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CHARACTERIZATION OF HYBRIDS BETWEEN *H. annuus* L. AND THE SUBSPECIES *Subrhomboideus* (Rydberg) Heiser OF PERENNIAL HEXAPLOID *H. pauciflorus*

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SUMMARY

The subspecies *Subrhomboideus* (Rydberg) Heiser of the perennial hexaploid species *Helianthus pauciflorus* (*rigidus*) was crossed with the cultivated sunflower *H. annuus* L. The crossability rate was high. Seeds and hybrid plants were obtained in both directions of crossing. The F_1 plants had an intermediate type of heritability, but they strongly resembled the wild parent in most important biomorphological characters. All plants were with the perennial cycle of growth. During the second and third years, a large number of plantlets emerged from sleeping buds on the root system of F_1 materials. The hybrid nature of the F_1 plants was confirmed through cytological, RAPD and electrophoretic analyses of seed storage proteins. Polymorphism of *H. annuus*, *H. pauciflorus* ssp. *subrhomboideus* and theirs F_1 hybrids was studied by RAPD. The results showed introgression of the subspecies *Subrhomboideus* in the hybrid progeny. It was established that the subspecies carried *Rf* genes for *cms* Pet-1. As a result of self-pollination, sib-pollination and back-crossing to cultivated sunflower, F_2, F_3, F_4 and BC_1 progenies were obtained. Some of the obtained hybrids were included in a program of development of lines for heterosis breeding in sunflower.

Key words: **cytology, *H. pauciflorus* (*rigidus*) ssp. *subrhomboideus*, interspecific hybridization, RAPD, seed storage proteins**

INTRODUCTION

The species *Helianthus pauciflorus* (*rigidus*) (Cass.) Desf. (2n=6×=102) belongs to the section *Divaricati*, series *Atrorubentes* (Schilling and Heiser, 1981).

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It has two subspecies: *rigidus* and *subrhomboideus*. The subspecies *subrhomboideus* was studied less than the former one.

Georgieva-Todorova (1971; 1976) investigated the meiosis and pollen fertility of *H. pauciflorus*. She analyzed F_1 plants of the cross *H. annuus* × *H. pauciflorus* and obtained a small number of hybrid plants, which differed by plant habit and biological parameters. Whelan, 1978 (unpublished data) obtained hybrids between *H. pauciflorus* and both wild and cultivated *H. annuus*. Pollen fertility was typically less than 5% and there existed large differences in grain size. In most of the hybrids, the chromosomes were clumped and they did not permit cytological study, but univalents and multivalents frequently occurred from diplotene to metaphase I. Subsequent to metaphase I, bridges with or without fragments, lagging chromosomes, and chromosome elimination were frequently observed. Despite these difficulties, a chromosome number of about 68 and not 102 could be counted in the hybrids (Jan, 1997).

Faure *et al*. (1998) used RAPD markers to characterize interspecific and intergeneric hybrids between cultivated sunflower and distant species from *Helianthus* genus and other related genera. Hristova-Cherbadzi (2004) used RAPD analysis to show the hybrid nature of the F_1 material obtained from the crossing of the species *H. annuus* and *H. neglectus*. The results confirmed polymorphism in the amplification PCR profiles.

Storage proteins have been used to characterize specific genotypes in many cultivars. Some publications proved the presence of polymorphism within storage proteins in the sunflower (Anissimova, 1988; Christov *et al.*, 1993; Ivanov and Christov, 1994; Ivanov *et al*., 1994). Sunflower storage proteins were subjected to scientific analysis, but many questions remain to be settled. The results explained only a few aspects of their biochemical and genetic nature and their functional properties.

MATERIALS AND METHODS

The investigation encompassed the period 1999 - 2004. Cultivated sunflower *H. annuus* and the subspecies *subrhomboideus* (Rydberg) Heiser of the perennial hexaploid species *Helianthus pauciflorus* (*rigidus*) (2n=6×=102) (M 002) were included. Cultivated sunflower in the combination *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus* was represented by lines 6116A, HA 89A and 2607A (sterile forms) and in the combination *H. pauciflorus* ssp. *subrhomboideus* × *H. annuus* by the line 6116B and the variety Peredovik.

Hybridization was carried out through reciprocal crosses realized under field conditions (1999-2000). Hybrid plants were grown and regular phenological observations were performed during growing season. Biometric parameters of the F_1 hybrids were analyzed and major morphological characters and biological peculiarities described. Directed selection was carried out as early as F_1.

Presence of sources of fertility restorer genes for *cms* Pet-1 was also registered. Female fertility of the plants was determined by the amount of seeds obtained after free pollination, and 1000 seed weight by measuring three samples, 50 seeds each. Oil content in seeds was also determined.

Cytological analyses were carried out during mitosis and meiosis. Chromosome number during the former stage was determined according to Georgieva-Todorova (1976). Chromosome number of pollen mother cells (PMC) was determined according to Atlagić (1990). Chromosome behavior was studied mainly in diakinesis, metaphase I, anaphase I and telophase II. Pollen viability was determined by a standard methodology (Owczarzak, 1952).

RAPD analysis was performed in order to determine the hybrid nature of the new F_1 sunflower hybrids. Total DNA was isolated from the youngest sunflower leaves by a modified method of Dellaporta *et al.* (1983). Kits for PCR analyses (Ready To Go PCR Beads, Amersham Pharmacia Biotech Inc.) and for amplification of random DNA sequence, RAPD decamer primers from Operon Technologies, USA, OPA-01, OPB-01, OPB-02 and OPB-07, were used. Amplified products were separated by electrophoresis and visualized on 2% agarose gel. The PCR program was: 5 min. at the temperature of 95°C; 45 cycles of 1 min. at 95°C, 1 min. at 36°C and 2 min. at 72°C; 5 min. at 72°C. DNA marker 50 bp Amersham Biosciences, USA was used.

Seed storage proteins were used also to determine the hybrid nature of the new F_1 sunflower hybrids. Protein extraction for electrophoretic analysis was performed, according to Laemmli (1970) with 5 M urea (Vladova *et al.*, 1989). Proteins were stained with Coomassie Brilliant Blue R-250.

RESULTS AND DISCUSSION

Crossability of cultivated sunflower *H. annuus* with the perennial hexaploid wild species *H. pauciflorus* ssp. *subrhomboideus*

The analysis of the results showed that the subspecies *subrhomboideus* (Rydberg) Heiser of the perennial hexaploid species *H. pauciflorus* (*rigidus*) can be crossed with cultivated sunflower and the crossability rate was comparatively high (Table 1). Success of crossing (percentage of fertilized heads) was 100% in the crosses and 60% in the backcrosses.

Table 1: Crossability of *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus*

Crosses	Pollinated inflorescences	Inflorescences with seed		Seed set		Hybrid plants	
		Number	%	m. n.	%	Number	%
H. annuus ×*H. pauciflorus*	6	6	100	17	1.3	24	23.5
H. pauciflorus ×*H. annuus*	5	3	60	14	12.6	9	21.4

Seeds and hybrid plants were obtained in both directions of crossing. In the combination *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus* 102 seeds and 24 F_1 hybrids were obtained. In the combination *H. pauciflorus* ssp. *subrhomboideus* × *H. annuus* 42 seeds and 9 hybrids were obtained.

Seed set per cultivated sunflower inflorescence after artificial pollination with pollen from the wild species was low (1.3%) and it was higher in the reciprocal crosses (12.6%). Seed set after free pollination of the parents was significantly higher: from 71.5 to 88.2% for lines HA 89, 6116, and 2607 and from 52.9 to 65.4% for the wild species. A slightly higher value of this indicator for the cross *H. pauciflorus* ssp. *subrhomboideus* × *H. annuus* was due to the difference of the size of disk flowers of cultivated and wild sunflowers and their numbers of seeds.

The percentages of germinating hybrid plants in both directions of crossing were similar. Higher percentages and numbers of hybrid plants were obtained when cultivated sunflower was used as a female parent.

Characterization of F_1 hybrids

1. Biomorphological characterization

The hybrid F_1 plants obtained from bidirectional crossing between *H. annuus* and the perennial hexaploid *H. pauciflorus* ssp. *subrhomboideus* had an intermediate type of heritability. The most important biomorphological characters (stem branches, anthocyanin coloration *etc*.) strongly resembled those of the wild species, even in those cases when these species were used as the male parent. All plants had the perennial life cycle. During the second and third years, a large number of plantlets emerged from sleeping buds on the root system of F_1 materials.

The vegetation period of the hybrids was shorter than those of the wild species and longer than that of the cultivated sunflower. Some differences were observed depending on the direction of crossing. The hybrids with *H. annuus* as the female component had a shorter vegetation period (Table 2).

F_1 plants of the combination *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus* (Figure 1) had similar phenotypic characteristics as the plants of the reciprocal crosses. All plants had erect and branched stems. They were colored dark-green and covered with sharp bristles. The branches were situated mostly in the lower part of the stem. The leaves were large, lanceolate, with sharp bristles. At the end of the vegetation period they acquired an anthocyanin coloration. The petiole had anthocyanin traces covered with short and coarse bristles. The size of seeds and the number of tubular florets, bracts and ray flowers were also under review. Ray flowers and stigmas were colored purple while pollen and ray florets were orange. The inflorescences of some plants did not produce pollen.

The presence of male fertile F_1 plants showed that the species *H. pauciflorus* ssp. *subrhomboideus* carried fertility restoration genes for *cms* Pet-1 because the female parent *H. annuus* was a male sterile form. All sterile florets formed seeds on

free pollination. Some fertile plants were self-pollinated. The plants from the cross *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus* formed 1 to 6 seeds. They were colored grayish-brown. The F_1 plants of the reciprocal crosses did not form seeds. The seed set was from 3.38 to 6.77% when the sterile analogue of the line HA 89A was pollinated with pollen from F_1 plants.

Figure 1: H. annuus × *H. pauciflorus ssp. subrhomboideus.*

Table 2: Some biological characteristics of F_1 hybrids and parents

Characteristic	*H. pauciflorus* ssp. *subrhomboideus* × *H. annuus*	*H. annuus* × *H.pauciflorus*	*H.pauciflorus* × *H. annuus*	*H. annuus* L. HA89
Morphological characteristic				
Plant height (cm)	176 - 180	65 - 165	145 - 270	105 - 110
Leaf length (cm)	21 - 24	6 - 17	25 - 26	26.8
Leaf width (cm)	8 - 9	3 - 9	13 - 14	25.0
Petiole length (cm)	0.2 - 0.3	2 - 6	2 - 5.5	
Leaf number	134	15 - 46	32 - 143	24 - 28
Branches, number	6 - 12	2 - 11	3 - 14	0
Branch length (cm)	56 - 143	21 - 130	19 - 140	0
Bracts (number)	27 - 33	19 - 30	21 - 25	52
Ray flowers (number)	13 - 16	10 - 20	13 - 17	36
Tubular florets (number)	98 - 114	113 - 298	117 - 270	1338
Head diameter (cm)	1.7 - 2.2	1.6 - 5.2	3.0 - 4.1	22 - 25
Physiological development				
Period of vegetation, days	185	141	162	114

2. Cytological characterization

Chromosome number (at mitosis) of *H. annuus* × *H. pauciflorus ssp. subrhomboideus*.

A total of 37 complete metaphase cells (Figure 2) from 7 germinated F_1 seeds of the cross *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus* were observed.

It was difficult to determine the exact chromosome number of the obtained F_1 plants. The expected result was 2n = 68, because the chromosome number of the parents was 2n = 34 for *H. annuus* and 2n = 102 for *H. pauciflorus* ssp. *subrhomboideus*.

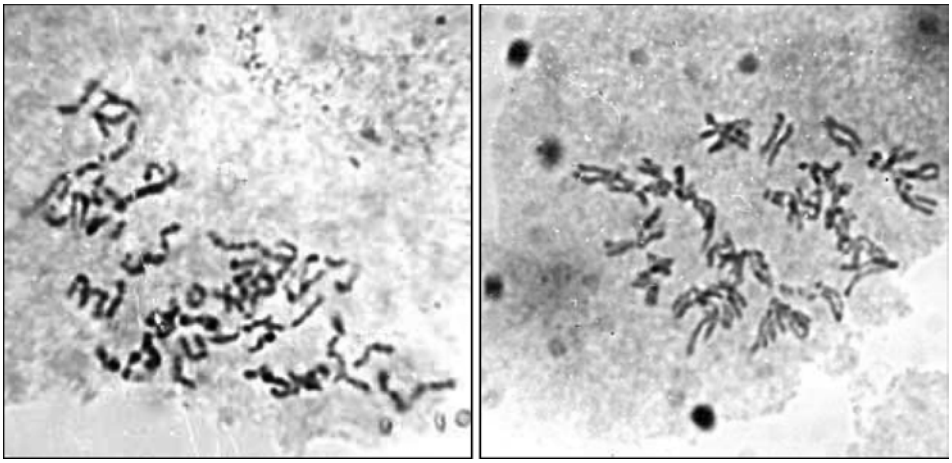


Figure 2: Metaphase cells of H. annuus H. pauciflorus ssp. subrhomboideus.

Meiosis of pollen mother cells (PMC)

Aberrations occur during the reduction division of PMC in both hybrids. The earliest meiotic phase observed in *H. pauciflorus* ssp. *subrhomboideus* × *H. annuus* was prophase, diplotene stage. Diakinesis stage was rarely observed, probably because of a high rate of progression of the process. Diakinesis stage was not observed in the hybrid seeds of the cross *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus*.

The analysis of PMC from F_1 plants (*H. pauciflorus* ssp. *subrhomboideus* × *H. annuus*) showed that there were 2.4% of cells with bivalents. Univalent and multivalent configurations were observed in 97.6% of the cells (Figure 3). During diakinesis, 1-4 multivalent configurations (quadrivalents and hexavalents) were registered in addition to bivalents in some cells. In other cells, 1-3 univalents and trivalents occurred in addition to bivalents. The presence of multivalent configurations in F_1, which were indicators for translocation and inversion**,** presumed the existence of differences in the chromosome structure of the parental genomes. The presence of univalents was a sign of an incomplete genome homology.

In metaphase I, metaphase lamella and fast chromosomes were seen in 75% of the cells. The number of fast chromosomes in these cells was from 1 to 3 for *H. pauciflorus* × *H. annuus* and 2 for *H. annuus* × *H. pauciflorus*. In the rest of the cells metaphase I was normal.

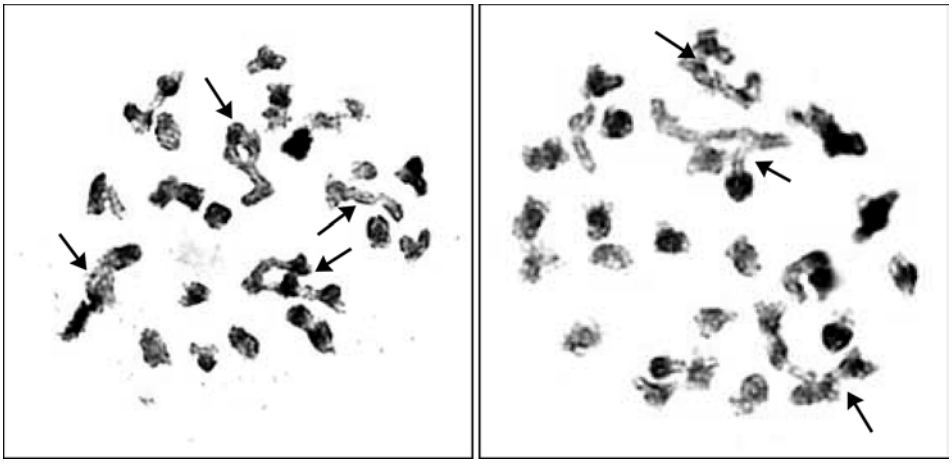


Figure 3: Diakinesis of H. pauciflorus ssp. subrhomboideus × H. annuus

In anaphase I, normal cells were seen as well as cells with 1 or 2 lagging chromosomes and one chromosomal bridge. Lagging chromosomes were a signal of disturbances in the spindle.

The reason for the high percentages of non-included chromosomes in metaphase I and anaphase I could be the different movement rates of the chromosomes. Perhaps in perennial hexaploid *H. pauciflorus* ssp. *subrhomboideus* the chromosomes move at a rate different from that of annual diploid *H. annuus*. Non-included chromosomes in metaphase I and anaphase I were prerequisite for the occurrence of micronuclei and polyads; however, only normal tetrads were observed in telophase II.

Pollen viability ranged from 29.95 to 44.95% for *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus,* and from 13.79 to 38.72% for *H. pauciflorus* ssp. *subrhomboideus* × *H. annuus*. The reduction of normal pollen viability showed an introduction of genes from the wild species to cultivated sunflower and lower fertility of female plants, which might be caused by other factors as well.

Meiosis of PMC of the species *H. pauciflorus* ssp. *subrhomboideus* was normal. Conjugation of chromosomes was observed in diakinesis. The larger part of the bivalents was open (with a single chiasma). Single cells with bivalents and quadrivalents were rare. Metaphase I and anaphase I also showed individual abnormalities: non-included chromosomes (1-6 chromosomes per cell) and chromosome bridges (up to 3 per cell), respectively. Formation of normal tetrads was observed during telophase II. Pollen viability ranged from 94.7 to 96.4%.

The results of cytological investigations of F_1 showed that there existed structural differences in the chromosomes of the parental genomes. The formation of different configurations was an indication of incomplete genome homology and disturbances in the spindle. Perhaps the chromosomes in the perennial hexaploid wild *H. pauciflorus* ssp. *subrhomboideus* move at a rate different from that of annual diploid *H. annuus*.

3. RAPD analysis

F_1 plants from the cross *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus* and the parents were analyzed in order to prove their hybrid nature. Analyses were carried out on those fragments that were well visible. The comparison of the amplification profiles of F_1 plants and parents was based on the presence or absence of fragments and the homology in their sizes.

Primer OPA-01 (Figure 4) allowed to amplify specific fragments for one of the parents and the hybrid, and fragments that were common for the three genotypes.

In the F_1 genotype there was one band specific only for the genotype of the wild species (its size over 1000 bp). There were also bands specific for the genotypes of cultivated sunflower and the hybrid, and bands represented in all three genotypes.

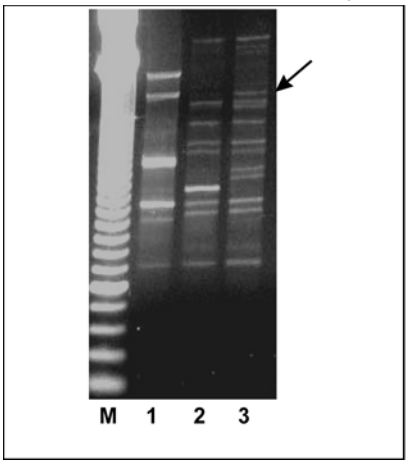


Figure 4: Electrophoretic spectra of amplification profiles of the three genotypes with OPA-01 primer
1. H. pauciflorus ssp. subrhomboideus-M 002
2. H. annuus-line 6116A
3. F_1-H. annuus × H. pauciflorus ssp. subrhomboideus

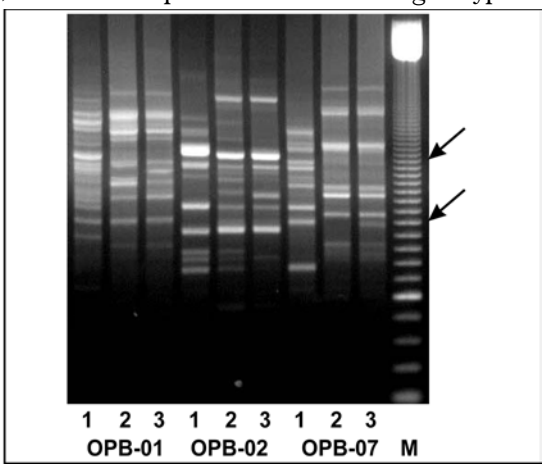
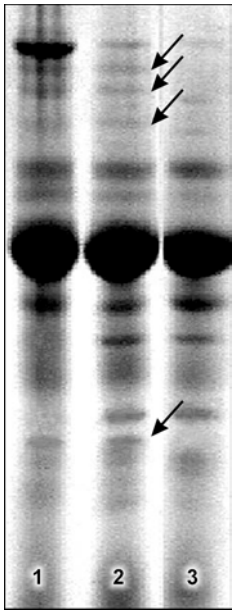


Figure 5: Electrophoretic spectra of amplification profiles of the three genotypes with OPB-01, OPB-02 and OPB-07 primers
1. H. pauciflorus ssp. subrhomboideus-M 002
2. H. annuus-line 6116A
3. F_1-H. annuus (L 6116A) × H. pauciflorus ssp. subrhomboideus (M 002)

Primers OPB-01, OPB-02 and OPB-07 (Figure 5) were also used for amplification. One of them (OPB-02) did not show fragments specific for the wild species and the hybrid. The other primers allowed to amplify two specific fragments- OPB-01 (800 bp) and OPB-07 (500 bp).

Of the four primers used for amplification of the parents and the hybrid, one (OPB-02) did not show fragments specific for the wild species and the hybrid. The others primers allowed amplifying 3 fragments specific for the wild species and the hybrid. The results confirm that there was a polymorphism in the amplification PCR profiles of *H. annuus, H. pauciflorus* ssp. *subrhomboideus* and *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus*, *i.e.*, the RAPD analysis confirmed the hybrid nature of the F_1 material obtained from crosses between the line 6116 and the wild species. Furthermore, the electrophoregram of the three genotypes indicated introgression of subspecies *subrhomboideus* in the hybrid generation.

4. Seed storage proteins



The specific protein fractions for one of parent genotypes in electrophoretic spectra (Figure 6) were marked. There were fractions, which were represented in all three genotypes.

From the electrophoretic spectra of parental genotypes and obtained hybrid population could be seen that the proteins fractions from the initial forms are superposed in the hybrid's genotype in different degree (as the high molecular fraction of wild species-11S globulin). The results confirm that there was a polymorphism in the spectra of *H. annuus, H. pauciflorus* ssp. *subrhomboideus* and *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus*.

Figure 6: Electrophoretic spectra of seed proteins from H. pauciflorus ssp. subrhomboideus, H. annuus and their F_1 hybrid
1. H. pauciflorus ssp. subrhomboideus - M 002
2. F_1 - H. annuus × H. pauciflorus ssp. subrhomboideus.
3. H. annuus - line 6116A

Characterization of the second and subsequent hybrid generations

The plants of second hybrid generations were with annual cycle of growth. All plants were branched. Both F_2 and BC_1 was observed different degree of anthocyanin traces on stem, branch and leaf stem.

The form of leaves for F_2 plants was from broadly lanceolate to elongated-cordate. Ray flowers and the stigmas were colored light purple or purple, and pollen and ray florets were orange. Seeds size varied and they were colored in gray-brown to black with anthocyanin. Statistic analyses are presented in table 3.

In F_2 there was a variability of the phenotype forms, because of segregation in the characters. In the table is represented a quantitative mark of transgression on degree (Td) and on frequency (Tf). The 100% positive transgress forms with degree

30.56% were reported on plant height. This was important indicator for selection on high sunflower. The values for transgression in relation to the better parents were negative as regards to head diameter.

BC_1 plants were with single branches, bigger sizes of leaves and inflorescences. The branches were situated mostly in the central part of stem. Big part of plants was sterile with large period of vegetation. The seeds were bigger than those of the wild parent and colored in gray-brown or form dark brown to black. Some biological characteristics were presented in table 3.

Plants without branches were registered among the variability of forms in third hybrid generation. The stems, branches and inflorescences were colored with light anthocyanin or without. The leaves, inflorescences and seeds sizes varied. The leaves were big with different dentate. The seeds were colored in gray-brown, dark brown, gray-black, brown-black, black and black with anthocyanin.

In part of plants from fourth hybrid generation by reason of applying on purposive selection very useful characteristics were collected. Branched forms with low stem, absence of anthocianin on the stems, leaves' stems and peel, a high oil content in seed, a finer peel *etc.*, were developed. They were included in a program for developing lines for heterosis breeding in sunflower.

Table 3: Some biological characteristics of hybrids

Characteristics	*H. annuus* × *H. pauciflorus*				*H. pauciflorus* × *H. annuus*		
	F_2	BC_1	BC_1F_1	BC_1F_2	BC_1	BC_1F_1	BC_1F_2
Morphological characteristic							
Plant height (cm)	205-240	145-180	175	170	170-230	130-260	165
Head diameter (cm)	7-11	14-21	16	15	8	13-21	19
Physiological development							
Period of vegetation (day)	139	135	129	123	142	134	128
Technological characteristic							
1000 seeds weight (g)	21.1	36.7	43.7	45.2	×	46.8	48.9
Oil (%)	×	41.7	39.1-42.6	44.3	×	38.1-46.0	46.0

CONCLUSIONS

The obtained results indicate that hybridization of the species *H. pauciflorus* ssp. *subrhomboideus* is possible in both directions. Better results were received when the cultivated sunflower was used as the female line. Use of right crosses increases the rate of changes in the nuclear material, which favors heterosis selection and use of cytoplasmic male sterility (*cms*) in the sunflower. Back crossing offers additional possibilities for changing sunflower properties controlled by cytoplasmic genes. Such crosses could be used for finding new *cms* sources. The crossability depended not only on the number of chromosomes in the species. There were probably other factors, which influence the forming of the zygote.

The existence of pollen viability in F_1 hybrids and transfer of Rf genes from the species *H. pauciflorus* to cultivated sunflower were confirmed. The introduction of genes from the species *H. pauciflorus* ssp. *subrhomboideus* to *H. annuus* caused a reduction of the normal pollen viability and lower fertility of female plants, which might be affected by other factors as well.

The results of cytological investigations of F_1 showed the existence of structural differences in the chromosomes of the parent genomes. The formation of different configurations was an indication of incomplete genome homology and disturbances in the spindle. Perhaps the chromosomes in the perennial hexaploid wild *H. pauciflorus* ssp. *subrhomboideus* move at a rate different from that of annual diploid *H. annuus*. Still it could be said that the F_1 progeny is hybrid material.

The results confirm that polymorphism occurred in the amplification PCR profiles of *H. annuus, H. pauciflorus* ssp. *subrhomboideus* and *H. annuus* × *H. pauciflorus* ssp. *subrhomboideus*, *i.e.*, the RAPD analysis confirmed the hybrid nature of the F_1 material obtained from crosses between the line 6116 and the wild species. Furthermore, the electrophoregrams of the three genotypes indicated introgression of the subspecies *subrhomboideus* in the hybrid progeny.

The electrophoretic analyses of seed storage proteins showed similar results, confirming the hybrid nature of the F_1 plants.

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CARACTERIZACIÓN DE LOS HÍBRIDOS ENTRE *H. annuus* L. Y LA SUBESPECIE *Subrhomboideus* (Rydberg) Heiser DE LA ESPECIE HEXAPLOIDE PERENNE *H. pauciflorus*

RESUMEN

La subespecie *subrhomboideus* (Rydberg) Heiser de la especie hexaploide perenne *Helianthus pauciflorus* (*rigidus*) ha sido cruzada con el girasol cultivado *H. annuus* L. El éxito del cruzamiento fue alto. La semilla y las plantas híbridas fueron obtenidas en las dos direcciones de cruzamiento. Las plantas F_1 tenían el tipo de herencia intermediaria, pero se parecían mucho al progenitor silvestre, por las principales características biomorfológicas. Todas las plantas tenían el ciclo de crecimiento de varios años. Durante el segundo y el tercer año, un gran número de brotes aparecieron de los capullos durmientes en el sistema radical del material F_1. La naturaleza híbrida de las plantas F_1 fue confirmada por los análisis citológicos, RAPD y electroforéticos de proteínas de reserva en la semilla. El polimorfismo de las especies *H. annuus*, *H. pauciflorus* spp. *subrhomboideus* y sus híbridos F_1 fue estudiado por medio de RAPD. Los resultados demostraron que había ocurrido la transferencia del material genético de la subespecie *subrhomboideus* en la descendencia híbrida. Fue determinado que la subespecie posea los genes *Rf* para *cms* Pet-1. Como resultado de autofertilización en semiparentesco y el cruzamiento recurrente con el girasol cultivado, fueron obtenidos las descendencias F_2, F_3, F_4 y BC_1. Algunos de los híbridos obtenidos, están incorporados en el programa de creación de las líneas para la selección heterótica del girasol.

CARACTÉRISATION DE L'HYBRIDE OBTENU PAR LE CROISEMENT DE *H. annuus* L. ET DE LA SOUS-ESPÈCE *subrhomboideus* (Rydberg) Heiser DE L'ESPÈCE HEXAPLOÏDE VIVACE *H. pauciflorus*

RÉSUMÉ

La sous-espèce *subrhomboideus* (Rydberg) Heiser de l'espèce vivace hexaploïde *Helianthus pauciflorus (rigidus)* a été croisée avec le tournesol de culture *H. annuus* L. Le résultat du croisement a été une grande réussite. Des semences et des plantes hybrides ont été obtenues dans les deux sens du croisement. Les plantes F_1 ont montré un type intermédiaire d'hérédité mais ressemblaient beaucoup au parent sauvage pour les caractéristiques biomorphologiques les plus importantes. Toutes les plantes avaient un cycle de croissance vivace. Au cours de la deuxième et de la troisième année un grand

nombre de pousses étaient issues des bourgeons dormants sur le système de racines du matériel F_1. La nature hybride des plantes F_1 a été confirmée par les analyses cytologiques, RAPD et électrophorétique des protéines de réserve dans la graine. Le polymorphisme des espèces *H. annuus*, *H. pauciflorus* spp. *subrhomboideus* et de leurs hybrides F_1 a été analysé à l'aide de RAPD. Les résultats ont montré que le matériel génétique avait été transmis de la sous-espèce *subrhomboideus* à la descendance hybride. Il a té démontré que la sous-espèce possédait le gène *Rf* pour *cms* Pet-1. L'autofécondation, la fécondation en consanguinité et le rétro-croisement avec le tournesol de culture a eu pour résultat l'obtention de descendants F_2, F_3, F_4 et BC_1. Certains des hybrides obtenus ont été inclus dans un programme de création de lignées pour la sélection hétérotique du tournesol.