2022 Progress for enhancing rust resistance in confection sunflower production through next-generation technologies

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Main causal organism: fungus Puccinia helianthi

 Greenhouse Screening
 Field Screening
 Image: Constraint of the streening
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Rust threats sunflower production

- One of the most serious diseases of sunflower in the world with an increasing importance in North America in recent years
- Number of North American (NA) rust races identified currently: 38
- Control: Registered fungicides and host resistance
- Using of resistant hybrids is most effective management tool (economic & environmental)

Genetics of rust resistance in sunflower

- Single dominant genes in sunflower control rust resistance
- A total of 19 rust resistance (R) genes have been discovered in sunflower
- New rust races make current R genes ineffective
 - Out of 19 rust R genes, 7 (R₁₁, R₁₂, R_{13a}, R_{13b}, and R₁₄-R₁₆) remain effective to all rust races

Research objectives (2022)

- Stack effective rust R genes to generate multi-disease resistant lines
- Construct high density SNP genetic map of R_{12}
- Develop diagnostic molecular markers for the R₁₂ gene
- Identify candidate genes associated with R_{12} in the sunflower genome

R genes stack



Marker selection of three gene pyramids in the F_2 populations

I. One homozygous F_2 with three genes $(R_{12}/R_{15}/PI_{Arg})$ was idetified from 188 F_2 plants of the cross HA-DM2/HA-R8



Fig. 1. 1: HA-DM2 (R_{12}/PI_{Arg}) , 2: HA-R8 (R_{15}) , 3: triple-gene plant $(R_{12}/R_{15}/PI_{Arg})$

Table 1 Marker confirmation of the selected homozygous triplegene F_2 plants from the cross of HA-DM2/HA-R8

		DNA marker for R ₁₂			DNA marker for Pl _{Arg}			DNA marker for R ₁₅		
Line No.	Gene	<i>C</i> 11_147312085	S11_189205190	<i>c</i> 11_149085167	NSA_001570	NSA_005063	NSA_005423	NSA_002851	SUN398	SUN406
HA-DM2	R ₁₂ /PI _{Arg}	Α	A	Α	Α	Α	Α	A	-	-
HA-R8	R ₁₅	-	-	-	-	-	-	-	В	В
20-087-187	$R_{12}/R_{15}/PI_{Arg}$	Α	A	Α	Α	Α	Α	Α	В	В

Note, "A" represents HA-DM2 marker allele and "B" represents HA-R8 marker allele. The marker used in the initial screen is red.

II. Four homozygous F_2 with three genes $(R_{13a}/R_{15}/PI_{17})$ were identified from 188 F_2 plants of the cross HA-DM3/HA-R8



Fig. 2. 1: HA-DM3 (R_{13a}/PI_{17}) , 2: HA-R8 (R_{15}) , 3-6: triplegene plants $(R_{13a}/R_{15}/PI_{17})$

Table 2 Marker confirmation of the selected homozygous triplegene F_2 plants from the cross of HA-DM3/HA-R8

		Ma	irker	for l	R _{1 3a}	Mark	ker fo	r <i>Pl₁₇</i>	Ma	arker	for	R ₁₅
Line No.	Gene	ORS316	c13_194268343	c13_194735854	НТ382	C4_5696413	SPB0001	SPB0007	SFW05824	SUN398	SUN406	S8_100385559
HA-DM3	R_{13a}/PI_{17}	A	Α	Α	A	A	Α	A	-	-	-	-
HA-R8	R ₁₅	-	-	-	-	-	-	-	В	В	В	В
20-088-38	$R_{130}/R_{15}/PI_{17}$	A	Α	Α	Α	A	Α	A	В	В	В	В
20-088-49	$R_{130}/R_{15}/PI_{17}$	A	Α	Α	H	A	Α	A	В	В	В	В
20-088-63	$R_{130}/R_{15}/PI_{17}$	A	Α	Α	A	Α	H	H	В	В	В	В
20-088-99	$R_{130}/R_{15}/PI_{17}$	A	Α	Α	Α	A	A	A	В	В	В	В

Note, "A" represents HA-DM3 marker allele, "B" represents HA-R8 marker allele, and "H" represents heterozygous. The marker used in the initial screen is red.

Downy mildew and rust evaluation of the released lines

Table 3 The results of downy mildew and rust tests for the released germplasms HA-R20 and HA-R21 along the parents and susceptible check

			DM scor rc	re (P. ace 73	halstedii 4)	Rust score (P. helianthi race 336)			
Lines	Plant No.	Genes	No. of plants tested	S	R	No. of plants tested	IT	Severity	
HA 89	18-1494-1	-	25	25	0	8	4	>40	
HA-R8	19-02	R ₁₅	8	0	8	8	1	0.1	
HA-DM2	16-1115	R ₁₂ /Pl _{Arg}	17	0	17	8	1	0.1	
HA-R20	21-1100	$R_{12}/R_{15}/PI_{Arg}$	62	0	62	98	0	0	
HA 89	18-1494-1	-	25	25	0	8	4	>40	
HA-R8	19-02	R ₁₅	8	0	8	8	1	0.1	
HA-DM3	17-023	R _{13a} /PI ₁₇	37	0	37	8	1	0.1	
HA-R21	21-1116a	$R_{13a}/R_{15}/PI_{17}$	57	0	57	119	0	0	

Marker validation of the released lines



Fig. 3. 1: HA-DM2 (R12/PlArg), 2: HA-R8 (R15), 3-48: 46 of 94 HA-R20 plants (R15/R12/PlArg) tested

Fig. 3. Marker validation of HA-R20. 1: HA-DM2 (R_{12}/PI_{Arg}) , 2: HA-R8 (R_{15}) , 3-48: 46 of 94 HA-R20 triple-gene plants $(R_{12}/R_{15}/PI_{Arg})$ tested



Fig. 3. Marker validation of HA-R21. 1: HA-DM3 (R_{13a}/PI_{17}), 2: HA-R8 (R_{15}), 3-48: 46 of 94 HA-R21 triple-gene plants ($R_{13a}/R_{15}/PI_{17}$) tested

Saturation mapping of R₁₂ (2021)

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SSR marker development

- Extracted 3.5 Mb sequence covering R₁₂ from the reference genome of HA412-HO
- Designed 58 pairs of SSR primers from the sequence
- Four SSR markers were mapped to the R₁₂ target region

SNP marker development

- Sequenced RHA 464 (R₁₂) with 40x genome coverage
- Selected 186 SNPs from the gene target region
- Screened the polymorphism between HA 89/RHA 464 (R₁₂)
- Genotyped 140 F₂ individuals with 52 polymorphic markers
- > Finally, 4 SSR and 24 SNP markers were mapped to the R_{12} target region



Fig. 5 Genetic maps of the rust resistance gene R_{12} . (a) R_{12} basic map, (b) R_{12} saturation map

Fine mapping of R_{12} (2021-2022)

- Two markers were used to screen recombinants from 2,004 individuals of a large population
- > Identified 63 recombinants
- Evaluated rust resistance of R₁₂ recombinant families
- > Genotyped R_{12} recombinants

Table 4 Summary of R_{12} fine mapping results

Marker	No.	Position in the fine	Physical position on XRQr1.0 assembly (bp)				
	recombination	map (cm)	Start	End			
C11_147312085	0	0.0000	147,311,885	147,312,285			
SUN129	5	0.1248	150,521,211	150,520,851			
C11_150451336	5	0.2496	150,451,136	150,451,536			
R ₁₂	2	0.2995	-	-			
S11_189205190	3	0.3744	150,450,528	150,450,748			
<i>C</i> 11_149180689	7	0.5491	149,180,489	149,180,889			
C11_149178667	1	0.5741	149,178,467	149,178,867			
<i>C</i> 11_149148043	2	0.6240	149,147,843	149,148,243			
<i>C</i> 11_149090985	5	0.7488	149,090,785	149,091,185			
<i>C</i> 11_149088669	0	0.7488	149,088,469	149,088,869			
C11_149085167	1	0.7738	149,084,967	149,085,367			
C11_149033221	3	0.8487	149,033,021	149,033,421			
S11_189699712	0	0.8487	149,033,186	149,032,786			
<i>C</i> 11_149028685	0	0.8487	149,028,485	149,028,885			
C11_149027585	0	0.8487	149,027,385	149,027,785			
NSA_001570	34	1.6970	148,685,411	148,685,130			

Reducing R_{12} interval from 2.4 Mb to 0.001 Mb



Fig. 6 Genetic maps of the rust resistance gene R_{12} . (a) R_{12} basic map, (b) R_{12} saturation map, and (c) R_{12} fine map.

Marker specificity test of R_{12}

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 $\begin{array}{c|c} & \text{NSA_003426 (146299234 bp)} \\ & 2.4 \text{ Mb} \\ & \text{NSA_001570 (148685411 bp)} \end{array} \begin{array}{c} & \text{R}_{12} \text{ (RHA 464)} \\ & \text{R}_{14} \text{ (PH3)} \end{array}$

A total of 24 SNP markers mapped to 2.4 Mb region were selected to test three lines, RHA 464, PH3, and HA 89





10 SNP markers showed the same PCR patterns in R_{12} /RHA 464 and R_{14} /PH3

Fig. 7 The PCR amplification pattern of SNP markers in the three lines, HA 89, R_{12} /RHA 464, and R_{14} /PH3

Eleven SNP markers were selected to test 96 sunflower lines, and three SNP markers can distinguish R_{12} from all other lines



Fig. 7 The PCR amplification pattern of SNP marker C11_149085167 diagnostic for R_{12} in the 96 selected sunflower lines. Lane 32, HA-DM2 confection sunflower line with R_{12} , and lane 49, RHA 464/ R_{12} . Lane 44, HA-R3 shows the heterozygous PCR pattern

Candidate gene for R_{12}

Table 5 Plant genes discovered in the R_{12} region of sunflower chromosome 11 in the XRQr1.0 assembly

Gene Name	Description	Position	Length
R12 interval		HanXRQChr11:150260000150460000	200,000 bp
HanXRQChr11g0348641	Putative papD-like; Ankyrin repeat-containing domain	HanXRQChr11:150278902150280515	1,614 bp
HanXRQChr11g0348651	Putative START-like domain; Bet v I type allergen	HanXRQChr11:150283162150283785	624 bp
HanXRQChr11g0348661	Putative NB-ARC; P-loop containing nucleoside triphosphate hydrolase; Leucine-rich repeat domain, L domain-like	HanXRQChr11:150349748150353942	4,195 bp

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