

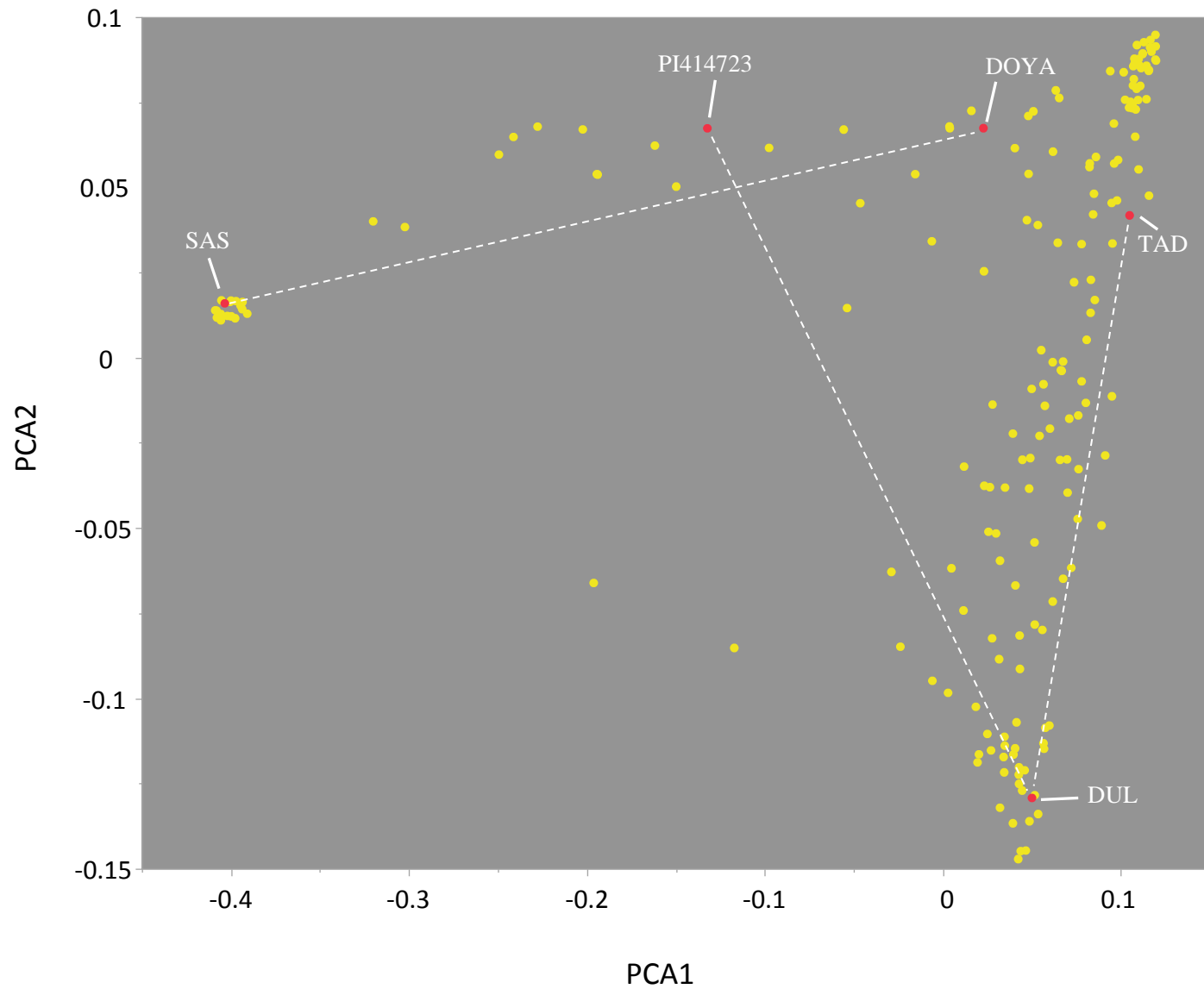
Supplementary information

Genome-Wide Linkage-Disequilibrium Mapping to the Candidate Gene Level in Melon (*Cucumis melo*)

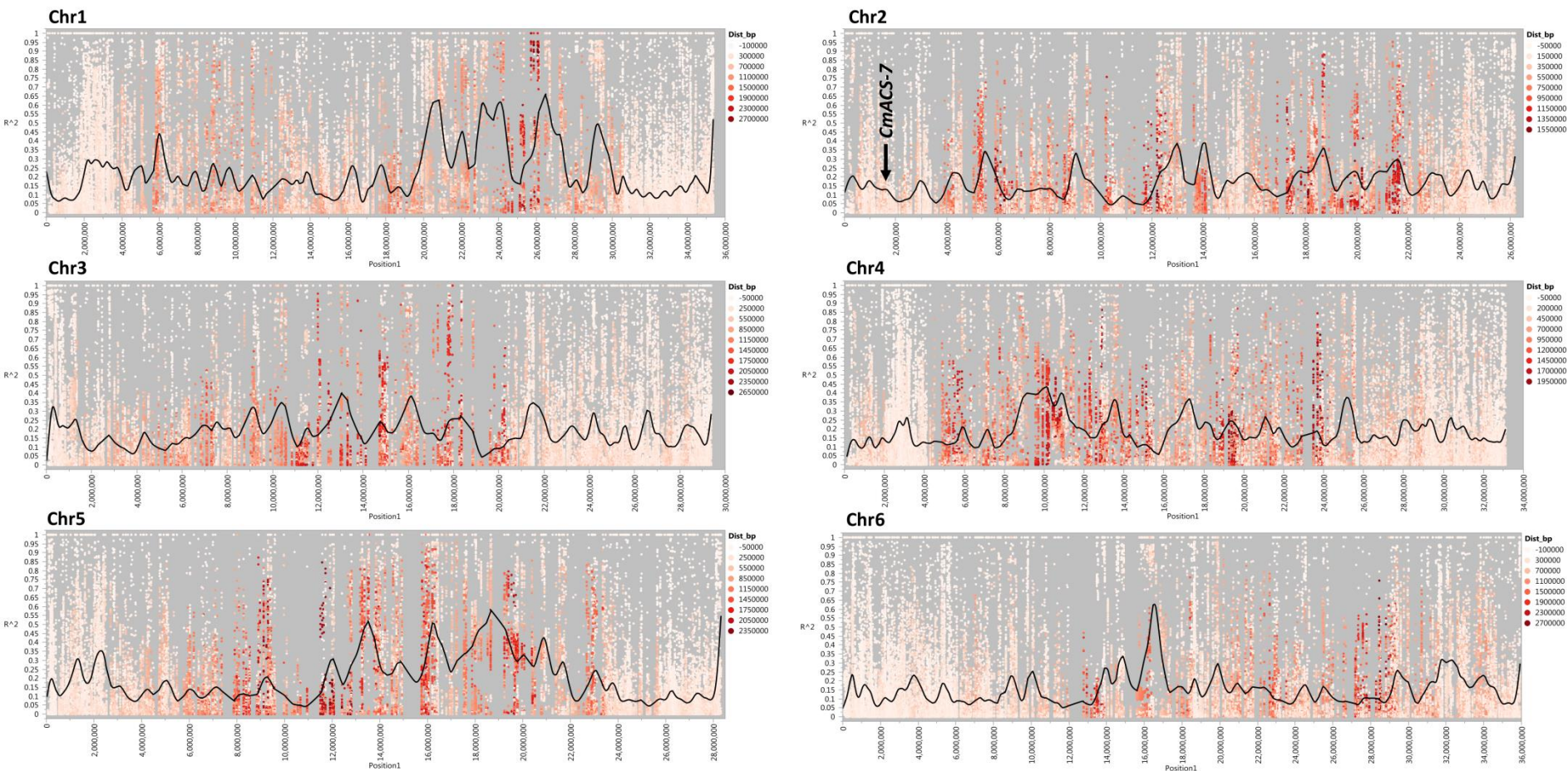
Amit Gur^{1*}, Galil Tzuri¹, Ayala Meir¹, Uzi Sa'ar¹, Vitaly Portnoy¹, Nurit Katzir¹, Arthur A. Schaffer², Li Li³, Joseph Burger¹ and Yaakov Tadmor¹

¹Plant Science Institute, Agricultural Research Organization, Newe Ya'ar Research Center, P.O. Box 1021, Ramat Yishay 3009500, Israel. ²Plant Science Institute, Agricultural Research Organization, The Volcani Center, P.O. Box 15159, Rishon LeZiyyon 7507101, Israel. ³Robert W Holley Center for Agriculture and Health, USDA-ARS, Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, New York 14853, USA.

*Correspondence should be addressed to A.G. (e-mail: amitgur@volcani.agri.gov.il)

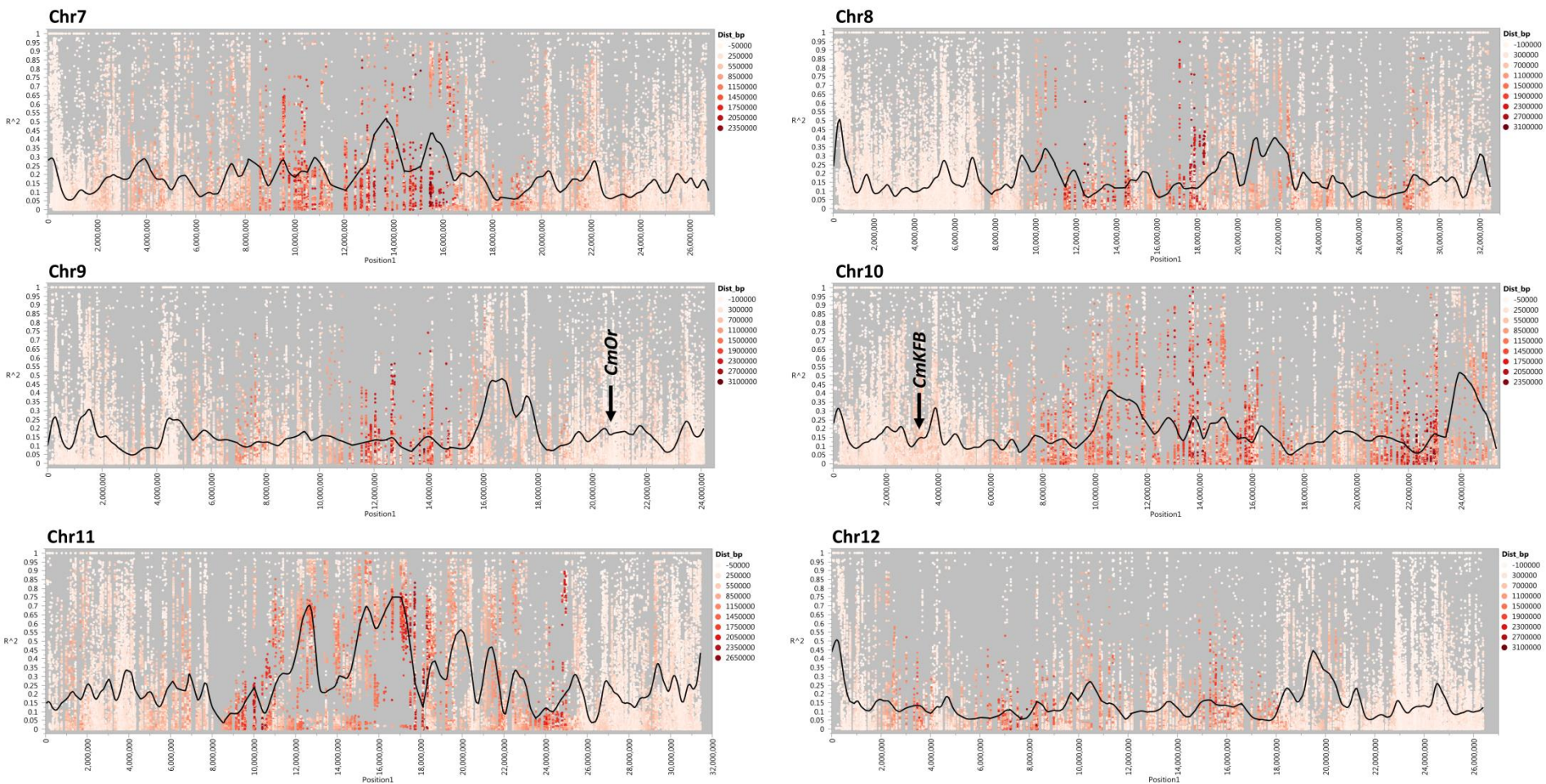


Supplementary Figure S1: Parental lines of the 3 bi-parental populations highlighted on the genetic PCA analysis. Crosses are shown in dashed white lines. SAS: *C. melo* ssp. *agrestis*, var *makuwa* . PI414723: *C. melo* ssp. *agrestis*, var *momordica*. DOYA: *C. melo* ssp. *melo*, var *flexuosus*. TADA: *C. melo* ssp. *melo*, var *inodorus*. DUL: *C. melo* ssp. *melo*, var *reticulatus*



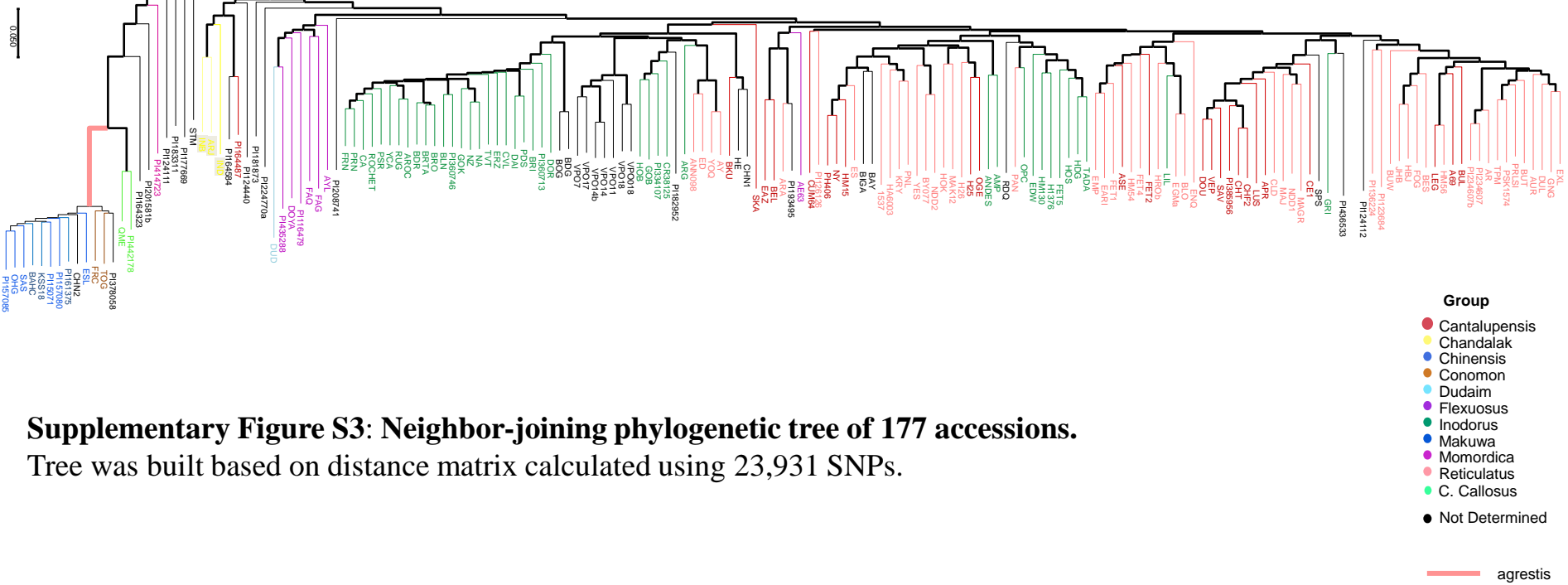
Supplementary Figure S2: LD (R^2) by physical position across 12 melon chromosomes.

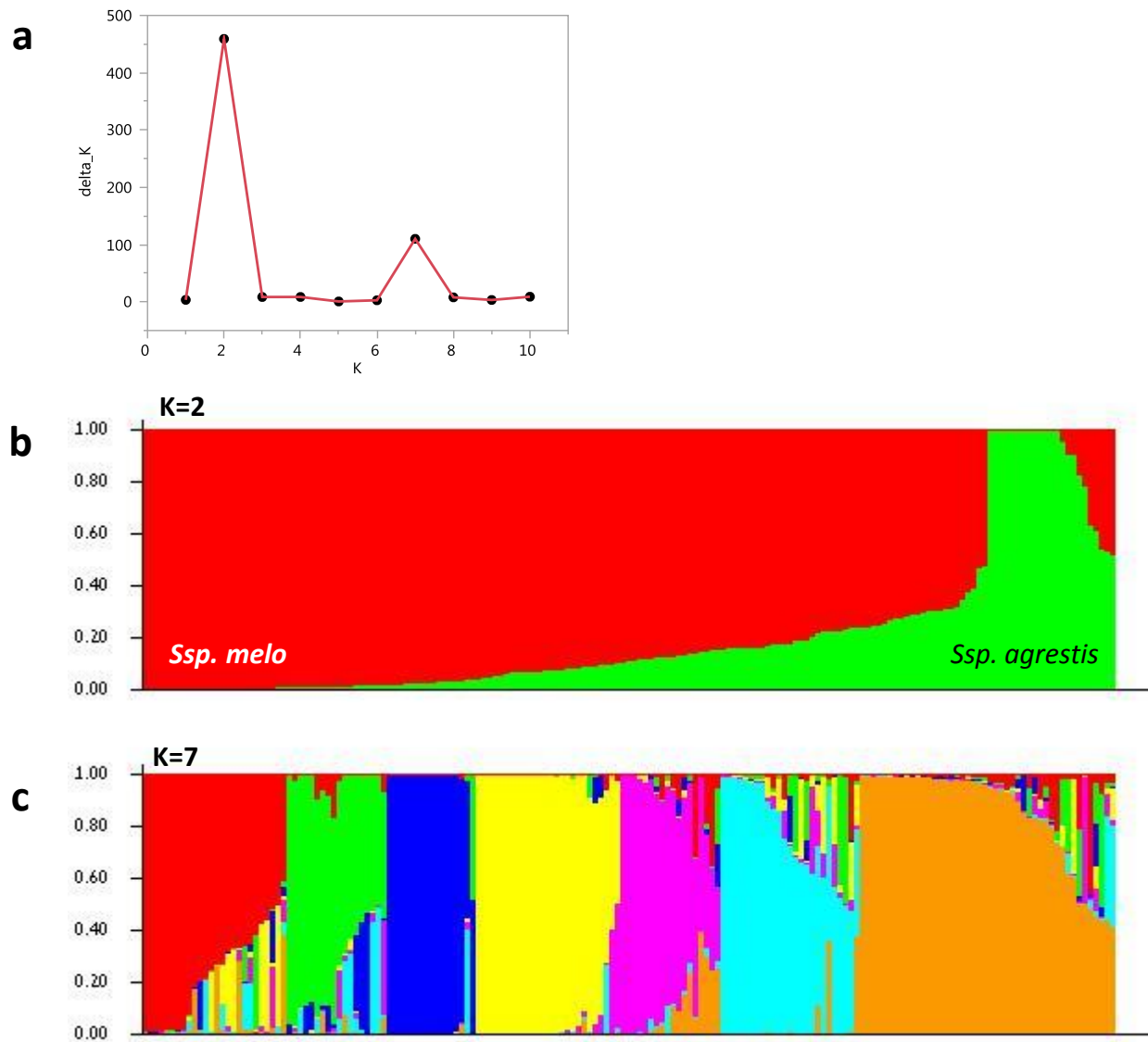
LD between intra-chromosomal SNP pairs is plotted (Y axis) by the physical genomic position of first SNP in each pair. Distance between SNPs in each pair is indicated using white-to-red color scale.



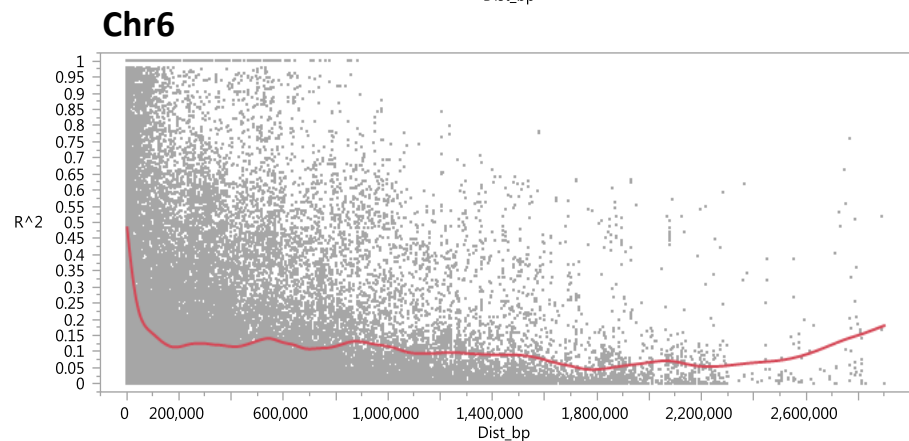
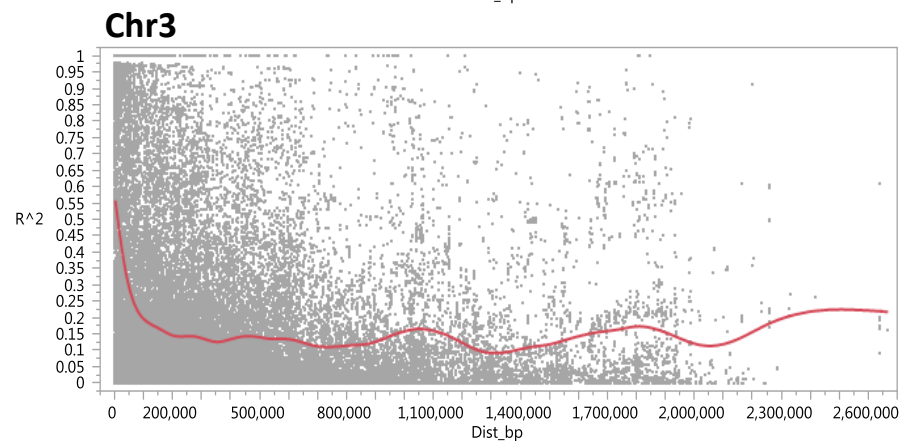
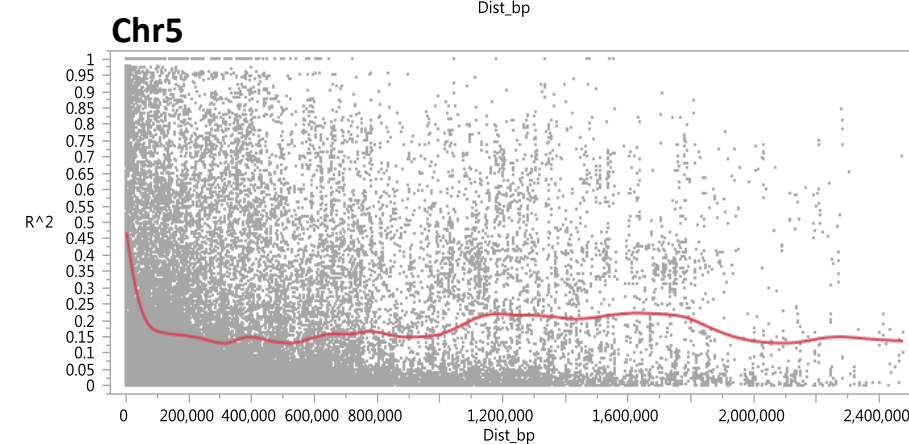
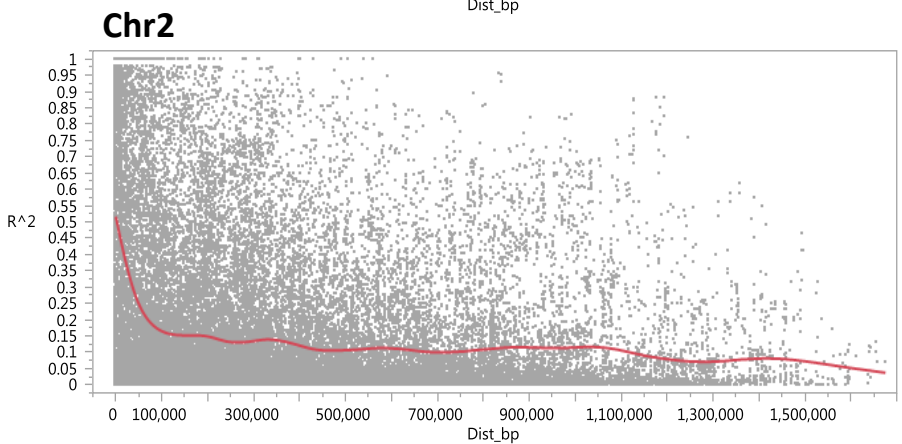
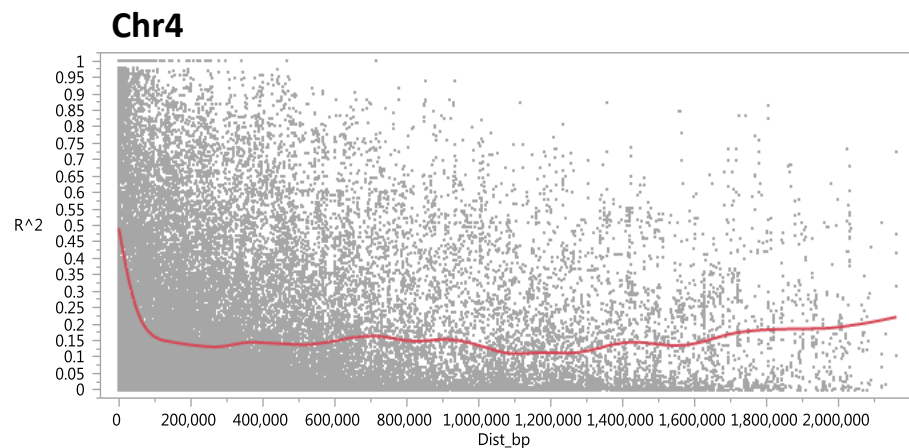
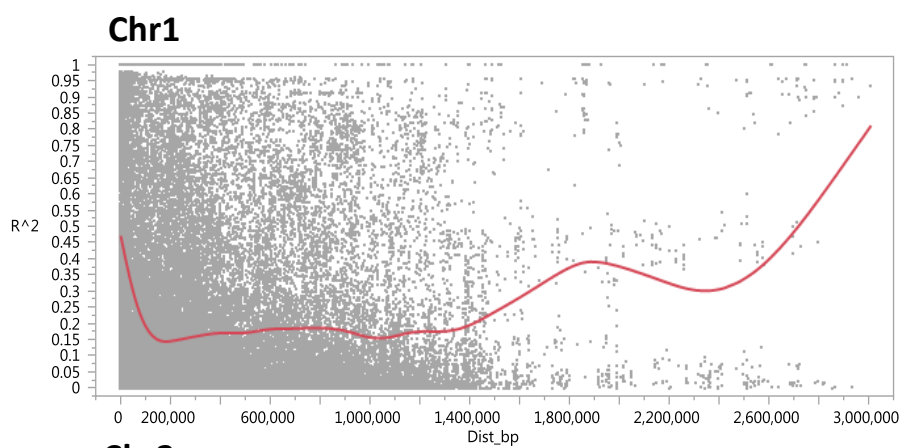
Supplementary Figure S2: LD (R^2) by physical position across 12 melon chromosomes.

LD between intra-chromosomal SNP pairs is plotted (Y axis) by the physical genomic position of first SNP in each pair. Distance between SNPs in each pair is indicated using white-to-red color scale.

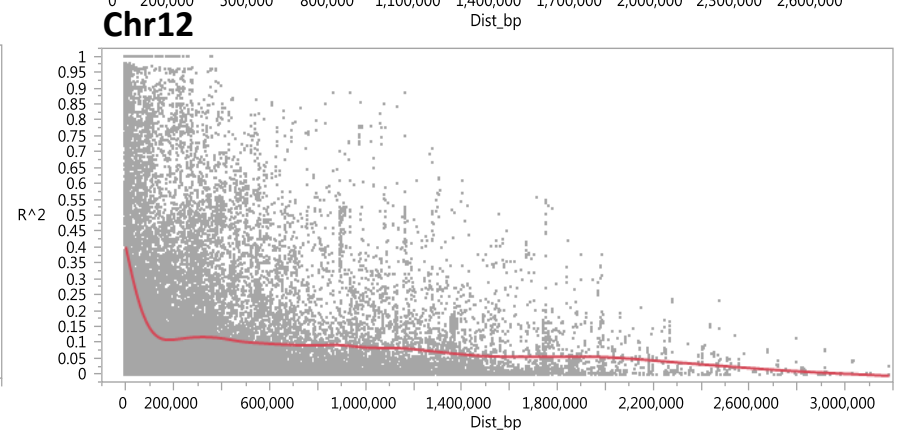
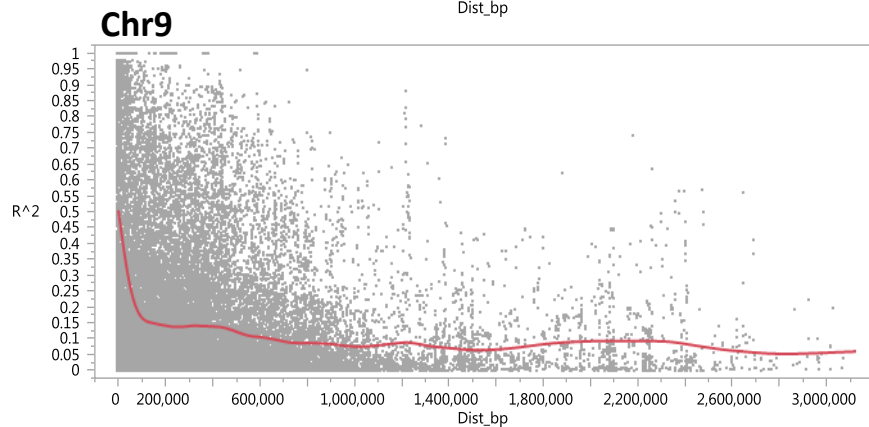
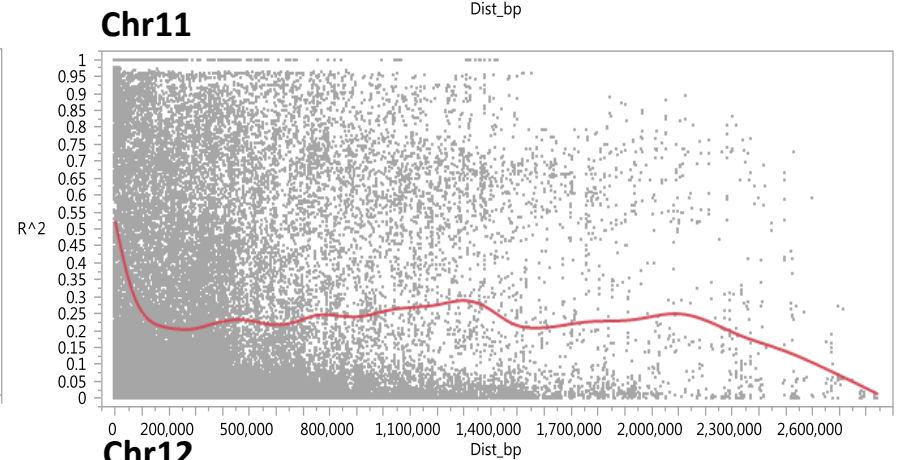
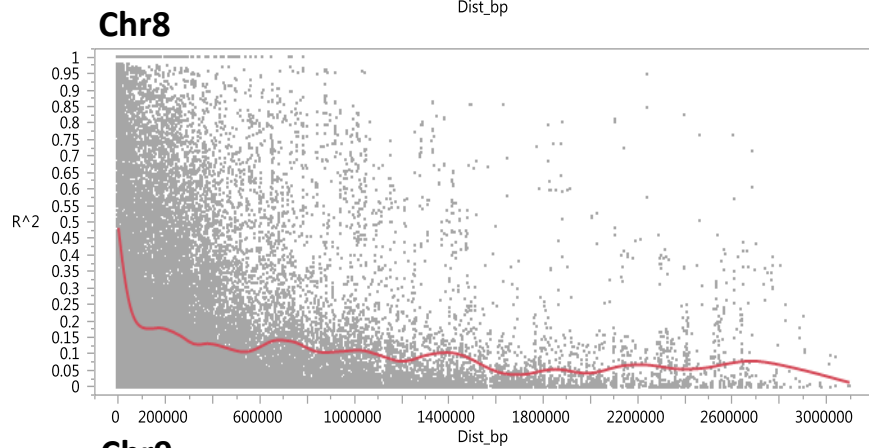
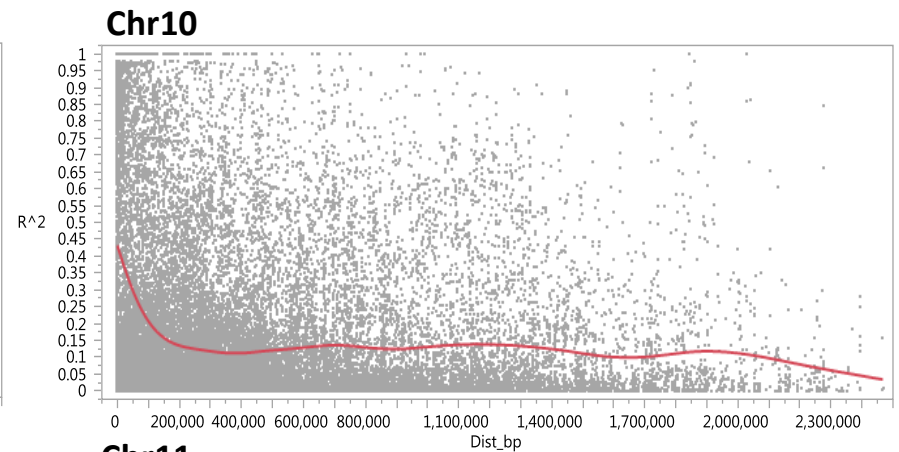
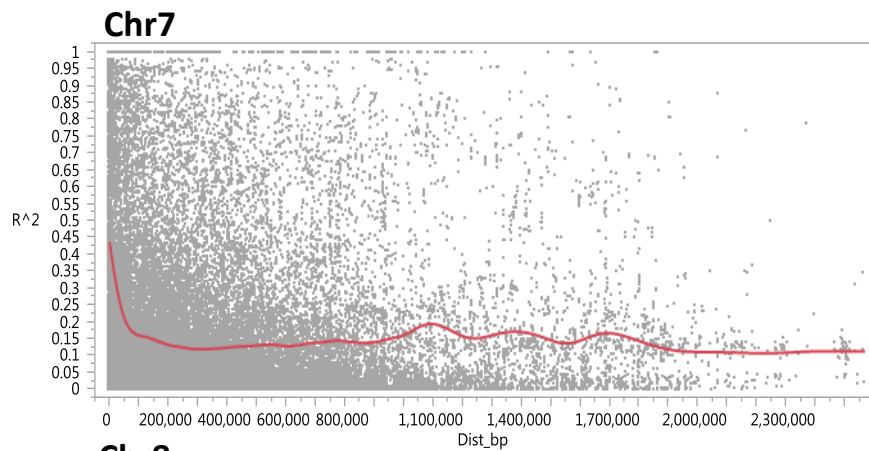




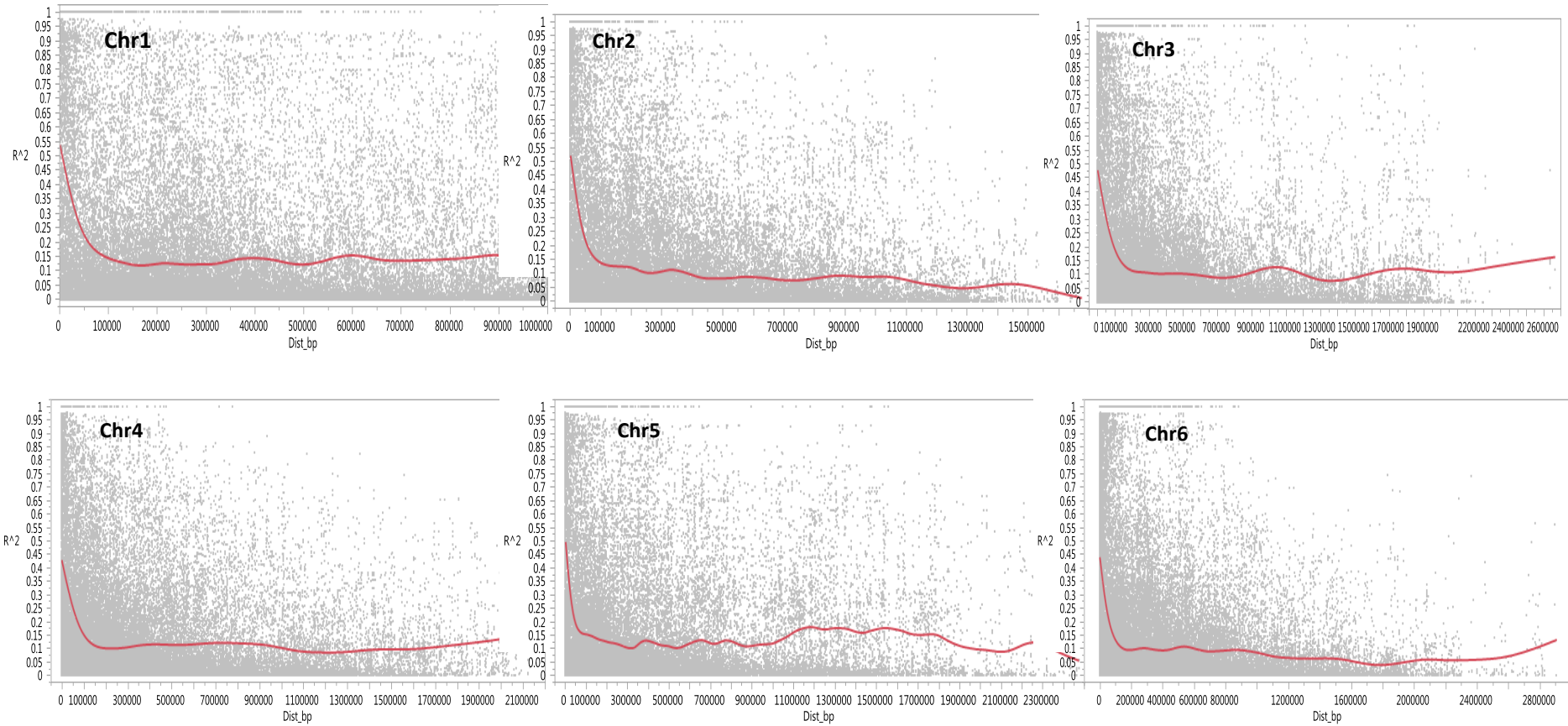
Supplementary Figure S4: Population structure analysis. Results are shown for the minimum number of subpopulations (K) which sufficiently define genetic variation. Each individual is represented by a vertical line. **(a)** detection of optimal subpopulations using the delat K method (Reference 62). Plot of delta K for K = 1–10. **(b)** Population structure for K=2, in consensus with the *spp. melo* and *spp. agrestis* division. **(c)** Population structure obtained at K=7.



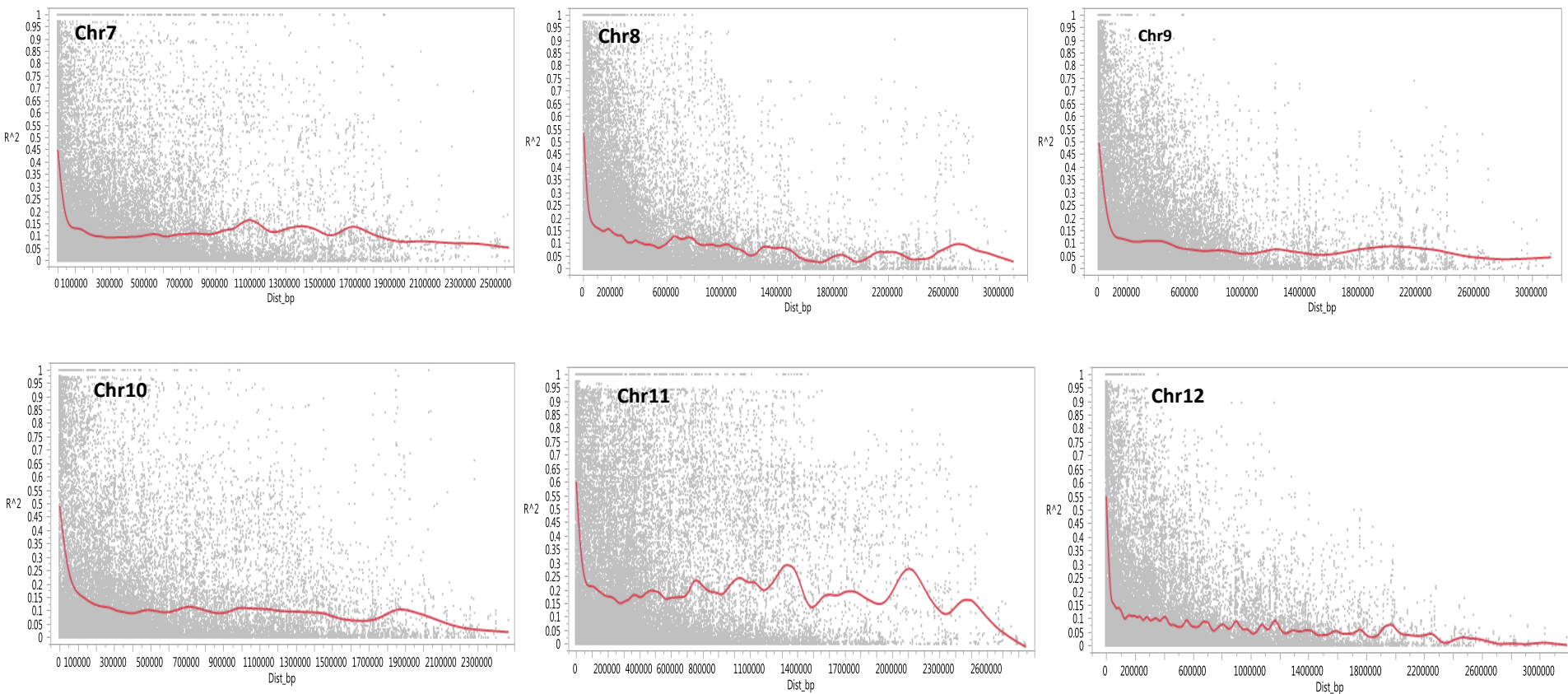
Supplementary Figure S5a: LD decay plots by chromosomes across 177 accessions. R^2 between intra-chromosomal marker pairs plotted against the physical distance between them.



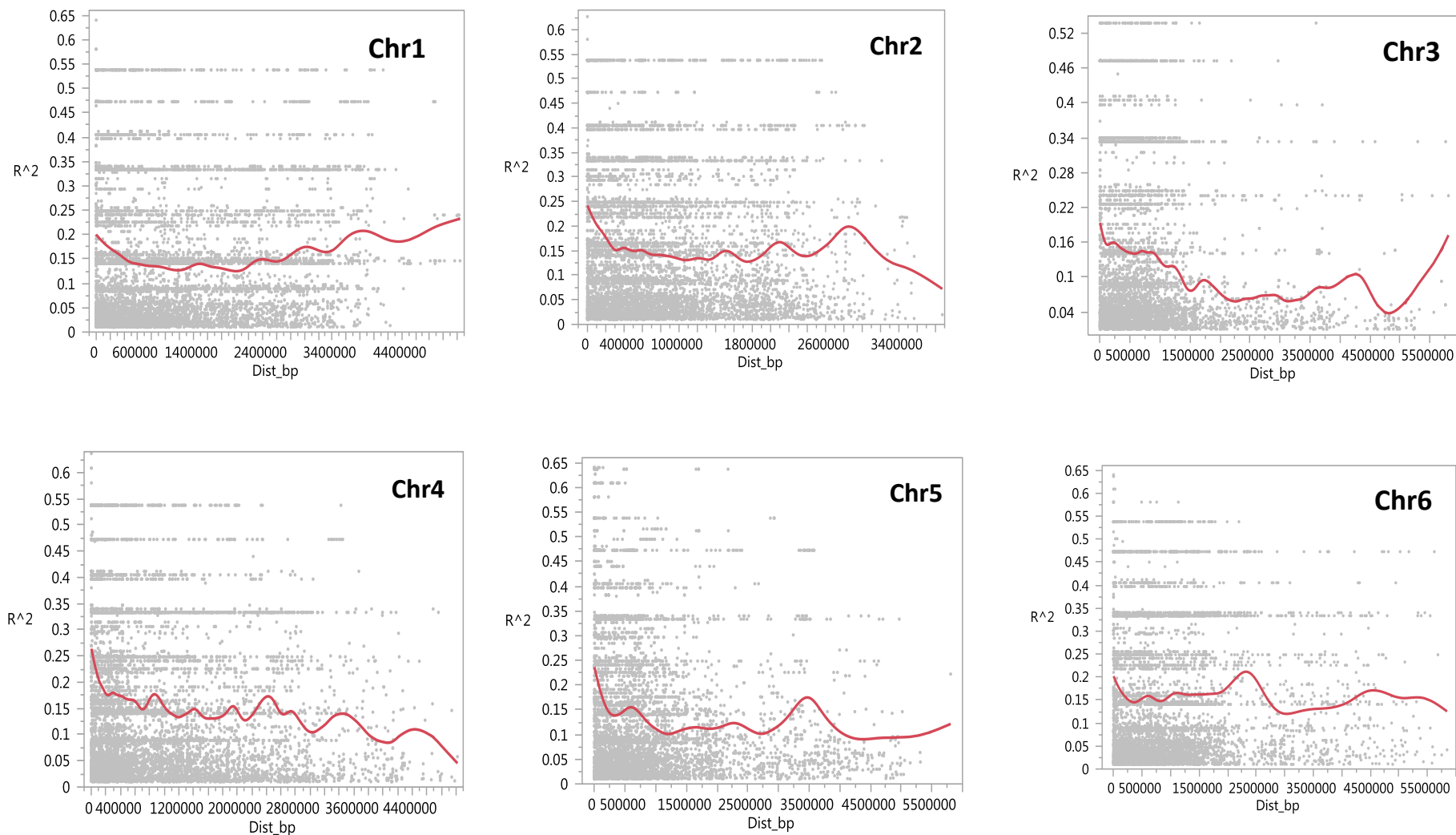
Supplementary Figure S5a: LD decay plots by chromosomes across 177 accessions. R² between intra-chromosomal marker pairs plotted against the physical distance between them.



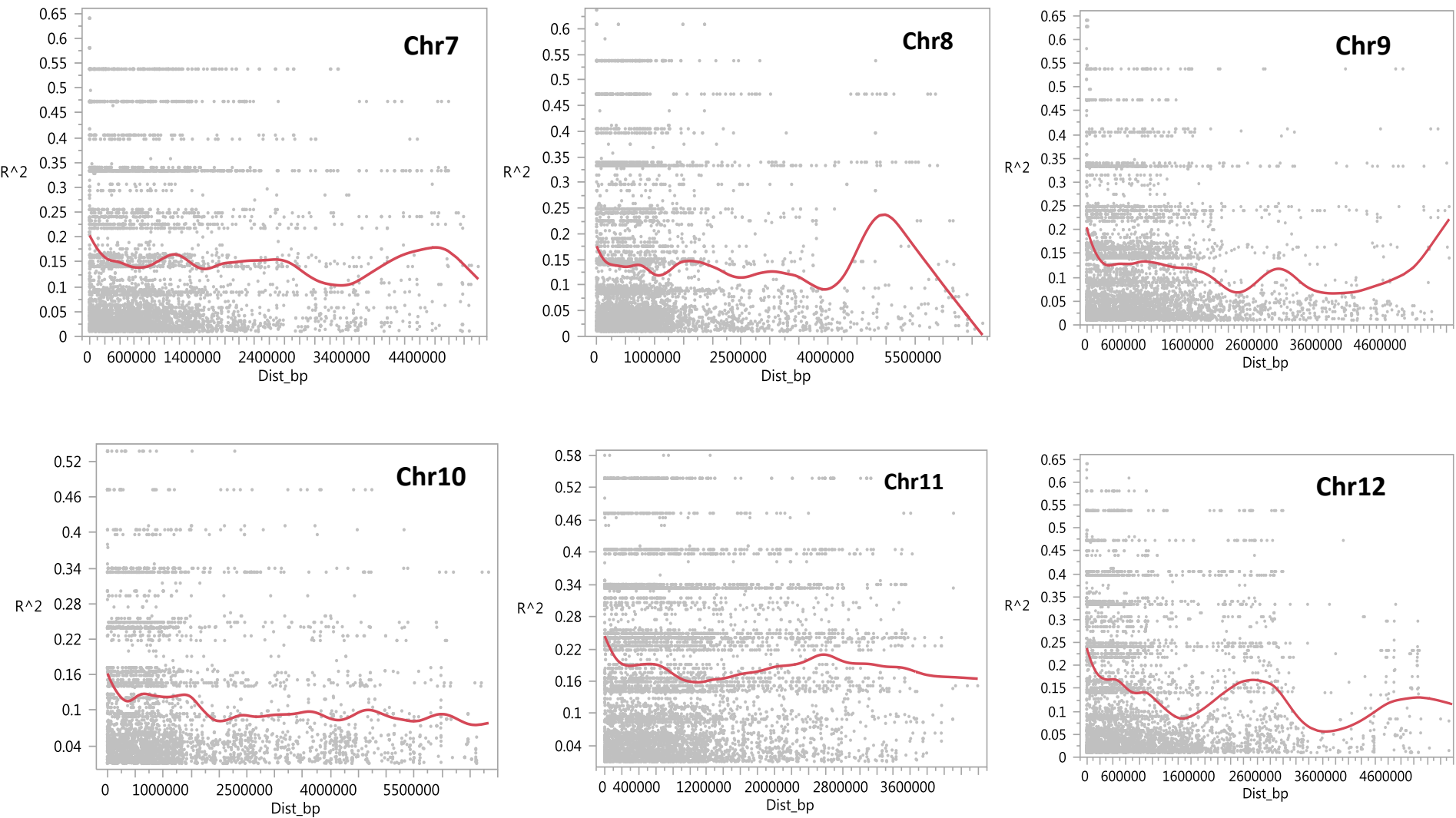
Supplementary Figure S5b: LD decay plots by chromosomes within the *ssp. melo* group. R^2 between intra-chromosomal marker pairs plotted against the physical distance between them.



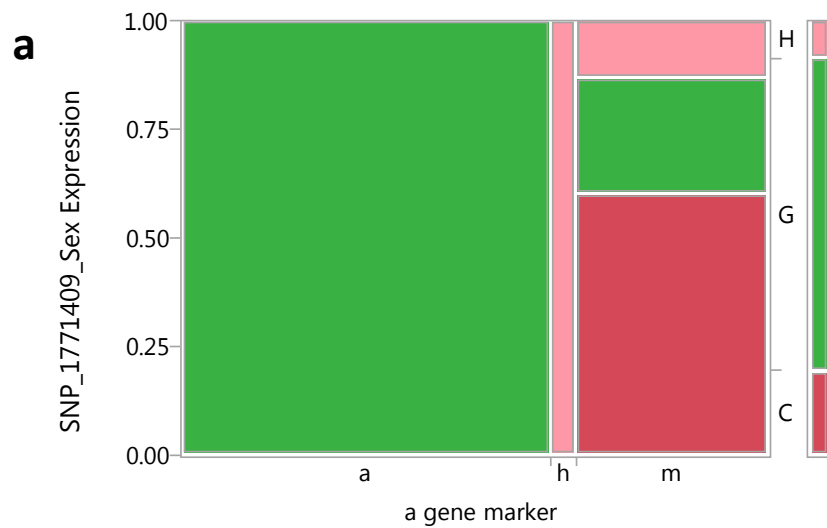
Supplementary Figure S5b: LD decay plots by chromosomes within the *ssp. melo* group. R^2 between intra-chromosomal marker pairs plotted against the physical distance between them.



Supplementary Figure S5c: LD decay plots by chromosomes within the *ssp. agrestis* group. R^2 between intra-chromosomal marker pairs plotted against the physical distance between them.



Supplementary Figure S5c: LD decay plots by chromosomes within the *ssp. agrestis* group. R^2 between intra-chromosomal marker pairs plotted against the physical distance between them.



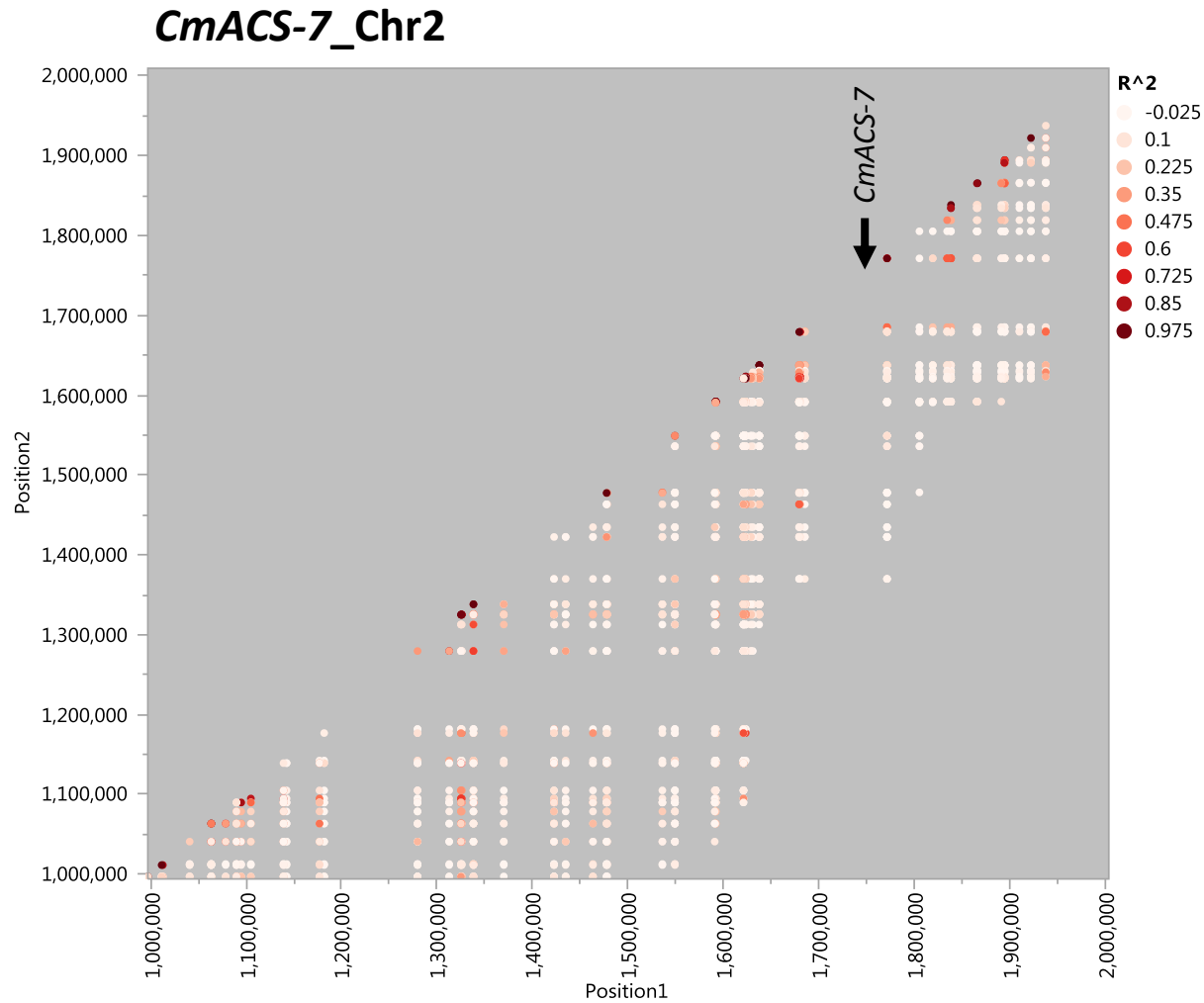
SNP_1771409_Sex Expression

b

	Count	C	G	H	Total
	Total %				
	Col%				
	Row %				
a		0	29	0	29
		0.00	63.04	0.00	63.04
		0.00	87.88	0.00	
		0.00	100.00	0.00	
h		0	0	2	2
		0.00	0.00	4.35	4.35
		0.00	0.00	50.00	
		0.00	0.00	100.00	
m		9	4	2	15
		19.57	8.70	4.35	32.61
		100.00	12.12	50.00	
		60.00	26.67	13.33	
Total		9	33	4	46
		19.57	71.74	8.70	

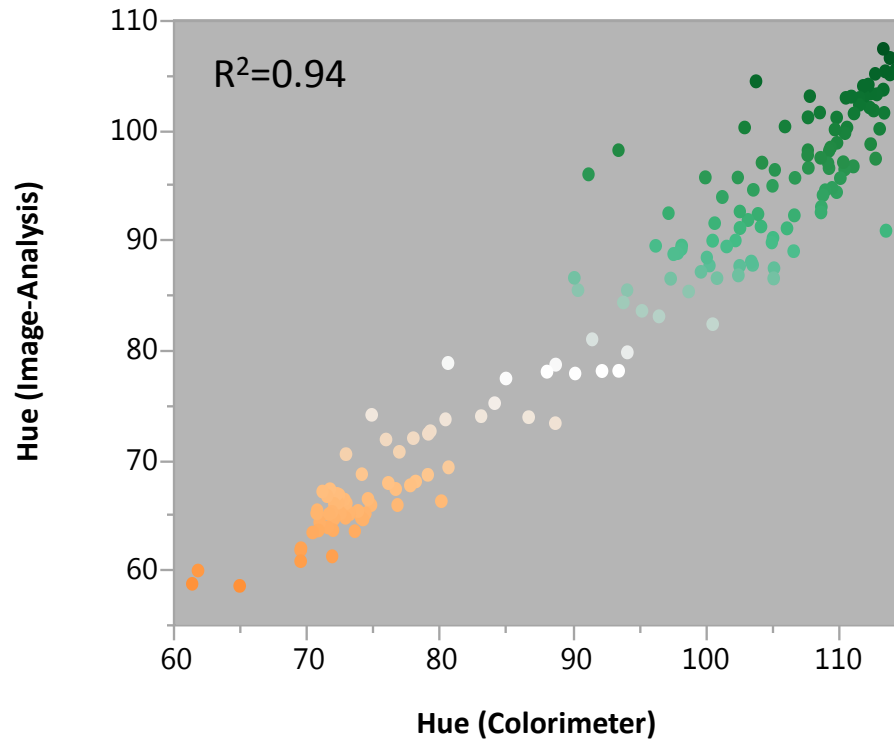
a gene marker

Figure S6: Validation of SNP_1771409 for flower sex-expression through alignment with PCR marker at the *CmACS-7* gene (MELO3C015444, Boualem et al. 2008). (a) Mosaic plot of segregation of the two markers. (b) contingency table of segregation data



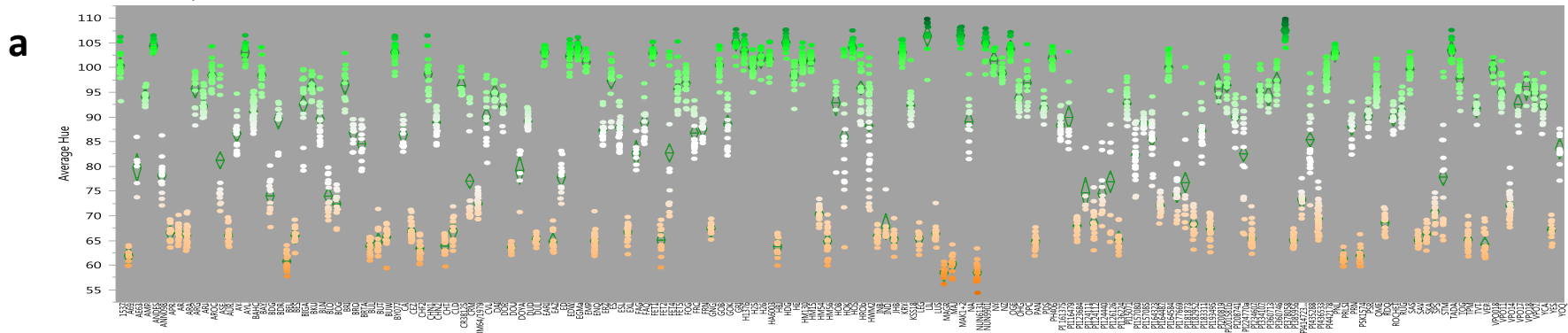
Supplementary Figure S7: LD distribution plot in a 1,000,000 bp interval surrounding the *CmACS-7* gene on chromosome 2.

LD is expressed as R² and color-coded using white-to-Red scale.

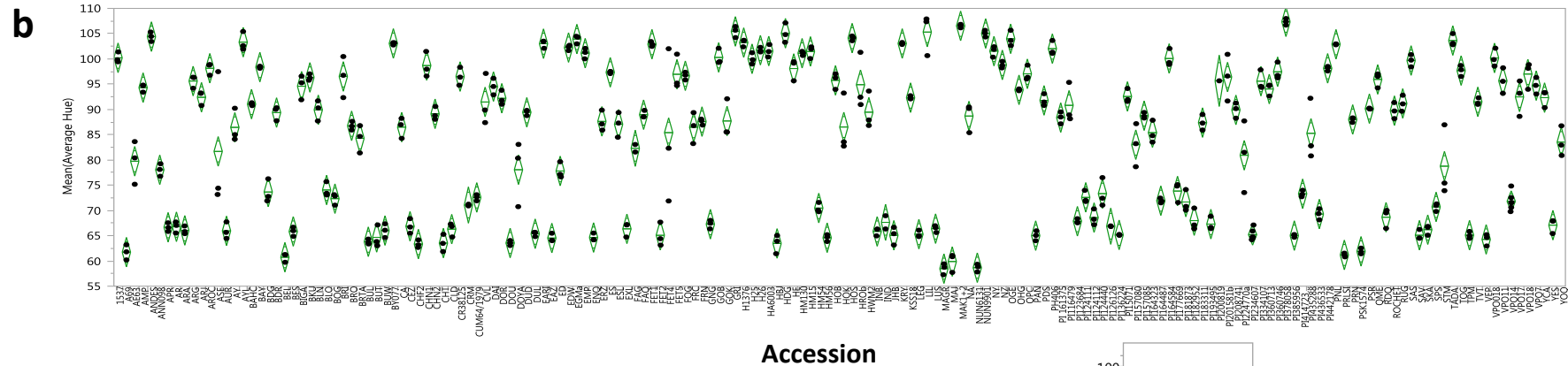


Supplementary Figure S8: Correlation between fruit flesh hue, measured using colorimeter (X axis) and through fruit image analyses (Y axis).

Fruit basis; $H^2=0.95$

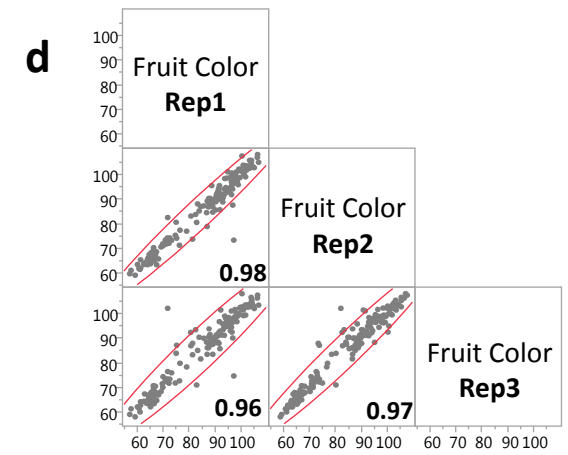


Plot basis; $H^2=0.96$

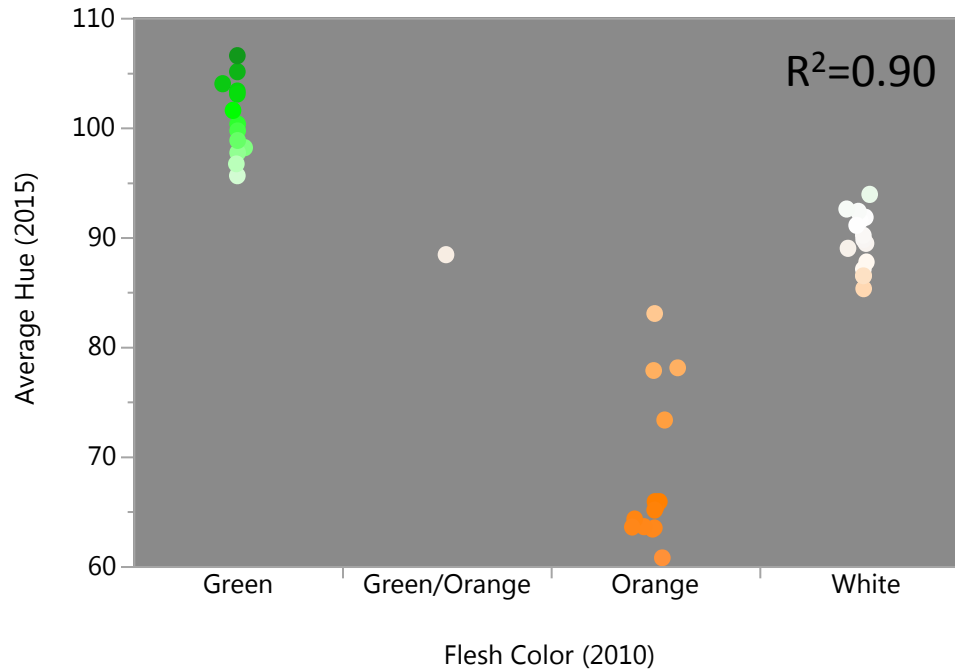


c

REML Variance Component Estimates						
Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Rep	0.005122	0.0613497	0.0741873	-0.084055	0.2067541	0.027
Accession	17.643397	211.32736	22.806405	166.62763	256.02709	94.610
Residual		11.977703	0.3211469	11.372371	12.632928	5.362
Total		223.36641	22.808432	184.62751	275.78996	100.000

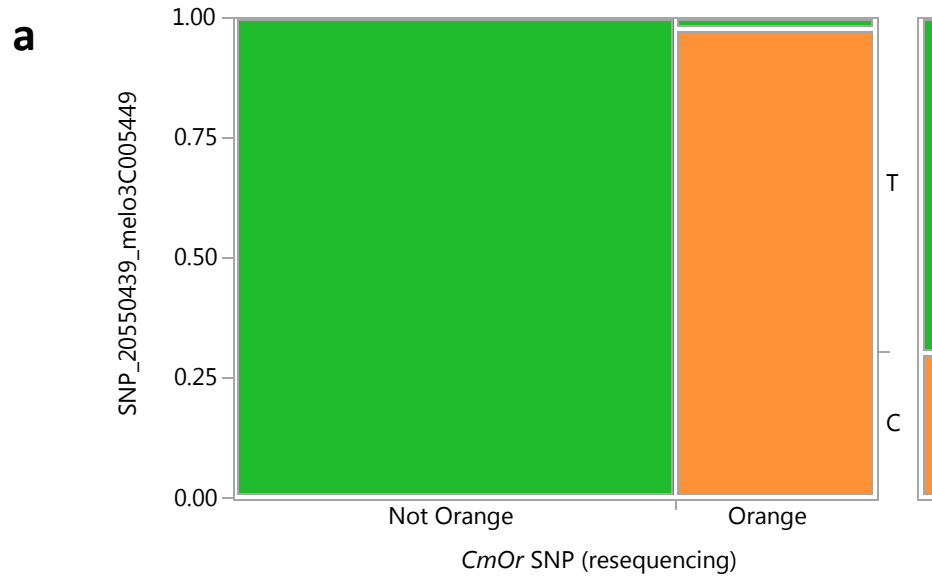


Supplementary Figure S9: Properties of flesh color variation . (a) one-way ANOVA at the fruit basis. (b) one-way ANOVA at the plot basis. (c) variation components table for the fruit basis ANOVA. (d) correlation between replications for plot means.



Supplementary Figure S10: repeatability of flesh color in two different years across 43 diverse accessions.

Each point represent accession mean. X axis are flesh color classes scores on the Summer 2010 experiment. On the Y axis are flesh color quantitative data collected from digital fruit images on Summer 2016 (see materials and methods).



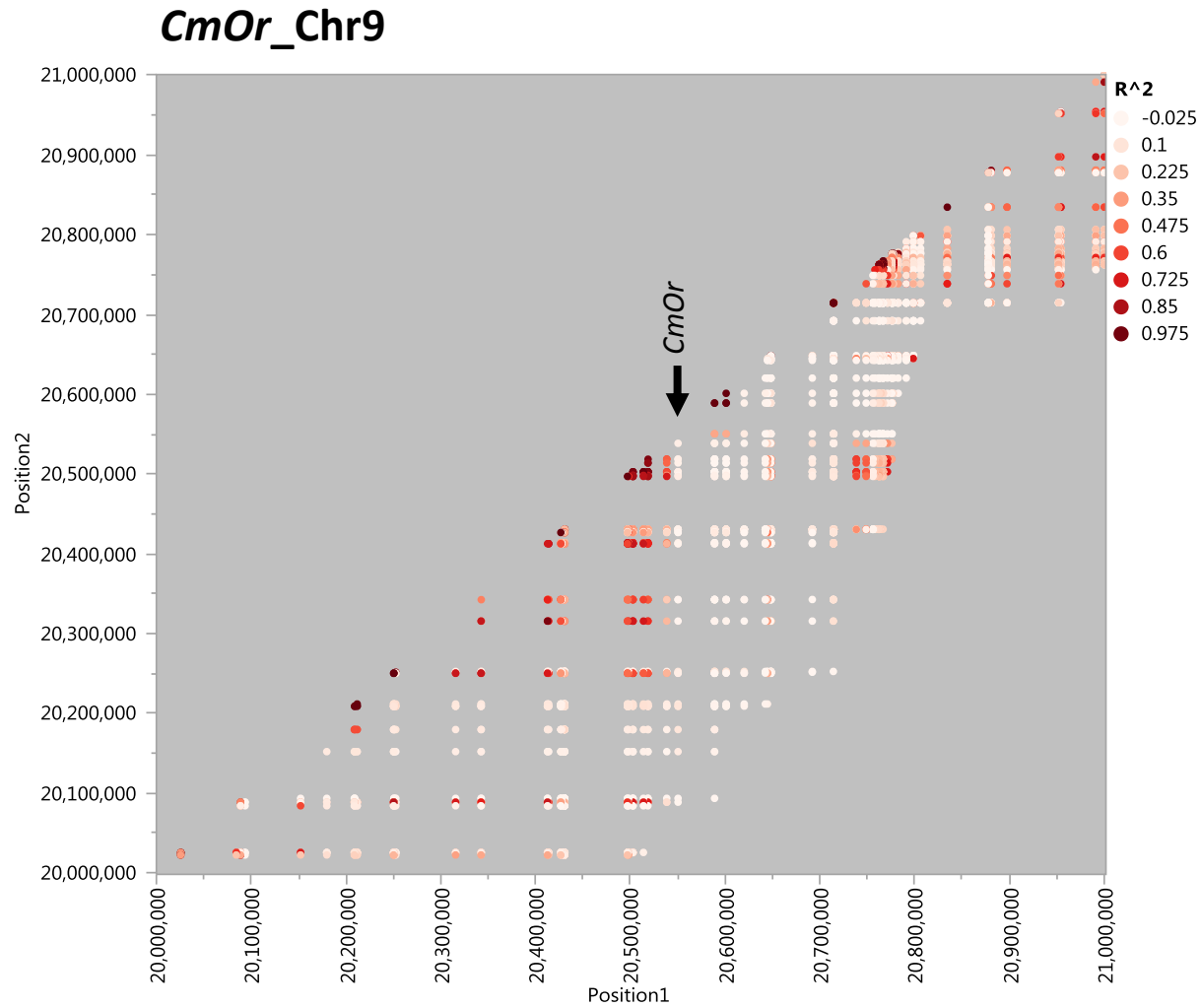
SNP_20550439_melo3C005449

b

Count	C	T	Total
Total %			
Col %			
Row %			
Not Orange	0 0.00 0.00 0.00	91 68.94 98.91 100.00	91 68.94
Orange	40 30.30 100.00 97.56	1 0.76 1.09 2.44	41 31.06
Total	40 30.30	92 69.70	132

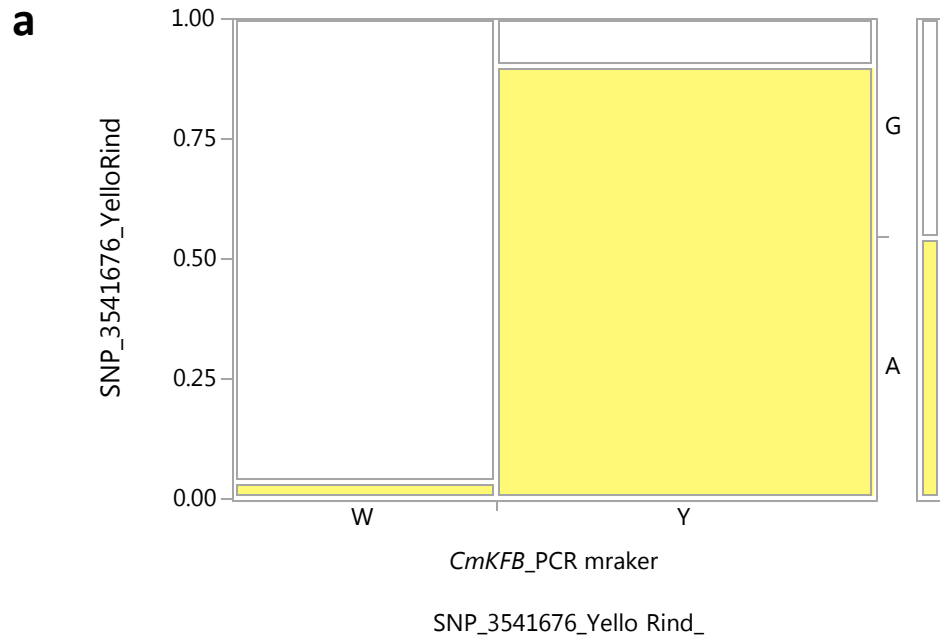
CmOr SNP (resequencing)

Figure S11: Validation of SNP_20550439 for flesh color through alignment with resequencing-based SNP at the *CmOr* gene (melo3C005449, Tzuri et al. 2015). (a) Mosaic plot of segregation of the two markers. (b) contingency table of segregation data



Supplementary Figure S12: LD distribution plot in a 1,000,000 bp interval surrounding the *CmOr* gene on chromosome 9.

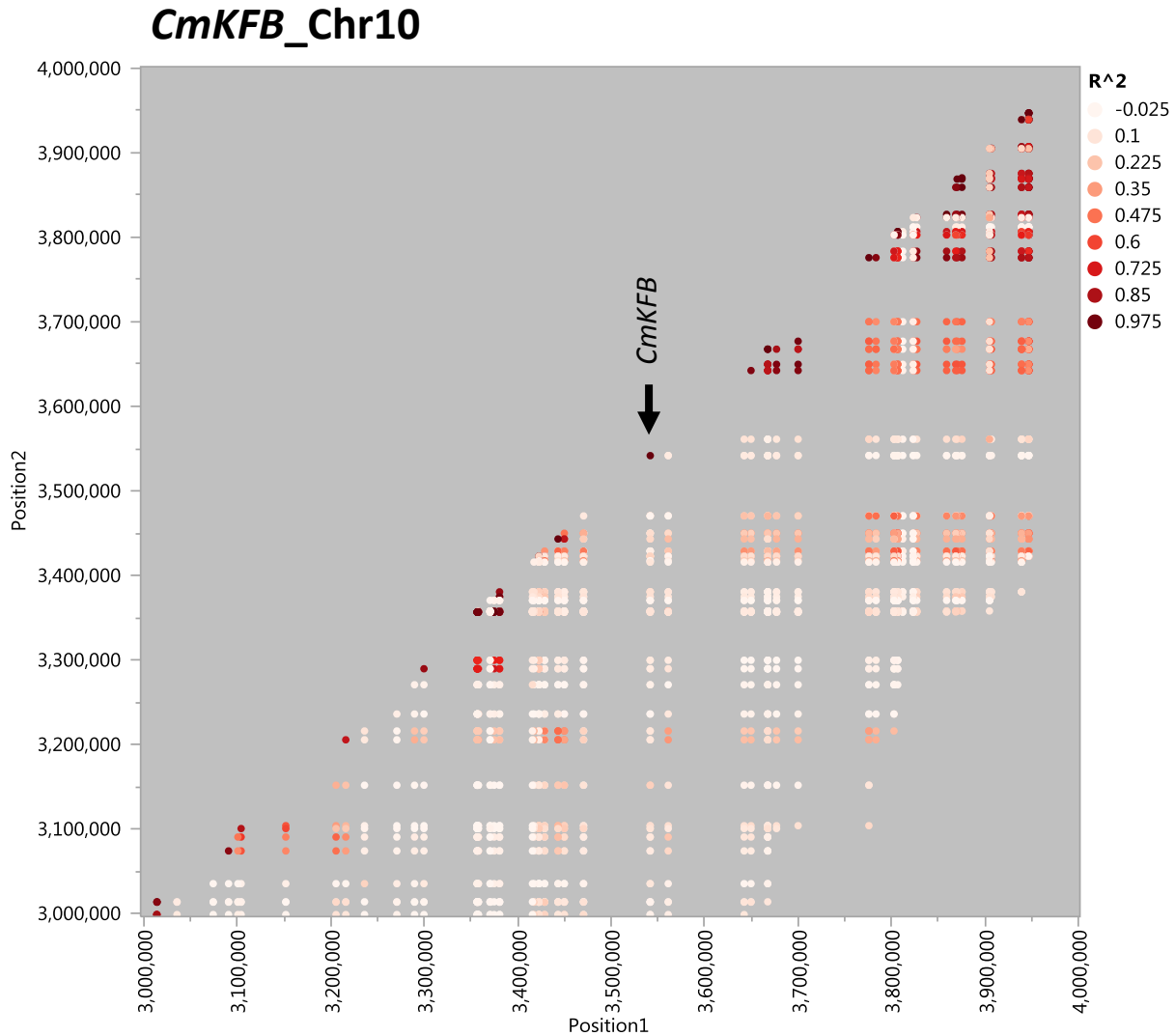
LD is expressed as R^2 and color-coded using white-to-Red scale.



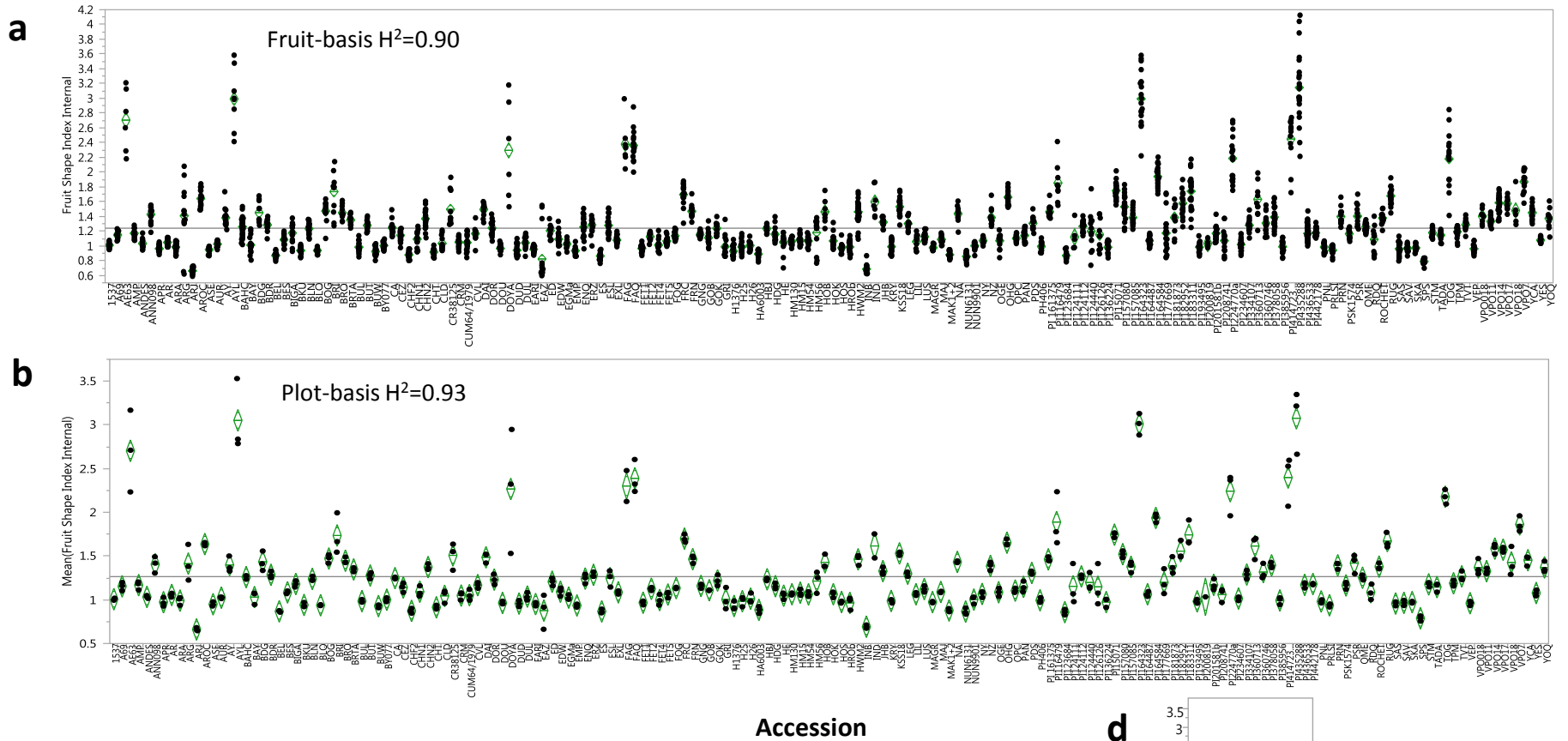
b

CmKFB_PCR Marker	Count			
	Total %	A	G	Total
	Col %			
	Row %			
W		1	27	28
		1.47	39.71	41.18
		2.70	87.10	
		3.57	96.43	
Y		36	4	40
		52.94	5.88	58.82
		97.30	12.90	
		90.00	10.00	
Total		37	31	68
		54.41	45.59	

Figure S13: Validation of SNP_3541676 for flesh color through alignment with PCR marker scoring an INDEL at the *CmKFB* gene (MELO3C11980, Feder et al. 2015). (a) Mosaic plot of segregation of the two markers. W=White Allele, Y=Yellow allele. (b) contingency table of segregation data

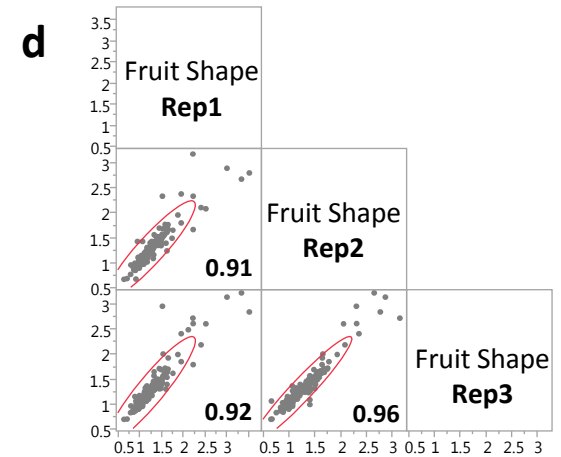


Supplementary Figure S14: LD distribution plot in a 1,000,000 bp interval surrounding the *CmKFB* gene on chromosome 10. LD is expressed as R^2 and color-coded using white-to-Red scale.

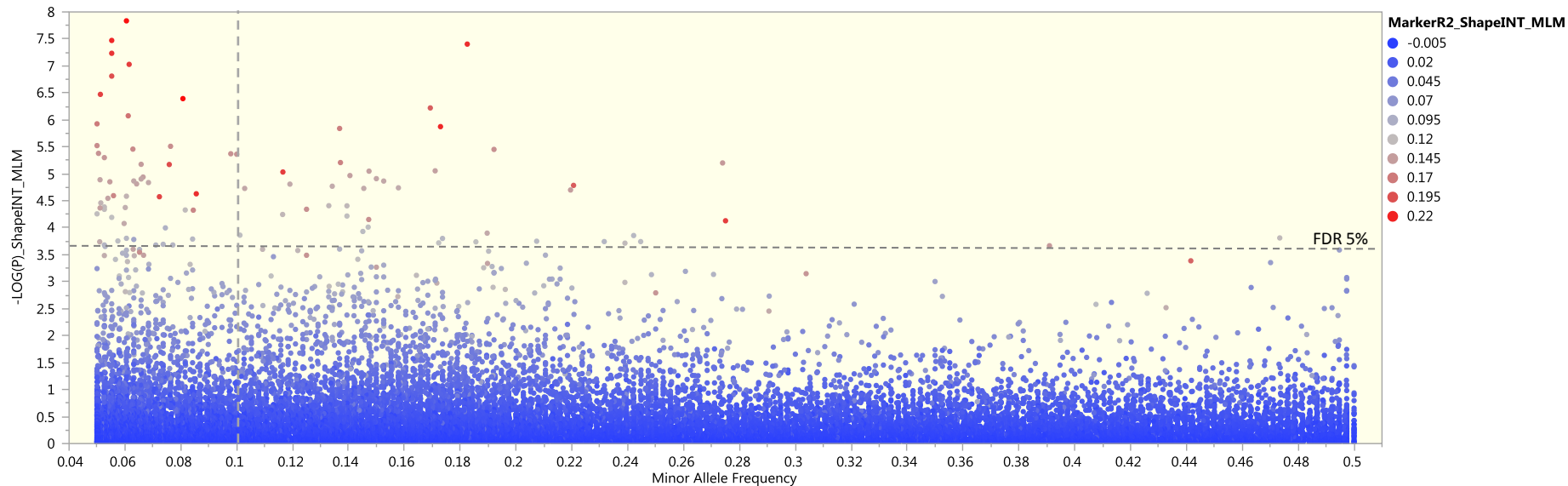


c

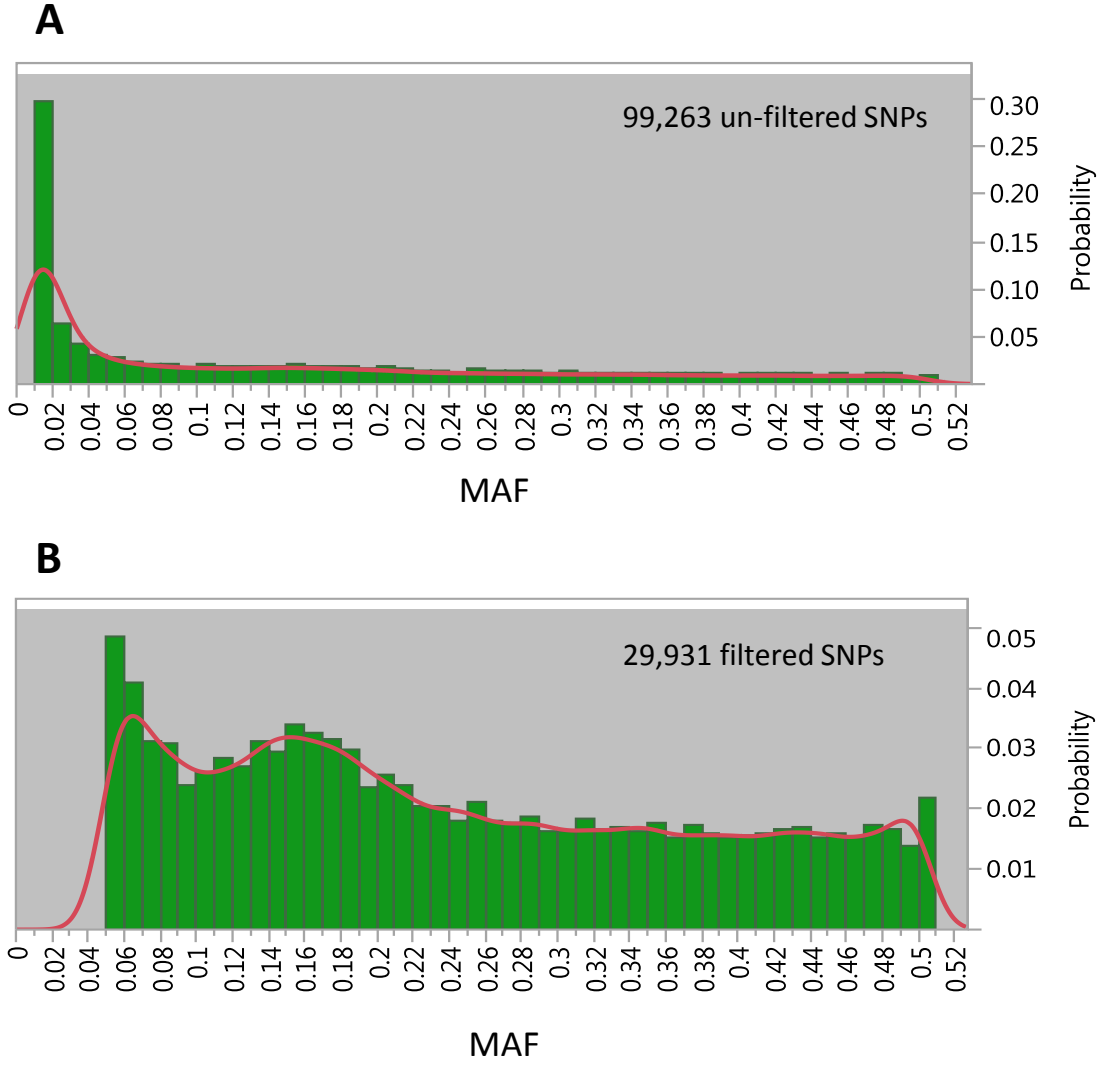
REML Variance Component Estimates						
Random Effect	Var					
	Var Ratio	Component	Std Error	95% Lower	95% Upper	Pct of Total
Rep	0.0064533	0.0001207	0.0001404	-0.000155	0.0003959	0.065
Accession	8.8655302	0.1657838	0.0179868	0.1305303	0.2010372	89.805
Residual		0.0186998	0.0004998	0.0177576	0.0197194	10.130
Total		0.1846043	0.0179929	0.1538541	0.2256451	100.000



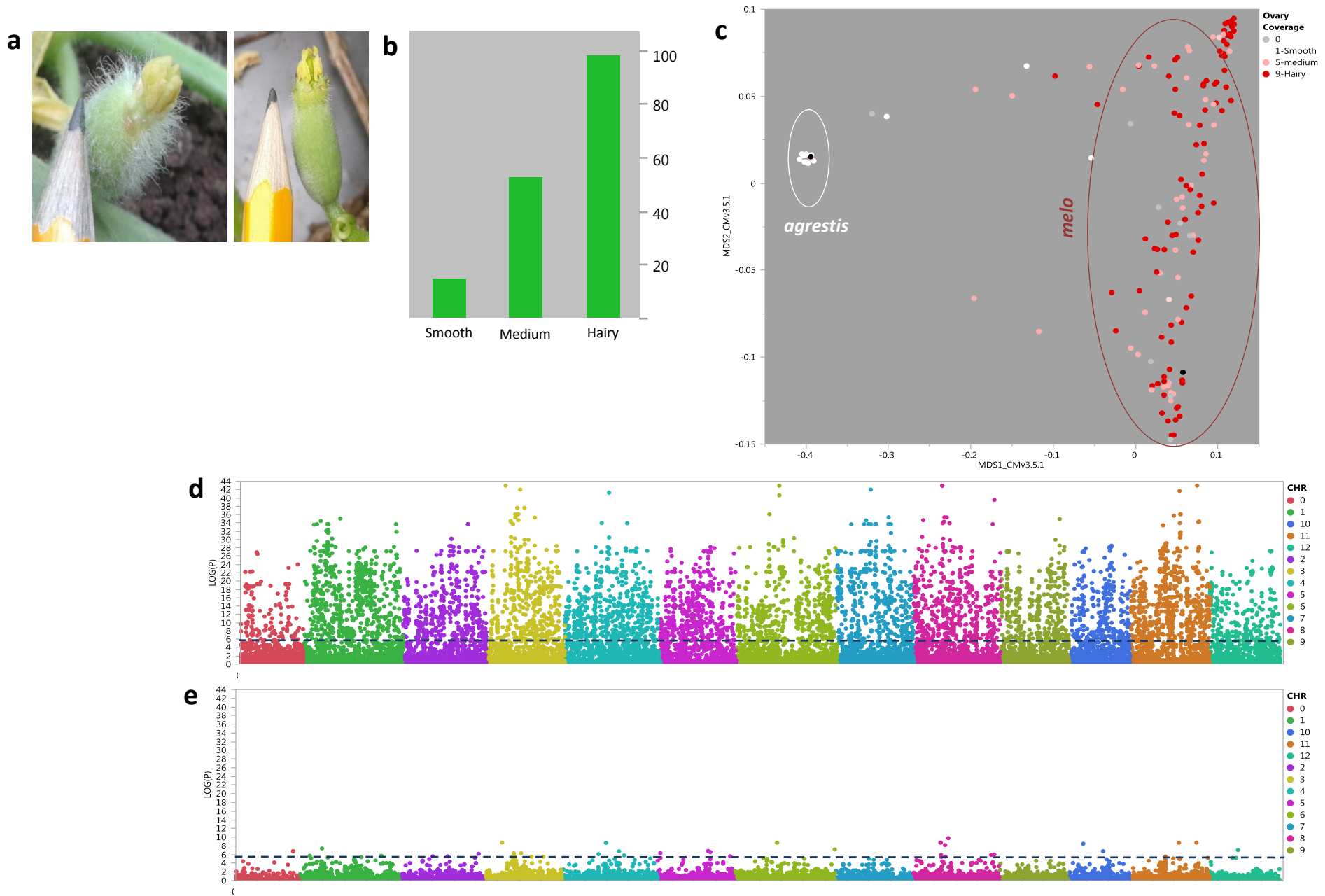
Supplementary Figure S15: Properties of fruit shape variation . (a) one-way ANOVA at the fruit basis. (b) one-way ANOVA at the plot basis. (c) variation components table for the fruit basis ANOVA. (d) correlation between replications for plot means.

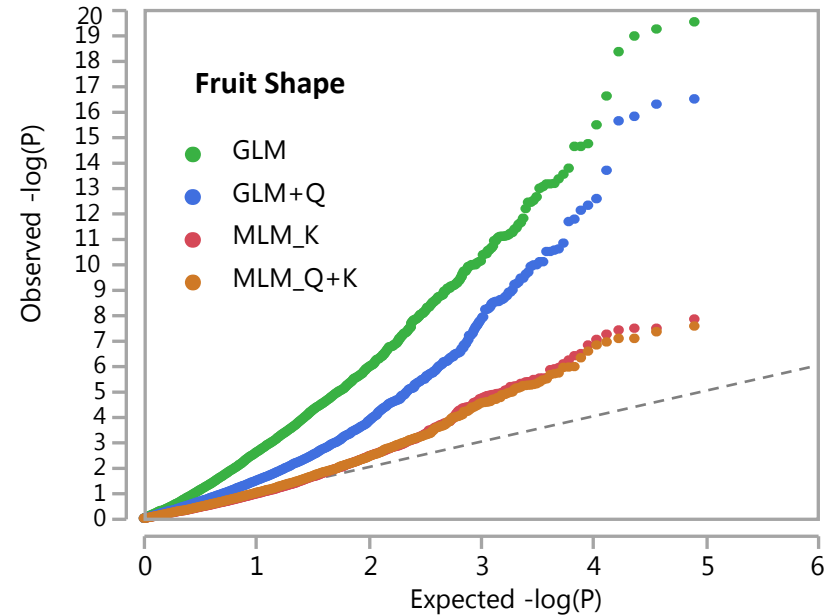
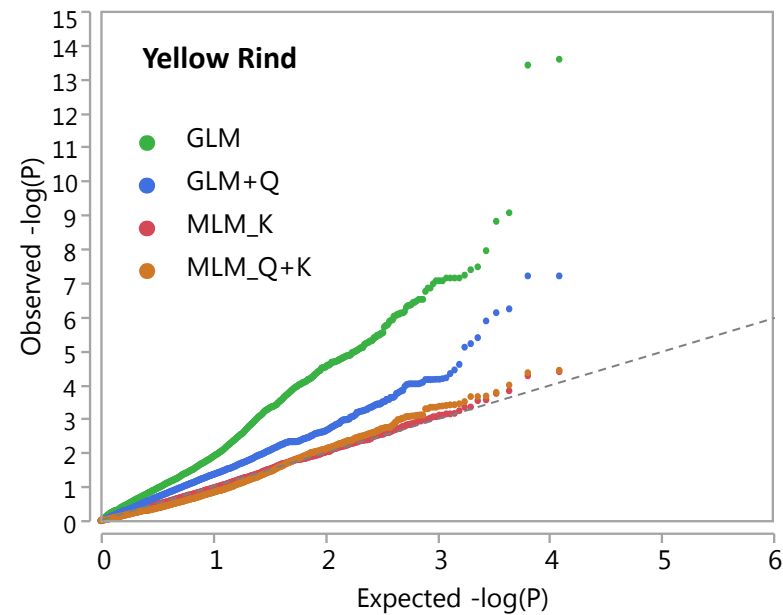
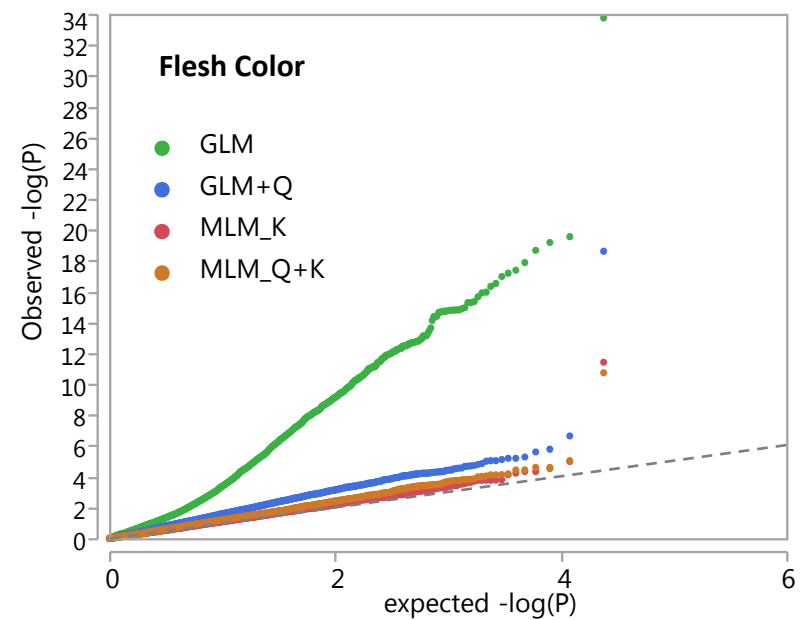
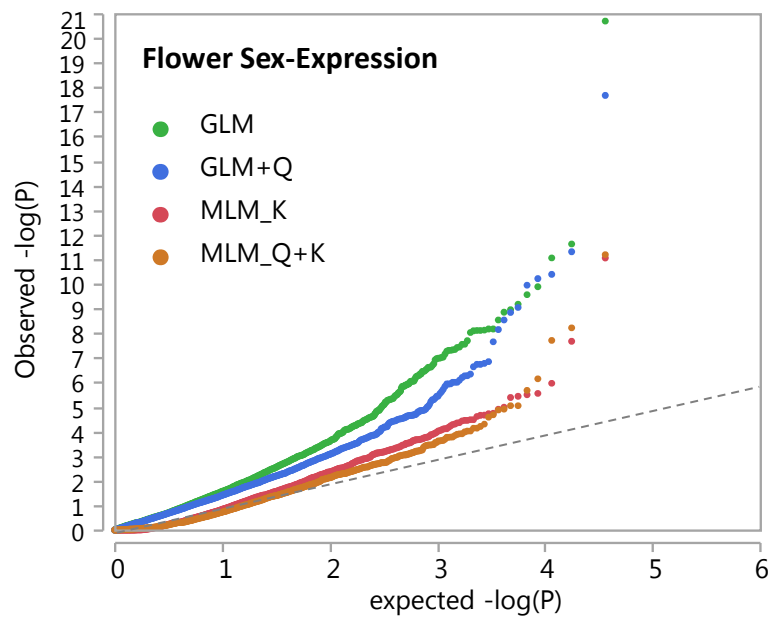


Supplementary Figure S16: Polymorphism effect size for fruit shape index and allele frequencies. SNP allele frequencies plotted against effects on fruit shape index (expressed as $-\text{Log } P$ values). Effects are also colored based on SNP R^2 .



Supplementary Figure S17: Frequency distribution of minor allele frequencies (MAF).
(A) Full set – 99,263 SNPs. (B) Filtered set - 23,931 SNPs.





Supplementary Figure S19: Quantile-quantile (Q-Q) plots comparing distribution of P values at the 4 statistical models used for GWA analyses. The negative logarithm of the observed (y axis) and the expected (x axis) P value is plotted for each SNP (dot), and the gray dashed line indicates the null hypothesis of no true association.