Seed Component Diversity of Hybrid Forms, Originated from Wild Helianthus Species

Daniela VALKOVA*, Nina NENOVA, Galin GEORGIEV, Valentina ENCHEVA, Emil PENCHEV, Julia ENCHEVA
Dobroudza Agricultural Institute, General Toshevo 9520, Bulgaria
*Corresponding author: valkova_d@abv.bg

Abstract
This study presents the evaluation of hybrid forms, obtained as a result of interspecific hybridization between four sunflower inbred lines and wild annual H. debilis and H. petiolaris accessions from the collection of wild Helianthus species at Dobrudzha Agricultural Institute, on various indices related to their seed oil and protein content. The selected accessions, used as paternal parents in the crosses and the obtained hybrid material, were previously tested and evaluated as resistant to leaves pathogens. The indices 1000 seed weight (g), kernel and hull (%), oil content in kernel (%) and in seed (%), and seed protein content were evaluated. The results showed very good statistical authenticity of the analytical hypothesis that the investigated hybrid forms were with different genetic potential on the studied indices, pointing to the existence of high variability among the used wild germplasm. Hybrid forms, resistant to leaves pathogens, with high oil and protein content were obtained.

Keywords: interspecific hybridization, Helianthus debilis, Helianthus petiolaris, seed oil content, seed protein content

Introduction
Wild Helianthus species have been and continue to be an invaluable source of new genes for the improvement of cultivated sunflower (Seiler G. & Gulya T., 2004). Genetic variability of the cultivated sunflower may be increased by interspecific hybridization with wild sunflower species. Wild species from the genus Helianthus possess not only considerable variability for most of the traits but also excellent survival environmental mechanisms (Thompson et al., 1981). They possess genes for resistance to diseases (biotic stress), tolerance to abiotic stresses (drought, cold, soil salinity, certain herbicides) and high quality of proteins and oil (Hladni N. and Miklič V. 2012). That's why they were widely used in sunflower breeding programs (Seiler, 1992; Skoric, 1992). Dorrell et al. (1978) have used H. annuus and H. petiolaris to transfer desirable agronomic characteristics to cultivated sunflower and their potential for improving oil quality characteristics. Seiler G. (1994) determined oil concentration and fatty acid composition in achenes of populations of two annual Helianthus species and 11 perennial species and pointed out that the highest average oil concentration was observed in annual H. petiolaris Nutt. with 28.8%, followed by H. annuus L. with 25.4%. The present worldwide interest in growing sunflowers as a crop is due to the increased yield of the new commercially available oilseed hybrids. This was obtained by increasing the portion of kernel to the whole achene. The decreasing of hull thickness led to facilitating the sunflower moth attacks and damages in process of harvest and post-harvest activities (Ivanov and Tsvetkova, 1984). Sunflower was used as a traditional source of proteins. According to Christov (1990) if sunflower breeding was conducted in that direction, it needed a wide diversity of initial materials. It means that some forms of the genus Helianthus can be used as initial genetic source. This study presents the evaluation of interspecific hybrid forms, originated from wild H. debilis and H. petiolaris accessions on various indices related to their seed oil and protein content.

Materials and Methods
The investigation was carried out in Dobrudzha Agricultural Institute, General Toshevo. Interspecific crosses cultivated sunflower x wild species were performed and the obtained hybrid plants were grown in field conditions. Six accessions of two wild annual species were picked and included in this study. They were E-012, E-137 (H. debilis, ssp. cucumerifolius), E-138, E-139 (H. debilis, ssp. silvestris), E-022 and E-142 (H. petiolaris, ssp. petiolaris). The chosen accessions were preliminary
tested and evaluated, with resistant type of reaction to the leaves pathogens (Encheva V. and Valkova D., 2012; Encheva V. et al., 2012). The cultivated sunflower was represented by four susceptible CMS lines – 217 A, 807 A, 1017 A and 3607 A. Seed oil content, kernel oil content, 1000 seed weight, percentage kernel and hull for each hybrid form were estimated according to Rushkovski (1957). The protein content analysis was carried out according to the methods of Nikolova (1987). The interaction between different indices was determined with dispersion and correlation analyses. The statistical package SPSS was applied.

Results

Two populations of the annual species \textit{H. petiolaris} and four populations of the annual species \textit{H. debilis} were crossed with cultivated sunflower lines. Cultivated lines were not branched except line 217A, in which plants with basal branching were found. All F1 interspecies hybrid populations obtained in this study were fully branched with or without central head. The plants were 110 cm to 170 cm tall. Leaves were mostly alternate, ovate and petiolate, slightly serrate on the margins, truncate at the base.

The crossability between \textit{H. petiolaris} accessions and cultivated sunflower varied from 65% to 100%. The results from hybridization showed that the seed set was lower and varied from 13,24\% for the hybrid 1017 A x E-022, to 19,24\% for the hybrid 217 A x E-142 (fig.1). Some differences in viability of hybrid seeds were established. The percentage of viable F$_1$ plants varied from 55\% for the hybrid 1017 A x E-022 to 78\% for the hybrid 217 A x E-142, which was distinguished also with the biggest number of seeds per head. The accession E-142 was characterized with better crossability and the biggest number of obtained hybrid seeds.

![Figure 1](image1.png)

\textbf{Figure 1.} Seed set (%), number of obtained seeds per head and viable hybrid plants (%) for crosses with the participation of \textit{H. petiolaris} accessions.

![Figure 2](image2.png)

\textbf{Figure 2.} Seed set (%), number of obtained seeds per head and viable hybrid plants (%) for crosses with the participation of \textit{H. debilis} accessions.
Figure 3. Seed set (%) and number of obtained seeds per head for crosses between cultivated sunflower and H. debilis and H. petiolaris accessions.

Figure 4. Coefficient of variation of the studied characters for crosses between cultivated sunflower and H. debilis and H. petiolaris accessions.
### Table 1. Correlation coefficients between characters, connected to seed oil and protein concentration for hybrids obtained with participation of *H. debilis* accessions.

<table>
<thead>
<tr>
<th>characters</th>
<th>1000 seed weight, g</th>
<th>kernel content, %</th>
<th>hull content, %</th>
<th>kernel oil content, %</th>
<th>seed oil content, %</th>
<th>protein in the defatted kernel, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>kernel content, %</td>
<td>-0.37</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hull content, %</td>
<td>0.37</td>
<td>-1***</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kernel oil content, %</td>
<td>-0.26</td>
<td>0.34</td>
<td>-0.34</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>seed oil content,%</td>
<td>-0.27</td>
<td>0.64**</td>
<td>-0.64**</td>
<td>0.85***</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>protein in the defatted kernel, %</td>
<td>0.22</td>
<td>-0.28</td>
<td>0.28</td>
<td>-0.60**</td>
<td>-0.59**</td>
<td>1</td>
</tr>
<tr>
<td>protein in the kernel, %</td>
<td>0.30</td>
<td>-0.36</td>
<td>0.36</td>
<td>-0.71**</td>
<td>-0.64**</td>
<td>0.77***</td>
</tr>
</tbody>
</table>

* Proved at $P = 0.05$; ** Proved at $P = 0.01$; *** Proved at $P = 0.001$.

### Table 2. Correlation coefficients between characters, connected to seed oil and protein concentration for hybrids obtained with participation of *H. petiolaris* accessions.

<table>
<thead>
<tr>
<th>characters</th>
<th>1000 seed weight, g</th>
<th>kernel content, %</th>
<th>hull content, %</th>
<th>kernel oil content, %</th>
<th>seed oil content, %</th>
<th>protein in the defatted kernel, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>kernel content, %</td>
<td>0.26</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hull content, %</td>
<td>-0.26</td>
<td>-1***</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kernel oil content, %</td>
<td>-0.80***</td>
<td>-0.24</td>
<td>0.24</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>seed oil content,%</td>
<td>-0.62**</td>
<td>0.36</td>
<td>-0.36</td>
<td>0.82***</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>protein in the defatted kernel, %</td>
<td>0.11</td>
<td>-0.11</td>
<td>0.11</td>
<td>-0.21</td>
<td>-0.29</td>
<td>1</td>
</tr>
<tr>
<td>protein in the kernel, %</td>
<td>0.78***</td>
<td>0.20</td>
<td>-0.20</td>
<td>-0.96***</td>
<td>-0.81***</td>
<td>0.46*</td>
</tr>
</tbody>
</table>

* Proved at $P = 0.05$; ** Proved at $P = 0.01$; *** Proved at $P = 0.001$
Discussion

The crossability between *H. debilis* accessions and cultivated sunflower varied from 44% to 100%. The results from hybridization showed that the seed set was much lower and varied from 4.3% for the hybrid 1017 A x E-138, to 8.4% for the hybrid 217 A x E-137 (fig.2). Some differences in viability of hybrid seeds were also established. The percentage of viable F₁ plants varied from 21.4 % for the hybrid 1017 A x E-138 to 47.1 % for the hybrid 807 A x E-139. The accessions E-137 and E-139 were characterized with better crossability, bigger number of obtained hybrid seeds and viable plants.

Comparing the results of crossability for all interspecific crosses we can conclude, that hybrid combinations with the participation of *H. petiolaris* accessions were characterized with bigger seed set and number of viable plants (fig.3).

Oil content depends on both percentage of hull and oil concentration in the kernel. The evaluation of variation and similarities of some characters connected to seed oil and protein content was done to compare the hybrid forms, obtained with participation of *H. debilis* and *H. petiolaris* accessions (fig.4). Coefficients of variation were determined at statistical authenticity level p=0.05. The most variable character was 1000 seed weight, followed by seed oil concentration, hull content and protein content in the kernels. Protein in the defatted kernel and kernel content were comparatively stable characters for both *H. annuus* (cult. line) x *H. debilis* and *H. annuus* (cult. line) x *H. petiolaris*.

The studied variables were determined through correlation analysis and thus the correlation dependence was proved (table1).

The correlation coefficients between characters for hybrids obtained with participation of *H. debilis* accessions signified their importance. There was higher negative correlation between protein and oil concentration in the kernel (r=-0.71). Seed oil content correlated positively with kernel content (r=0.64) and oil content in it (r=0.85). The indices percentage of hull and percentage of kernel were in inverse proportion.

The presence of correlation dependences between characters for hybrids obtained with participation of *H. petiolaris* accessions were also proved (table 2). The highest negative correlations were determined for the characters protein and oil content in the kernel (r=-0.96); seed oil content and protein content in the kernel (r=-0.81); 1000 seed weight and oil content in the kernel (r=-0.80). Positive correlation was determined between characters 1000 seed weight and protein content in the kernel (r=0.78). The hybrid combinations 217 A x E-142, 3607 A x E-142 and 217 A x E-022 were distinguished for the highest seed oil content. There was the highest negative correlation (r=-0.984**) between percentage of hull and percentage of kernel and lower negative correlation between percentage of hull and 1000 seed weight. There was a significant positive correlation only between percentage of kernel and 1000 seed weight.

Conclusion

According to Christov (1990) in the annual species, the differentiation of a genotype is possible as a result of self-pollination of a single plant in natural or artificial conditions. In that way, each species was represented by a different number of stabilized genotypes which, in total, expressed the diversity of the given species. It is known that within each *Helianthus* species, there is a great diversity in form and the value of individual traits.

The present study represented the seed component diversity of interspecific hybrid forms. As a results of this some hybrid forms, resistant to leaves pathogens with higher seed and protein content were selected for the needs of sunflower breeding programs.

References


Biotechnology and Biodiversity”, Novi Sad, Serbia.