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Response of sunflower genotypes to mid-stalk rot caused by *Sclerotinia sclerotiorum*

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Abstract

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Phytopathological investigations of wild annual *Helianthus annuus* L. accessions and some hybrid progenies, derived from them were carried out to determine their reaction to two isolates of *S. sclerotiorum* under artificial inoculation of stems. Plants infection was carried out at the flowering stage. The analysis of variance for Stem disease score (SDS) and Lesions size (LS) showed a statistically significant interaction between isolate and genotype at P<0.05%. Differences in genotypes responses depending upon the isolates were established. The *genotype × isolate* interaction for the included wild accessions was significant, indicating that the wild sunflower genotypes differentially responded to both *S. sclerotiorum* isolates. The wild accessions E-129, E-115, E-110 and E-154 were consistent in terms of their responses, showing moderate resistance to both isolates. Five of the studied interspecific hybrids gave the lowest SDSs, which determined the isolate SS1914 as less aggressive than SS1941 isolate. Higher SDSs of isolate SS1941 were specified for hybrids, although they ranged to a certain extent, and determined the various degrees of hybrids susceptibility. Existence of certain interactions between studied genotypes and fungal isolates can influence the efficiency of breeding programs.

Keywords: S. sclerotiorum; isolate; wild *Helianthus annuus;* Stem disease score; Lesions size; cluster analysis

Introduction

S. sclerotiorum is a necrotrophic ascomycete fungus, which infects more than 600 plant species, including important crops such as sunflower, soybean, oilseed rape, dry bean, peanut, lentil, various vegetables and numerous weeds (Davar et al., 2011; Liang & Rollins, 2018) and can survive in the soil for up to 8 years in the form of sclerotia (Liu et al., 2017). Sclerotia of the pathogen may germinate carpogenically producing apothecia and ascospores that infect host plants (sexual reproduction), or myceliogenically to produce mycelia (asexual reproduction) depending on the geographic origin of the isolates, soil moisture, presence of exogenous nutrients and the temperature for formation of inoculum, mycelia or sclerotia (Bardin & Huang, 2001). White rot, caused by *S. sclerotiorum*, is a major yield-limiting factor in sunflower in temperate regions of the world. Rapid drying of the leaves and development of lesions on the taproots and basal portions of stems cause plants to die within a few days after the onset of wilting (Dorrell & Huang, 1978). *S. sclerotiorum* causes infection of sunflower plants at any growth stage and results in so-called Sclerotinia wilt or basal stalk rot (BSR), mid-stalk rot (MSR), and head rot (HR) diseases (Gulya et al., 1997; Qi et al., 2016). Yield losses can reach 100% when the climatic conditions are favorable for the fungus (Sackston, 1992; Schwartz & Singh, 2013). Breeding for resistance to this pathogen is challenging, since no immune germplasm has been identified in sunflower or its close relatives. Nevertheless, various studies have demonstrated that resistance performance of diverse sunflower germplasms differs considerably (Bazzalo et al., 1991; Gulya et al., 2010; Talukder et al., 2014) and the resistance is

conditioned by multiple genes, each having a small effect (Davar et al., 2010; Amouzadeh et al., 2013; Talukder et al., 2016). The use of resistant genotypes is considered as the most important method to control this disease; however, the development of these genotypes requires information on the aggressiveness of the pathogen in the region and interaction of fungal isolates with host genotypes. Davar et al. (2011) and Ekins et al. (2007) showed that *S. sclerotiorum* isolates differed in their aggressiveness on sunflower plants. The high genetic variability for pathogenicity in *S. sclerotiorum* requires simultaneous incorporation of several genes for resistance to remain effective in cultivars used over a large area (Amoozadeh et al., 2015). Davar et al. (2012) determined the aggressiveness of isolates of *S. sclerotiorum* on a single cultivar of sunflower and identified new sources of resistance to *Sclerotinia* basal stem disease in the genotypes selected from the mutant sunflower population. The authors concluded that isolate-specific and isolate-nonspecific partial resistant genotypes could be used in crossing programs for the breeding of durable resistance to *Sclerotinia* basal stem disease.

The objectives of this study were to determine the reaction of 25 sunflower genotypes (wild *Helianthus annuus* accessions and their interspecific hybrids) to two isolates of *S. sclerotiorum* isolated from sclerotia, under artificial inoculation of stems.

Material and Methods

The investigation was carried out in Dobrudzha agricultural institute under field conditions in 2019.

Plant material. Ten accessions of wild *Helianthus annuus* L., from the collection of wild sunflower species, maintained at DAI-General Toshevo as populations, were included in the investigation, together with 15 interspecific hybrid combinations, derived from crosses with cultivated line 712 A, used as a maternal parent and susceptible to *S. sclerot-*

Fig. 1. Nine-degree scale for the evolution of stem disease score (SDS)

iorum. The studied material was planted in rows. The rows spacing was 0.7 m and 0.3 m within the plants.

Fungal isolates and Experimental design. Two isolates of *S. sclerotiorum* isolated from sclerotia, collected from diseased common bean (SS1914) and sunflower (SS1941) plants in the region of North-East Bulgaria with typical symptoms were included in the investigation. They were previously preserved as sclerotia at 4^0C. On the base of their mycelium compatibility, the isolate SS1941was referred to MCG–MCBG 5, and the isolate SS1914 – to MCBG 1 (Kiryakov & Zhecheva, 2019). Both isolates were distinguished with different level of aggressiveness to the dry bean cultivar GTB Blyan (Kiryakov & Zhecheva, 2019) and sunflower hybrid Dara (unpublished data). Plants infection was carried out at the flowering stage. The artificial inoculation method, developed by Encheva & Kiryakov (2002), was used in the present study to apply standardized infection. For this purpose, the mid-height leaf petiole was cut 2 cm from its base. A unilaterally closed plastic straw (6 x 25 mm) containing agar disc of 3 days old culture of the respective isolate on potato dextrose agar (PDA) was put into the piece of straw. Four plants per genotype (hybrid/accession) were infected with both isolates respectively. Two petioles, next to each other, of each plant were infected. The checks were inoculated on the same dates in the same way with pure agar disc. The size of lesions (in mm) was measured 14 days after inoculation. The stem disease score (SDS) was recorded 14 days after inoculation by 9-degree scale (Figure 1). According to middle disease score (MDI) of reaction, the genotypes were grouped as follow: High resistant – 1.0; Resistant –1.1 to 3.0; Moderately resistant –3.1 to 5.0; Susceptible –5.1 to 7.0; High susceptible– above 7.1.

Statistical analysis. Analysis of variance (ANOVA) was performed for determination of the main effects of genotypes and isolates, as well as their interactions (SPSS). The correlation coefficient between SDS and the size of lesions was calculated. Cluster analysis was used as a statistical method to group similar genotypes into respective categories. The goal of its performing was to sort different genotypes into groups in a manner that the degree of association between studied materials was high if they belong to the same group, and low if they belong to different groups.

Results and Discussion

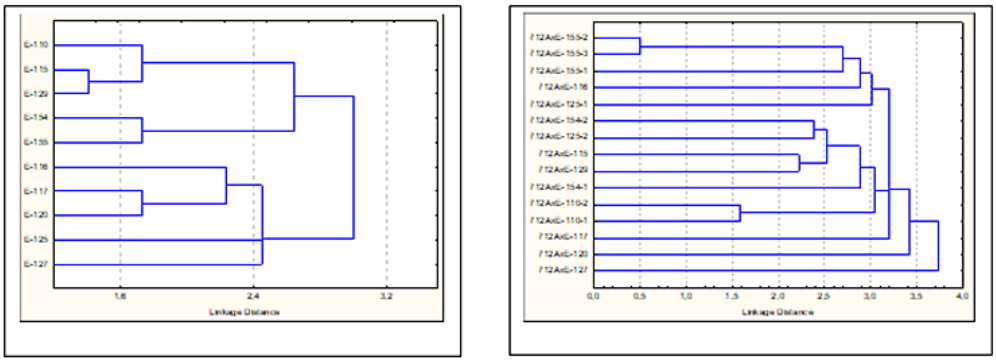
The analysis of variance for Stem disease score (SDS) and Lesions size (LS) showed a statistically significant interaction between isolate and genotype at P<0.05% (Table. 1). The positive correlation between SDS and LS (0.865) at P = 0.01% was also established.

Table 1.Analysis of variance for disease evaluation and size of lesions for 25 sunflower genotypes after inoculation with two isolates of *S. sclerotiorum*

Source	df	MS	F	P
SDS				
Isolate (I)	1	51.005	20.553	.000
Genotype (G)	24	15.948	6.426	.000
I x G	24	6.161	2.483	.000
Error	150	2.482		
LS				
Isolate (I)	1	68931.845	22.911	.000
Genotype (G)	24	31855.707	10.588	.000
I x G	24	8373.710	2.783	.000
Error	150	3008.692		

According to the obtained SDS results, the isolate SS1941 was more aggressive than SS1914 (Table 2). The wild accessions E-129, E-115, E-110 and E-154 were consistent in terms of their responses, showing moderate resistance to both isolates. Differences in responses of genotypes depending upon the isolates were established in Table 2. Wild accessions E-117, E-120 and E-155 showed susceptible response to isolate SS1941 and moderate resistance to isolate SS1914. In contrast was determined for the wild accession E-116, while E-125 and E-127 were susceptible to both isolates. According to the results, presented on Table 2, the reaction of accession E-127 was statistically different from all other accessions included in the study except E-125, and the accession E-125 was different from others, except E-116, E-117, E-120 and E-127. The *genotype* × *isolate* interaction for the included wild accessions was significant, indicating that the wild sunflower genotypes differentially responded to both *S. sclerotiorum* isolates. On the base of MDS for SDS the studied accessions were grouped in two main clusters. Five accessions were included in each cluster. (Figure 2A). Very similar responses to both isolates demonstrated the accessions E-115 and E-129, followed by E-117 and E-120.

The analysis of variance was used to compare the mean of selected lines and the mean of their parents (Rachid Al-Chaarani et al., 2004; Rachid Al-Chaarani et al., 2005; Abou Al Fadil et al., 2007). This analysis was used to compare the SDSs among the interspecific hybrids after inoculation with two *S. sclerotiorum* isolates at the 5% level of significance. As expected, the studied genotypes showed a wide variation of SDSs (Figure 2B). Five of the hybrids (H1, H4, H7, H8 and H14) gave the lowest SDSs, which determined the isolate SS1914 as less aggressive than SS1941 isolate. Higher SDSs of isolate SS1941 were specified for hybrids, although they ranged to a certain extent, and determined the various



A **B**

Fig. 2. Dendrogram using Euclidean distances computed for 10 accessions of wild *H. annuus* (A) and for 15 interspecific hybrids, derived from them (B), inoculated with two isolates of *S. sclerotiorum* on the base of SDS

Table 2. Stem disease score (SDS) of 25 sunflower genotypes after inoculation with 2 *S. Sclerotiorum* isolates

Acc. №	Hybrid/Accession	SS1941			SS1914			Average per genotype
		Min.	Max.	Avr.	Min.	Max.	Avr.	
H1	712 A x E-155-2	7	8	7.8	2	4	3.0	5.4
H2	712 A x E-116	7	8	7.8	2	7	4.0	5.9
H3	712 A x E-154-2	4	8	5.8	3	7	4.5	5.1
H4	712 A x E-115	5	7	5.5	3	5	4.0	4.8
H5	712 A x E-125-2	4	9	6.0	3	9	5.3	5.6
H6	712 A x E-110-2	3	6	3.5	2	7	3.8	3.6
H7	712 A x E-129	4	7	4.8	2	4	2.8	3.8
H8	712 A x E-155-1	7	8	7.5	4	5	4.5	6.0
H9	712 A x E-120	3	8	6.3	8	8	8.0	7.1
H10	712 A x E-125-1	8	8	8.0	2	9	6.0	7.0
H11	712 A x E-127	8	8	8.0	8	9	8.5	8.3
H12	712 A x E-154-1	4	8	5.8	5	8	6.8	6.3
H13	712 A x E-110-1	2	7	4.0	2	7	4.3	4.1
H14	712 A x E-155-3	7	8	7.3	2	4	3.0	5.1
H15	712 A x E-117	8	8	8.0	5	8	6.5	7.3
A3	E-110	3	5	3.8	2	4	3.0	3.4
A5	E-115	4	5	4.3	3	5	3.8	4.0
A8	E-116	2	7	3.8	5	7	5.8	4.8
A10	E-117	4	8	5.5	2	7	4.0	4.8
A17	E-120	3	7	6.0	3	7	4.0	5.0
A22	E-125	3	9	6.3	5	8	6.3	6.3
A23	E-127	4	9	7.0	7	9	8.0	7.5
A33	E-129	3	4	3.3	3	5	3.5	3.4
A47	E-154	2	7	3.8	3	3	3.0	3.4
A49	E-155	3	8	5.3	3	4	3.3	4.3
Average per isolate				5.78			4.77	

LSD 5% - 0.44 for isolate; 1.56 for genotype and 2.05 for Isolate x Genotype

degrees of hybrids susceptibility. According to the results, presented in Table 2, the hybrids H11, H6, H7, H15, H9, H10 and H13 were the most statistically different among all others included in the study. The wild accessions E-129, E-115, E-110 and the hybrids, derived from them, were consistent in terms of their responses, showing moderate resistant reactions against both isolates. Variation in the mean responses of other studied accessions and derived from them hybrids

to the infection caused by both isolates of *S. sclerotiorum* was identified. Their distributions were slightly skewed towards the higher values (susceptibility). Davar et al. (2012) established that some recombinant inbred lines produced a lower disease severity than their parents, but others produced higher disease severity than the parents and suggested that transgressive segregation for resistance occurred in the studied crosses. Transgressive segregation in sunflower had previously been reported by Micic et al. (2005) and Davar et al. (2011) for partial resistance to *S. sclerotiorum,* as well as by Darvishzadeh et al. (2007) for partial resistance to Phoma black stem. Davar et al. (2011) pointed out that differences in aggressiveness of *S. sclerotiorum* isolates suggested the existence of *genotype x isolate* interactions in the sunflower/*S. sclerotiorum* pathosystem. Existence of any interactions between sunflower genotypes and fungal isolates can influence the efficiency of breeding programs. Understanding differential responses of sunflower genotypes to different *S. sclerotiorum* isolates is useful for development of durable resistance and for plant breeding, providing breeders knowledge, which types of *S. sclerotiorum* existed in the geographic areas, they were breeding for. Amouzadeh et al. (2015) concluded that the high genetic variability for pathogenicity in *S. sclerotiorum* required simultaneous incorporation of several genes for resistance into host genotypes.

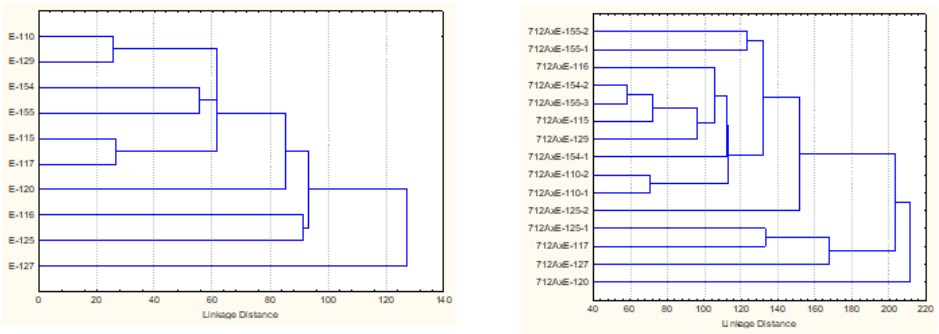
Variation in the mean responses of studied genotypes to the infection caused by two isolates of *S. sclerotiorum* was also determined for the size of lesions (Table 3). Statistically was proved the bigger aggressiveness of isolate SS1941. The LSD test in this investigation, used to compare the LS among the wild accessions of *Helianthus annuus* after inoculation with two *S. sclerotiorum* isolates at the 5% level of significance, showed statistically proved difference of E-127 among all other accessions except E-125. Accession E-125 differed significantly also from E-129 and E-154. Differences between other accessions were not significant.

The *genotype x isolate* interaction for LS was significant, indicating that wild sunflower populations differen-

Table 3. Lesions size (mm) on 25 sunflower genotypes after inoculation with two *S. sclerotiorum* isolates

Acc. №	Hybrid/Accession	SS1941			SS1914			Average per genotype
		Min.	Max.	Avr.	Min.	Max.	Avr.	
H1	712 A x E-155-2	150	325	213.8	20	70	37.5	125.6
H2	712 A x E-116	190	210	200.0	20	165	73.8	136.9
H3	712 A x E-154-2	108	220	152.5	30	120	78.8	115.6
H4	712 A x E-115	130	160	137.5	60	120	86.3	111.9
H5	712 A x E-125-2	70	330	152.5	30	220	102.5	127.5
H6	712 A x E-110-2	30	130	55.0	30	170	62.5	58.8
H7	712 A x E-129	75	140	106.3	20	70	35.0	70.6
H8	712 A x E-155-1	230	290	260.0	65	105	85.0	172.5
H9	712 A x E-120	30	330	183.3	270	270	270.0	226.6
H10	712 A x E-125-1	210	290	243.3	20	330	148.3	195.8
H11	712 A x E-127	220	295	268.3	260	270	265.0	266.6
H12	712 A x E-154-1	60	210	130.0	100	170	127.5	128.8
H13	712 A x E-110-1	20	190	85.0	20	170	78.8	81.9
H14	712 A x E-155-3	130	200	155.0	20	80	50.0	102.5
H15	712 A x E-117	230	310	275.0	120	350	222.5	248.8
A3	E-110	35	70	59.0	20	65	42.5	50.8
A5	E-115	70	110	90.0	30	110	72.5	81.3
A8	E-116	20	80	40.0	80	150	106.3	73.1
A10	E-117	65	132	90.8	20	110	62.5	76.6
A17	E-120	30	170	106.3	30	175	66.3	86.3
A22	E-125	30	150	92.5	90	130	116.3	104.4
A23	E-127	60	170	132.5	110	230	170.0	151.3
A33	E-129	30	65	38.8	30	75	41.3	40.0
A47	E-154	20	115	48.8	25	30	28.8	38.8
A49	E-155	30	141	73.3	25	70	38.8	56.0
Average per isolate				135.9			98.7	

LSD 5% - 15.56 for isolate; 54.30 for genotype and 76.79 for *Isolate x Genotype*



A **B**

Fig. 3. Dendrogram using Euclidean distances computed for 10 accessions of wild *H. annuus* (A) and for 15 interspecific hybrids, derived from them (B), inoculated with two isolates of *S. sclerotiorum* on the base of LS

tially responded to both isolates of the pathogen (Figure 3A). Smallest dissimilarities were established for accessions E-110 and E-129 as well as for E-115 and E-117. The dendrogram suggested that E-154 and E-155 were much closer to each other than were E-120, E-116 and E-125. E-127 appeared to be quite different from any of accessions, included in this study. Analysis of variance showed significant interactions between interspecific hybrids and *S. sclerotiorum* isolates for LS suggesting that the response to the pathogen of a given genotype relative to others varied between isolates (Figure 3B). For both isolates, especially for isolate SS1941, the distribution of LS was skewed toward susceptibility, indicating that SS1941 was more aggressive than SS1914 isolate on the studied genotypes. The differences regarding LS for hybrids H5 and H10, as well for H8 and H14 were statistically significant although the male parents were the same. This was because the wild accessions were maintained as populations.

Conclusion

The main effects of genotypes (accessions of wild *Helianthus annuus* and interspecific hybrids, derived from them), and *S. sclerotiorum* isolates SS1941 and SS1914, as well as their interactions, were determined on the base of analysis of variance for Stem disease score (SDS) and Lesions size (LS) and showed statistically significant interactions. Variation in the mean responses of the studied accessions and derived from them hybrids to pathogen infection was identified. Their distributions were slightly skewed towards the higher values (susceptibility). Existence of certain interactions between studied genotypes and fungal isolates can influence the efficiency of breeding programs.

References

Abou Al Fadil, T. A., Kiani, S. P., Dechamp-Guillaume, G., Genzbittel, L. & Sarrafi, A. (2007). QTL mapping of partial resistance to Phoma basal stem and root necrosis in sunflower (*Helianthus annuus* L.), *Plant Science, 172,* 815–823.

Amoozadeh, M., Darvishzadeh, R., Davar, R., Abdollahi Mandoulakani, B., Haddadi, P. & Basirnia, A. (2015). Quantitative trait loci associated with isolate specific and isolate non-specific partial resistance to *Sclerotinia sclerotiorum* in sunflower. *J. Agr. Sci. Tech., 17,* 213-226

Amouzadeh, M., Darvishzadeh, R., Haddadi, P., Aabdollahi Mandoulakani, B. & Rezaee Danesh, Y. (2013). Genetic analysis of partial resistance to basal stem rot (*Sclerotinia sclerotiorum*) in sunflower. *Genetika, 45,* 737–748.

Bardin, S. D. & Huang, H. C. (2001). Research on biology and control of *Sclerotinia* diseases in Canada. *Canadian Journal of Plant Pathology, 23(1),* 88-98.

Bazzalo, M. E., Dimarco, P., Martinez, F. & Daleo, G. R. (1991). Indicators of resistance of sunflower plant to basal rot (*Sclerotinia sclerotiomm*): Symptomatological, biochemical, anatomical, and morphological characters of the host. *Euphytica, 57,* 195–205.

Darvishzadeh, R., Poormohammad Kiani, S., Dechamp-Guillaume, G., Gentzbittel, L. & Sarrafi, A. (2007). Quantitative trait loci associated with isolate specific and isolate nonspecific partial resistance to *Phoma macdonaldii* in sunflower. *Plant Pathology, 56,* 855–861.

Davar, R., Darvishzadeh, R. & Jahanbakhsh-Godehkahriz, S. (2012). Analysis of aggressiveness among isolates of *Sclerotinia sclerotiorum* from sunflower. *AWERProcedia Information Technology & Computer Science, 1,* 543-546.

Davar, R., Darvishzadeh, R. & Majd, A. (2011). Genotype-isolate interaction for resistance to *Sclerotinia sclerotiorum* in sunflower. *Phytopathol. Mediterr., 50,* 442–449.

Davar, R., Darvishzadeh, R., Majd, A., Ghosta, Y. & Sarrafi, A. (2010). QTL mapping of partial resistance to basal stem rot in sunflower using recombinant inbred lines. *Phytopathol. Mediterr., 49,* 330–341.

Dorrell, D. G. & Huang, H. C. (1978). Influence of *Sclerotinia* wilt on seed yield and quality of sunflower wilted at different stages of development. *Crop Science, 18,* 974–978**.**

Ekins, M. G., Aitken, E. A. B. & Goulter, K. C. (2007). Aggressiveness among Isolates of *Sclerotinia sclerotiorum* from Sunflower. *Australas. Plant Pathol., 36,* 580–586.

Encheva, V. & Kiryakov, I. (2002). A method for evaluation of sunflower resistance to *Diaporthe/Phomopsis helianthi* Munt. Cvet. et al. *Bulg. J. Agric. Sci., 8, 219-222.*

Gulya, T. J., Marek, L. F. & Gavrilova, V. (2010). Disease resistance in cultivated sunflower derived from public germplasm collections. In: *Proc. of the International Symposium "Sunflower Breeding on Resistance to Diseases, Krasnodar, Russia, The International Sunflower Association, Paris,* 7–18.

Gulya, T. J., Rashid, K. & Masirevic, S. (1997). Sunflower diseases. In: Schneiter AA (ed). *Sunflower technology and production.* Soil Science Society of America Inc., Madison, WI, 263–379.

Kiryakov, I. & Zhecheva, K. (2019). Mycelial compatibility and aggressiveness of Bulgarian *Sclerotinia sclerotiorum* isolates. *Field Crops Studies, XII (3),* 9-22.

Liang, X. & Rollins, A. (2018) Mechanisms of broad host range necrotrophic pathogenesis in *Sclerotinia sclerotiorum*. *Phytopathology, 108(10),* 1128–1140.

Liu, J., Zhang, Y., Meng, Q., Shi, F., Ma, L. & Li, Y. (2017). Physiological and biochemical responses in sunflower leaves infected by *Sclerotinia sclerotiorum. Physiological and Molecular Plant Pathology, 100,* 41-48.

Micic, Z., Hahn, V., Bauer, E., Melchinger, A. E., Knapp, S. J., Tang, S. & Schön, C. C. (2005). Identification and validation of QTL for *Sclerotinia* mid-stalk rot resistance in sunflower by selective genotyping. *Theoretical and Applied Genetics, 111,* 233–242.

Qi, L., Long, Y., Talukder, Z. I., Seiler, G. J., Block, C. C. & Gulya, T. J. (2016). Genotyping-by-Sequencing uncovers the introgression alien segments associated with Sclerotinia basal stalk rot resistance from wild species-I. *Helianthus argophyllus* and *H. petiolaris. Front. Genet., 7,* 219. https://doi.org/10.3389/fgene.2016.00219

Rachid Al-Chaarani, G., Gentzbittel, L., Huang X. Q. & Sarrafi, A. (2004). Genotypic variation and identification of QTLs for agronomic traits, using AFLP and SSR markers in RILs of sunflower (*Helianthus annuus* L.). *Theoretical and Applied Genetics, 109,* 1353–1360.

Rachid Al-Chaarani, G., Gentzbittel, L., Wedzony, M. & Sarrafi, A. (2005). Identification of QTLs for germination and seedling development in sunflower (*Helianthus annuus* L.). *Plant Science, 169,* 221–227.

Sackston, W. E. (1992). On a treadmill: Breeding sunflowers for resistance to disease. *Annual Review of Phytopathology, 30,* 529–551.

Schwartz, H. F. & Singh, Sh. P. (2013). Breeding common bean for resistance to white mold: A review. *Crop Sci., 53,* 1832–1844.

Talukder, Z. I., Hulke, B. S., Marek, L. F. & Gulya, T. J. (2014). Sources of resistance to sunflower diseases in a global collection of domesticated USDA plant introductions. *Crop Sci., 54,* 694–705.

Talukder, Z. I., Seiler, G. J., Song, Q., Ma, G. & Qi, L. L. (2016). SNP discovery and QTL mapping of *Sclerotinia* basal stalk rot resistance in sunflower using genotyping-by-sequencing. *Plant Genome, 9(3).* DOI: 10.3835/plantgenome2016.03.0035.

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