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Influence of the content of antioxidants (phenols and flavonoids) in sunflower hybrids and the degree of attack by pests

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Abstract

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The cultivated sunflower is one of the most significant oil-producing crops globally, and its cultivation in Bulgaria, as a primary strategic crop, currently lacks an alternative. One of the factors that can reduce yield and quality in sunflower crops is the presence of pests.

The present study evaluated the relationship between the antioxidant content of interspecific, intergeneric hybrids and one oilseed sunflower variety and their resistance to pests, with a view to including the plant materials in breeding programmes.

The trials were carried out during the 2019–2021, at the experimental field of the Institute of Plant Physiology and Genetics – BAS, Lozen village near Sofia. The plant material tested included interspecific and intergeneric hybrid sunflower lines, as well as one cultivated sunflower variety NA 1114. The content of antioxidants (phenols and flavonoids) in the plant materials wear quantified by spectrophotometric methods. Entomological studies were evaluated under field conditions by observing and assessing the natural attack of the experimental plant material by existing local populations of sunflower pests.

The species composition of the pests and the indifferent fauna of the tested sunflower lines and variety was determined. The data suggest that the differences in the degree of pest attack were due to antioxidant potential. The findings indicate that the sunflower variety HA 1114, the result of distant interspecific hybridization, displays an acceptable level of genetic resistance to pests.

Keywords: sunflower; hybrids; variety; antioxidants; pests

Introduction

The genetic diversity of plant species represents a crucial component of human nutrition. The improvement and diversification of numerous agricultural crops is dependent upon the utilisation of wild relatives, which serve as a source of genetic resources. In the pursuit of methods to enhance the diversity of cultivated sunflowers, the integration of genetic material from wild species within the genus *Helianthus,* represents a logical and effective approach to achieving this objective. The genus *Helianthus* (Asteraceae) comprises 51 species, of which 37 are perennial (diploid, tetraploid and hexaploid) and 14 are annuals (diploid 2n = 2x = 34). The wild relatives of *Helianthus annuus* L. have been identified as a source of potential resistance and tolerance to diseases and pests, as well as oleic fatty acids and other vital components of vegetable sunflower oil (vitamin E) and others.

The pests that infest sunflowers represent a significant factor influencing the yields and quality of the resulting products. The changes that have occurred in the sunflower cultivation technology and in the climatic conditions have resulted in the rapid multiplication and expansion of the geographical range of these pests, as well as the appearance of new species. The major insect pests of sunflower crop include the cotton boll-

worm (*Helicoverpa armigera* (Hubner 1886)) (Lepidoptera: Noctuidae), the European Sunflower Moth (*Homoeosoma nebulella* (Denis & Schiffermüeller 1775)) (Lepidoptera: Pyralidae), the sunflower Long-horned beetle (*Agapanthia dahli* (Richter, 1821)) (Coleoptera: Cerambycidae), and sucking insect pests such as aphids and others (Prasifka, 2015).

A mas reproduction of sunflower pests has been observed in Bulgaria in the last few years. In 2022, a significant infestation of the meadow moth (*Loxostege sticticalis* L.) was documented in the eastern and some central regions of Bulgaria (Andreev, 2022). This species is a common pest of sunflower crops in Bulgaria, with a nationwide distribution. Popov (1976) notes that the population is usually small and stable, but there are years when it increases significantly. In their study, Ivanov and Angelova (2023) identified 17 species of harmful sunflower entomofauna belonging to the orders Hemiptera, Coleoptera, Lepidoptera, and Blatodea in the Tutrakan region. Various sunflower hybrid cultivars were tested by Dimitrov and Koleva (2022). The authors identified the following aphid species: *Aphis fabae* subsp. *evonymi* Fabricius, *Sipha maydis* Passerini, *Brachycaudus helichrysi* (Kaltenbach) and *Myzus persicae* Sulzer. The dominant species were *A. fabae* subsp. *evonymi* and *S. maydis*. The results demonstrated that the hybrid cultivars had different degrees of host suitability for aphids.

The terpene components released by the glandular trichomes in the flowers of some of the wild species of the genus *Helianthus* are known to kill or sharply slow down the development of sunflower moth larvae (Prasifka et al. 2015, Prasifka and Hulke, 2020). The sesquiterpene lactones and diterpene compounds present in *Helianthus annuus* have been observed to exhibit strong insect antifeedant, antifungal and allelopathic activities. These biological activities suggest that *H. annuus* has great potential for use in natural crop protection (Galisteo et al., 2023).

Several studies have reported the risks of the multiplication of the pest due to the intensification of crop production and the extensive use of hybrid varieties (Horváth, Hatvani, 2001). A correlation was established between non-compliance with the growing technology, due to the specifics of new hybrid varieties, and the extent of the damage (Gornovska and Fedorenko, 2014). In the scientific literature, aphids are defined as a vector that transmits and spreads various viral diseases on sunflower plants. The most frequently referenced is the sunflower mosaic virus (SuMV) (Raj and Wati 2005; Singh et al., 2005; Bhat and Reddy, 2016). It has been established that the sunflower mosaic virus is transmitted mechanically in sap and by several species of aphids. The most significant vectors are *Aphis gossypii* Glover, *Aphis craccivora* Koch, *Aphis malvae* Williams and *Rhopalosiphum maidis* (Fitch), which transmit the virus in a non-persistent manner, both in laboratory settings and in natural field conditions. Transmission of this virus by the green peach aphid, *M. persicae* and the Russian olive aphid, *Capitophorus elaegni* (Del Guercio), is also successful (Gulya et al. 2002).

In recent years in Bulgaria has experienced a significant increase in the number of pests, which has highlighted the need for a more comprehensive investigation into their biological characteristics and the development of effective and innovative control strategies. Research into the resistance of interspecies hybrids to biotic stress factors and the causes of the specific response of the genotypes under study implies the accumulation of information on the possibilities of using wild species as donors of valuable economic qualitie. The provides direction for complex research in the field of theoretical and applied entomology, genetics and breeding. A review of the literature reveals a dearth of data on the content of antioxidants in interspecific hybrids and the relationship between this indicator and their resistance to pests. Notwithstanding the inherent difficulties associated with interspecific hybridization, the potential for gene transfer to confer specific resistance to diseases and pests makes the preliminary studies investigating the suitability of interspecific sunflower genotypes as hosts for these organisms a worthwhile endeavour.

The objective of the present study was to examine the antioxidant content of a single oilseed sunflower variety and interspecific hybrids, with a view to establishing a relationship between this indicator and their resistance to arthropod pests. The results must be evaluated to ascertain their suitability for incorporation into breeding programmes.

Materials and Methods

Characteristics of the experimental area

The investigates were carried out in 2019–2021, at the experimental field of the Institute of Plant Physiology and Genetics-BAS, Lozen village near Sofia (42.635815 N, 23.450931 E), with a predominant soil type of leached cinnamon forest soil. The sowing was conducted out in May after the previous crop: corn (2018) and green beans (2019). Plant protection activities using pesticides have not been applied.

Characteristics of plant material

The observations included interspecific and intergeneric hybrid sunflower lines, as well as one variety of sunflower HA 1114, created in IPPG, BAS and certified with a patent in 2017.

The hybrid lines were obtained by using conventional methods of crossing between cultivated sunflower (male sterile line HA 89) and wild annual and perennial species of

Helianthus, which are pollen donors /interspecific crosses/, and wild relatives of the family Asteraceae, representatives of the genera *Verbesina*, *Echinacea*, *Tagetes*. In order to obtain a homogeneous hybrid line resulting from wide hybridisation, a series of breeding and selection processes were carried out on the F1 hybrids obtained, as well as a series of backcrosses and sister pollinations. The following hybrid lines were included in the study:

1. Interspecific crosses with perennial wild species

Helianthus annuus x *Helianthus mollis* (2n = 2x = 34). Both lines demonstrated resistance to drought conditions. Their antioxidant potential was analysed, as well as their agronomic characteristics (Vassilevska-Ivanova R. et al., 2012).

Helianthus annuus x *Helianthus nuttallii* (2n = 2x = 34). The preliminary analyses have indicated that as a consequence of the introgression, a probable alteration in the fatty acid constitution and the quantities of tocopherol (vitamin E) is to be expected (Vassilevska-Ivanova et al., 2018).

2. Intergeneric crosses Helianhus x wild relative from Compositae

The following species: *Verbesina encelioides* and *Echinacea purpurea* were included in the IPPG breeding programme. All species involved in the hybridisation process have a set of genes that, when incorporated into the genome of the cultivated sunflower, can lead to the emergence of new characteristics.

Helianthus x *Echinacea*

Some of the obtained recombinant lines were described by Vassilevska-Ivanova R. et al. (2014a, 2014b, 2015, 2016a, 2016b).

Helianthus x *Verbesina*

This recombinant line was described by Vassilevska-Ivanova R. et al. (2013).

Oilseed sunflower variety "HA 1114" The oilseed sunflower variety has successfully completed the requisite technical tests and, as documented in the final report, was subsequently included in the official variety list of Bulgaria in 2017. The variety is of medium height (179 cm), exhibits a high level of resistance to lodging (rated at 9 points on the scale), has a weight per 1000 seeds of 59.16 g, and displays a high level of resistance to *Orobanche* spp., *Sclerotinia*, *Phoma* and *Phomopsis*. The oil content is 46.96 %.

The content of antioxidants (phenols and flavonoids) in the plant materials was determined spectrophotometrically. The total flavonoids were determined in accordance with the methodology proposed by Zhishen et al. (1999), while the flavonoids present in the plant material were quantified using the approach outlined by Lamaison et al. (1990). The total antioxidant activity was assessed through the ferric reducing antioxidant power (FRAP) assay, as described by Benzie and Strain (1999) and Benzie and Szeto (1999). Additionally, the antiradical activity was evaluated using the methodology established by Brand-Williams et al. (1995).

Experimental design

Entomological studies were carried out under field conditions by observing and accounting for natural attacks on experimental plant material by existing local populations of sunflower pests.

The experimental plots were located in such a way that the various genotypes were situated in close proximity to one another, thereby enabling the pests to select between the individual hybrids and the cultivated sunflower at the outset of the attack.

The following various interspecific hybrid lines and a sunflower variety were subjected to study:

Cultivated sunflower *H. annuus* variety 1114 and hybrid lines: *H. annuus* x *Echinacea purpurea*; *H. annuus* x *Helianthus mollis*; *H. annuus* x *Helianthus nuttallii* and *H. annuus* x *Verbesina encelioides*.

Field observations and methods for evaluations

Following the commencement of the intensive growth of the sunflower (BBCH 30-39), observations and readings were conducted at 10-day intervals. The population dynamics of insect pests in the test lines and cultivated sunflower were monitored by direct visual. The reports have been carried out on 30 plants placed randomly in each hybrid. Assault from aphids was reported with a modified approximate scale from 0 to 7 (Schnelle 1995). The data were obtained from the average number of individuals per plant, counted during all growth stages of the phenological development of the sunflower.

Statistical analysis

The mathematical processing of the results was carried out using the statistical computer program SYSTAT 13.1. The CHAID method (chi-squared automatic interaction detection) was used to compare the empirically determined frequencies (number of pests in each hybrid line and variety) with the expected frequency distribution. Analysis of variance (ANOVA) was used to determine the influence of the factors tested. Means were compared using Tukey's test.

Results and Discussion

Table 1 presents the antioxidant contents (ascorbic acid, phenols flavonoids and vitamin E) and the number of individual pests and the degree of attack in the tested genotypes.

Table 1. Antioxidant content, number of pest and degree of attack in the tested hybrid sunflower lines and cultivated sunflower

Hybrid sunflower lines and cultivated sunflower	Antioxidants				Pests			
					Aphididae	Leafhoppers	*L.pratensis*	*Aceria sp.*
	Ascorbic acid µm g $^{dw-1}$	Phenols mg g dw^{-1}	Flavonoids µm g dw^{-1}	Vitamin E µm g dw^{-1}	Degree of attack (0–7)	Mean number ind./plant.		
Helianthus annuus, V.*1114*	93.63±4.68a	18.86±0.94a	1.97±0.10a	3.69±0.18a	7.9±0.10b	29.3±0.50a	0±0.00c	0±0.00c
H. annuus x *E. purpure*a	113.72±5.69b	18.60±0.93a	2.31±0.11b	1.92±0.10b	6.3±0.60a	2.1±0.30b	0±0.00c	24.30±0.00a
H. annuus x *H. nuttalli*i	113.04±7.40b	40.70±0.50b	33.62±0.32c	1.87±0.09b	0±0.00c	0±0.00d	0±0.00c	5.30±0.90b
H. annuus x *V. encelioides*	113.01±6.40b	40.01±0.07b	33.02±0.03c	1.85±0.07b	6.1±0.30a	58.6±0.70c	105.10±0.30a	0±0.00c
H. annuus x *H. mollis*	128.53±6.45c	18.55±0.92a	2.15±0.11b	2.13±0.50b	6.9±0.10a	0±0.0d	5.1 ±0,90b	0±0.00c

Mean ± SE; different letters in columns = significant differences for each genotype vs. each pest species or pest group; $P < 0.05$; Tukey test

It should be noted that the attack by natural populations of pests was reported under field conditions. The rate of settlement on the plants was found to be relatively slow. In the highest density the pests have been ascertained during the phenophases BBCH 59–71.

The data obtained for the period of the observations carried out, show different degrees of attack by pests. The studied interspecific and intergeneric hybrid lines and the cultivated sunflower *H. annuus* were strongly attacked by aphids (Table 1), with the highest degree reported for the cultivated sunflower (7.9±0.1) and the lowest (6.1±0.3) for the hybrid line *H. annuus* x *V. encelioides*. No aphid infestation was reported in the interspecific hybrid *H. annuus* x *H. nuttallii*. The lowest number of leafhoppers was found in the line *H. annuus* x *E. purpurea* (2.1±0.3ind./plant) and the highest in *H. annuus* x *V. encelioides* (58.6±0.7 ind./plant). Cultivated sunflower was intermediate as a suitable host (29.3±0.5 ind./plant) (Table 1).

The first adult herbivorus bugs were observed in the hybrid line *H. annuus* x *V. encelioides*, with a mean number of bugs per plant of 105.1±0.3; later, in *H. annuus* x *H. mollis*, a much lower mean number of bugs per plant of 5.1±0.9 was reported. During this period, the hybrid lines studied were in phenophases BBCH 47÷69. Adult individuals of the new generation met up to the full maturity of the sunflower – BBCH 89. The eriophid mite damage observed in the interspecific hybrid lines *H. annuus* x *E. purpurea* (24.3±0.0 mean number of erineums/plant) and *H. annuus* x *H. nuttallii* (5.3±0.9 mean number of erineums/plant) was of particular interest.

The data on antioxidant content showed significant differences between the genotypes studied (Table 1). The highest value of ascorbic acid was observed in the hybrid line *H. annuus* x *H. mollis* (128.53 ± 6.45), while the lowest value was recorded in the cultivated sunflower *H. annuus* (93.63 ± 4.68). Statistical analysis showed that there was a significant difference in ascorbic acid content between cultivated sunflower and the other hybrid lines. The observed differences in phenolic content between cultivated sunflower and the hybrid lines *H. annuus* x *E. purpurea* and *H. annuus* x *H. mollis* were not reliable. However, they were statistically significant when compared to the hybrid lines *H. annuus* x *H. nuttallii* and *H. annuus* x *V. encelioides*. The differences in flavonoid content between the hybrid lines *H. annuus* x *H. nuttallii* (33.62±0.32) and *H. annuus* x *V. encelioides* (33.02±0.03) were significantly higher than those observed in the hybrid lines *H. annuus* x *E. purpurea*, *H. annuus* x *H. mollis* and cultivated sunflower *H. annuus*. The vitamin E content varied from 3.69 ± 0.18 to 1.85 ± 0.07 and the content of this vitamin was significantly higher in cultivated sunflower than in any of the hybrid lines tested.

The hybrid lines are characterised by specific characteristics that are intermediate in relation to the parental forms. They are therefore easily recognisable morphologically throughout the growing season, except for the first few days of seed germination. These differences have also been observed in relation to a number of biochemical indicators, such as antioxidant capacity. It can be assumed that the content of ascorbic acid, phenolics, flavonoids and vitamin E /α-tocopherol/ is directly related to the attack by certain pests. The observed differences between the lines in terms of the degree of attack and damage observed are probably partly due to the antioxidant content of the plants. The study of correlations and the influence of genotype in relation to herbivory is an important issue that will be analysed in future studies.

Sunflower lines resulting from distant hybridisation (intergeneric and interspecific) and cultivated sunflower are characterised by different levels of antioxidant metabolites. Natural antioxidants are secondary metabolites found in plants. The complex antioxidant system includes reduced glutathione, ascorbic acid (vitamin C), α-tocopherol /vitamin E/, carotenoids and enzymes, as well as phenolic compounds, which protect the plant against oxidative damage to cells as a result of abiotic and biotic stress. The results obtained showed a significant difference in the total antioxidant potential, measured by the DPPH method, between the cultivated sunflower and the hybrid lines. In the *H. annuus* x *H. mollis* cross, the concentration of ascorbic acid in the leaves of the plants was higher than in the cultivated sunflower (Table 1). The three lines *H. annuus* x *E. purpurea*, *H. annuus* x *H. nuttallii* and *H. annuus* x *V. encelioides* did not differ significantly in ascorbic acid content. The higher level of ascorbic acid may result in a relatively higher stress resistance of these lines compared to cultivated sunflower. Based on this, it can be assumed that the higher aphid (7.9±0.1) and leafhopper (29.3±0.5) damage is due to the lower ascorbic acid content in cultivated sunflower. Several studies have reported a high degree of correlation between phenolic compounds and total antioxidants (Tepe et al., 2006). The total phenolic content of the hybrid lines and cultivated sunflower is shown in the Table 1. It was found that the phenolic content was significantly higher in the two lines *H. annuus* x *H. nuttallii* and *H. annuus* x *V. encelioides* than in the cultivated sunflower *H. annuus* and the hybrid lines *H. annuus* x *E. purpurea* and *H. annuus* x *H. mollis*. The data obtained suggest that the high antioxidant potential of the first two lines was a prerequisite for the differences found in the degree of attack by the different pests. The highest flavonoid content was observed in the interspecific hybrids *H. annuus* x *H. nuttallii* and *H. annuus* x *V. encelioides*, while the lowest was found in the leaves of cultivated sunflower. A notable distinction was evident in the α-tocopherol content between cultivated sunflower and the lines, with the latter exhibiting significantly lower levels compared to *H. annuus*.

Plants that are the product of distant hybridisation are characterised by an intermediate phenotype, with some showing new characteristics as a result of the combination of two distant genomes. It has been found that the manifestation of new characters mainly affects the reproductive system of the plants and the leaf apparatus. Among the lines studied, a form with a modified architecture of the whole plant has also been described, which creates selection advantages when sowing a larger number of plants per unit area (Vassilevska-Ivanova and Tcekova, 2005). At the same time, the extent to which the external manifestation of these traits depends on and influences the degree of attack by certain pests and the suitability of the plants as hosts is to be determined. Of particular interest is the relationship between the plant /host/ and the pests attacking it, in order to determine the parameters of this interaction, which will make it possible to predict the results of the harvest.

The research is the first of its kind, and further studies are expected to show the relationship between the levels of natural antioxidants and sunflower pests, and which antioxidants are crucial to sunflower resistance to pests. Subsequently, there will be an analysis of the morphological characteristics that attract or repel pests. The created interspecific hybrid lines are an extremely important research tool with the potential to provide initial plant material for genetic and breeding purposes as donors of resistance to pests.

Conclusions

Based on the results obtained, the following can be concluded:

The antioxidant potential of plants has a positive effect on both their health status and the reactions of the harmful fauna surrounding them;

The data for the period studied show different levels of herbivory, suggesting that there is a relationship between the genotype of the plants and the attack density;

From the results obtained it can be concluded that the sunflower variety HA 1114, the result of distant interspecific hybridisation, has satisfactory genetic resistance arthropod pests.

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