.63	20.13 ***
1	CT259	0.58	0.42	6.94 **	4	S75487	0.43	0.57	7.02 **	7	P14/M51-F-455-P1	0.37	0.63	22.97 ***
1	ID155	0.42	0.58	8.19 ***	4	TMS22	0.35	0.65	31.92 ***	7	P15/M62-F-073-P1	0.39	0.61	14.85 ***
1	SODCC	0.41	0.59	10.71 ***	4	P11/M48-F-079-P2	0.35	0.65	31.63 ***	7	P15/M62-F-228-P2	0.42	0.58	9.84 ***
1	P11/M48-F-300-P1	0.39	0.61	15.81 ***	4	P11/M48-F-127-P2	0.32	0.68	42.77 ***	8	SSR38	0.38	0.62	21.75 ***
1	P11/M51-F-097-P2	0.43	0.57	6.62 *	4	P11/M48-F-247-P2	0.30	0.70	53.45 ***	8	ID200-2	0.42	0.58	9.39 ***
1	P11/M51-F-175-P1	0.42	0.58	8.96 ***	4	P11/M50-F-092-P1	0.33	0.67	40.05 ***	8	ID322	0.40	0.60	12.32 ***
1	P11/M51-F-185-P2	0.41	0.59	11.70 ***	4	P11/M50-F-158-P1	0.34	0.66	34.31 ***	8	P11/M48-F-221-P1	0.41	0.59	11.17 ***
1	P11/M51-F-269-P1	0.43	0.57	6.12 **	4	P11/M50-F-187-P1	0.43	0.57	6.26 *	8	P11/M50-F-197-P2	0.35	0.65	30.86 ***
1	P13/M47-F-500-P2	0.58	0.42	7.96 ***	4	P11/M50-F-290-P1	0.29	0.71	52.85 ***	8	P11/M50-F-211-P1	0.34	0.66	30.59 ***
1	P13/M49-F-211-P2	0.42	0.58	8.48 ***	4	P11/M51-F-290-P1	0.35	0.65	29.90 ***	8	P11/M50-F-307-P1	0.35	0.65	30.67 ***
1	P13/M49-F-380-P2	0.57	0.43	6.82 **	4	P11/M60-F-100-P1	0.28	0.72	65.58 ***	8	P11/M50-F-340-P1	0.34	0.66	32.26 ***
1	P14/M50-F-237-P1	0.42	0.58	9.28 ***	4	P11/M60-F-239-P1	0.42	0.58	9.67 ***	8	P11/M51-F-170-P1	0.35	0.65	29.73 ***
1	P14/M51-F-088-P2	0.64	0.36	22.38 ***	4	P11/M60-F-438-P1	0.38	0.62	20.51 ***	8	P11/M60-F-097-P2	0.35	0.65	30.07 ***
1	P14/M51-F-281-P1	0.41	0.59	11.25 ***	4	P11/M62-F-083-P2	0.43	0.57	6.05 *	8	P13/M47-F-099-P1	0.33	0.67	37.78 ***
1	P14/M51-F-407-P1	0.44	0.56	3.91 *	4	P11/M62-F-215-P2	0.45	0.55	4.15 *	8	P13/M47-F-136-P2	0.38	0.63	22.00 ***
1	P14/M60-F-111-P1	0.38	0.62	16.23 ***	4	P13/M47-F-158-P2	0.36	0.64	28.90 ***	8	P13/M47-F-179-P1	0.38	0.62	21.13 ***
1	P14/M60-F-145-P2	0.58	0.42	8.45 ***	4	P13/M47-F-243-P1	0.29	0.71	60.28 ***	8	P13/M61-F-156-P1	0.40	0.60	14.98 ***
1	P15/M62-F-133-P1	0.40	0.60	11.69 ***	4	P13/M47-F-424-P1	0.36	0.64	26.78 ***	8	P13/M61-F-532-P2	0.43	0.57	6.41 *
2	LEHMG2A	0.32	0.68	46.02 ***	4	P13/M49-F-273-P1	0.34	0.66	33.59 ***	8	P14/M50-F-058-P2	0.35	0.65	28.62 ***
2	SSR356	0.37	0.63	22.75 ***	4	P13/M61-F-321-P2	0.37	0.63	21.25 ***	8	P14/M50-F-213-P1	0.35	0.65	31.44 ***
2	SSR40	0.35	0.65	31.56 ***	4	P14/M50-F-073-P2	0.33	0.67	37.33 ***	8	P14/M50-F-305-P2	0.43	0.57	7.16 **
2	ID285-3	0.37	0.63	20.51 ***	4	P14/M50-F-107-P1	0.43	0.57	6.78 **	8	P14/M60-F-200-P1	0.35	0.65	30.96 ***
2	P11/M48-F-082-P1	0.39	0.61	17.48 ***	4	P14/M50-F-126-P2	0.38	0.62	17.72 ***	8	P15/M62-F-113-P1	0.34	0.66	33.84 ***
2	P11/M48-F-137-P1	0.37	0.63	21.75 ***	4	P14/M51-F-093-P1	0.41	0.59	10.19 ***	8	P15/M62-F-158-P1	0.39	0.61	16.59 ***
2	P11/M48-F-244-P1	0.43	0.57	7.18 **	4	P14/M51-F-233-P1	0.32	0.68	43.27 ***	8	P15/M62-F-302-P2	0.41	0.59	10.07 ***
2	P11/M48-F-462-P2	0.41	0.59	11.05 ***	4	P14/M60-F-261-P1	0.30	0.70	55.38 ***	9	LED6	0.44	0.56	5.47 *
2	P11/M50-F-140-P2	0.29	0.71	54.79 ***	4	P14/M60-F-434-P2	0.43	0.57	5.58 *	9	LEWIPIG	0.43	0.57	6.47 *
2	P11/M50-F-216-P2	0.43	0.57	6.13 *	4	P15/M62-F-324-P2	0.42	0.58	7.58 **	9	P11/M51-F-214-P1	0.42	0.58	7.28 **
2	P11/M50-F-387-P2	0.38	0.63	18.50 ***	4	P15/M62-F-582-P1	0.33	0.67	39.81 ***	9	P11/M51-F-491-P1	0.41	0.59	9.62 ***
2	P11/M51-F-085-P2	0.33	0.67	32.89 ***	6	P11/M62-F-443-P2	0.56	0.44	4.78 *	9	P11/M62-F-165-P2	0.45	0.55	2.94 *
2	P11/M51-F-103-P2	0.31	0.69	50.95 ***	7	SSR52	0.44	0.56	4.96 *	9	P11/M62-F-167-P1	0.43	0.57	7.10 **
2	P11/M51-F-115-P2	0.39	0.61	15.92 ***	7	SSR45	0.39	0.61	18.08 ***	9	P13/M49-F-420-P1	0.39	0.61	15.83 ***
2	P11/M51-F-274-P2	0.34	0.66	31.80 ***	7	P11/M48-F-111-P2	0.41	0.59	11.84 ***	9	P14/M50-F-174-P2	0.45	0.55	3.03 ***
2	P11/M51-F-296-P1	0.30	0.70	54.72 ***	7	P11/M48-F-171-P1	0.42	0.58	9.12 ***	9	P14/M51-F-209-P1	0.41	0.59	10.53 ***
2	P11/M62-F-217-P1	0.42	0.58	8.96 ***	7	P11/M48-F-432-P1	0.37	0.63	18.63 ***	9	P15/M62-F-549-P1	0.42	0.58	9.22 ***
2	P11/M62-F-415-P2	0.37	0.63	22.28 ***	7	P11/M50-F-090-P2	0.44	0.56	4.91 *	10	P13/M47-F-440-P2	0.59	0.41	10.38 ***
2	P13/M47-F-176-P1	0.42	0.58	9.01 ***	7	P11/M50-F-125-P2	0.43	0.57	5.88 *	10	P15/M62-F-510-P1	0.43	0.57	5.92 *
2	P13/M47-F-187-P2	0.32	0.68	44.44 ***	7	P11/M51-F-254-P2	0.42	0.58	8.38 ***	11	LE20592	0.44	0.56	4.49 *
2	P13/M47-F-274-P2	0.39	0.61	18.18 ***	7	P11/M51-F-304-P2	0.43	0.57	7.27 **	11	ID329	0.39	0.61	10.67 ***
2	P13/M49-F-435-P1	0.36	0.64	26.45 ***	7	P11/M60-F-218-P2	0.42	0.58	8.53 ***	12	ID120	0.67	0.33	22.45 ***
2	P14/M50-F-135-P2	0.41	0.59	9.95 ***	7	P11/M62-F-352-P2	0.43	0.57	7.10 **	11	P11/M50-F-099-P1	0.44	0.56	4.35 *
2	P14/M50-F-192-P2	0.43	0.57	7.35 **	7	P11/M62-F-358-P1	0.43	0.57	7.68 **	11	P11/M51-F-324-P1	0.41	0.59	9.74 ***
2	P14/M50-F-243-P2	0.30	0.70	54.08 ***	7	P11/M62-F-400-P1	0.41	0.59	10.26 ***	11	P11/M60-F-247-P1	0.44	0.56	5.66 *
2	P14/M51-F-169-P2	0.34	0.66	33.52 ***	7	P13/M47-F-125-P1	0.44	0.56	5.10 *	11	P13/M47-F-364-P1	0.40	0.60	12.33 ***
2	P14/M60-F-155-P2	0.38	0.62	18.25 ***	7	P13/M49-F-128-P1	0.42	0.58	7.82 ***	11	P14/M51-F-360-P1	0.42	0.58	8.50 ***
2	P14/M60-F-388-P1	0.42	0.58	8.24 ***	7	P13/M49-F-153-P1	0.42	0.58	7.82 ***	12	P11/M62-F-229-P2	0.57	0.43	6.05 *
2	P15/M62-F-173-P1	0.31	0.69	50.30 ***	7	P14/M50-F-070-P1	0.41	0.59	10.65 ***	12	P13/M47-F-406-P1	0.55	0.45	3.88 *
2	P15/M62-F-185-P1	0.37	0.63	23.96 ***	7	P14/M50-F-096-P2	0.43	0.57	6.90 **	12	P14/M51-F-182-P2	0.58	0.42	9.67 ***
2	P15/M62-F-373-P1	0.42	0.58	9.39 ***	7	P14/M50-F-169-P2	0.41	0.59	10.65 ***	12	P14/M60-F-545-P2	0.59	0.41	10.85 ***
4	ASR3	0.29	0.71	54.45 ***	7	P14/M50-F-354-P1	0.43	0.57	6.78 **					

Chr = chromosome.

Asterisks indicated significance level of *P* value χ^2 test. **P* < 0.05. ***P* < 0.01. ****P* < 0.005. *****P* < 0.001. ******P* < 0.0005. *******P* < 0.0001.

Table 3. SSR, SNP, and AFLP markers showing deviation from expected genotype frequency in *Solanum lycopersicum* cv. Solentos × *S. arcanum* LA2157 F_2 population.

Chr	Marker	Genotype frequency			χ^2 value	Chr	Marker	Genotype frequency			χ^2 value
		p^2	q^2	2pq				p^2	q^2	2pq	
1	SSR135	0.19	0.64	0.18	13.11 *****	8	P11/M51-F-170-P1	0.09	0.53	0.38	3.89 *
1	P11/M51-F-097-P2	0.14	0.57	0.28	5.13 *	8	P11/M60-F-097-P2	0.08	0.54	0.38	6.25 *
1	P11/M62-F-106-P2	0.16	0.60	0.24	7.73 **	8	P13/M47-F-099-P1	0.06	0.54	0.40	8.30 ***
1	P11/M62-F-322-P2	0.15	0.59	0.26	6.11 *	8	P13/M61-F-532-P2	0.09	0.67	0.24	20.31 ******
1	P14/M50-F-503-P2	0.19	0.60	0.21	6.58 *	8	P14/M50-F-058-P2	0.07	0.55	0.37	7.34 **
1	P14/M51-F-281-P1	0.11	0.60	0.29	9.50 ***	8	P14/M50-F-213-P1	0.08	0.55	0.38	7.09 **
1	P14/M51-F-407-P1	0.15	0.59	0.26	5.42 *	8	P14/M60-F-200-P1	0.08	0.53	0.39	4.69 *
1	P14/M51-F-580-P1	0.21	0.65	0.14	12.97 *****	8	P15/M62-F-113-P1	0.07	0.55	0.39	8.10 ***
1	P15/M62-F-205-P2	0.17	0.59	0.24	5.80 *	9	LED6	0.13	0.62	0.25	11.15 ****
2	P11/M48-F-244-P1	0.14	0.58	0.28	5.98 *	9	P11/M48-F-065-P1	0.16	0.57	0.26	4.25 *
2	P11/M50-F-387-P2	0.09	0.56	0.34	5.71 *	9	P11/M50-F-292-P2	0.21	0.66	0.13	14.24 *****
2	P11/M51-F-296-P1	0.06	0.49	0.45	4.42 *	9	P11/M50-F-408-P2	0.18	0.68	0.14	18.37 ******
2	P13/M49-F-352-P2	0.14	0.63	0.23	10.67 ***	9	P11/M51-F-491-P1	0.09	0.66	0.26	20.29 ******
2	P14/M60-F-155-P2	0.10	0.55	0.35	4.77 *	9	P11/M60-F-159-P2	0.15	0.61	0.24	8.71 ***
4	P11/M50-F-187-P1	0.14	0.58	0.28	5.58 *	9	P11/M62-F-110-P2	0.17	0.58	0.24	4.88 *
4	P11/M62-F-215-P2	0.16	0.58	0.26	5.32 *	9	P11/M62-F-115-P1	0.15	0.60	0.25	7.55 **
4	P13/M49-F-273-P1	0.07	0.54	0.39	6.87 **	9	P11/M62-F-165-P2	0.15	0.61	0.24	9.11 ***
4	P13/M49-F-509-P2	0.20	0.65	0.15	12.23 *****	9	P13/M47-F-211-P2	0.15	0.62	0.23	11.27 ****
5	PRF1	0.38	0.26	0.36	9.24 ***	9	P13/M49-F-420-P1	0.08	0.64	0.29	19.14 ******
6	P11/M51-F-407-P1	0.15	0.61	0.23	8.58 ***	9	P14/M50-F-060-P2	0.15	0.62	0.23	10.30 ***
6	P14/M60-F-160-P2	0.22	0.60	0.18	6.71 **	9	P14/M50-F-081-P1	0.16	0.58	0.26	4.83 *
6	P14/M60-F-362-P2	0.22	0.66	0.12	18.27 ******	9	P14/M50-F-174-P2	0.14	0.62	0.24	10.89 ****
7	P11/M50-F-090-P2	0.13	0.61	0.26	8.87 ***	9	P14/M51-F-209-P1	0.12	0.58	0.30	6.48 *
7	P11/M62-F-352-P2	0.13	0.61	0.27	10.22 ***	9	P15/M62-F-202-P2	0.15	0.61	0.24	9.76 ***
7	P11/M62-F-358-P1	0.12	0.61	0.27	11.41 ****	9	P15/M62-F-549-P1	0.14	0.56	0.30	4.40 *
7	P14/M50-F-354-P1	0.15	0.56	0.29	3.95 *	11	ID329	0.04	0.70	0.26	24.94 ******
7	P14/M51-F-455-P1	0.07	0.60	0.34	13.00 *****	11	P11/M50-F-099-P1	0.15	0.58	0.27	5.63 *
8	P11/M50-F-197-P2	0.07	0.56	0.38	8.43 ***	11	P11/M60-F-247-P1	0.15	0.57	0.28	4.03 *
8	P11/M50-F-211-P1	0.08	0.54	0.39	5.41 *	11	P11/M62-F-118-P2	0.17	0.59	0.24	6.35 *
8	P11/M50-F-307-P1	0.08	0.53	0.39	5.19 *	11	P13/M47-F-364-P1	0.11	0.58	0.32	5.92 *
8	P11/M50-F-340-P1	0.07	0.54	0.39	5.92 *						

Chr = chromosomes. Markers in italics also showed deviation in allele frequency homogeneity.
Asterisks indicated significance level of *P* value χ^2 test. *P < 0.05. **P < 0.01. ***P < 0.005. ****P < 0.001. *****P < 0.0005. ******P < 0.0001.

Analysis of Distorted Marker

Of the 192 distorted markers, the allele frequencies of 152 marker loci deviated significantly from the expected homogeneity allele frequency (χ^2 = 2.94 to 65.58; P<0.05 to P<0.001), indicating that their segregation may be caused by gametophytic selection (Table 2). On the other hand, 61 markers deviated from the expected random mating distribution in F_2 population (χ^2 = 3.89 to 24.94; P<0.05 to P<0.001), which indicate that their segregation may be influenced by zygotic selection. These markers were observed on all chromosomes, except on chromosome 3 (Table 3). Among those distorted markers, the segregation of 37 markers may be influenced by gametophytic and zygotic selection simultaneously since they showed deviation from the expected homogeneity allele frequency and random mating distribution of F_2 genotypes (Table 3).

The map positions of distorted markers in our map were often nearby to each other, as observed on chromosomes 1, 2, 4, 7, 8, and 9 (Figure 1). Cluster of distorted markers might become candidate genes for viability selection as has been reported in *Arabidopsis*. By using visible recessive markers, Grini *et al*. (1999) were able to identify gametophytic mutations in *Arabidopsis* that could be recognized by the segregation distortion of the nearby markers.

The calculation of allele and genotype proportions of those distorted markers provides initial inference about the genetic factors contributing segregation distortion. Identification of loci and dissection of mechanisms underlying SD require extensive multiple mapping studies (Reflinur, 2014; Xu *et al*., 2013). Severe segregation of *segregation distortion* 1 (*sed1*) locus that was fine-mapped to a region of 450 kb in maize has been demonstrated to be contributed by both gametophytic and zygotic selection (Xu *et al*., 2013). Five SD loci distributed in five chromosomes of rice were identified by using F_2 and BC_1F_1 populations of an interspecific cross. These loci were influenced by both gametophytic and zygotic selection (Reflinur *et al*., 2014).

CONCLUSIONS

Out of 371 mapped markers, segregation of 192 markers deviated from the expected F_2 ratio. Among the distorted markers, 152 showed deviation from the

expected homogeneity allele frequency ($p = q$), whereas 61 markers deviated from the expected F_2 genotype distribution (p^2:2pq:q^2), indicating that their segregation might be affected by gametophytic and zygotic selection, respectively. Thirty seven of the distorted markers showed deviation from both the expected homogeneity allele frequency and genotype frequency in an F_2 population. Distorted markers can be retained in linkage analysis since early blight fungus resistance loci were also identified in chromosomal regions containing distorted markers.

ACKNOWLEDGEMENTS

This study was part of the first author's Ph.D. research supported by the Royal Netherlands Academy of Arts and Sciences (KNAW) in the framework of the Scientific Programme Indonesia-The Netherlands. We are indebted to the late Prof. Piet Stam for his excellent supervision and encouraging advice during the research.

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