

Fig. S1. Distribution of the yield associated traits in the BILs and BILHs. (A and B) Frequency distribution for plant weight (PW) in the BILs and BILHs. (C and D) Frequency distribution for fruit weight (FW) in the BILs and BILHs. (E and F) Frequency distribution for Brix (BX%) in the BILs and BILHs.

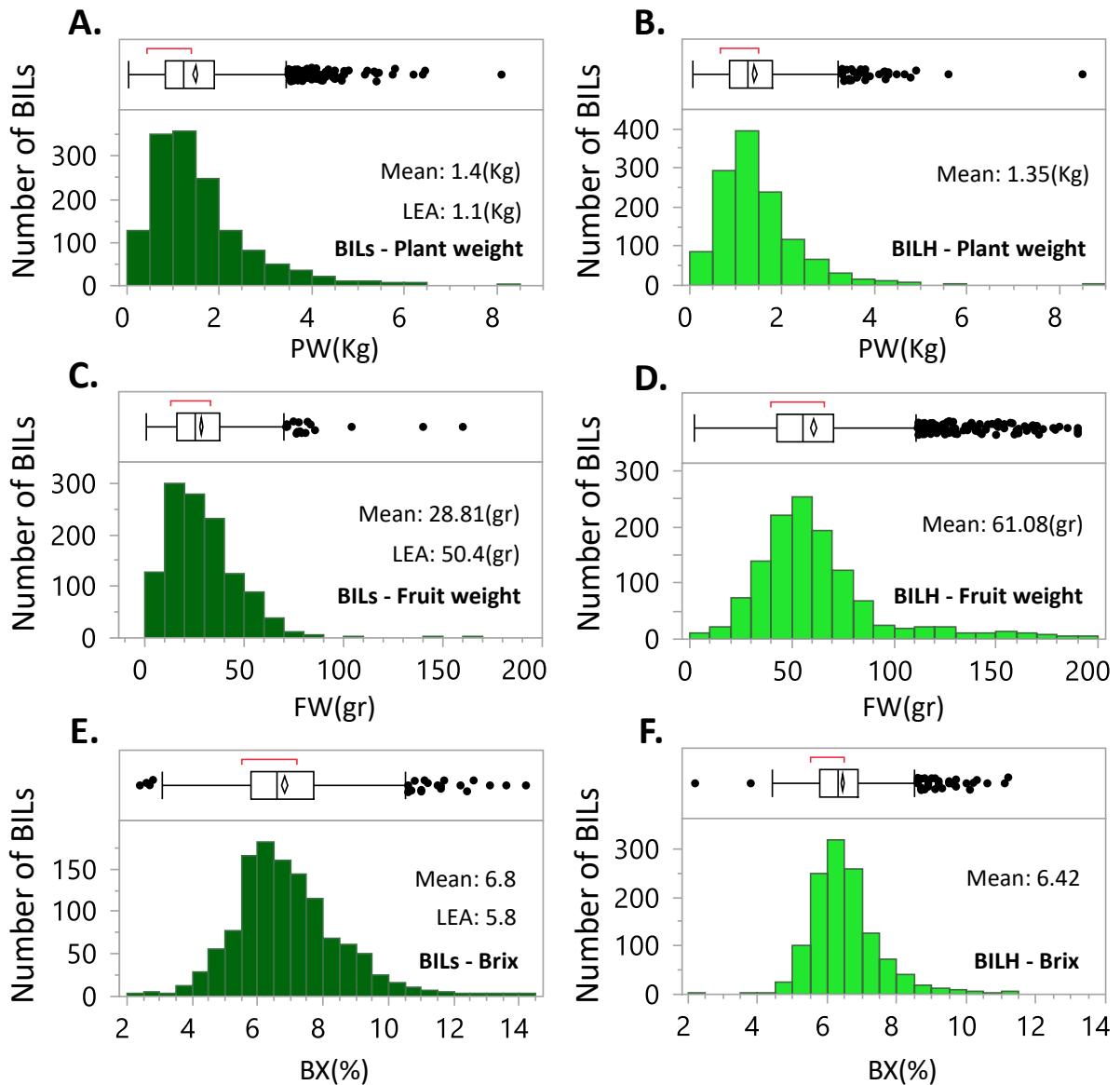


Fig. S2. Single marker analysis of yield associated traits in the BIL and BILHs.

(A and B) QTL analysis for plant weight (PW) in the BILs and the BILHs. (C and D) QTL analysis of fruit weight (FW) in the BILs and BILHs. (E and F) QTL analysis for total soluble solids (BX%) in the BIL and BILH. LOD scores for each of the marker effects was calculated by Haley-Knott regression and the LOD threshold was determine by 1000 permutation tests. All the effect values are in percent of LEA.

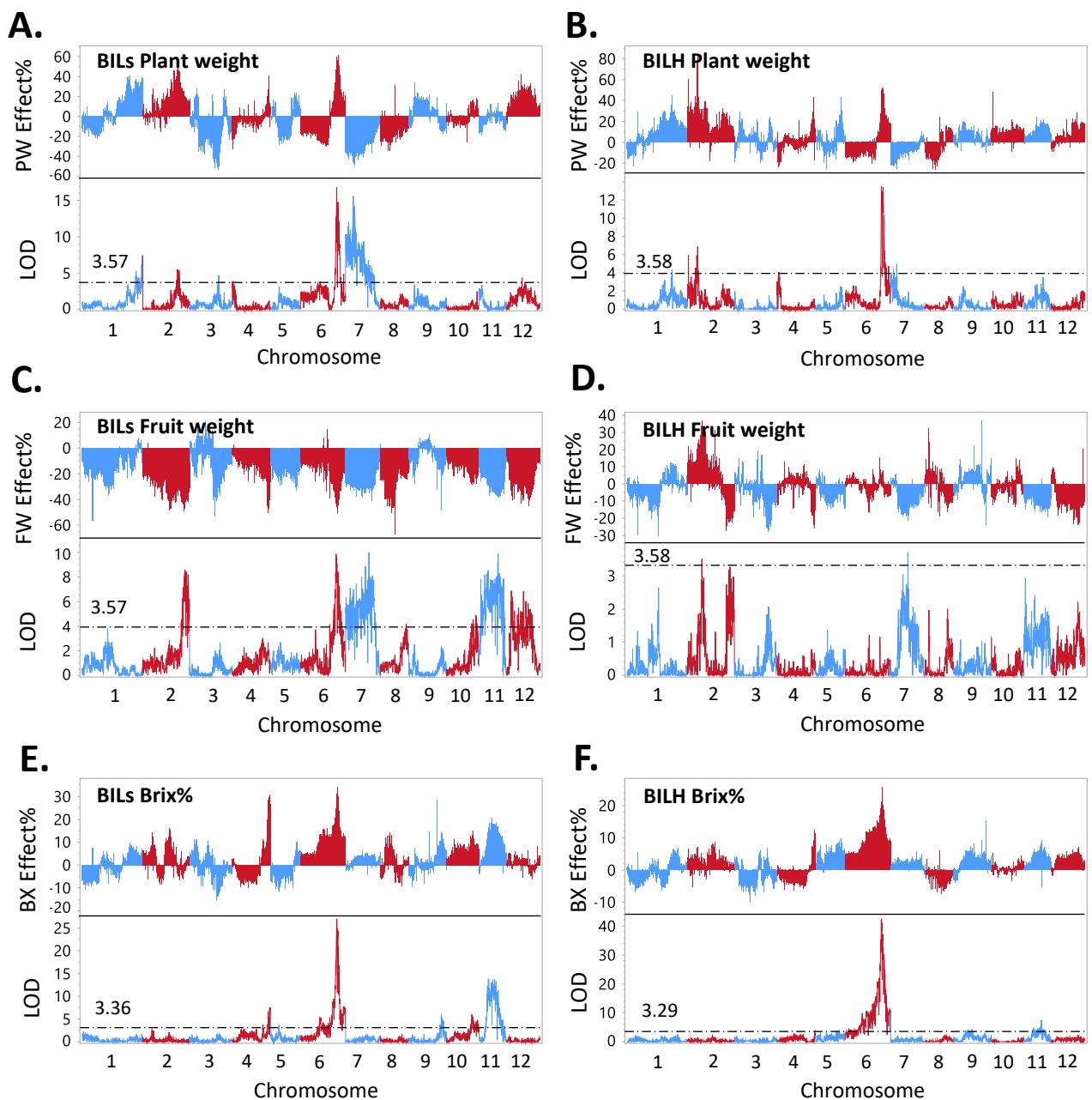


Fig. S3. Validation analysis of chromosome 4 for the effect on total yield. (A) Initial detection of the yield QTL that increases TY by 20% in BILH (Akko 2018). (B) The BILH heterozygous for the chromosome 4 QTL was crossed to eight processing tomato inbreds and the progenies were genotyped for the chromosome 4 marker. The two genotypic groups were compared with a t-test and * indicates a significant difference ($P<0.05$). C) A test of the pooled total yield (TY) data from all the eight genetic backgrounds shows an 18% yield increase due to the chromosome 4 QTL.

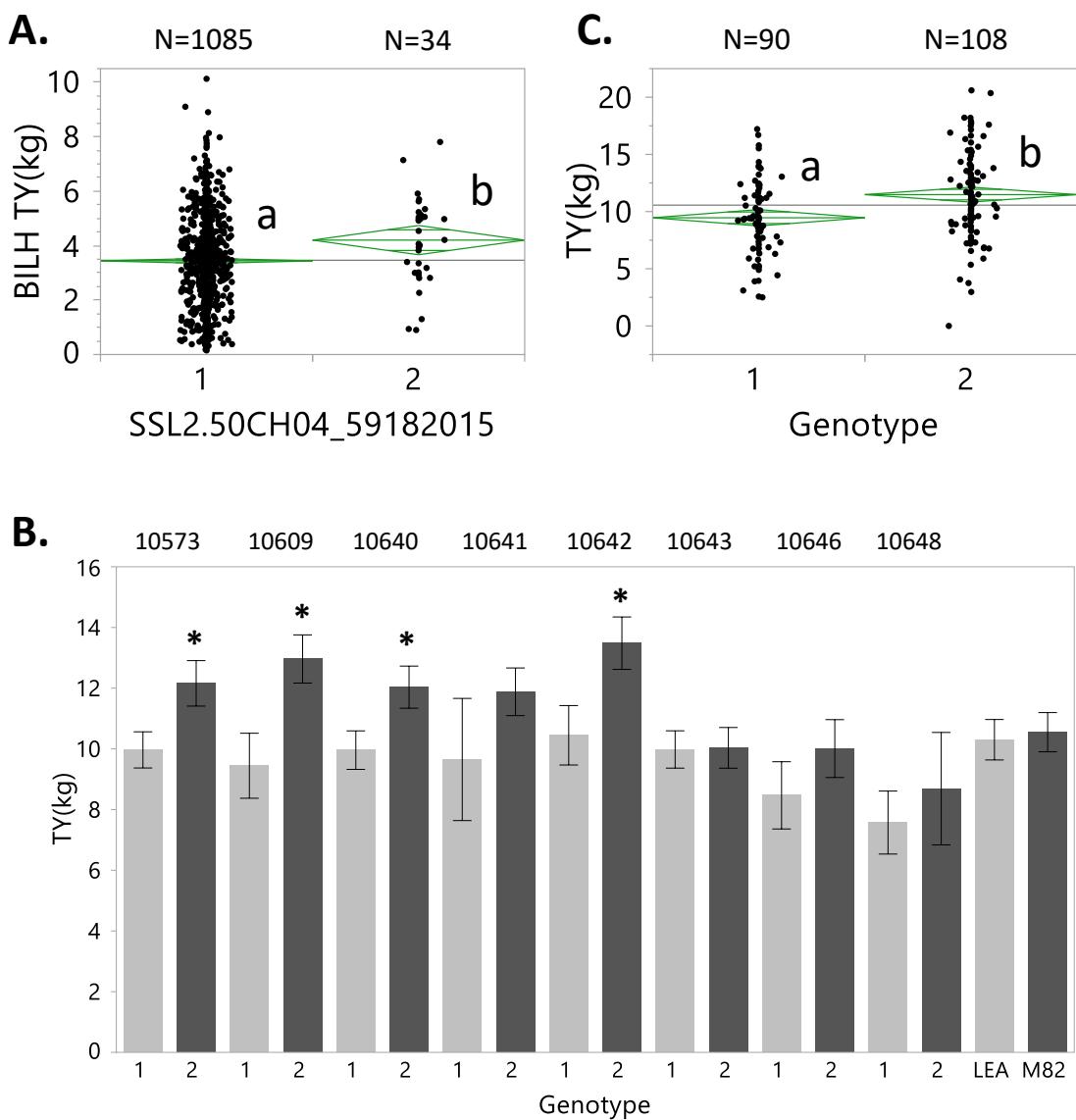


Fig. S4. Validation of the epistatic QTL in wide spacing. Yield components (Akko 2022) measured using replications of 1 plant per m². We present the results of the four genotypic groups with respect to the markers on chromosomes 1 (SSL2.50CH01_95261222) and 7 (SSL2.50CH07_65737800): 1) Homozygous for the cultivated tomato alleles in chromosomes 1 and 7 (1_1). 2) Heterozygous for the chromosome 1 introgression (2_1). 3) Heterozygous for the chromosome 7 introgression (1_2). 4) Heterozygous for both introgressions (2_2). Genotypic groups' means showing the same letters are not significantly different at the 5% level based on the Tukey-Kramer test. The traits measured were plant weight (A), total yield (B), fruit weight (C) and the estimated fruit number (D).

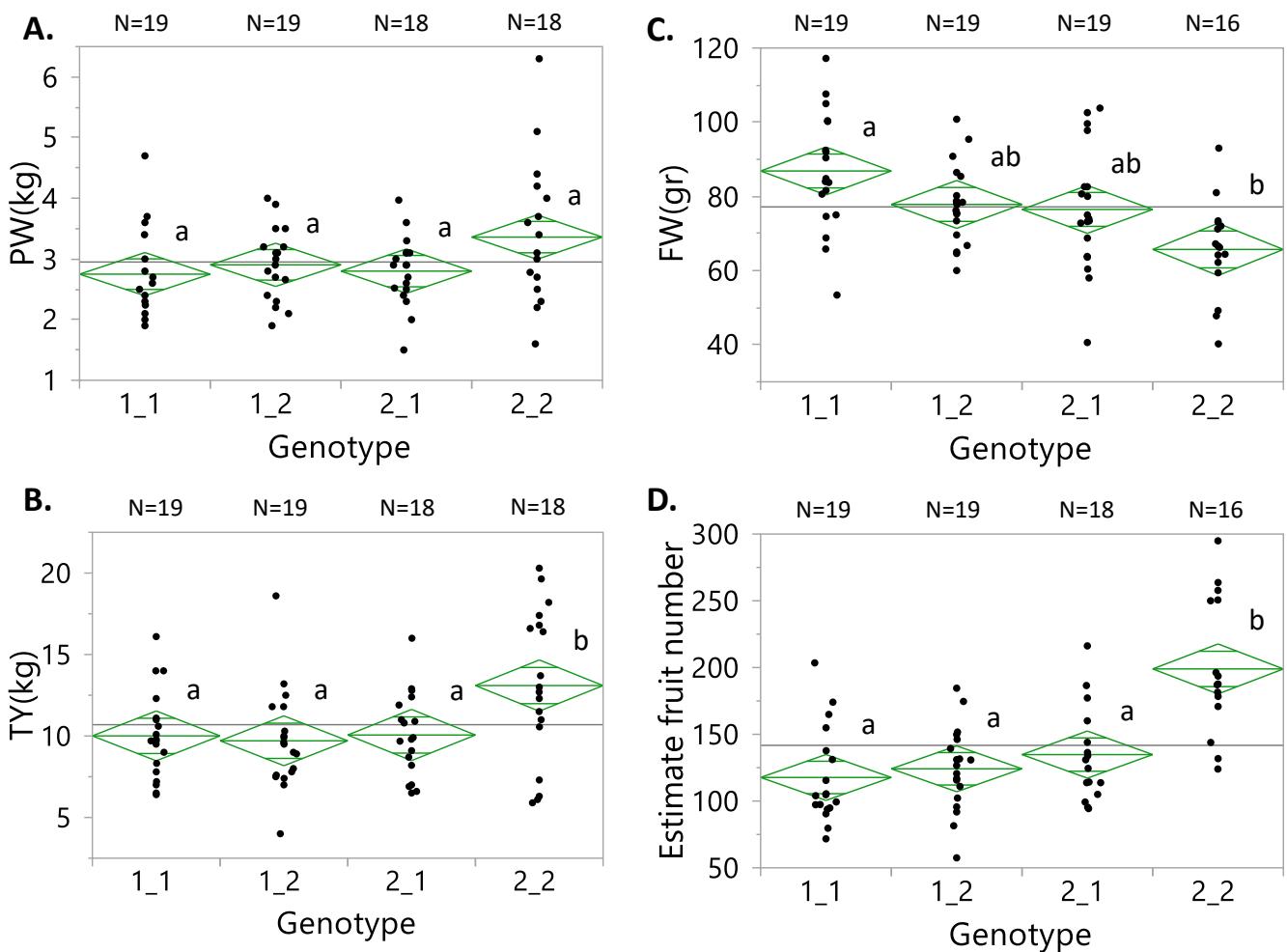


Fig. S5. Number of inflorescences. (A) Number of inflorescences along the main stem of plants heterozygous for the heterotic QTL on chromosomes 1 and 7 in the LEA background (2_2) compared to the genotypes 1_1; 1_2 and 2_1. (B) Number of inflorescences along the main stem of the plants heterozygous for the heterotic QTL on chromosomes 1 and 7 in the 10640 and 10643 background compared to the genotypes 1_1; 1_2 and 2_1.

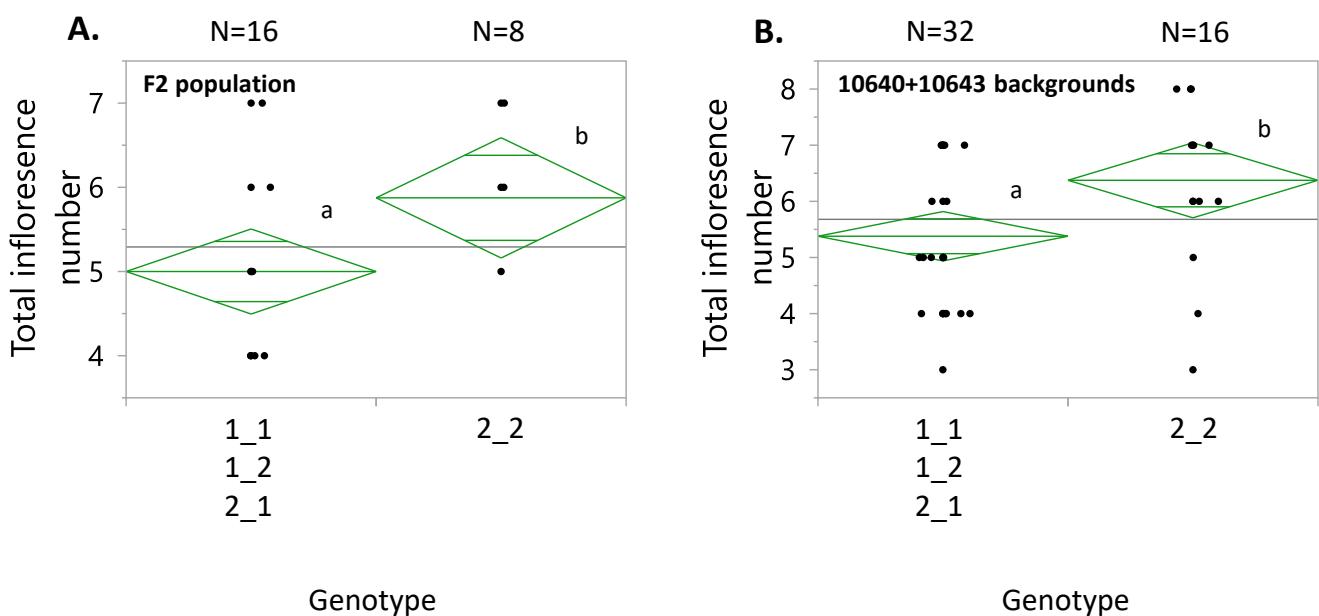


Fig. S6. Fine mapping scheme for the QTL involved in the epistasis. For fine mapping of the epistatic QTL on chromosome 1 and 7, we will use the two introgressions that create the epistasis and the mapped recombinant BILs for each of the chromosomes. The BILH for the chromosome 1 QTL (green chromosome) are crossed to homozygous BILs that are recombinant in chromosome 7. The progeny of such crosses would produce nearly isogenic hybrids of two genotypes: with or without the QTL on chromosome 1. The yield of the isogenic hybrids would be compared and if the hybrids with the chromosome 1 QTL would have higher yield, then the assumption is that the recombined segment of the BIL on chromosome 7 carries the second QTL needed to generate heterosis. A comparison of the values of the two genotypic groups using multiple recombinants BILs would indicate the location of the chromosome 7 QTL. To map the QTL on chromosome 1 which is involved in the interaction, we will cross the BILH of the chromosome 7 QTL (orange chromosome) to recombinants BILs of chromosome 1 and follow the scheme described above.

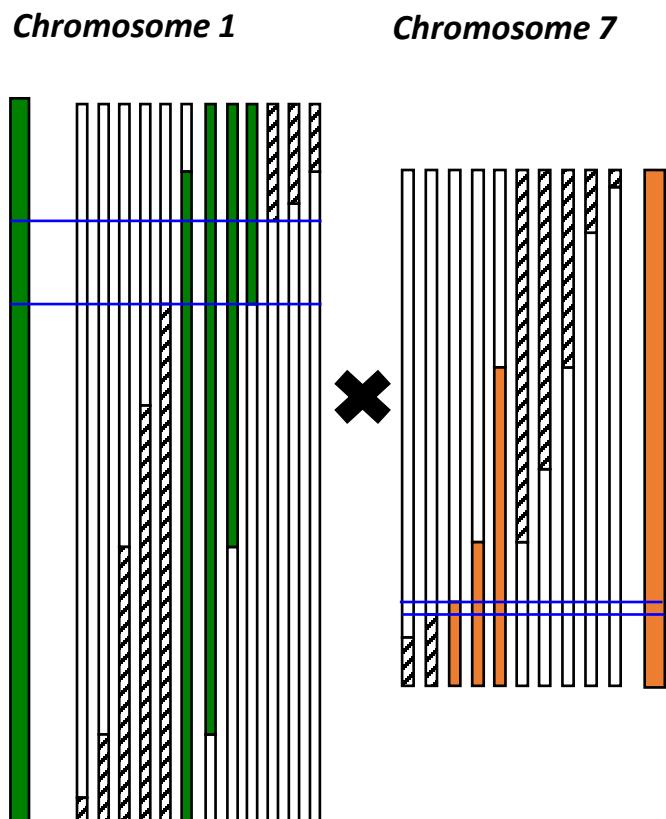


Table S1. Two-dimensional epistasis analysis for 80 significant yield components in the BILs (A) and BILHs (B). QTL analysis was conducted using the Scantwo command in R software using the Rqtl package¹⁶. Epistatic interactions were called when LOD.fv1>3. In the epistasis analysis we included only digenic-scans with at least ten plants that carried both QTL alleles derived from the wild species. The QTL genetic positions (cM) of the first and second epistatic QTL are indicated. For each genotypic group we include the trait phenotypic values in the BILs and the BILHs. (1_1) indicates lines that are homozygous in both QTL for the LEA allele, (2_1) heterozygous for the first QTL in the BILH or homozygous for the *S. pennellii* allele in the first QTL in the BILs, (1_2) heterozygous for the second QTL in the BILH or homozygous for the *S. pennellii* allele in the second QTL in the BILs, and (2_2) heterozygous for both QTL in the BILHs or homozygous for the *S. pennellii* alleles in the BILs. The effect of the QTL represents the phenotypic value of the (2_2) genotype relative to LEA and to the best parent (2_1/1_2).

Trait	Population	1st QTL	2nd QTL	First QTL position(CM)	Second QTL position(CM)	Phenotypic values					Phenotypic values relative to 1_1				Mode of epistasis	
						1_1	3_1	1_3	3_3	p.value(ANOVA)	3_1	1_3	sum of QTls effect	3_3	More/less than additive	
PW(kg)	BILs	1	1	89.578	127.042	1.39	2.08	1.96	1.98	0.0001	0.69	0.57	1.26	0.59	Less	
PW(kg)	BILs	1	4	127.042	0	1.45	1.99	1.09	1.74	0.0001	0.54	-0.36	0.18	0.29	More	
PW(kg)	BILs	1	7	121.029	15.981	1.47	2.09	0.74	1.14	0.0001	0.62	-0.73	-0.11	-0.33	More	
PW(kg)	BILs	3	6	16.291	80.55	1.37	1.44	2.44	1.51	0.001	0.07	1.07	1.14	0.14	Less	
PW(kg)	BILs	4	5	0.323	31.735	1.52	1.12	1.04	1.14	0.0001	-0.4	-0.48	-0.88	-0.38	Less	
PW(kg)	BILs	5	5	39.948	67.131	1.48	0.93	1.98	1.43	0.0001	-0.55	0.5	-0.05	-0.05	Less	
PW(kg)	BILs	5	6	83.406	80.55	1.37	1.6	2.34	1.77	0.0001	0.23	0.97	1.2	0.4	Less	
PW(kg)	BILs	6	6	57.236	79.59	1.41	1.15	2.52	1.48	0.0001	-0.26	1.11	0.85	0.07	Less	
PW(kg)	BILs	6	7	80.55	15.981	1.47	2.36	0.8	1.35	0.0001	0.89	-0.67	0.22	-0.12	Less	
PW(kg)	BILs	11	4	0	2.956	1.57	1.13	1.27	1.4	0.0001	-0.44	-0.3	-0.74	-0.17	Less	
PW(kg)	BILs	11	5	30.558	15.253	1.54	1.06	1.27	1.65	0.0001	-0.48	-0.27	-0.75	0.11	More	
PW(kg)	BILs	11	6	80.55	18.933	1.39	2.32	1.31	2.02	0.0001	0.93	-0.08	0.85	0.63	Less	
PW(kg)	BILs	11	9	27.193	2.655	1.47	1.93	1.26	1.15	0.0001	0.46	-0.21	0.25	-0.32	Less	
PW(kg)	BILs	11	12	13.622	60.118	1.47	1.26	1.96	1.35	0.0001	-0.21	0.49	0.28	-0.12	Less	
PW(kg)	BILs	12	4	0	54.689	1.47	1.09	1.87	1.86	0.0001	-0.38	0.4	0.02	0.39	More	
PW(kg)	BILs	12	7	15.981	54.689	1.49	0.8	1.97	1.16	0.0001	-0.69	0.48	-0.21	-0.33	More	
PW(kg)	BILs	12	9	26.736	60.118	1.4	1.78	1.79	1.78	0.0001	0.38	0.39	0.77	0.38	Less	
PW(kg)	BILs	12	12	49.808	54.689	1.41	2.1	2.25	1.67	0.0001	0.69	0.84	1.53	0.26	Less	
FW(g)	BILs	1	7	39.339	37.879	30.2	21.1	19.53	14.49	0.0001	-9.1	-10.67	-19.77	-15.71	Less	
FW(g)	BILs	1	10	39.339	52.374	30.33	20.4	22.57	20.63	0.0001	-9.93	-7.76	-17.69	-9.7	Less	
FW(g)	BILs	2	7	72.401	37.803	30.8	19.26	20.41	12.4	0.0001	-11.54	-10.39	-21.93	-18.4	Less	
FW(g)	BILs	2	8	68.515	35.608	30.53	16.5	23.21	18.3	0.0001	-14.03	-7.32	-21.35	-12.23	Less	
FW(g)	BILs	6	6	55.708	77.434	29.5	32.64	17.5	14.54	0.0001	3.14	-12	-8.86	-14.96	Less	
FW(g)	BILs	7	8	37.803	33.426	30.55	19.58	23.57	18.04	0.0001	-10.97	-6.98	-17.95	-12.51	Less	
FW(g)	BILs	10	2	72.401	52.374	30.83	17.18	22.7	20.58	0.0001	-13.65	-8.13	-21.78	-10.25	Less	
FW(g)	BILs	10	4	136.347	54.907	30.01	22.86	23.06	25.9	0.0001	-7.15	-6.95	-14.1	-4.11	Less	
FW(g)	BILs	10	7	37.803	52.374	30.85	20.07	23.4	16.7	0.0001	-10.78	-7.45	-18.23	-14.15	Less	
FW(g)	BILs	10	8	35.608	53.931	30.69	22.6	22.86	20.09	0.0001	-8.09	-7.83	-15.92	-10.6	Less	
FW(g)	BILs	10	11	66.853	87.021	31.6	23.79	17.8	24.1	0.0001	-7.81	-13.8	-21.61	-7.5	Less	
FW(g)	BILs	11	7	37.803	101.921	31.1	19.55	18.7	18.62	0.0001	-11.55	-12.4	-23.95	-12.48	Less	
FW(g)	BILs	11	8	37.106	87.021	30.9	23.9	19.59	21.6	0.0001	-7	-11.31	-18.31	-9.3	Less	
FW(g)	BILs	12	8	37.106	58.629	29.9	24.4	20.22	18.8	0.0001	-5.5	-9.68	-15.18	-11.1	Less	
Brix(%)	BILs	3	7	13.004	38.56	6.74	7.35	7.14	5.75	0.0001	0.61	0.4	1.01	-0.99	Less	
Brix(%)	BILs	4	4	116.214	132.214	6.83	5.86	8.18	6.9	0.0001	-0.97	1.35	0.38	0.07	Less	
Brix(%)	BILs	10	11	54.907	80.496	6.6	7.3	8.1	7.38	0.0001	0.7	1.5	2.2	0.78	Less	
Brix(%)	BILs	11	11	52.855	77.412	6.68	7.04	6.58	8.11	0.0001	0.36	-0.1	0.26	1.43	More	
TY(kg)	BILs	3	3	113.83	117.058	1.48	2.77	2.4	0.8	0.0001	1.29	0.92	2.21	-0.68	Less	
TY(kg)	BILs	4	7	126.681	30.564	1.67	1.05	0.68	0.77	0.0001	-0.62	-0.99	-1.61	-0.9	Less	
TY(kg)	BILs	6	6	38.004	80.55	1.66	0.92	0.56	0.43	0.0001	-0.74	-1.1	-1.84	-1.23	Less	
TY(kg)	BILs	6	7	79.59	30.564	1.7	0.62	0.7	0.47	0.0001	-1.08	-1	-2.08	-1.23	Less	
TY(kg)	BILs	6	8	79.59	32.601	1.68	0.62	1	0.43	0.0001	-1.06	-0.68	-1.74	-1.25	Less	
TY(kg)	BILs	7	8	30.564	35.532	1.69	0.7	1.04	0.44	0.0001	-0.99	-0.65	-1.64	-1.25	Less	
TY(kg)	BILs	8	9	35.532	153.315	1.64	0.97	0.91	0.94	0.0001	-0.67	-0.73	-1.4	-0.7	Less	
TY(kg)	BILs	10	8	32.601	18.171	1.64	1.01	0.87	0.4	0.0001	-0.63	-0.77	-1.4	-1.24	Less	
TY(kg)	BILs	10	10	13.041	54.907	1.61	1.01	1.25	0.36	0.0001	-0.6	-0.36	-0.96	-1.25	Less	
TY(kg)	BILs	10	11	18.171	58.877	1.67	0.93	1	0.23	0.0001	-0.74	-0.67	-1.41	-1.44	Less	
TY(kg)	BILs	11	4	3.906	82.649	1.67	1.09	0.72	0.67	0.0001	-0.58	-0.95	-1.53	-1	Less	
TY(kg)	BILs	11	5	36.508	58.877	1.69	0.63	0.97	0.48	0.0001	-1.06	-0.72	-1.78	-1.21	Less	
TY(kg)	BILs	11	6	79.59	12.383	1.73	0.61	1.15	0.41	0.0001	-1.12	-0.58	-1.7	-1.32	Less	
TY(kg)	BILs	11	7	30.564	58.877	1.73	0.69	0.96	0.63	0.0001	-1.04	-0.77	-1.81	-1.1	Less	
TY(kg)	BILs	11	8	35.532	83.112	1.66	0.97	0.89	0.69	0.0001	-0.69	-0.77	-1.46	-0.97	Less	
TY(kg)	BILs	11	11	12.383	69.245	1.71	1.16	0.87	0.95	0.0001	-0.55	-0.84	-1.39	-0.76	Less	
TY(kg)	BILs	12	7	30.564	57.066	1.67	0.73	0.81	0.39	0.0001	-0.94	-0.86	-1.8	-1.28	Less	
TY(kg)	BILs	12	8	35.532	52.74	1.65	1.03	0.74	0.3	0.0001	-0.62	-0.91	-1.53	-1.35	Less	
Trait	Population	1st QTL	2nd QTL	First QTL position(CM)	Second QTL position(CM)	Phenotypic values					Phenotypic values relative to 1_1				Mode of epistasis	
						1_1	2_1	1_2	2_2	p.value(ANOVA)	2_1	1_2	sum of QTls effect	2_2	More/less than additive	
PW(kg)	BILH	4	7	9.573	9.549	1.46	1.14	1.1	0.71	0.0001	-0.32	-0.36	-0.68	-0.75	Less	
PW(kg)	BILH	6	6	70.75	80.55	1.36	3.95	2.36	1.77	0.0001	2.59	1	3.59	0.41	Less	
PW(kg)	BILH	11	4	2.09	90.576	1.4	1.17	1.77	1.1	0.0001	-0.23	0.37	0.14	-0.3	Less	
FW(g)	BILH	4	9	35.706	181.868	61.29	54.38	57.99	91.24	0.0006	-6.91	-3.3	-10.21	29.95	More	
Brix(%)	BILH	1	1	76.651	83.791	6.4	6.35	7.67	6.4	0.0001	-0.05	1.27	1.22	0	Less	
Brix(%)	BILH	1	4	82.538	12.651	6.44	6.31	6.31	7.5	0.0004	-0.13	-0.13	-0.26	1.06	More	
Brix(%)	BILH	1	9	39.339	105.562	6.39	6.2	6.66	7.59	0.0001	-0.19	0.27	0.08	1.2	More	
Brix(%)	BILH	3	9	19.349	71.874	6.37	6.47	6.66	7.27	0.0002	0.1	0.29	0.39	0.9	More	
Brix(%)	BILH	5	5	19.782	67.131	6.4	6.48	6.56	7.72	0.0001	0.08	0.16	0.24	1.32	More	
Brix(%)	BILH	5	6	60.491	80.55	6.32	6.3	7.4	7.8	0.0001	-0.02	1.08	1.06	1.48	More	
Brix(%)	BILH	5	9	60.491	97.359	6.36	6.65	6.59	7.4	0.0001	0.29	0.23	0.52	1.04	More	
Brix(%)	BILH	6	6	37.459	80.992	6.28	6.6	7.33	8.49	0.0001	0.32	1.05	1.37	2.21	More	
Brix(%)	BILH	6	9	80.55	64.421	6.28	7.4	6.62	7.41	0.0001	1.12	0.34	1.46	1.13	Less	
Brix(%)	BILH	9	9	105.562	162.84	6.37	6.65	6.46	7.36	0.0001	0.28	0.09	0.37	0.99	More	
Brix(%)	BILH	10	3	19.349	25.457	6.41	6.48	6.42	7.27	0.0055	0.07	0.01	0.08	0.86	More	
Brix(%)	BILH	10	4	125.801	20.067	6.43	6.17	6.4	6.78	0.0514	-0.26	-0.03	-0.29	0.35	More	
Brix(%)	BILH	10	6	80.55	52.686	6.3	7.58	6.49	7.02	0.0001	1.28	0.19	1.47	0.72	Less	
Brix(%)	BILH	10	9	105.562	18.171	6.37	6.65	6.4	7.24	0.0002	0.28	0.03	0.31	0.87	More	
Brix(%)	BILH	11	11	78.121	82.03	6.36	6.95	7.5	6.9	0.0001	0.59	1.14	1.73	0.54	Less	
Brix(%)	BILH	12	12	79.404	81.247	6.4	8.96	5.95	6.57	0.0001	2.56	-0.45	2.11	0.17	Less	
TY(kg)	BILH	1	7	114.409	50.702	3.45	3.75	3.09	5.57	0.0001	0.3	-0.36	-0.06	2.12	More	
TY(kg)	BILH	4	4	3.682	121.087	3.44	3.29	4.39	2.79	0.0001	-0.15	0.95	0.8	-0.65</td		

Table S2. Trait correlations in the dense spacing (Fig 5) and wide spacing (Fig S4) experiments

Experiment	Corralation tests between traits	r	r²
Dense spacing	Av.fw(gr)/Total yield(kg)	-0.17	0.03
Dense spacing	Plant weight(kg)/Total yield(kg)	0.77*	0.6*
Dense spacing	Plant weight(kg)/Av.fw(gr)	-0.1	0.01
Wide spacing	Av.fw(gr)/Total yield(kg)	0.2	0.04
Wide spacing	Plant weight(kg)/Total yield(kg)	0.76*	0.59*
Wide spacing	Plant weight(kg)/Av.fw(gr)	0.2	0.04

*P<0.0001

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