Studies on some biochemical characters of hybrid forms, originated from wild *Helianthus* species

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Abstract
The study presents the evaluation of hybrid forms, obtained as a result of interspecific hybridization between three sunflower inbred lines and wild perennial species *H. maximiliani* (accession M-175) and *H. tuberosus* (accession M-146) from the collection of wild *Helianthus* species at Dobrudzha Agricultural Institute, on various indices related to the content of oil, protein and fatty acids in the seed. Interspecific F_1 plants were obtained by *embryo rescue* techniques and classical breeding methods. BC2 and F3 were obtained as a result of self-pollination and backcrossing with cultivated sunflower. The linoleic acid concentration for the obtained two hybrid forms, originated from *H. maximiliani* averaged 60.5 % and those originated from *H. tuberosus* – 70.8%. This is approximately equal to 69% for cultivated sunflower with linoleic type of oil. The combined saturated palmitic and stearic fatty acids concentration varied from 8.6 % to 11.2% which is less or about equal to traditional cultivated sunflower oil that averaged 11.5%. The obtained hybrid material could be a potential source of genes for increasing linoleic acid concentration in traditional sunflower oil. Hybrid forms could be used as sources for reducing the concentration of saturated acids in traditional cultivated sunflower oil. They will be included in sunflower breeding programs for the diversification of initial genetic material.

Key words: interspecific hybridization; *Helianthus maximiliani*; *Helianthus tuberosus;* seed oil content; fatty acids concentration

INTRODUCTION

Genetic resources are the biological basis of global food security. Preservation of cultivars, landraces, and wild relatives of important plant species provides the foundation to sustain and promote agriculture (Seiler, 1986). Edible vegetable oils are the principal source of fats in human diets. Sunflower seeds are a good source of protein and fiber in the diet (Willett, 1994). The residual cake is a high-protein supplement. Decorticated seed cake contains 44 % protein. The fatty acid composition, one of the key factors determining the physical and chemical properties of vegetable oils, is subject to physiological regulation and highly variable owing to environmental factors (Sarmiento et al., 1998) and the genetic background (Knowles, 1988). The range of applications for sunflower oil may be extended to industrial applications if ‘designer oil’ with specific fatty acid composition could be produced. Many hybrids, producing oil with particular fatty acid composition, have been obtained by conventional breeding techniques (Ivanov et al., 1988, Miller & Vick, 1999).

The genus *Helianthus* consists of 52 species and 19 subspecies with 14 annual and 38 perennial species (Schilling, 2006). *Helianthus* species have been included in sunflower breeding programs in DAI, General Toshevo mainly as donors for resistance to diseases. Transfer of genes, controlling the resistance, into cultivated sunflower lines, allowed diversification of cultivated sunflower and broadening its gene pool (Christov, 1996). *Helianthus* species were distinguished with their great diversity, different quantity and quality of seed oil, and seed protein content (Valkova et al., 2014). Tavoljanskiy et al. (2004) pointed that the low oil percentages in achenes of wild sunflower species are not limiting factors for use of these species in breeding programs, since it can be easily increased by repeated crossings with cultivated forms. It is better to use sunflower seed oil with high linoleic acid content (more than 70%) in the margarine industry.

Recent emphasis on the concentration and fatty acid composition of sunflower oil has increased interest in using wild species in breeding programs, but introgression of low oil concentration and quality from the wild species into cultivated sunflower has limited their use in applied breeding programs (Jan et al., 2008). The fatty acids composition of cultivated sunflower determines its uses and health effects on humans, while oil content determines the price, paid to producers (Table 1). The farmers aspired to use hybrids, distinguished with high seed yield, ability to overcome different stress factors as ecological changes in the environment and diseases and pests attacks (Georgiev et al., 2012a; Georgiev et al., 2012b; Encheva & Georgiev, 2009).

Sunflower oil contains both saturated and unsaturated fatty acids, either mono- or polyunsaturated. Unsaturated fatty acids comprise approximately 900 g kg^{-1} of the oil and include oleic acid (18:1) and linoleic acid (18:2). Saturated fatty acids such as palmitic acid (16:0) and stearic acid (18:0) may constitute another 70 to 110 g kg^{-1} of the oil (Friedt et al., 1994; Pierson, 1994; Skoric et al., 2008). Increased consumption of saturated fatty acids significantly increases the risks of heart attack, stroke and other health problems in humans. Consumption of oils with a high concentration of unsaturated fatty acids has been found to have a positive effect on human health (Jing et al., 1997; Hu et al., 2001).

The main purpose of this investigation was obtaining of interspecific hybrids and study some of their biochemical characters related to their fatty acids concentration to be included as initial material in the sunflower breeding program of DAI, General Toshevo.

MATERIAL AND METHODS

This study presents the evaluation of hybrid forms, obtained as a result of interspecific hybridization between three sunflower inbred lines (583 B, 712 B, 197 B) and wild perennial species *H. maximiliani* (accession M-175) and *H. tuberosus* (accession M-146) from the collection of wild *Helianthus* species at Dobrudzha Agricultural Institute. The selected accessions, used as paternal parents in the crosses, were previously tested and evaluated as resistant to phomopsis (Encheva et al., 2006). Interspecific F_1 plants were obtained by *embryo rescue* techniques (Nenova, 2002) and classical breeding methods (Christov, 1990). BC_3F_2 plants were obtained as a result of self-pollination and backcrossing with cultivated sunflower. The female plants were emasculated manually and fresh pollen from wild sunflower accession was used for pollination, applying the method of Petcu & Pacureanu (2011). Seed oil and protein content was determined by the method of Rushkovskii (1957) and fatty acids content – by the method of gas chromatography using “Perkin Elmer”.

RESULTS AND DISCUSSION

The results of fatty acids concentration in wild perennial accessions were presented in Table 2. The

Table 1. Types of sunflower oil available in the industry (NSA, online-published data)

Types of Sunflower Oil	Oleic acid, %	Linoleic acid, % (polyunsaturated)	Saturated acids, %	
	(monounsaturated)	(polyunsaturated)	stearic acid	palmitic acid
High Oleic	82	9	5	4
Nutrisun™ High Stearic/ High Oleic (HSHO) oils	72	5	18	5
NuSun® or Mid-Oleic	65	26	5	4
High Linoleic	20	69	4	5

average oil content was 27%, considerably lower than cultivated sunflower, which averaged 45% and higher. The low oil content can be increased by backcrossing with cultivated sunflower. The average linoleic acid concentration approached 69.4% is approximately equal to 69% expected for cultivated sunflower linoleic type. The corresponding oleic acid concentration is higher for accession M-175 (*H. maximiliani*). The combined saturated palmitic and stearic fatty acid concentrations for both populations averaged 7.7% and it was lower than the cultivated sunflower linoleic type.

Interspecific F_1 plants were obtained by *embryo rescue* techniques, and BC_3F_1 plants were obtained as a result of backcrossing with cultivated sunflower and self-pollination (Figure 1).

Seiler & Brothers (1999) examined oil concentration and fatty acid composition in oil of four perennial species (32 populations) from the Prairie Provinces of Canada and established that *H. maximiliani* Schrad. had the highest average oil concentration with 31.1% and *Helianthus tuberosus L.* had the highest average palmitic acid concentration with 8.0%. The highest average linoleic acid authors observed in *H. maximiliani* (77.4%). The certain differences with our data were because accessions M-146 and M-175 were maintained in the conditions of southeast Bulgaria for a long time. These two accessions can be successfully included in breeding programs for obtaining initial material, distinguished with a low concentration of saturated acids.

Table 2. Seed oil content and fatty acids concentration of wild *Helianthus* species

Species/ Accession	Seed oil content, %	Linoleic acid, %	Oleic acid, %	Stearic acid, %	Palmitic acid, %
H. maximiliani - M-175	27,5	65,4	27	2,2	5,4
H. tuberosus - M-146	26,5	73,3	18,9	2,3	5,5

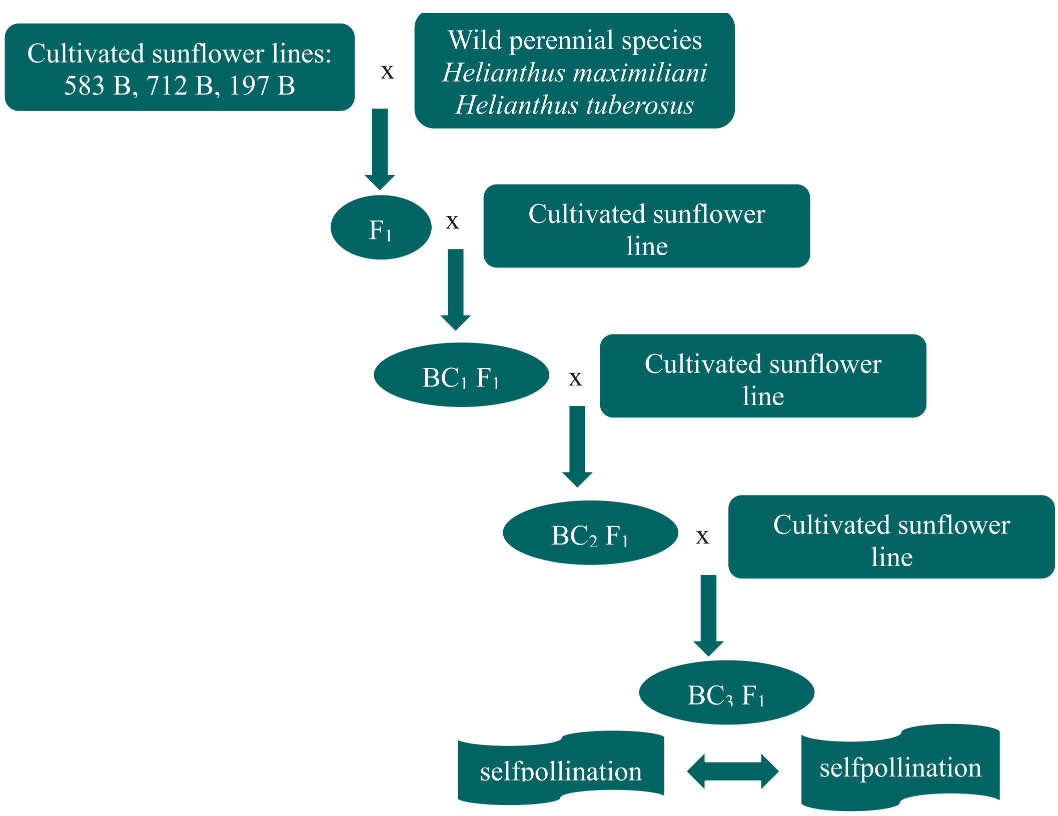

Figure 1. Crossing scheme of cultivated lines and wild sunflower accessions

For overcoming the difficulties in applying classical breeding methods, connected to the incompatibility of cultivated sunflower and to obtain the maximum number of hybrid plants, the method of *embryo rescue* was applied. The initial crosses were done in field conditions. The obtained 15 embryos from hybrid combinations were cultivated on firm plant tissue culture. The most suitable period for detachment of embryos was 10-12 days after pollination (Nenova, 2002). F_1 hybrid plants were obtained from all crosses. This showed that *embryo rescue* could be successfully applied for obtaining of interspecific hybrids. As a result of the applied crossing scheme and self-pollination five inbred lines were obtained.

The results of fatty acids content of obtained hybrid material were presented in Tables 3 and 4.

The linoleic acid concentration of hybrid forms, originated from *H. maximiliani* averaged 60.5% and those originated from *H. tuberosus* – 70.8%. This was approximately equal to 69% for cultivated sunflower with a linoleic type of oil. Similar results were published for sunflower lines obtained from interspecific hybrid material derived from perennial species *H. ciliaris*, accession M-092, from the Collection of DAI (Nenova et al., 2014).

The combined saturated palmitic and stearic fatty acids concentration varied from 8.6 % to 11.2%, which was less or about equal to traditional cultivated sunflower oil that averaged 11.5%.

Seiler (1986) and De Haro & Fernandez-Martinez (1991) established that high temperatures during flowering, achene filling, and maturation favor a low linoleic acid concentration and a high oleic acid concentration. It is also a function of planting date, Nitrogen rate, and hybrid (Zheljazkov, 2009).

CONCLUSIONS

The obtained hybrid material with different genetic potential could be a potential source of genes for increasing linoleic acid concentration in traditional sunflower oil when grown in a warm climate. They could be used as sources for reducing the concentration of saturated acids in traditional cultivated sunflower oil. The future studies of wild sunflower germplasm will ensure its preservation for the future, and will greatly increase the available genetic diversity for improving the cultivated sunflower, keeping it as a competitive global oilseed crop.

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Table 3. Results of fatty acids content of hybrid material originated from *H. maximiliani*

Accession	Seed oil content, %	Linoleic acid, %	Oleic acid, %	Stearic acid, %	Palmitic acid, %
14-MAX-1	41,2	61,2	27,8	2,7	5,9
14-MAX-2	42,2	59,8	28,2	3,2	6,5

Table 4. Results of fatty acids content of hybrid material originated from *H. tuberosus*.

Accession	Seed oil content, %	Linoleic acid, %	Oleic acid, %	Stearic acid, %	Palmitic acid, %
14-TUB-1	43,2	71,4	14,6	3,7	7,5
14-TUB-2	43,9	70,9	17,2	3,3	6,9
14-TUB-3	45,2	70,1	19,6	4,1	5,9

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