Molecular mapping of new downy mildew resistance genes, Pl_{37} and Pl_{38}, in sunflower

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Background

❖ **Main causal organism:** *Plasmopara halstedii,*
the most destructive foliar disease in sunflower

Fig. 1a Chlorosis on upper leaf surface

Fig. 2 Stunting of downy mildew infected sunflower plants (in yellow oval) as compared to healthy plants.

Fig. 1b Abundant white sporulation on leaf surface

Background-cont.

- ❖ Prior to 1980, only two DM races were known, one each in Europe and North America
- ❖ To date, at least 24 DM races were identified in Europe and 40 races in North America
- ❖ Control: Registered fungicides and host resistance
- ❖ Host resistance provides an economically and environmentally friendly method of controlling downy mildew

Background-cont.

❖ A total of 37 DM R genes (Pl_1–Pl_{36}, Pl_{Arg}) have been reported in sunflower

❖ Many DM *R* genes in the sunflower gene pool have become ineffective in the field due to ever-changing pathogen populations rendering most of the world's sunflower production susceptible to devastating epidemics

❖ The identification of new resistance genes is necessary to successfully counter the continuous evolution of the DM pathogen

Materials and Methods

I. Mapping populations

Pop1: BC_1F_2 and BC_1F_3 from the cross of HA 89/*H. annuus* PI 435417

Pop2: BC_1F_2 and BC_1F_3 from the cross of HA 89/*H. praecox* PRA-417

II. DM evaluation of the BC_1F_3 populations

Pop1: 140 families (30 seedlings/family)
Pop2: 122 families (30 seedlings/family)

Materials and Methods-cont.

III. Genotyping of the BC_1F_2 populations

Pop1: using an Optimal GBS AgriSeq™ Panel with 768 mapped SNP markers at BDI
Pop2: Genotyping-by-sequencing at UMN

IV. Saturation mapping

Pop1: additional 161 SNPs in the Pl_{37} gene target region
Pop2: additional 35 SNPs in the Pl_{38} gene target region

Results

I. Mapping of the DM *R* gene *Pl*$_{37}$ from the wild *H. annus* PI 435417

❖ **DM phenotyping**

A total of 140 BC$_1$F$_3$ families were phenotyped with DM hot race 734.

36 homozygous susceptible

72 heterozygous resistant

32 homozygous resistant

❖ The DM resistance derived from PI 435417 is controlled by a single dominant gene named as *Pl*$_{37}$
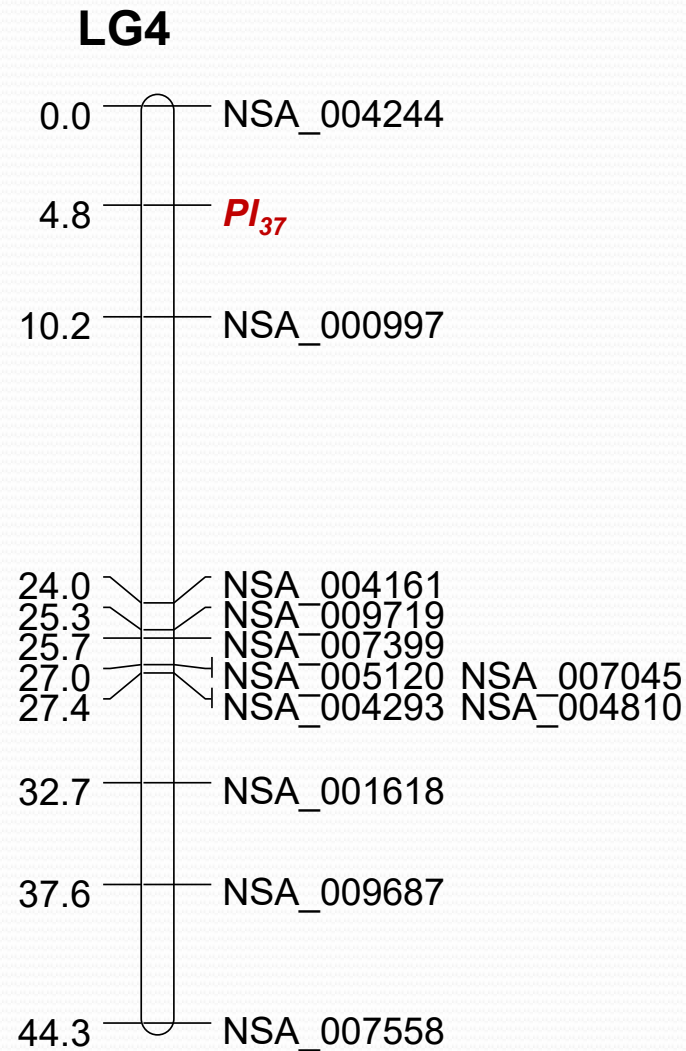
Results-cont.

❖ **Genotyping of Pop1**

Table 1 Distribution of polymorphic SNPs in the sunflower genome in the population of HA 89/*H. annuus* PI 435417

Chromosome	No. polymorphic SNP
1	3
2	6
3	3
4	12
5	10
6	0
7	3
8	4
9	0
10	5
11	8
12	4
13	0
14	1
15	9
16	9
17	1
Total	78/768

Results-cont.

❖ **Linkage analysis**

Fig. 1 Genetic map of PI_{37} on sunflower chromosome 4

LG4

0.0	NSA_004244
4.8	PI_{37}
10.2	NSA_000997
24.0	NSA_004161
25.3	NSA_009719
25.7	NSA_007399
27.0	NSA_005120 NSA_007045
27.4	NSA_004293 NSA_004810
32.7	NSA_001618
37.6	NSA_009687
44.3	NSA_007558

Results-cont.

❖ Saturation mapping of the Pl_{37} region

Pl_{37} was mapped to LG4 close to Pl_{17} and Pl_{33}. A total of 161 SNPs from the fine maps of Pl_{17} and Pl_{33} were used to saturate the Pl_{37} region and 19 were mapped to Pl_{37} target region

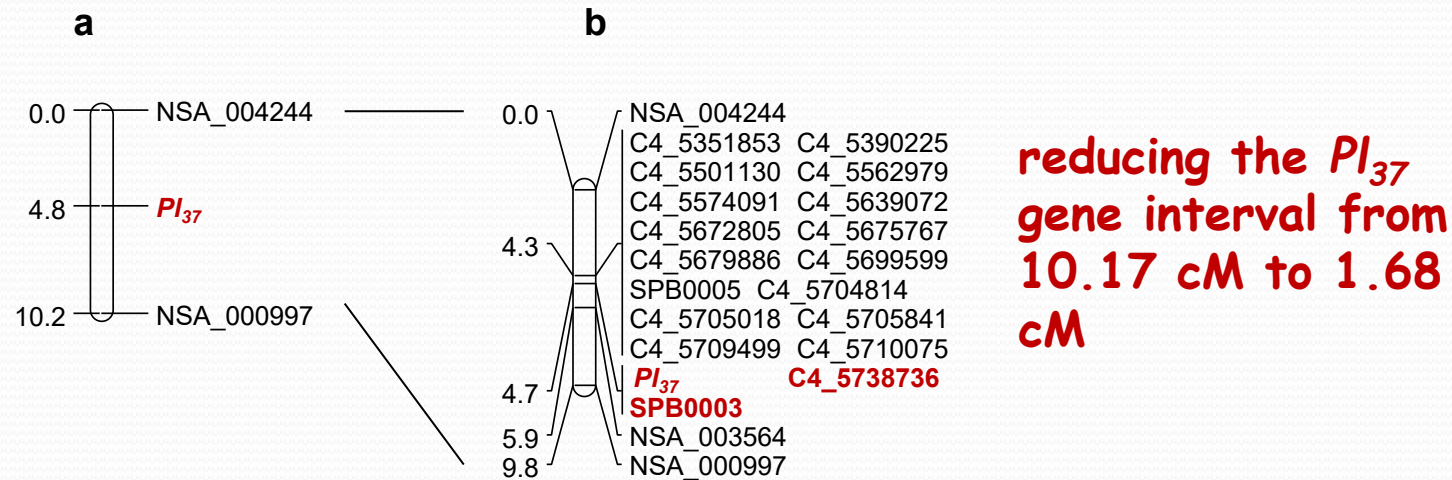reducing the Pl_{37} gene interval from 10.17 cM to 1.68 cM

Fig. 2 Genetic maps of Pl_{37} on sunflower chromosome 4.
a) basic map, b) saturation map

Results-cont.

❖ Pl_{37} is different from Pl_{17} and Pl_{33}

1. A common marker SPB005 is mapped downstream of Pl_{17} and Pl_{33}, but upstream of Pl_{37}

2. The diagnostic marker alleles specific to the Pl_{17} and Pl_{33} genes were not detected in the plant with Pl_{37}
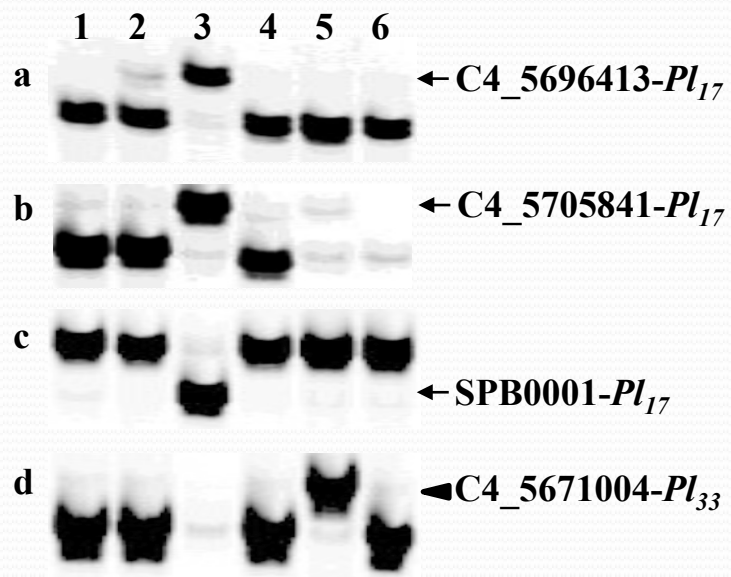

Fig. 3 PCR patterns of SNP markers diagnostic for Pl_{17} and Pl_{33} in six sunflower lines, HA 434 (lane 1), HA 89 (lane 2), HA 458/Pl_{17} (lane 3), HA-DM5/Pl_{19} (lane 4), TX16R/Pl_{33} (lane 5), and 17-32-33/Pl_{37} (lane 6)

Results-cont.

II. Mapping of the DM R gene Pl_{38} from the wild *H. praecox* PRA-417

❖ DM phenotyping

A total of 122 BC_1F_3 families were phenotyped with DM hot race 734

28 homozygous susceptible

68 heterozygous resistant

26 homozygous resistant

❖ The DM resistance derived from PRA-417 is controlled by a single dominant gene named as Pl_{38}
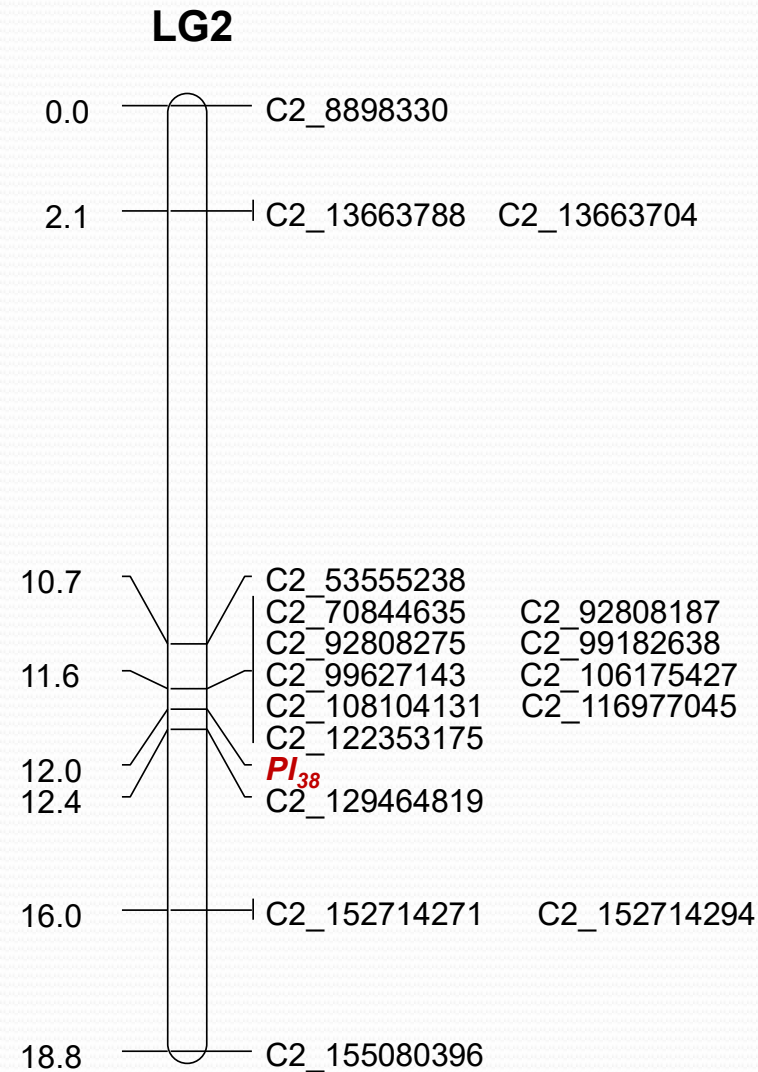
Results-cont.

❖ Genotyping of Pop2

Table 2 Distribution of polymorphic SNPs in the population of HA 89/*H. praecox* PRA417 by genotyping-by-sequencing

Chromosome	Total No. of polymorphic SNPs	No. of distorted SNPs/(%)	No. of non-distorted SNPs
1	59	51 (86%)	8
2	997	466 (47%)	531
3	88	78 (89%)	10
4	92	76 (83%)	16
5	115	109 (95%)	6
6	125	70 (57%)	55
7	78	67 (86%)	11
8	247	109 (44%)	138
9	73	70 (96%)	3
10	141	109 (77%)	32
11	61	46 (75%)	15
12	125	65 (52%)	60
13	105	87 (83%)	18
14	166	114 (69%)	52
15	139	101 (73%)	38
16	216	114 (53%)	102
17	173	105 (61%)	68
Total	3000	1837 (61%)	1163

Results-cont.

❖ **Linkage analysis**

Fig. 4 Genetic maps of the DM *R* gene *Pl$_{38}$* on sunflower LG2

LG2

Position	Markers
0.0	C2_8898330
2.1	C2_13663788 C2_13663704
10.7	C2_53555238
	C2_70844635 C2_92808187
	C2_92808275 C2_99182638
11.6	C2_99627143 C2_106175427
	C2_108104131 C2_116977045
	C2_122353175
12.0	*Pl$_{38}$*
12.4	C2_129464819
16.0	C2_152714271 C2_152714294
18.8	C2_155080396

Results-cont.

❖ **Saturation mapping of the Pl_{38} region**

Pl_{38} was mapped to LG2 close to Pl_{18}. A total of 35 SNPs from the public SNP map and Pl_{18} fine map were used to saturate the Pl_{38} region and 8 were mapped to Pl_{38} target region

a

10.7	C2_53555238
	C2_70844635 C2_92808187
	C2_92808275 C2_99182638
11.6	C2_99627143 C2_106175427
	C2_108104131 C2_116977045
	C2_122353175
12.0	*Pl_{38}*
12.4	C2_129464819
16.0	C2_152714271 C2_152714294
18.8	C2_155080396

b

10.7	C2_53555238
	C2_70844635 C2_92808187
	C2_92808275 C2_99182638
11.5	C2_99627143 C2_106175427
	C2_108104131 C2_116977045
	C2_122353175
	SFW03013 SFW04107
	SFW04108 S2_128982087
11.9	C2_128624983 C2_128872074
	C2_128916238 *Pl_{38}*
12.3	C2_129464819
13.6	SFW03883
16.0	C2_152714294 C2_152714271
18.6	C2_155080396

reducing the Pl_{38} gene interval from 7.1 Mb to 0.55 Mb

Fig. 5 Genetic maps of Pl_{38} on sunflower chromosome 2. a) basic map, b) saturation map, the new SNP markers are underlined

Results-cont.

❖ Pl_{38} is different from Pl_{18}

1. Pl_{38} and Pl_{18} locate in a small region on LG2. Out of 23 SNP markers selected from the Pl_{18} map, only four markers were mapped to the Pl_{38} region

2. The 12 diagnostic marker alleles specific to the Pl_{18} genes were not detected in RPA-417 with Pl_{38}
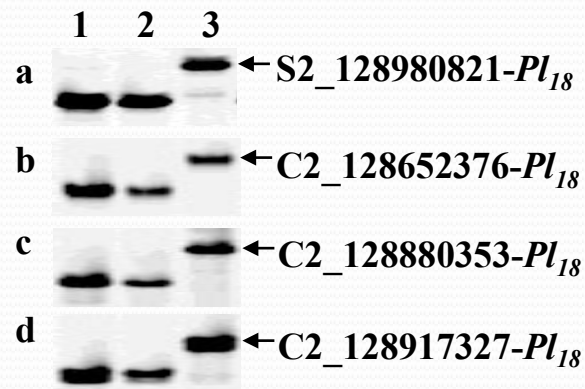
Fig. 6 PCR patterns of the SNP markers diagnostic for Pl_{18} in the three sunflower lines, HA 89 (lane 1), RPA-417/Pl_{38}(lane 2), and HA-DM1/Pl_{18} (lane 3)

Results-cont.

❖ Downy mildew resistance spectra *of Pl$_{37}$* and *Pl$_{38}$*

Table 3 Downy mildew resistant tests of the homozygous families of *Pl$_{37}$* and *Pl$_{38}$* with seven *P. halstedii* races

Line	Material	P. halstedii races												
		700		707		714		730		734		770		774	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R
HA 89	S-check	15	0	15	0	14	0	12	0	14	0	18	0	14	0
HA-DM1	R-check	0	15	0	15	0	15	0	13	0	15	0	15	0	12
17-32-177	*Pl$_{37}$* homo BC$_1$F$_3$ family	0	21	0	31	0	29	0	26	0	27	0	19	0	30
17-32-213	*Pl$_{37}$* homo BC$_1$F$_3$ family	0	28	0	29	0	30	0	16	0	18	0	24	0	30
17-32-249	*Pl$_{37}$* homo BC$_1$F$_3$ family	0	20	0	31	0	26	0	16	0	15	0	21	0	12
19-06-3	*Pl$_{38}$* homo plant	0	21	0	30	0	29	0	17	0	23	0	23	0	22
19-06-4	*Pl$_{38}$* homo plant	0	27	0	31	0	29	0	29	0	28	0	27	0	30
19-06-5	*Pl$_{38}$* homo plant	0	25	0	28	0	29	0	25	0	27	0	22	0	25

Results-cont.

❖ **Germplasm development in oilseed sunflower with** Pl_{37} **or** Pl_{38}

1. HA-DM15/Pl_{37} was selected from the BC_1F_3 population of the cross HA 89//NMS HA 89/*H. annus* PI 435414

2. HA-DM16/Pl_{38} was selected from the RF PRA-417 line which was originally derived from CMS HA 89/*H. praecox* PRA-417

58 RF PRA-417

12 resistant   46 susceptible

3 homozygous resistant

HA-DM16/Pl_{38}
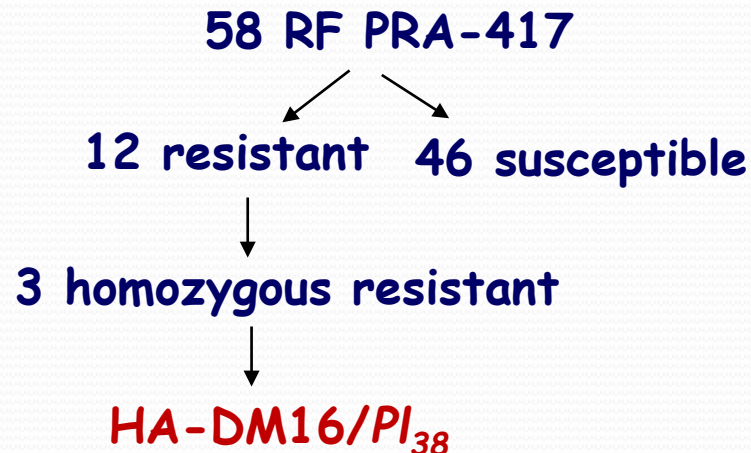
Summary of genetic mapping of 21 *R* genes (2010-2022)

Table 4 The 10 DM R genes mapped by Qi's lab

Gene	Linkage Group	R gene donor line	Publications
Pl_{17}	4	HA 458	2015 Theor Appl Genet 128:757–767
Pl_{18}	2	*H. argophyllus* PI494573	2016 Theor Appl Genet 129:741–752
Pl_{19}	4	*H. annuus* PI	2017 Theor Appl Genet 130:29–39
Pl_{20}	8	*H. argophyllus* PI494573	2017 Theor Appl Genet 130:1519–1529
Pl_{33}	13	TX16R	2019 Mol Breeding 39:19
Pl_{34}	13	RHA 428	2019 Frontiers in Genetics 10:216
Pl_{35}	1	*H. argophyllus* PI494573	2019 Theor Appl Genet 132:2553–2565
Pl_{36}	13	803-1	2022 Mol Breeding 42:8
Pl_{37}	4	*H. annuus* PI 435414	2023 Theor Appl Genet (in review)
Pl_{38}	2	*H. praecox* PRA-417	2023 Theor Appl Genet (in review)

Table 5 The 11 rust R genes mapped by Qi's lab

Gene	Linkage Group	R gene donor line	Publications
R_4	13	HA-R3	2011 Theor Appl Genet 123:351-358
R_{11}	13	Rf ANN-1742	2012 Theor Appl Genet 125:921-932
R_5	2	HA-R2	2012 Mol Breeding 30:745-756
R_{12}	11	RHA 464	2013 Theor Appl Genet 126:93-99; 2014 PLOS ONE 7:e98628
R_{13a}	13	HA-R6	2013 Theor Appl Genet 126:2039-2049
R_{13b}	13	RHA 397	2013 Theor Appl Genet 126:2039-2049
R_2	14	MC29	2015 Theor Appl Genet 128:477-488
R_{15}	8	HA-R8	2018 Theor Appl Genet 31:1423-1432
R_{16}	13	TX16R	2019 Mol Breeding 39:19
R_{17}	13	KP193	2022 Theor Appl Genet 134:2291-2301
R_{18}	13	KP199	2022 Theor Appl Genet 134:2291-2301

Summary of 37 released germplasms (2013-2022)

Table 6 The 12 released germplasms with one R gene from Qi's lab

Name	Type	Resistance gene	Year released
HA-R9	Oilseed	R_{11}	01-2013
HA-R10	Confection	R_5	12-2013
HA-R11	Confection	R_4	12-2013
HA-R18	Oilseed	R_{17}	10-2020
HA-R19	Oilseed	R_{18}	10-2020
HA-DM1	Oilseed	Pl_{18}	06-2015
HA-DM5	Confection	Pl_{19}	06-2017
HA-DM6	Confection	Pl_{35}	07-2019
HA-DM7	Oilseed	Pl_{20}	07-2019
HA-DM8	Oilseed	Pl_{35}	03-2020
HA-DM15	Oilseed	Pl_{37}	in process
HA-DM16	Oilseed	Pl_{38}	in process

Table 7 The 17 released germplasms with multi-R genes from Qi's lab

Name	Type	Resistance gene	Year released
HA-R12	Confection	R_2/R_{13a}	07-2014
HA-R13	Confection	R_5/R_{13a}	07-2014
HA-R14	Oilseed	$R_4/R_{12}/Pl_{Arg}$	01-2020
HA-R15	Oilseed	$R_5/R_{12}/Pl_{Arg}$	01-2020
HA-R16	Oilseed	$R_{13b}/R_{12}/Pl_{Arg}$	01-2020
HA-R17	Oilseed	R_{13b}/R_{15}	01-2020
HA-R20	Confection	$R_{12}/R_{15}/Pl_{Arg}$	08-2022
HA-R21	Confection	$R_{13a}/R_{15}/Pl_{17}$	08-2022
HA-DM2	Confection	Pl_{Arg}/R_{12}	06-2017
HA-DM3	Confection	Pl_{17}/R_{13a}	06-2017
HA-DM4	Confection	Pl_{18}/R_{13a}	06-2017
HA-DM9	Oilseed	Pl_8/Pl_{17}	08-2021
HA-DM10	Oilseed	Pl_8/Pl_{18}	08-2021
HA-DM11	Oilseed	Pl_{17}/Pl_{18}	08-2021
HA-DM12	Oilseed	$Pl_8/Pl_{Arg}/R_{12}$	08-2021
HA-DM13	Oilseed	$Pl_{17}/Pl_{Arg}/R_{12}$	08-2021
HA-DM14	Oilseed	$Pl_{18}/Pl_{Arg}/R_{12}$	08-2021

Table 8 The 8 released germplasms with BSR resistance from Qi's lab

Name	Type	Resistance gene	Year released
HA-BSR1	Oilseed	BSR resistance from HA441/RHA439	08-2016
HA-BSR2	Oilseed	BSR resistance from *H. petiloris* + Pl_{17}	10-2017
HA-BSR3	Oilseed	BSR resistance from *H. argophyllus* + Pl_{17}	10-2017
HA-BSR4	Oilseed	BSR resistance from *H. argophyllus* + Pl_{17}	10-2017
HA-BSR5	Oilseed	BSR resistance from *H. argophyllus*	10-2017
HA-BSR6	Oilseed	BSR resistance from *H. pracoxes* + Pl_{17}	10-2017
HA-BSR7	Oilseed	BSR resistance from *H. pracoxes* + Pl_{17}	10-2017
HA-BSR8	Oilseed	BSR resistance from *H. pracoxes* + Pl_{17}	10-2017

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