Identification, characterization and validation of cultivated sunflower lines with resistance to Sclerotinia basal stalk rot

Angidi Srushtideep[1], Julie S. Pasche[1] and William Underwood[2].
[1] Dept of Plant Pathology, North Dakota State University, Fargo, ND, USA.
[2] USDA-ARS, Sunflower & Plant Biology Research Unit, Fargo, ND, USA.

Introduction

Basal stalk rot (BSR) of cultivated sunflower (*Helianthus annuus L.*) is caused by necrotrophic fungal pathogen *Sclerotinia sclerotiorum* (Lib) de Bary and is an economically vital disease limiting sunflower production in the Northern Great Plains region of the USA. The mode of infection starts as a root infection and moves into the base of the stem, causing basal stem lesions, plant wilting, and premature senescence or death, resulting in reduced yield. Known resistance to *S.sclerotorium* is quantitative and no major gene is known for resistance (1). Therefore, the improvement of host plant resistance is an important goal for sunflower breeding because producers have limited options for managing this disease.

Objectives

1. Distinguish between highly and moderately resistant genotypes, by re-evaluating 60 cultivated sunflower genotypes that have shown some degree of resistance in field trials.
2. Molecular marker analysis for BSR resistance from previous identified QTL mapping to characterize resistant genotypes.
3. Evaluation of Genotype X isolates interactions with multiple fungal isolates of *S.sclerotiorum* to check broad spectrum resistance.

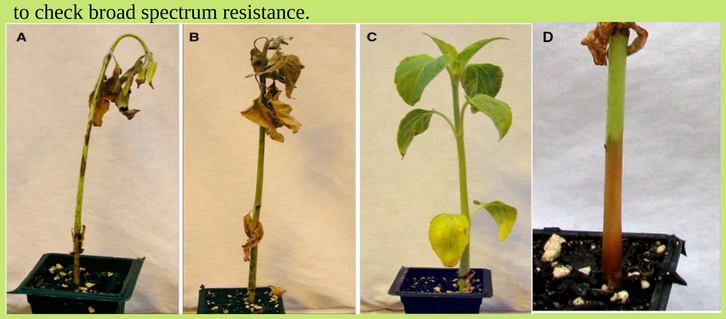

Figure 1: Sunflower Basal stalk rot symptoms observed in greenhouse. **A.** Plant exhibiting terminal wilt at 14 dpi. **B.** Plant exhibiting desiccation at 14 dpi. **C.** Plant exhibiting few or no symptoms at 14dpi. **D.** Lesions of BSR on stem observed after 14 dpi. (2)

Methodology

1. Five-week-old root bound plants were inoculated by removing plant and root mass from the pot and placing millet inoculum in the cup bottom and returning root bound plant to the pot. After inoculation they were evaluated for 28 days based on terminal wilt and plant desiccation.
2. The greenhouse phenotyping experiment was conducted three times, resulting in days to death data for 48 plants per accession, replicated three times.
3. The Levene's test was performed to confirm homogeneity of variance and the combined experiments were later analyzed by a generalized linear model. The post- hoc, Tukey test was used to identify significant differences among the lines.

Results and Discussion

Re-evaluation of 60 cultivated sunflower genotypes to distinguish between highly and moderately resistant genotypes

Table 1: Greenhouse evaluation of BSR resistance for 60 cultivated Sunflower inbred lines. Moderate resistance inbred line check RHA 801 (green in colour), moderate susceptible inbred line check HA 89 and OPV check Cabure 1004 (red in color). Lines exhibiting significantly higher difference than RHA 801 are indicated in bold.

Accession Name	PI	Avg Days to Death	Statistical Grouping	Accession Name	PI	Avg Days to Death	Statistical Grouping
A-1499	**413050**	**20.6**	**a**	No. 7728	172904	13.5	cdefghijk
HA 124	**599775**	**20.2**	**a**	MN17	650392	13.5	cdefghijk
No. 9121	**175733**	**18.9**	**ab**	Ireoi Korai Csikos	650409	13.4	cdefghijk
Short Russian	650379	17.1	abc	Kustanajskij 01	650364	13.2	cdefghijk
Olea	650369	16.9	abc	RHA 276	599761	13.2	cdefghijk
Zelenka	650831	16.8	abc	S8 SM 10/2-2	650519	13.1	cdefghijk
FS-a-3	480471	16.6	abcd	PL 7957-91	650477	12.9	defghijk
HA 61	599771	16.5	abcd	Polstar	650372	12.8	defghijk
Voshod Elite 7	650458	16.4	abcde	Sratovskij P-10	650377	12.7	defghijkl
RHA 408	603989	16.4	abcde	VK-1	650461	12.7	defghijkl
VIR 160	497250	16.3	abcde	RHA 391	603987	12.6	defghijkl
CMG-3	650400	16.3	abcde	Karlik	650558	12.5	defghijkl
Romsun V3355 AC	650498	16.0	bcdef	VK-10	650464	12.4	defghijkl
A-1405	380562	15.9	bcdefg	Guaran	650810	12.3	efghijkl
Lengyel A	531366	15.8	bcdefg	40-44 VK-25	431528	12.3	efghijkl
PO 6/4-2	431560	15.5	bcdefg	HA 410	603991	12.2	fghijkl
HZ.SM 27.208	531359	15.4	bcdefgh	RHA 392	603988	12.1	ghijkl
VK-53	650468	15.4	bcdefgh	Romsun V-8740	650540	12.1	ghijkl
HA 390	603986	15.3	bcdefghi	D-75-11	431543	12.0	ghijkl
D-75-10	431542	15.2	cdefghi	**HA 89**	**599773**	**11.8**	**hijkl**
Franslever	650405	15.0	cdefghi	VK-6	650463	11.7	hijkl
Pioner Sibiri	497933	15.0	cdefghi	Ostonne	650371	11.6	ijkl
Jugovostocnyj	650412	14.9	cdefghi	HA 411	603992	11.5	jkl
RHA 801	**599768**	**14.8**	**cdefghi**	Ames 102	490282	11.4	jkl
VIR 130M	497249	14.8	cdefghi	PL 7968-84	650476	11.0	jkl
IREGI HNK 81	531361	14.5	cdefghij	HA 304	599782	10.8	jkl
VIR 117	650485	14.2	cdefghijk	CO-PB 105	600714	10.8	jkl
Slovenska siva	650380	14.2	cdefghijk	Primrose flpl	490320	10.7	kl
S8 V8883 4/2-1	650520	14.1	cdefghijk	VIR 110	650536	10.5	kl
Cakinskij 269	497930	13.9	cdefghijk	VIR 119	497248	10.4	kl
Ames 101	490281	13.7	cdefghijk	**Cabure 1004**	**650798**	**8.8**	**l**
Iregi Napraforgo	650410	13.6	cdefghijk				

1. A total of 16 lines displayed a significantly higher level of resistance than the moderately susceptible inbred check HA 89.
2. Three lines (**HA 124/PI 599775, A-1499/PI 413050, and No. 9121/PI 175733**) exhibited significantly higher resistance levels than the previous most resistant material, inbred line **RHA 801**.
3. A total of 20 tested lines were not significantly different than the highly susceptible check variety Cabure 1004.

Evaluation of Genotype X isolates interactions with multiple fungal isolates of *S.sclerotiorum*

Table 2 : BSR Greenhouse Evaluation of Sclerotinia isolate JS757

Accession Name	PI	Statistical grouping	Avg days to death
HA 124	599775	a	27
RHA 801	599768	b	19
Short Russian	650379	bc	18
Zelenka	650831	bcd	18
RHA 408	603989	bcd	17
HA 61	599771	bcde	17
HIR 34	650613	bcde	17
A-1499	413050	bcde	17
No. 9121	175733	cdef	16
HA 390	603986	cdef	15
HA 441	639164	efg	14
RHA 439	639162	fg	14
FS-a-3	480471	fg	14
VIR 160	497250	fg	14
Romsun V3355 AC	650498	fg	13
CMG-3	650400	gh	12
Voshod Elite 7	650458	gh	12
Cabure 1004	650798	hi	11
HA 89	599773	hi	10
RHA 373	560141	i	9

Table 3 : BSR Greenhouse Evaluation of Sclerotinia isolate BN166

Accession Name	PI	Statistical grouping	Avg days to death
HA 124	599775	a	18
HIR 34	650613	a	17
Romsun V3355 AC	650498	ab	17
A-1499	413050	bc	15
HA 61	599771	bc	15
No. 9121	175733	cd	15
Zelenka	650831	cd	14
RHA 408	603989	cde	14
RHA 801	599768	cde	14
VIR 160	497250	def	13
HA 390	603986	def	13
FS-a-3	480471	def	13
CMG-3	650400	efg	12
Short Russian	650379	efg	12
Voshod Elite 7	650458	fg	12
HA 441	639164	g	11
RHA 439	639162	g	10
RHA 373	560141	h	9
HA 89	599773	hi	8
Cabure 1004	650798	i	7

* Statistically grouped lines indicated by same letter are not significantly different (α =0.05)

1. **With JS 577 isolate** , one inbred line displayed significantly higher level of resistance and six lines were not significantly different than RHA 801.
2. **With BN 166 isolate**, three lines showed significantly higher resistance and ten lines were not significantly different from RHA 801.
3. All lines exhibited higher resistance than susceptible control line check HA 89, Cabure 1004, RHA 373 with both the isolates.

Molecular marker analysis for BSR resistance from previous identified QTL mapping to characterize resistant genotypes.

1. Quantitative trait loci (QTL) for BSR resistance were identified in a sunflower recombinant inbred line (RIL) population derived from the cross HA 441 X RHA 439.
2. Resistance allele of the QTL on **LGs 4, 9, and 17** were contributed by the **HA 441** parent, while the resistance alleles of the QTL on **LGs 10, 11, and 16** were contributed by the **RHA 439** parent. Identified QTL's are converted in to length polymorphic markers.

Table 2 : Comparison of the amplification of selected inbred lines with primer derived from codominant parent lines (HA441 and RHA 439) which are moderately resistant for Sclerotonia BSR.

Primers	HA 124	A-1499	No. 9121	Short Russian	Zelenka	FS-a-3	HA 61	Voshod Elite 7	RHA 408	VIR 160	CMG-3	R V3355 AC	HA 390	RHA 801	HIR 34	HA 89	RHA 373	Cabure 1004
S4_135190076	S	X	X	S	S	R	S	S	S	S	S	S	S	R	R	R	S	S
S10_288646223	S	S	R	R	S	S	S	S	R	R	S	S	S	R	S	S	R	R
S17_228661362	S	S	S	S	S	R	R	S	R	S	R	S	R	S	S	R	R	R
S16_157591485	S	S	S	S	S	R	R	R	R	R	R	R	S	S	R	S	R	S

Where,
R = carrying resistance allele
S= carrying susceptible allele
X= No amplification

Summary and Future work.

- One inbred line showed significantly higher level of resistance to the isolates resulting in broad spectrum resistance.
- Future work includes molecular marker evaluation of these inbred line with remaining QTL markers developed from cross between HA 441 and RHA 439 to determine the likelihood of novel resistance for resistance assessment and genetic mapping .
- Testing additional isolates and assessing genotype x isolate interactions by two-way ANOVA to determine if these interactions are an important consideration for breeding resistance.
- Release of new inbred line with high and broad-spectrum level resistance.

References

1.Talukder ZI, Hulke BS, Marek LF, Gulya TJ (2014) Sources of resistance to sunflower diseases in a global collection of domesticated USDA plant introductions. Crop Sci. 54:694-705.

2.Underwood, W., Misar, C. G., Block, C. C., Gulya, T. J., Talukder, Z. I., Hulke, B. S., et al. (2020). A greenhouse method to evaluate sunflower quantitative resistance to basal stalk rot caused by *Sclerotinia sclerotiorum*. *Plant Dis.* doi: 10.1094/pdis-08-19-1790-re

Acknowledgement-The authors thank National Sclerotinia Initiative for financial support for this research.