Fine mapping of a major quantitative trait locus *qSS.C9* associated with seeds per silique in *Brassica napus* L.

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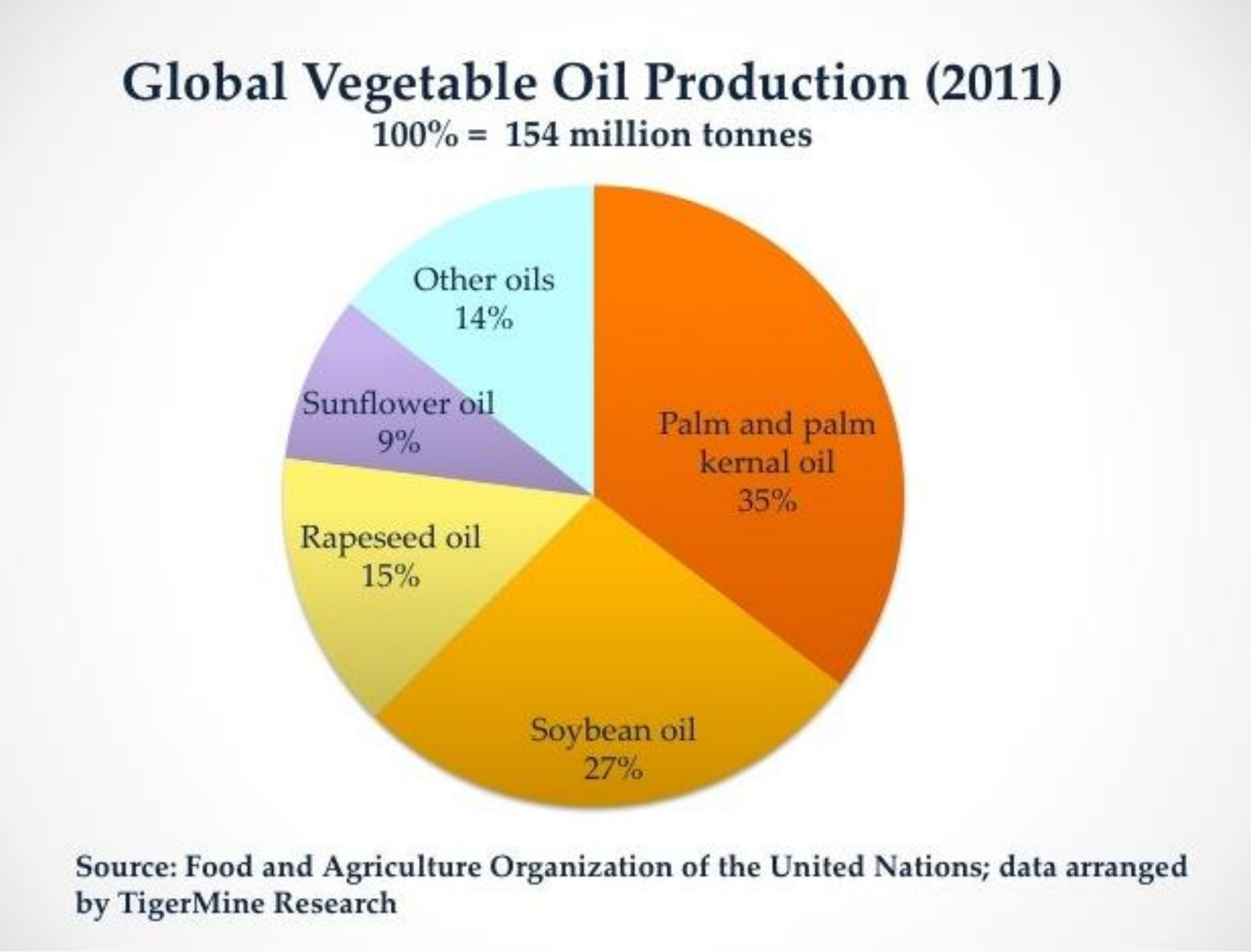
Objectives

- Background
- Plant materials and method
- Results

Background



Global Vegetable Oil Production (2011)

100% = 154 million tonnes

Source: Food and Agriculture Organization of the United Nations; data arranged by TigerMine Research

Background

- Seeds per silique (SS) is one of the three direct components (silique per plant, seeds per silique and seed weight) of the seed yield per plant in rapeseed.
- Seeds per silique (SS) is controlled by nuclear genes (Wei et al., 2000) and has a high inheritability(Shi et al.,2009 and Zhang et al., 2011)

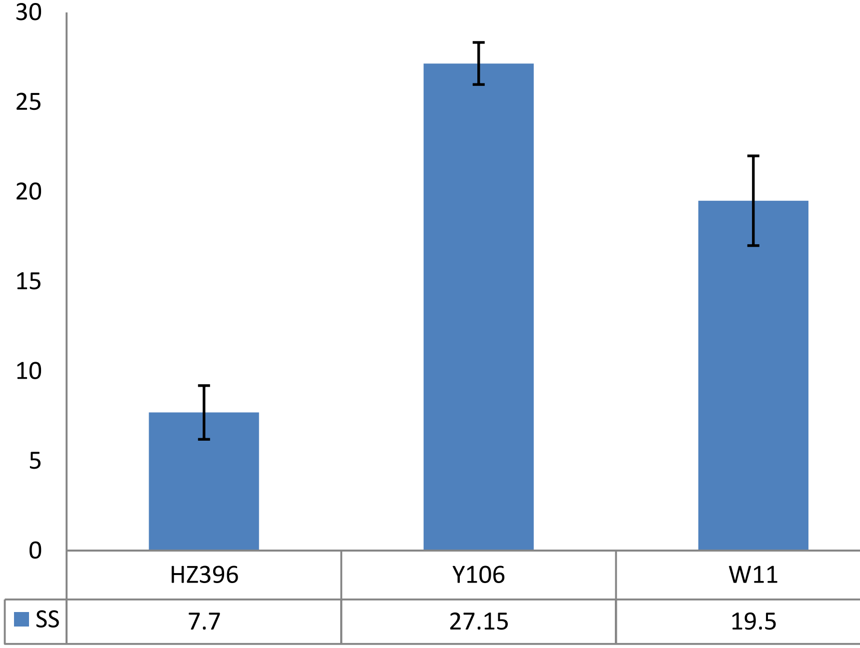
Background

Population parental lines	Population size	Population type	References
Express617×R53	250	DH	Radoev et al.2008
Tapidor×Ningyou7	202	DH	Shi et al. 2009
HZ396×Y106	140	DH	Zhang et al.2011
Quantum×No.2127-17	258	DH	Chen et al.2011
8008×4942C-5	181	DH	Qi et al.2014
Zhongyou 821 ×No. 2127-17	192	RIL	Cai et al.2014

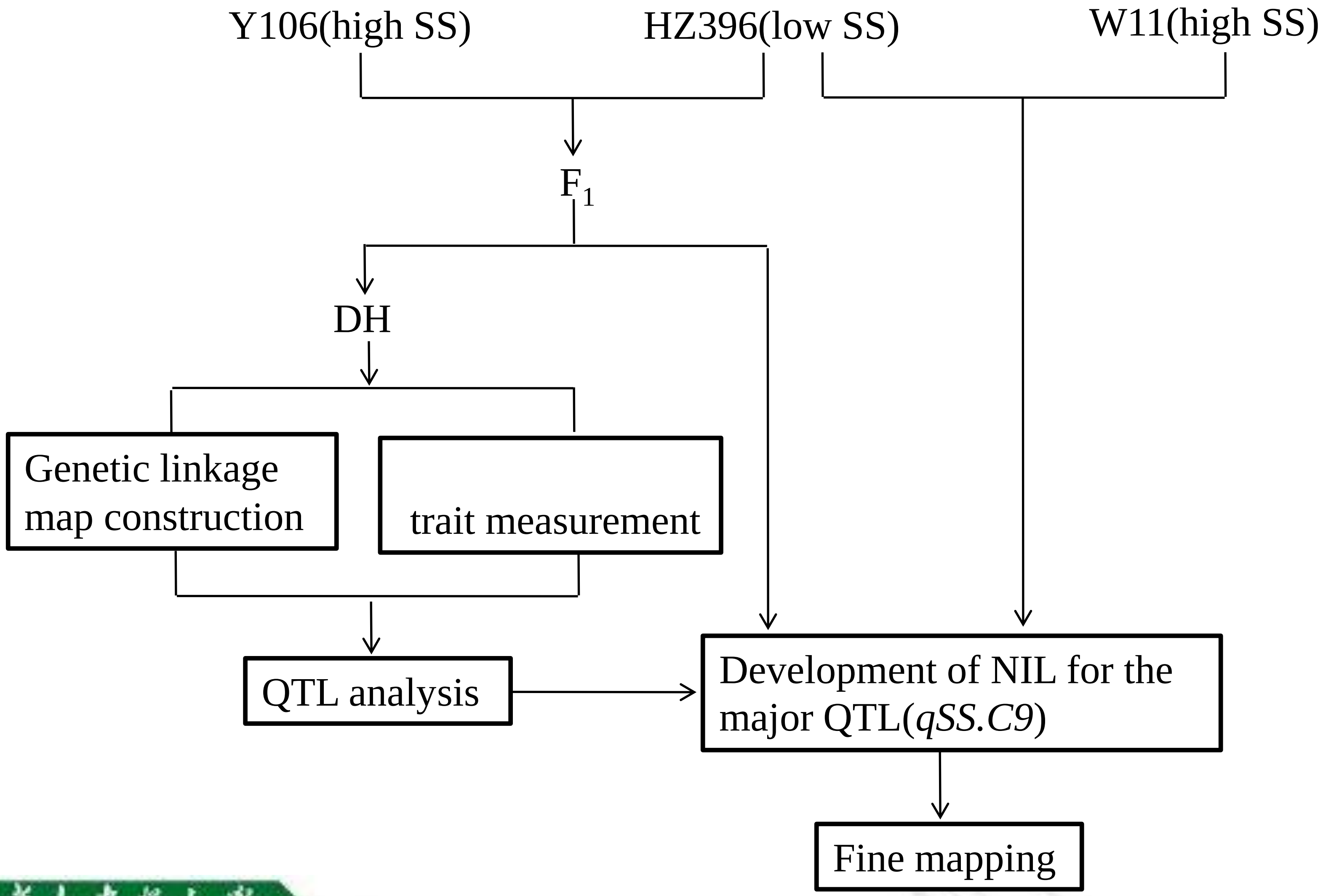
- Up to now, 24 quantitative trait loci (QTLs) related to seeds per silique have been reported, but none of these QTLs has been cloned thus far.

Plant materials and method

HZ396 Y106 W11



Results


Y106(high SS)

HZ396(low SS)

W11(high SS)

F_1

DH

Genetic linkage map construction

trait measurement

QTL analysis

Development of NIL for the major QTL(*qSS.C9*)

Fine mapping

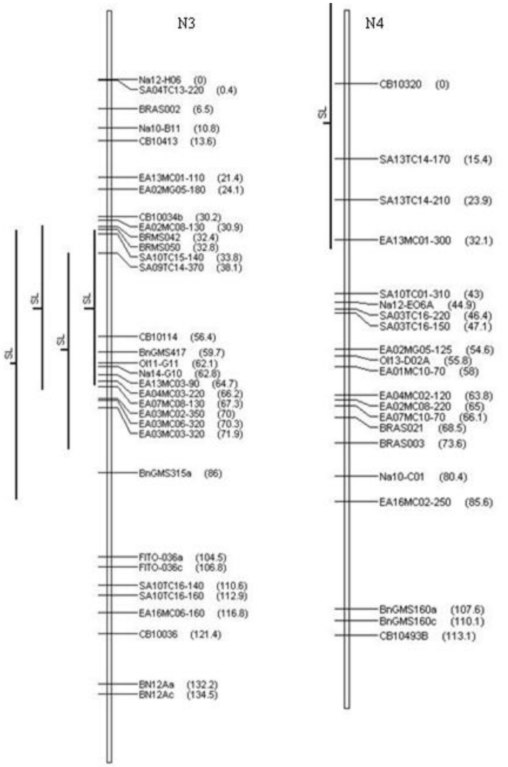
Genetic and correlation analysis of silique-traits in *Brassica napus* L.by quantitative trait locus mapping

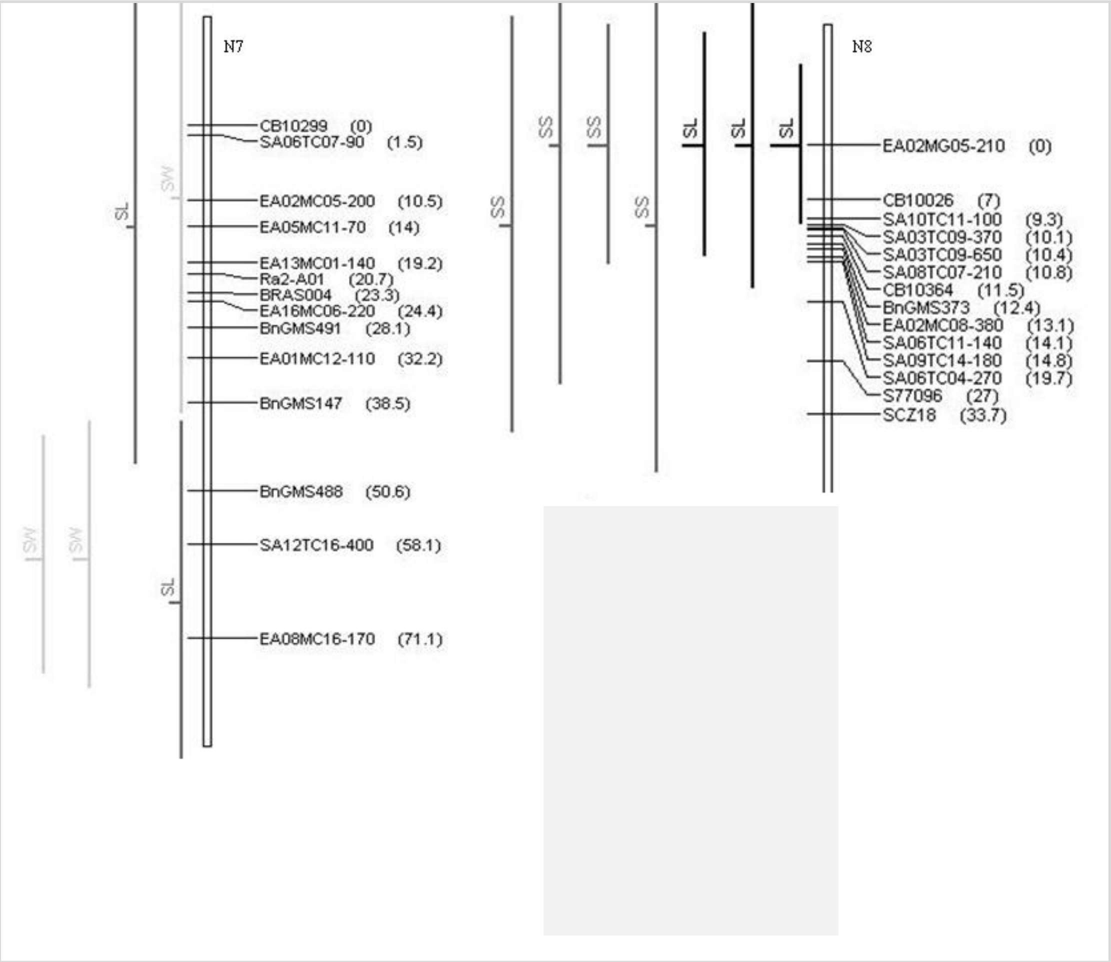
- A F_1 plant derived from two inbred lines(HZ396 and Y106) differing in seeds per silique were used to generate 140 double haploid lines.
- The phenotypic performances for the parental lines and the DH population were carried out over 3 years and two locations
- A linkage map comprising 150 SSR and 195 AFLP markers covering 1,759.6 cM was constructed

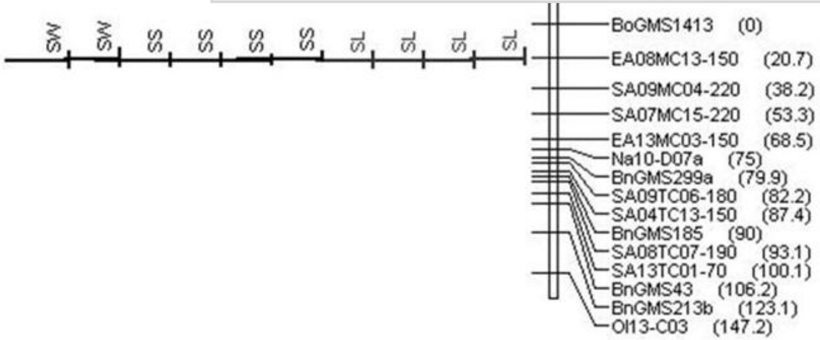
Phenotypic variation for silique length and seeds per silique as well as seed weight in the DH population

			Parents			DH population				
Years	Locations	Traits	HZ396 Mean ± s.d.	Y106 Mean ±s.d.	T-test for parents	Range	Mean ± s.d.	CV (%)	Skew	Kurt
2007	Wuhan	*SS*	11.0±2.6	27.3±3.7	19.14*	4.5-31.5	21.7±5.9	27.26	-0.76	-0.25
		SL	3.64±0.60	5.78±0.39	13.63*	2.99-6.30	4.79±1.11	23.17	-0.19	-0.25
		SW	4.46±0.41	4.31±0.15	5.63	1.52-5.20	3.32±0.71	21.39	0.41	-0.37
2008	Wuhan - Ⅰ	*SS*	11.5±2.3	26.7±2.0	28.59*	4.4-32.6	21.28±7.29	34.26	-0.61	-0.87
		SL	3.39±0.28	6.12±0.60	13.01*	2.57-6.00	4.63±0.74	15.98	-0.31	-0.6
		SW	4.11±0.22	3.89±0.17	5.79	2.09-5.1	3.23±0.72	22.29	0.74	-0.28
	Wuhan - Ⅱ	*SS*	7.7±3.1	25.90±2.1	21.28*	4.0-31.8	20.46±6.44	31.48	-0.67	-0.32
		SL	3.38±0.43	5.31±0.45	13.92*	2.78-6.60	4.7±0.73	15.53	-0.22	-0.11
2009	Gansu	*SS*	13.2±2.4	29.0±1.7	14.95*	3.7-35.3	23.44±8.11	34.60	-0.70	-0.73
		SL	3.69±0.14	6.84±0.45	16.73*	2.71-7.40	5.07±0.86	16.96	-0.30	0.09

Trait	*SS*	*SL*	*SW*
SS		0.56	0.29
SL	0.76**(0.73**)		0.19
SW	-0.57**(-0.62**)	-0.44**(-0.37**)	
h^2 (%)	82.5	76.9	87.5


N3
Na12-H06 (0)
SA04TC13-220 (0.4)
BRAS002 (6.5)
Na10-B11 (10.8)
CB10413 (13.6)
EA13MC01-110 (21.4)
EA02MG05-180 (24.1)
CB10034b (30.2)
EA02MC08-130 (30.9)
BRMS042 (32.4)
BRMS050 (32.8)
SA10TC15-140 (33.8)
SA09TC14-370 (38.1)
CB10114 (56.4)
BnGMS417 (59.7)
Ol11-G11 (62.1)
Na14-G10 (62.8)
EA13MC03-90 (64.7)
EA04MC03-220 (66.2)
EA07MC08-130 (67.3)
EA03MC02-350 (70)
EA03MC06-320 (70.3)
EA03MC03-320 (71.9)
BnGMS315a (86)
FITO-036a (104.5)
FITO-036c (106.8)
SA10TC16-140 (110.6)
SA10TC16-160 (112.9)
EA16MC06-160 (116.8)
CB10036 (121.4)
BN12Aa (132.2)
BN12Ac (134.5)
SL
SL
SL
SL
N4
CB10320 (0)
SA13TC14-170 (15.4)
SA13TC14-210 (23.9)
EA13MC01-300 (32.1)
SA10TC01-310 (43)
Na12-EO6A (44.9)
SA03TC16-220 (46.4)
SA03TC16-150 (47.1)
EA02MG05-125 (54.6)
Ol13-D02A (55.8)
EA01MC10-70 (58)
EA04MC02-120 (63.8)
EA02MC08-220 (65)
EA07MC10-70 (66.1)
BRAS021 (68.5)
BRAS003 (73.6)
Na10-C01 (80.4)
EA16MC02-250 (85.6)
BnGMS160a (107.6)
BnGMS160c (110.1)
CB10493B (113.1)
SL


N7
CB10299 (0)
SA06TC07-90 (1.5)
EA02MC05-200 (10.5)
EA05MC11-70 (14)
EA13MC01-140 (19.2)
Ra2-A01 (20.7)
BRAS004 (23.3)
EA16MC06-220 (24.4)
BnGMS491 (28.1)
EA01MC12-110 (32.2)
BnGMS147 (38.5)
BnGMS488 (50.6)
SA12TC16-400 (58.1)
EA08MC16-170 (71.1)
SL
SW
SW
SW
SL
SS
SS
SS
SS
SL
SL
SL
N8
EA02MG05-210 (0)
CB10026 (7)
SA10TC11-100 (9.3)
SA03TC09-370 (10.1)
SA03TC09-650 (10.4)
SA08TC07-210 (10.8)
CB10364 (11.5)
BnGMS373 (12.4)
EA02MC08-380 (13.1)
SA06TC11-140 (14.1)
SA09TC14-180 (14.8)
SA06TC04-270 (19.7)
S77096 (27)
SCZ18 (33.7)


SW
SW
SS
SS
SS
SS
SL
SL
SL
SL
BoGMS1413 (0)
EA08MC13-150 (20.7)
SA09MC04-220 (38.2)
SA07MC15-220 (53.3)
EA13MC03-150 (68.5)
Na10-D07a (75)
BnGMS299a (79.9)
SA09TC06-180 (82.2)
SA04TC13-150 (87.4)
BnGMS185 (90)
SA08TC07-190 (93.1)
SA13TC01-70 (100.1)
BnGMS43 (106.2)
BnGMS213b (123.1)
Ol13-C03 (147.2)

Mapping QTL for silique length , seeds per silique and seed weight

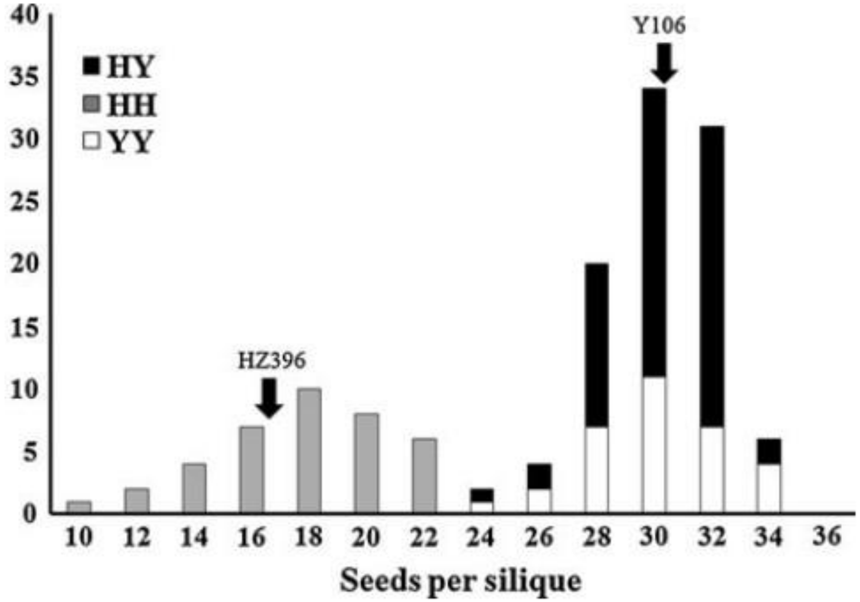
			2007			2008						2009		
						- I			- II					
QTL	a)连锁群 Linkage group	QTL位置 Position (cM)	LOD	b)A	c)PVE (%)	LOD	A	PVE (%)	LOD	A	PVE (%)	LOD	A	PVE (%)
qSL.A3-1	A3	42.2-56.4				2.84	0.25	11.16	2.82	0.28	9.16	2.79	0.29	10.70
qSL.A3-2	A3	56.5-61.7				3.54	0.23	8.85						
qSL.A3-3	A3	62.1-67.2										2.63	0.22	6.48
qSL.A4	A4	0.6-18.7	2.19	-0.19	7.08									
qSL.A7-1	A7	11.1-18.0							2.08	0.18	5.71			
qSL.A7-2	A7	57.9-68.1	2.81	0.19	7.90									
qSL.A8-1	A8	0.0-3.8	8.15	-0.30	18.80	4.11	-0.24	10.41	4.56	-0.37	13.14	3.52	-0.27	8.93
qSL.A8-2	A8	16.7-26.6	2.16	0.28	7.71									
qSL.C2	C2	43.1-55.3	3.06	0.18	7.09	3.48	0.24	9.6						
qSL.C3-1	C3	0.0-5.5	3.31	-0.18	7.02							3.14	-0.25	7.83
qSL.C3-2	C3	0.0-15.8				2.24	-0.18	5.86						
qSL.C4	C4	53.8-65.6										2.31	0.21	5.67
qSL.C6	C6	0.0-8.6	3.72	0.20	7.92							2.77	0.24	6.87
qSL.C9	C9	19.3-24.1	10.9	0.34	23.45	15.0	0.46	36.04	12.5	0.48	36.70	8.7	0.42	20.38
qSS.A8-1	A8	0.0-2.7				2.11	-1.82	6.04	4.38	-3.10	12.23	2.14	-2.05	6.16
qSS.A8-2	A8	9.3-10.8	2.36	2.71	10.60	2.37	3.40	10.15				2.92	4.07	7.28
qSS.A8-3	A8	17.5-25.9							2.90	2.73	9.63			
qSS.A8-4	A8	19.7-26.3	2.52	2.35	8.35	2.41	2.83	8.27						
qSS.C3-1	C3	0.0-5.2	3.59	-1.77	10.72							5.79	-3.21	15.21
qSS.C3-2	C3	0.0-11.4				3.42	-2.31	9.70	3.33	-2.06	9.83			
qSS.C9	C9	19.7-24.2	22.2	4.3	47.70	27.1	6.2	65.35	19.5	5.4	60.99	22.1	6.8	57.03
*qSW*A2-2	A2	0.0-19.0				2.25	0.22	8.34						
*qSW*A2-2	A2	24.0-24.8				3.67	0.42	10.98						
qSW.A2-3	A2	27.4-32.3	3.23	0.46	13.59									
qSW.A7-1	A7	4.1-17.8				2.06	-0.20	6.28						
qSW.A7-2	A7	47.5-66.7	3.11	0.23	10.38	3.20	0.27	11.70						
qSW.C3	C3	0.0-10.4	2.01	0.39	7.30	2.13	0.19	7.10						
qSW.C9	C9	19.8-24.5	12.1	-0.46	37.38	13.78	-0.47	37.21						

Development of NIL for the major QTL(*qSS.C9*) for seeds per silique (SS) on linkage groups C9

- Near-isogenic lines (BC_3F_2) for *qSS.C9* was produced by successively backcrossing the high SS parent Y106 with the recurrent parent HZ396.
- Construction of the local linkage map using SSR and AFLP markers linked to the *qSS.C9* locus
- QTL analysis in the BC_3F_2 population of HZ396×Y106

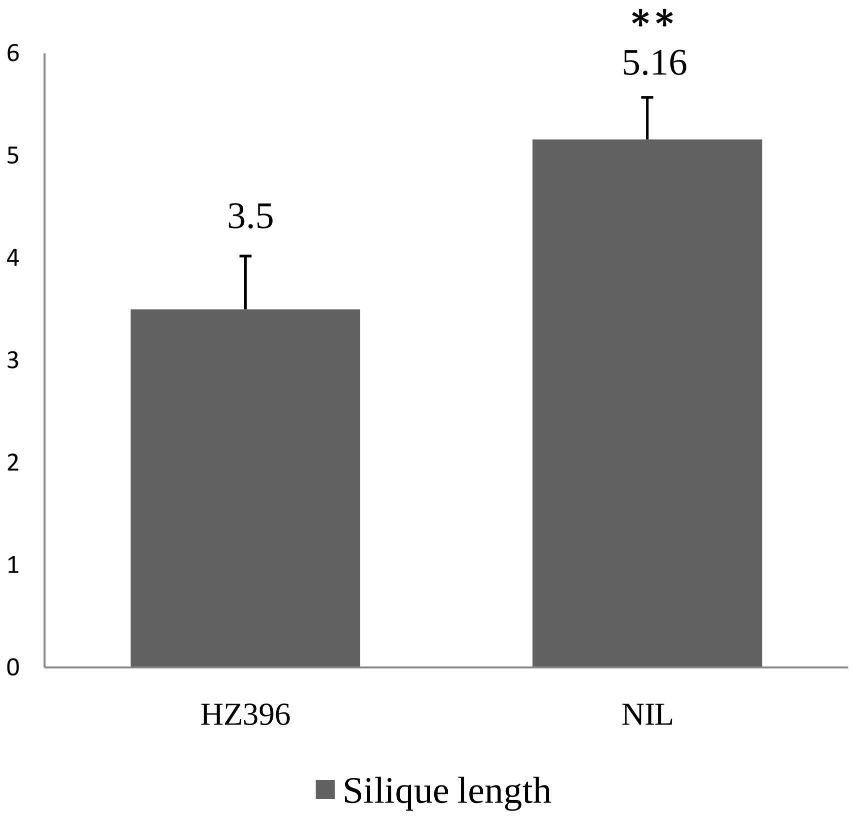
Phenotypic variations in the BC$_3$F$_2$ population

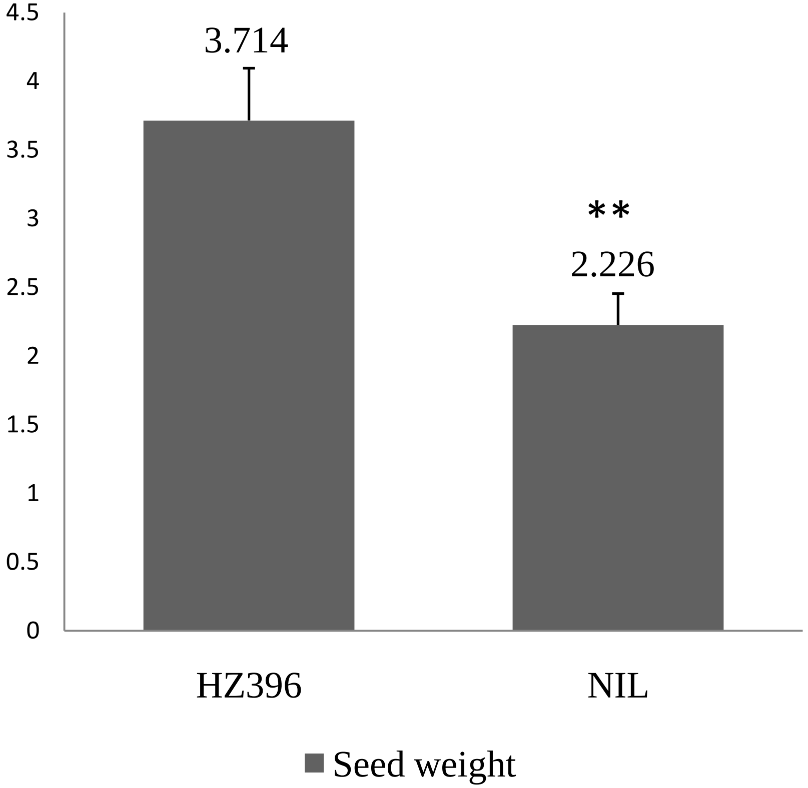
HZ396 NIL Y106



genotype	NO.of plant	SS
HH(HZ396)	40	17.5±2.72
HY(Y106/HZ396)	77	29.3±1.83
YY(Y106)	35	29.7±2.32

$\chi 2=0.41$

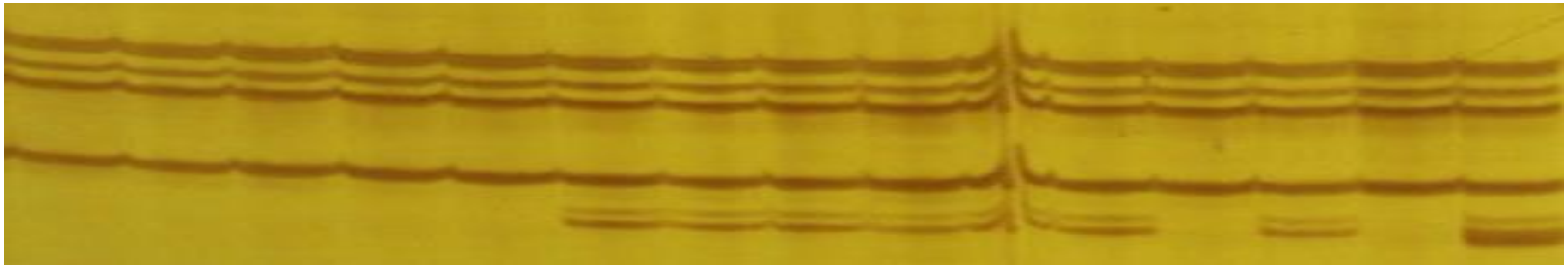

**
5.16
3.5
6
5
4
3
2
1
0
HZ396
NIL
■ Silique length


3.714
**
2.226
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
HZ396
NIL
■ Seed weight

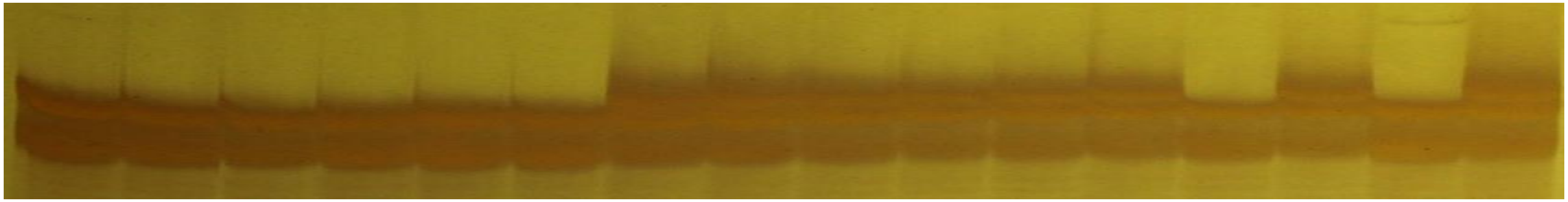
Development of AFLP and SSR markers linked to *qSS.C9*

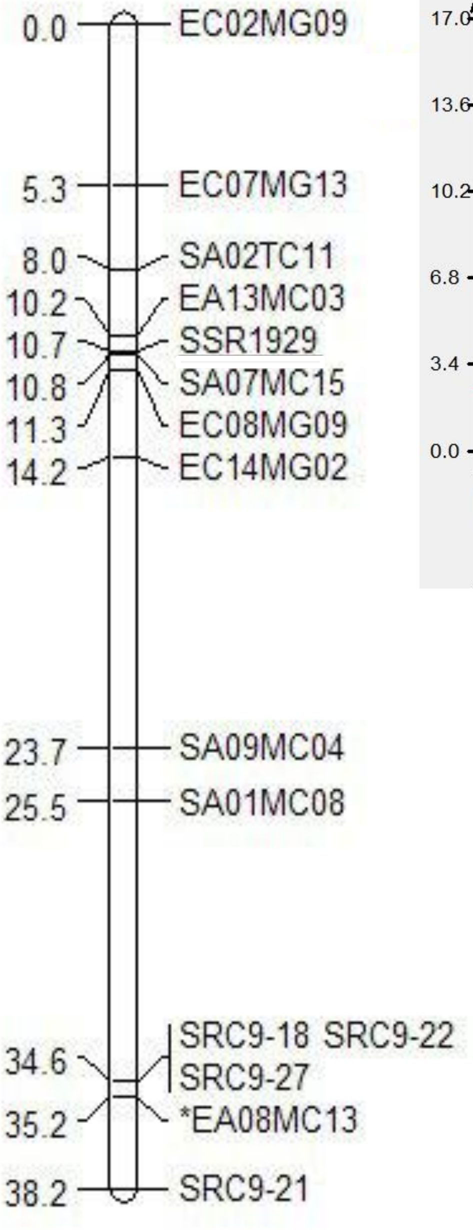
- To identify molecular markers linked to *qSS.C9*. Ten AFLP and five SSR markers linked to the locus were identified by bulked segregation analysis

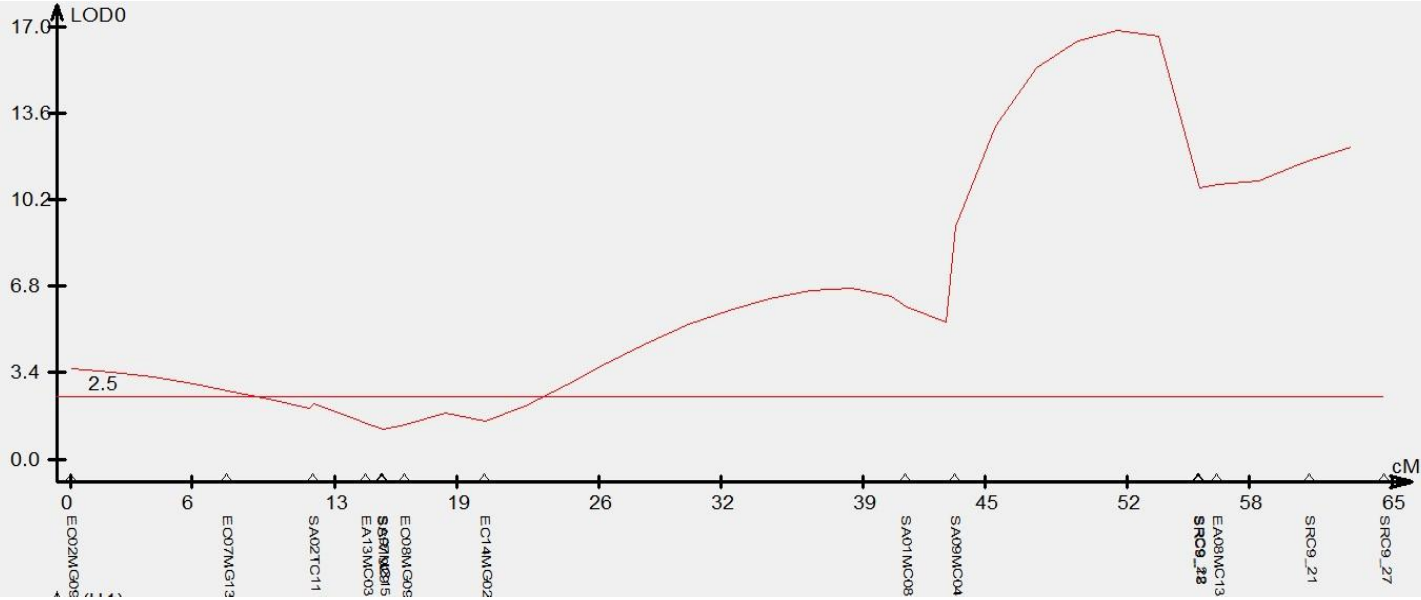
Individuals of low-SS bulks　Individuals of high-SS bulks　BL　BH　PL　PH



Individuals of low-SS bulks　Individuals of high-SS bulks　BL　BH　PL　PH

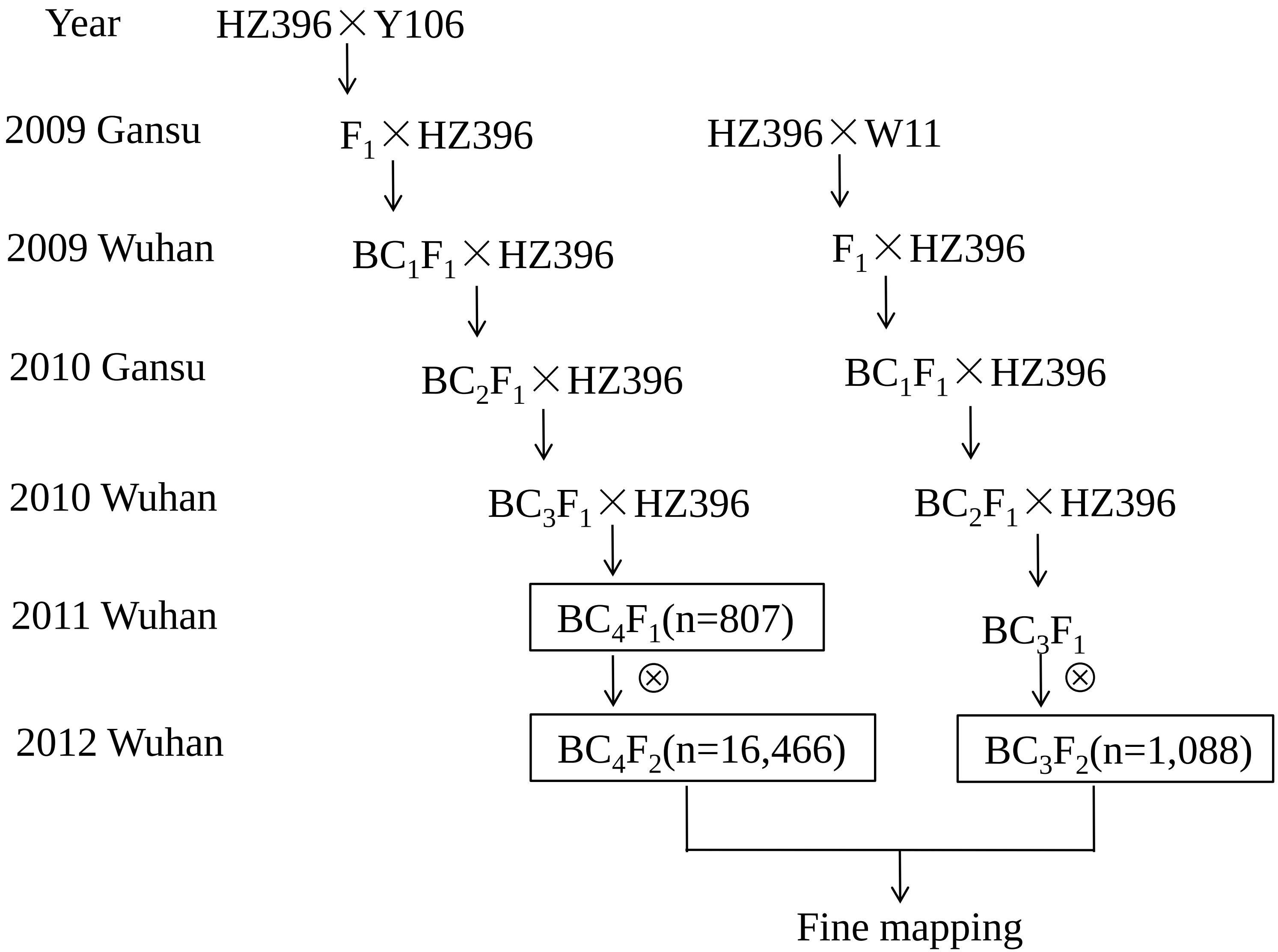






QTL analysis based on the BC_3F_2 population showed that this locus explained 85.8% of phenotypic variance with additive and dominant effects of 6.1 and 5.7 SS, respectively.

Fine mapping of *qSS.C9*



Year HZ396×Y106

2009 Gansu F_1×HZ396 HZ396×W11

2009 Wuhan BC_1F_1×HZ396 F_1×HZ396

2010 Gansu BC_2F_1×HZ396 BC_1F_1×HZ396

2010 Wuhan BC_3F_1×HZ396 BC_2F_1×HZ396

2011 Wuhan BC_4F_1(n=807) BC_3F_1

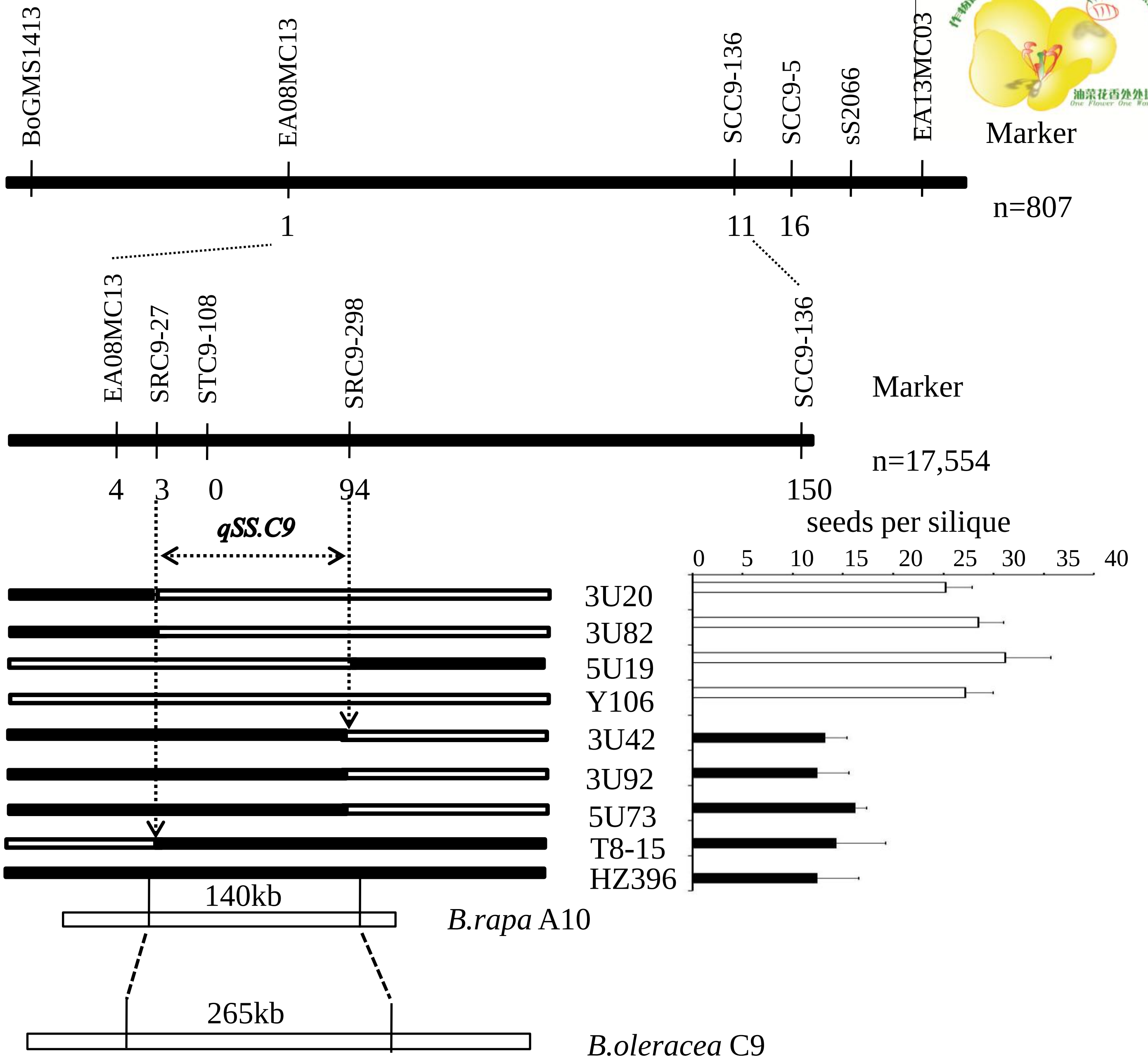
⊗ ⊗

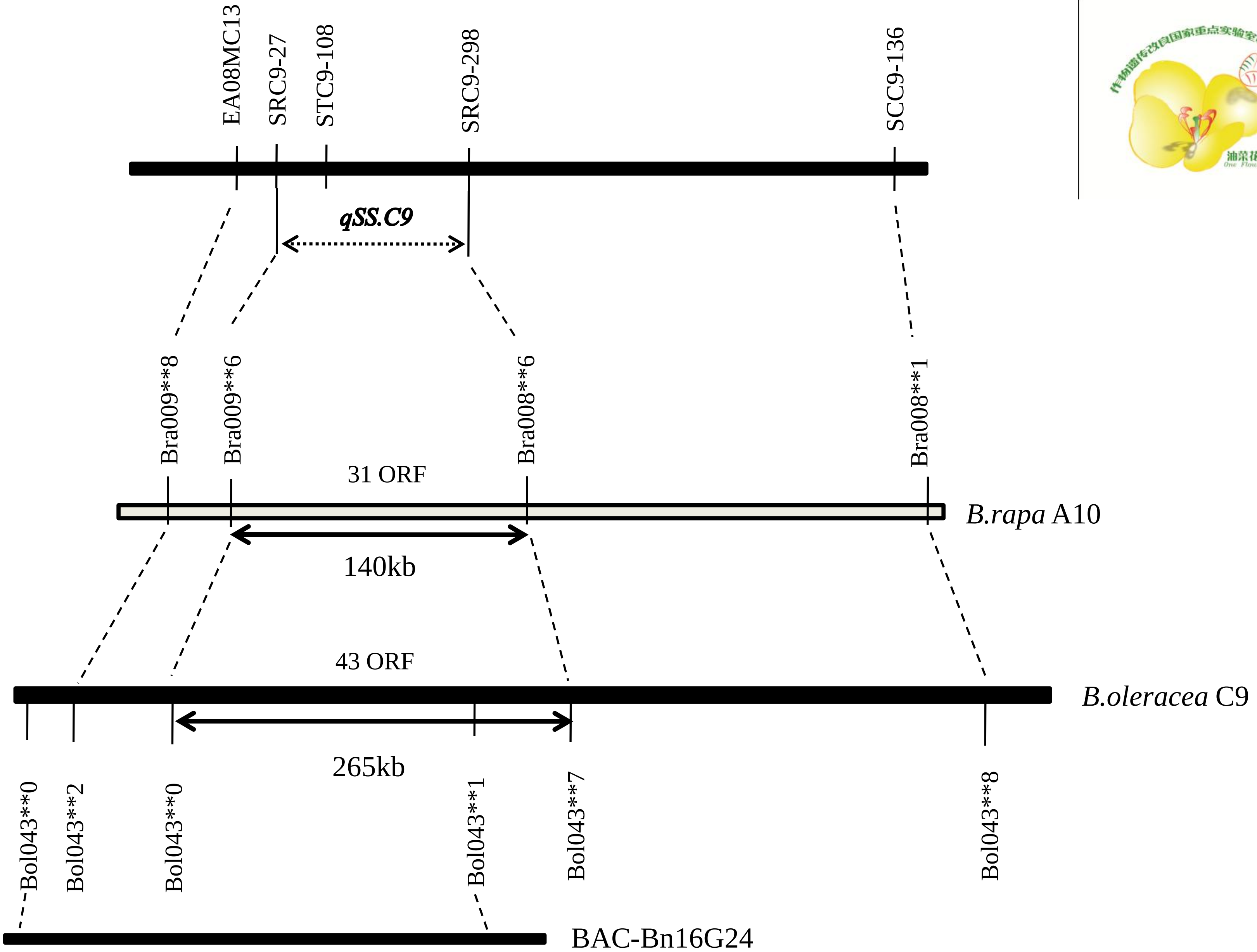
2012 Wuhan BC_4F_2(n=16,466) BC_3F_2(n=1,088)

Fine mapping

the mapping population of the *qSS.C9*

Year	Population	NO.of plant
2011	BC_4F_1(HZ296×Y106)	807
2012	BC_4F_2(HZ296×Y106)	16,466
	BC_3F_2(HZ296×W11)	1,088


BoGMS1413
EA08MC13
SCC9-136
SCC9-5
sS2066
EA13MC03
Marker
1
11
16
n=807
EA08MC13
SRC9-27
STC9-108
SRC9-298
SCC9-136
Marker
4
3
0
94
150
n=17,554
qSS.C9
seeds per silique
0
5
10
15
20
25
30
35
40
3U20
3U82
5U19
Y106
3U42
3U92
5U73
T8-15
HZ396
140kb
B.rapa A10
265kb
B.oleracea C9



- Comparative sequence analysis between parental lines showed that two genes would be likely the candidate genes of *qSS.C9*.


漫山遍野・油菜花

[Photo By Terry · TerryVision]

Thank You