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Evaluation of *Septoria* leaf spot (*Septoria helianthi*) alone and in combination with other foliar fungal spots on sunflower

https://doi.org/10.1515/helia-2022-0003
Received January 21, 2022; accepted August 30, 2022; published online September 23, 2022

Abstract: In recent years, *Septoria* leaf spot has taken a strong place in the list of economically important diseases of sunflower. It may be a favorable factor for the development of other diseases on sunflower plants too. The aim of the research was to evaluate the frequency and degree of infection of sunflower plants with *Septoria helianthi* as well as possibility of simultaneous infection with this pathogen and other fungi which cause foliar spots, such as downy mildew, *Alternaria* leaf spot and *Phoma* black stem under conditions of stationary infectious nursery. The year 2021 turned out to be extremely favorable for the development of fungal leaf spots on sunflower. Assessment of fungal diseases of sunflower plants was performed in F_2 families obtained after crossing ZL22A, ZL58A, ZL70A, ZL78A, and ZL169A lines of Zaporozhye breeding (Ukraine) with HAR7 line (originating from the USA). Each F_2 family (sample) was obtained from self-pollination of one F_1 plant with varying severity of Septoria disease. It was found that in the conditions of 2021, sunflower was quite affected by Septoria disease. The percentage of injured plants in F_2 samples ranged from 41.0 to 100.0%. The severity of disease was different. Less resistant genotypes included plants with lesions on sunflower leaves of lower, middle, and upper tiers in approximately equal numbers, while more resistant genotypes had almost no plants with injured leaves of upper tiers. It was established that the development of *Phoma* disease occurred both on plants with *Septoria* leaf spot and on healthy plants. On average, among the plants infected with *Phoma macdonaldii*, the proportion of plants jointly affected by *Phoma* and *Septoria* diseases was 2/3. However, the frequency of simultaneous infection of plants with *S. helianthi* and *P. macdonaldii* in genotypes with different resistance to *Septoria* leaf spot differed significantly and ranged from 80% in less resistant to

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40% in more resistant accessions. Most plants (about 90%) infected with *Plasmopara halstedii* were simultaneously infected with *S. helianthi*. That is, both of these pathogens can simultaneously develop on the same plant and lead to a decline in its basic physiological functions. The rate of simultaneous damage of sunflower by *Alternaria* and *Septoria* was quite low compared to plant damage by *Alternaria* alone and was less than 30%. This may mean that the pathogen of *Alternaria* leaf spot avoids plants with existing *S. helianthi* fungus.

Keywords: *Alternaria* leaf spot; downy mildew; *Phoma* black stem; *Septoria* leaf spot; simultaneous infection; sunflower.

Introduction

Sunflower is expanding its area in national and world agriculture, as well as being an economically important technical and fodder crop in the Ukraine. In the production of sunflower a significant violation in crop rotation is observed, which leads to a serious deterioration of the phytosanitary situation. Sunflower is a crop prone to various diseases (Gulya et al. 1997), and in recent years the level of disease losses has almost doubled. The most notable diseases of sunflower are caused by fungi. Leaf spot diseases of fungal origin, *Alternaria*, *Phoma*, and *Septoria* in particular, have become widespread (Irum 2009; Melnichuk et al. 2020).

The species of fungi that cause *Septoria* leaf spot are among the most common and widespread throughout the world. They cause leaf spot in many cultivated and wild plants (Babayants and Babayants 2014; Das et al. 2020; Eyal et al. 1987; Gul et al. 2016; Gusellaa et al. 2021; Kovács et al. 2020). According to Melkumov and Brazhnikova (2018), in the Voronezh region of the Russian Federation most pathogens of *Septoria* disease are found on the vegetative organs of plants from the families *Compositae, Labiatae, Leguminosae, Rosaceae, Umbelliferae, Caryophyllaceae*.

Sunflower *Septoria* disease is caused by the fungus *Septoria helianthi* Ellis & Kellerm. The leaves of the lower tier are the first to be affected. On the cotyledon leaves there are brown spots embedded in the leaf tissue on the lower surface and convex – on the upper surface. In adult plants the spots on the leaves are initially small, rounded or irregularly shaped, dark brown, lighter at the edges with edging. Necrotic leaf tissue falls out in rainy weather, numerous holes are formed, which is one of the characteristics of *S. helianthi*. In the center of the spots pycnidia of the fungus are developed with hyaline pycniospores. Gradually the spots merge and the leaves wither, then dry up. Afterwards the infection spreads to the upper tiers of leaves (Irum 2009; Marques et al. 2011; Vypritskaya et al. 2012).

Under the action of the pathogen the content of chlorophyll in the leaves of plants decreases. Destruction of chloroplasts and, consequently, reducing the size of the assimilation surface leads to a decrease in the basic physiological functions of the plant organism – the intensity of photosynthesis by 4–9 times and the intensity of respiration by 5–17%, reduced phytoassimilates production and, consequently, reduced yields (Brand et al. 2020; Melkumov and Brazhnikova 2018).

The disease develops at an optimal temperature of 22–25 °C. The primary source of infection is crop residues, and initial inoculation occurs with pycnospores. Their dissemination is facilitated by rains or raindrops in combination with moderate air temperatures (Retman et al. 2020; Vipritskaya et al. 2012).

Sunflower *Septoria* leaf spot is widespread in many countries in Europe, Africa, North and South America, Asia, Australia (Irum 2009). In China, India, and Thailand, *Septoria* disease is a significant factor for crop failure (Liu and Li 2007). In Brazil, *Septoria* disease is one of the diseases that cause the greatest damage of sunflower. The results of research by scientists in Brazil show that the greater the severity of *Septoria* leaf spot on sunflower, the less will be its height, head size, and shorter the period before flowering, i.e. premature maturation of plants is observed. In wet years, *Septoria* disease can reduce sunflower yields by up to 100% (Brand et al. 2018; Nechet et al. 2017).

In the Ukraine, in two regions for the period of 2017–2020, *Septoria* leaf spot was detected on the leaves in all sunflower crops with a spread of up to 100%, and under the conditions of irrigation of research plots in the Kiev region, the spread of the disease reached between 65 and 80.6%. Scientists claim that in years with favorable weather conditions, *Septoria* disease can threaten sunflower crops in Ukraine and cause significant losses (Retman et al. 2020).

It is known that the damage of plants by one disease is often accompanied by the damage of another (Vypritskaya and Kuznetsov 2019). The effect of two pathogens, such as *Alternaria helianthi* and *S. helianthi*, on sunflower yield has been studied in Brazil. Sunflower yield decreased when affected by both *Alternaria* and *Septoria* diseases (Brand et al. 2020).

The aim of the research was to evaluate the frequency and degree of infection of sunflower plants with *S. helianthi* as well as possibility of simultaneous infection with this pathogen and other fungi which cause foliar spots, such as downy mildew, *Alternaria* leaf spot and *Phoma* black stem under conditions of the stationary infectious nursery.

Materials and methods

The research was conducted in the sowing season of 2021 on a stationary artificial infectious plot of the Institute of Oilseed Crops of NAAS. The stationary infectious plot was established in the field crop rotation of the Institute of Oilseed Crops in 2005 to evaluate the breeding material of oilseeds for a complex of diseases. Every year it is enriched with infected plant residues collected in different regions of the Ukraine.

Assessment of fungal diseases on sunflower plants was performed in F_2 families obtained from crossing ZL22A, ZL58A, ZL70A, ZL78A, ZL169A lines of Zaporozhye breeding with HA-R7 line (originating from the USA). Each F_2 family was derived from self-pollination of one F_1 plant with varying severity of *Septoria* leaf spot.

Seeds of the selected samples were sown on 3–4 row plots with 20 hole rows, with a distance between rows of 70 cm and between holes of 35 cm, two seeds per hole. Samples within each cross combination (genotype) can be considered as replicates.

The weather conditions of the vegetation period of 2021 were quite favorable for the development of fungal diseases (Table 1).

Table 1 shows that the sum of temperatures during the growing season of sunflower was moderate, which was typical of that area, while the amount of precipitation was increased. The average daily temperature in May reached 17.4 °C, and in June –22.0 °C. The temperature regime in July and August was much warmer and temperature reached 25.6 and 25.4 °C, respectively. During the growing season of sunflower (May–August) in 2021 the amount of precipitation equaled to 307.8 mm, which is much higher than the usual average annual figures (211 mm). Analysis of climate data obtained during the observation period showed that June and July were the wettest months. During that period, 229 mm of precipitation fell, which is quite a high figure for the area. In general, weather conditions in 2021 were characterized by moderate air temperatures, but differed from long-term averages by increased rainfall, especially in the middle of the first and in the early second half of the sunflower growing season. Such weather conditions were favorable not only for growing sunflower, but also for the development of fungal diseases on sunflower plants, and especially leaf spots.

Plant damage by *Septoria* disease was determined in the early flowering phase. Assessment of the severity of plant damage was performed by visual inspection of all leaves using a modified scale proposed by Babayants and Babayants (2014) for wheat: – no lesions on all leaves; ± only the lower leaves are affected (minor lesions); + affected leaves of the lower and middle part of the plant (average lesions); ++ lesion is present on all leaves (severe lesions).

Infection of plants with *Alternaria alternate* and *Phoma macdonaldii* was determined in the phase of early maturation. Assessment of the severity of infection of sunflower plants with *Phoma* disease was performed similarly to *Septoria* leaf spot. With regard to *Alternaria* leaf spot, the presence or absence of characteristic symptoms was taken into account.

Initially, the assessment was performed on V8 stage, as single leaves of the lower tier dried up suffering from *Septoria* disease. The final assessment was performed during the assessment of plant damage by *Septoria* disease, i.e. at the beginning of flowering by a method similar to *Septoria* leaf spot (Babayants and Babayants 2014).

Damage to samples by fungal diseases was defined as a proportion of affected plants (in percent) to the total number of plants. The severity of disease was calculated as a proportion of plants with lower, middle, and upper tier leaves infected (in percent) to the actual number of plants in each sample. Simultaneous infection of sunflower plants with *S. helianthi* and pathogens of

Table 1: Weather conditions during growing season of sunflower, 2021.

Decade	Month							
	May		June		July		August	
	Average daily temperature t °C	Rainfall mm	Average daily temperature t °C	Rainfall mm	Average daily temperature t °C	Rainfall mm	Average daily temperature t °C	Rainfall mm
I	14.6	17.8	17.2	87.5	24.7	2.0	26.8	16.0
II	17.5	14.0	22.1	38.5	27.2	45.0	25.0	23.0
III	20.2	8.0	26.6	51.0	24.8	5.0	24.4	0.0
Average temperature	17.4	–	22.0	–	25.6	–	25.4	–
Total rainfall	–	39.8	–	177.0	–	52.0	–	39.0
Average long-term rates	16.0	40.0	19.4	62.0	22.6	58.0	21.2	51.0

other fungal spots was determined as the proportion of plants with symptoms of *Septoria* leaf spot among plants affected by the other leaf spot diseases.

Statistical processing of the obtained data was performed using the Microsoft Excel 2010 software package (Volkova and Shipunov 2008).

The percentage error was determined by the formula:

$$S_p = \sqrt{\frac{P*(100-P)}{n}},$$

where s_p is the percentage error; P is the percentage of affected plants; n – the total number of plants analyzed (Rokitsky 1973).

Results and discussion

The damage of sunflower accessions by *S. helianthi* and other fungal pathogens was assessed in the stationary infectious nursery. *Septoria* leaf spot lesions were detected alone and in combination with downy mildew (*Plasmopara halstedii* Berl. and De Toni) (Figures 1 and 2), *Alternaria* leaf spot (*A. alternate* Keissl.) and *Phoma* black stem (*P. macdonaldii* Boerema) (Figure 3) (Harveson et al. 2016).

In the course of the study it was found that sunflower was quite affected by *Septoria* leaf spot (Table 2). The percentage of affected plants in F_2 families ranged from 41.0 to 100.0%. The severity of disease was different – from minor to severe, depending on the sample and genotype.

The largest number of plants affected by *Septoria* leaf spot was observed among samples of the ZL58A × HA-R7 crossing combination. The number of affected plants in 10 samples of this genotype varied from 48.3 to 100%. With

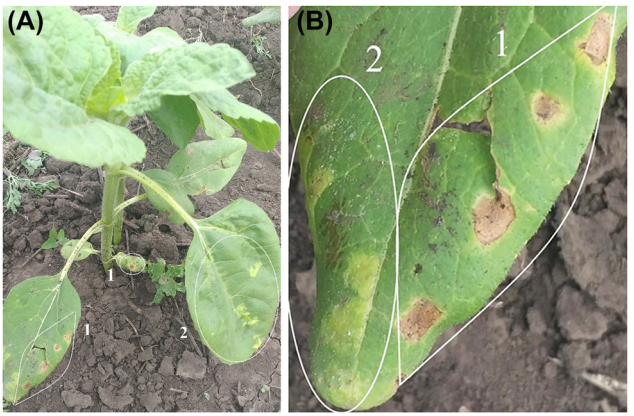

Figure 1: Appearance of *Septoria* leaf spot (1) and downy mildew (2) on the first true leaves of a sunflower (A, B).

Figure 2: *Septoria* leaf spot and downy mildew on the same sunflower plant.

regard to the severity of damage, the proportion of plants with minor, average, and severe damage amounted to 23.9, 28.5, and 24.5%, respectively.

F_2 samples of the ZL78A × HA-R7 and ZL22A × HA-R7 combinations were affected by *Septoria* leaf spot similar to the samples of the ZL58A × HA-R7 genotype. Among the plants affected by *Septoria* disease were plants with damaged leaves of the lower, middle and upper tiers in approximately equal proportions.

Samples of ZL70A × HA-R7 and ZL169A × HA-R7 crossing combinations were affected by *Septoria* leaf spot to a much lesser extent than the above. Moreover, there were almost no plants with injured leaves of the upper tier (1.5 and 1.1%).

Studies have shown that sunflower samples can be affected by *Septoria* leaf spot and black stem fungus simultaneously (Table 2). *Phoma* disease affected plants with varying severity. Black stem damage in the samples of the ZL58A × HA-R7 crossing combination varied from 26.8 to 94.3%. The majority of plants (60.8%) had an average lesion area, of which 78.6% were attributed to

Figure 3: *Septoria* leaf spot (1) and black stem (2) on the same sunflower plant.

Table 2: Assessment of *Septoria* leaf spot infection alone and in combination with *Phoma* black stem on the sunflower samples.

Sample	Number of F_2 plants	*Septoria* leaf spot					Black stem				
		Infected plants	Severity of infection				Infected plants	of them with *Septoria* leaf spot	Severity of infection		
			–	±	+	++			–	±	+	++
1.1	103	91	12	14	26	51	72	64	31	10(9)	62(55)	–
1.2	131	79	52	35	26	18	78	61	53	3	72(61)	3
1.3	59	35	24	9	14	12	26	14	33	1	25(14)	–
1.4	60	29	31	8	15	6	29	21	31	3(3)	26(18)	–
1.5	82	59	23	18	24	17	22	12	58	–	21(12)	1
1.6	96	53	43	36	12	5	58	29	38	16(8)	42(21)	–
1.7	133	114	19	38	56	20	108	92	5	–	108(92)	–
1.8	106	94	12	55	33	6	100	89	6	–	100(89)	–
1.9	121	121	–	15	35	71	106	106	20	5(5)	101(101)	–
1.10	114	100	14	13	46	41	56	48	58	1(1)	55(47)	–
2.1	114	93	21	28	40	25	107	81	14	6	100(80)	1(1)
2.2	103	55	48	28	18	9	90	45	13	5(1)	84(43)	1
2.3	89	48	41	12	19	17	59	17	30	–	59(17)	–
2.4	74	46	28	21	17	8	51	29	23	–	51(29)	–
3.1	116	98	18	33	42	23	72	54	53	11(11)	59(43)	2
3.2	106	71	35	25	9	37	56	21	50	42(16)	5(1)	9(4)
4.1	97	68	29	34	31	3	32	8	65	7	25(8)	–
4.2	100	41	59	32	9	–	87	36	13	3(2)	81(33)	3(1)
5.1	99	44	55	34	10	–	77	29	22	59(24)	17(5)	1
5.2	70	42	28	11	29	2	44	19	26	31(15)	12(4)	1

In parentheses – the number of plants infected with *Septoria helianthi*. Samples 1.1–1.10 correspond to F_2 (ZL58A × HA-R7); 2.1–2.4 – F_2 (ZL78A × HA-R7); 3.1–3.2 – F_2 (ZL22A × HA-R7); 4.1–4.2 – F_2 (ZL70A × HA-R7); 5.1–5.2 – F_2 (ZL169A × HA-R7).

Septoria leaf spot. Only 3.8% of plants demonstrated a slight damage by *Phoma* fungi, of which 77.8% of plants with obvious *Septoria* leaf spot.

In the genotype ZL70A × HA-R7 with black stem symptoms, a damage to the leaves of the middle tier was most often, and in ZL169A × HA-R7 – the lower tier leaves. Among plants of these samples affected by black stem rot, plants affected by *Septoria* leaf spot accounted for only 38.3%.

It should be noted that in most samples the pathogen of black stem affected the leaves of the lower and middle tiers of plants. There were almost no sunflower plants with severe *Phoma* disease. This may indicate that the infection had not spread to the upper tiers of plants. Apparently, weather conditions, namely the lack of precipitation in the second half of the sunflower growing season (Table 1), were the reason for this.

It was found that black stem disease advances on plants, both with existing *Septoria* leaf spot and on the intact plants. In some cases, *Phoma* disease affected sunflower more than *Septoria* one. Thus, in several F_2 samples of ZL58A × HA-R7, ZL70A × HA-R7, and ZL78A × HA-R7 crossing combinations, the number of plants infected with the black stem pathogen was significantly higher than with the *Septoria* leaf spot pathogen.

Table 3 shows that sunflower samples were also simultaneously affected by both *Septoria* leaf spot and downy mildew or *Alternaria* leaf spot. Those diseases were found on plants of the F_2 family of ZL58A × HA-R7 and ZL78A × HA-R7 crossing combinations. The infection with *P. halstedii* in these samples reached 16.8%. Almost all of them were infected by *S. helianthi* at the same time. The severity of downy mildew blight was strong in most plants, and only a few plants showed an average damage.

Some F_2 samples of these genotypes included up to 37.2% of *Alternaria*-infected plants. Of these, only one-third of plants demonstrated *Septoria* leaf spot symptoms as well. It should be noted that the samples of the ZL58A × HA-R7 crossing combination were more affected by *Alternaria* leaf spot than those of other genotype.

Table 4 presents the results of the joint development of *Septoria* leaf spot together with other leaf spots on sunflower plants in F_2 samples. The number of plants simultaneously affected by black stem fungus and *Septoria* leaf spot amounted to 66.4%. At the same time, more than 30% of plants affected by *Phoma* disease showed no symptoms of *Septoria* disease. That is, the development of black stem rot occured both on plants infected with *S. helianthi* and on healthy plants.

However, the frequency of simultaneous infection of plants by *Septoria* leaf spot and black stem pathogens in genotypes with different resistance to *Septoria* disease was significantly different. In the samples of the ZL58A × HA-R7 crossing that were most susceptible to *Septoria* leaf spot, the proportion of plants

Table 3: Assessment of *Septoria* leaf spot infection alone and in combinations with downy mildew and *Alternaria* leaf spot on the sunflower samples.

Sample	Number of F_2 plants	*Septoria* leaf spot	Downy mildew		*Alternaria* leaf spot	
		Infected plants	Infected plants	Of them with *Septoria* leaf spot	Infected plants	Of them with *Septoria* leaf spot
1.1	103	91	9	9	17	12
1.2	131	79	8	8	41	8
1.3	59	35	1	1	22	1
1.4	60	29	5	4	–	–
1.5	82	59	4	2	–	–
2.1	114	93	14	14	1	–
2.2	103	55	6	5	2	–
2.3	89	48	15	13	–	–
2.4	74	46	4	3	9	5

Samples 1.1–1.5 correspond to F_2 (ZL58A × HA-R7); 2.1–2.4 – F_2 (ZL78A × HA-R7).

Table 4: Infection frequency with *Septoria helianthi* among sunflower plants simultaneously infected by other foliar fungal pathogens.

Pathogen	Infected plants, total	Infected with *Septoria helianthi*	
		pcs.	%
Phoma macdonaldii	1330	875	$65.7 \pm 1.30^{b,c}$
Plasmopara halstedii	66	59	$89.3 \pm 3.80^{a,c}$
Alternaria alternata	92	26	$28.2 \pm 4.69^{a,b}$

[a, b, c] – Differences between pathogens are significant at the 0.1% level of significance.

simultaneously infected with two pathogens has reached more than 80%, while in the samples of the ZL70A × HA-R7 and ZL169A × HA-R7 crossings that were more resistant to *Septoria* disease, this indicator did not exceed 40% (Table 2).

The number of plants affected in parallel by downy mildew and *Septoria* leaf spot was about 90% of the total number of plants infected with the downy mildew pathogen. This suggests that both *S. helianthi* and *Plasmopara helianthi* can develop on the same plant and reduce its basic physiological functions.

The rate of simultaneous infection of sunflower with *Alternaria* leaf spot and *Septoria* leaf spot pathogens was quite low as compared to infection of plants with *Alternaria* pathogen alone, and did not reached 30%. This may mean that *Alternaria* leaf spot pathogen avoids plants with existing *Septoria* fungus, i.e. there exists a certain antagonism between the two pathogens.

It should be noted that pathogen that causes *Septoria* leaf spot may be a favorable factor for the development of other diseases on sunflower plants, including various spot infections. Its effect may be associated with a decrease in the host resistance, as a result they become susceptible to other diseases.

Obviously, this can explain the different frequency of simultaneous infection of plants with pathogens of *Septoria* leaf spot and *Phoma* black stem in genotypes with different resistance to *Septoria* disease. More resistant genotypes to *Septoria* leaf spot, in contrast to less resistant ones, were characterized by a lower severity of damage, which was expressed in the absence of plants with injured leaves of the upper tier (Table 2). A lower intensity of *Septoria* infection could eventually provide them with a better protection against infection with *Phoma* fungi. The weakening of the plant's defenses as a result of infection with the *Septoria* pathogen could be the cause of infection of the same plant with the downy mildew pathogen. In this case, the symptoms of *Septoria* leaf spot in the form of necrotic lesions were observed even on the cotyledon leaves (Figure 1A), which, of course, could already affect the normal metabolism and lead to a weakening of the protective forces.

The literature describes some cases of interaction of *Septoria* leaf spot disease with other diseases on the same host plant. Thus, the effect of this disease on the oat plants was studied and the relationship between *Septoria* leaf spot and *Phoma* black stem diseases was found. It has been established that the *Septoria avenae* pathogen contributed to the intensive development of black stem disease in oat plants (Clark 1980).

An interaction between the virus and *Septoria apiicola* has been reported to have a positive effect on celery plants. Infection of plants with viruses stimulates the production of phytoalexins and furanocoumarins, and high concentrations of furanocoumarins reduce the germination of *S. apiicola* spores. This decreases further infection. Plants affected by the viral pathogen were injured by *Septoria* disease by only 17–19%, and non-affected – by 57% (Ataga et al. 1999).

Interactions between *Septoria glycinea* and *Pseudomonas glycinea* were also observed in soybean samples. When soybean plants were infected with the both pathogens, the leaves showed characteristic symptoms and a decreased chlorophyll content was noted (Fucikovsky 1972). According to other researchers, both pathogens competed with each other for the same leaf area. Plant yields in the presence of both pathogens did not decrease significantly (Basu and Butler 1988). Williams and Nyvall (1980) also noted the lack of effect on plant yields when co-infected by both pathogens.

Thus, as a result of the studies, it was shown that in the conditions of excessive moisture and saturation of the growing environment with pathogens that cause various leaf spots, including *Septoria* leaf spot, infection of individual sunflower samples with *S. helianthi* can reach 100% with injured leaves not only of the lower and middle, but also the upper tiers of the plant. It was revealed not only the damage of sunflower plants with *Septoria* alone, but also in combination with downy mildew, *Phoma* black stem and *Alternaria* leaf spot. It has been established that the possibility of simultaneous infection of sunflower plants by the causative agent of *Septoria* leaf spot and the pathogens of other leaf spots is different. Among plants infected with *P. halstedii* almost all of them were infected with *S. helianthi*, and among plants infected with *Alternaria* only a third was affected by *Septoria*. Taking into account that *S. helianthi* pathogen inoculates plant at the earliest stages of its development, one can suggest that injuring the plant with *Septoria* fungus is a more favorable factor for the development of downy mildew than for *Phoma* black stem, and even to a larger extent for *Alternaria* leaf spot.

Acknowledgments: The authors thank Ekaterina Vedmedeva and Natalya Kutishcheva from the Institute of Oilseed Crops of the National Academy of Agrarian Sciences (Zaporozhye, Ukraine) for providing the source material of sunflower lines.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

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