

PRELIMINARY INVESTIGATIONS ON INTERSPECIFIC HYBRIDS BETWEEN *Helianthus annuus* L. AND PERENNIAL *Helianthus* L. SPECIES

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SUMMARY

Interspecific hybridization was made between the cultivated sunflower *Helianthus annuus* L. and two perennial diploid species, *Helianthus divaricatus* L. and *Helianthus mollis* Lambert. Reciprocal crosses were made. Conventional breeding methods were used. Six inbred lines and one variety, Peredovik, were included in the hybridization. Hybrid plants were produced in both directions.

The hybrid plants originating from crosses between the perennial species and cultivated sunflower had a perennial habit. Such plants were also produced from reciprocal crosses: line 1234 A x *Helianthus mollis* and line 3064 A x *Helianthus divaricatus*. Second hybrid and BC₁ generations were produced from the F₁ material, showing great diversity of phenotypes.

Key words: *Helianthus divaricatus* L., *Helianthus mollis* Lambert, *Helianthus annuus* L., hybridization, perennial growth habit, F₁ material.

INTRODUCTION

Interspecific hybridization between cultivated sunflower and other *Helianthus* species has been of considerable interest because it offers a prospective of making use of the immense diversity of the genus (Skoric, 1988). This is the reason that has stimulated many researchers to carry out interspecific hybridization, investigate the relationships between the different species in the genus *Helianthus* and transfer useful characters from wild species into the cultivated sunflower.

Schilling and Heiser (1981) reported relationships of *Helianthus* species, based on crossing results. Many of the species in the genus, particularly the perennial ones, have never been successfully hybridized with the cultivated sunflower. According to Georgieva - Todorova, 1984 almost complete incompatibility exists between the cultivated sunflower and diploid (2n=34) perennial species,

such as *H.mollis*, *H.divaricatus*, *H.giganteus*, *H.nuttallii* and *H.angustifolius*. Usage of diploid perennial species in hybridization with the cultivated sunflower has been reported also by Pustovoit (1975), Whelan (1981), Laferriere (1986).

Our investigations were carried out with the purpose of studying the possibilities of interspecific hybridization between *H.mollis*, *H.divaricatus* and *H.annuus* and to obtain genetic variation in forms, which would be of interest for the sunflower breeding process.

MATERIALS AND METHODS

This investigation was carried out at IWS "Dobroudja" during the period 1993 - 1995. Six sunflower inbred lines - 1607A, 1607B, 2607A, 2607B, 1234A, 1234B, 3064A, 3064B, HA-300A, HA-300B, HA-821B and one variety, Peredovik, were used in the hybridization process together with two diploid perennial *Helianthus* species, *Helianthus divaricatus* L. (GT-M-015) and *Helianthus mollis* Lambert (GT-M-082). *Helianthus divaricatus* and *Helianthus mollis* belong to section *Divaricati*, series *Divaricati*. Both species are diploid with perennial growth habit. Both accessions of the perennial species were maintained and reproduced in the Collection of Wild *Helianthus* Species at IWS.

First crossings were made in 1993 and repeated in 1994. Conventional breeding methods were used only. The six inbreds, Peredovik, and the two diploid species were used both as female parents or pollinators. Hybrid plants were produced in both directions. A - lines (CMS) as well as B - lines of the cultivated sunflower were used. The inflorescence of the B - lines were treated with gibberellic acid and those of the wild species were emasculated.

Phenotypic, phenologic and reproductive traits were the main criteria considered to define the "hybrid status" of the F_1 , F_2 and BC_1 plants issued from the crossing program.

RESULTS AND DISCUSSION

The results of the crossings (Tables 1 and 3) showed that it is possible to successfully hybridize both species with the cultivated sunflower, in both directions. Hybrid plants were produced in crosses *H.annuus* x *H.mollis* and *H.annuus* x *H.divaricatus* as well as in crosses *H.mollis* x *H.annuus* and *H.divaricatus* x *H.annuus*. The seed set was very low when the wild species were used as female parents in the crosses. The seed set for the hybridization with *H.divaricatus* was higher and the number of seeds obtained varied from 1 to 38, while for *H.mollis* it was lower and resulted in no more than one or two seeds per pollination. These results correspond to some extent to the self-pollination of these species (Table 2), where the seed set per head for *H.divaricatus* was 25.7% and for *H.mollis* - 0%.

Table 1: Hybridization between *H.divaricatus*, *H.mollis* and cultivated inbred lines

Species	Pollinator											
	line 1607			line 2607			line 1234			line 3064		
	Np	Ns	NF ₁	Np	Ns	NF ₁	Np	Ns	NF ₁	Np	Ns	NF ₁
1994												
<i>H.divaricatus</i>	5	59	0	5	20	0	5	77	0	5	6	0
<i>H.mollis</i>	5	3	0	5	1	2	5	1	1	5	3	0
1995												
<i>H.divaricatus</i>	0	0	0	7	3	0	7	8	0	0	0	0
<i>H.mollis</i>	0	0	0	5	1	1	5	0	0	0	0	0

Table 1: Hybridization between *H.divaricatus*, *H.mollis* and cultivated inbred lines

Species	Pollinator											
	line HA-300			line HA-821			Peredovik cv			Mixed pollen		
	Np	Ns	NF ₁	Np	Ns	NF ₁	Np	Ns	NF ₁	Np	Ns	NF ₁
1994												
<i>H.divaricatus</i>	5	102	2	5	77	0	5	34	0	5	67	0
<i>H.mollis</i>	5	3	1	5	0	0	5	2	0	4	0	0
1995												
<i>H.divaricatus</i>	5	50	0	5	38	0	5	7	0	5	0	0
<i>H.mollis</i>	5	0	0	5	0	0	5	1	0	5	0	0

Np - Number of pollinations; Ns - Number of seeds; NF₁ - Number of F₁ plants.

From the total amount of 442 seeds obtained in 1994 from crosses with *H.divaricatus* just 2 hybrid plants were produced, while in 1995 no hybrid plants were produced from the 106 seeds obtained. The number of putative F₁ hybrid seeds, produced when *H.mollis* was used as the female parent, was much smaller - 13 for 1994 and just 2 for 1995. Nevertheless, 4 F₁ hybrid plants were grown in 1994 and 1 in 1995. Similar results were reported by Pustovoit (1975), Georgieva - Todorova (1976), Christov (1991).

Table 2: Self pollination of *H.divaricatus* and *H.mollis* accessions

Species	Accession No	Self-pollination				
		No. of inflor. self-pollinated	No. of selfs obtained	No. of seeds	Average no. of seeds per head	Seed set per head (%)
1994						
<i>H.divaricatus</i>	GT - M-015	10	0	0	0	0
<i>H.mollis</i>	GT - M-082	10	0	0	0	0
1995						
<i>H.divaricatus</i>	GT - M-015	10	1	25	25	25.7
<i>H.mollis</i>	GT - M-082	10	0	0	0	0



Figure 1. BC_1 plants, [Peredovik x *H.divaricatus*] x HA 300

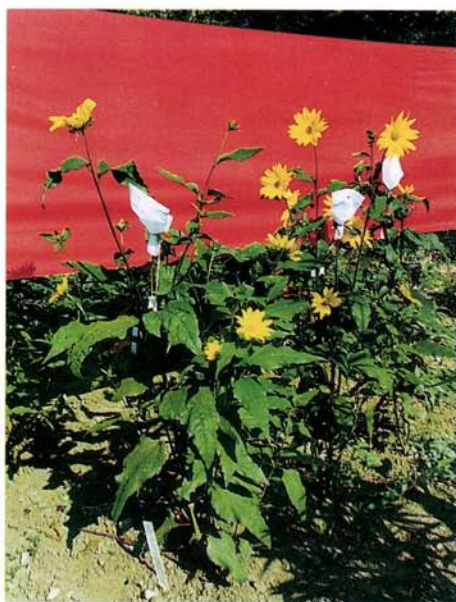


Figure 2. F_1 plants, *H.divaricatus* x *H.annuus* (HA 300)



Figure 3. F_2 plants, *H.annuus* (2607) x *H.mollis*



Figure 4. F_1 plants, *H.annuus* (1234A) x *H.mollis*

The results from the reciprocal crosses are summarized in Table 3. In almost every cross combination hybrid seed and hybrid plants were obtained for both wild species. The plants from combinations 1234A x *H.mollis* and 3064A x *H.divaricatus* had a perennial growth habit, whereas the remainder of the F₁ plants were annual. Unfortunately great part of the putative F₁ hybrid seeds could not germinate at all.

Table 3: Interspecific hybridization between cultivated sunflower and an accession of *H.divaricatus* and *H.mollis*

Cultivated sunflower	Pollinator					
	<i>H.divaricatus</i> - GT - M - 015			<i>H.mollis</i> - GT - M - 082		
	Np	Ns	NF ₁	Np	Ns	NF ₁
1994						
1607	5	17	6	4	6	2
2607	4	21	4	4	14	4
1234	4	247	11	4	1	1
3064	4	32	2	4	11	0
HA-300	4	3	3	4	39	3
HA-821	2	1	1	2	0	0
Peredovik	2	29	1	2	254	0
1995						
1607	2	0	0	2	0	0
2607	4	5	2	5	11	3
1234	4	9	1	4	10	2 γ
3064	2	60	3+14 γ	2	3	1
HA-300	4	16	14	4	54	12
HA-821	3	11	5	3	20	8
Peredovik	4	91	1	4	64	3

Np - Number of pollinations; Ns - Number of seeds; NF₁ - Number of F₁ plants; γ - Plants with perennial growth habit.

Helianthus annuus x *Helianthus divaricatus*

The F₁ hybrid material produced by crossing different cultivated sunflower inbreds with *H.divaricatus* contained both annual and perennial plants. They were all characterized by well-expressed heterosis, found in plant height, stem thickness, the size of the leaves, and the inflorescence. Most of the F₁ plants had the annual habit of *H.annuus*, but all of them were branched, a feature common

Table 4: Morphology of some characters of F1 plants (1995)

Material	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	No. of I class branches	Length of the longest branch (cm)	Stem thick ness (cm)	Head diameter (cm)	Head thick ness (cm)	No. of bract leaves	Length of bract leaves (cm)	Width of bract leaves (cm)	No. of ray floret	Length of ray florets (cm)	Width of ray florets (cm)
Hybrid combinations															
2607 x M-015	245	39.0	42.5	23.5	6	132.6	5.2	25.5	2.8	74	4.9	2.7	41	5.3	2.0
2607 x M-082	240	42.3	46.3	26.7	25	131.7	4.7	43.3	3.3	78	6.3	3.3	53	6.7	2.6
1234 x M-015	150	45.0	45.0	29.0	2	121	5.2	47.0	4.0	73	7.5	2.4	47	8.3	2.3
1234 x M-082 *	160	19.5	15.0	0	11	119.7	1.9	11.5	1.5	61	2.2	2.3	35	5.5	2.4
3064 x M-015	145	33.8	41.8	18.3	6	92.5	4.1	30.5	2.8	75	6.5	3.3	53	8.8	2.4
*	193	24.9	17.5	3.4	15	144.4	1.5	5.4	1.4	43	3.8	0.9	21	4.5	1.4
3064 x M-082	205	33.0	36.0	20.0	0	0	3.9	34.0	3.0	77	6.1	3.1	57	7.5	2.0
HA-300 x M-015	237	30.2	34.6	22.2	25.2	97.7	3.9	17.2	1.6	77	4.6	2.5	55	6.1	2.0
HA-300 x M-082	170	27.4	25.0	14.8	16	58.2	2.6	17.8	1.7	61	5.4	2.4	45	6.2	2.0
HA-821 x M-015	168	33.5	35.0	19.0	1	85	3.7	33.0	2.3	77	4.5	2.8	51	6.5	1.9
HA-821 x M-082	208	25.8	23.8	17.3	0	0	2.9	27.5	2.1	57	5.4	2.0	39	5.3	2.2
P-k x M-015	270	25.0	23.0	18.0	25	42.3	3.2	15.0	1.0	60	4.8	2.2	54	6.2	2.3
P-k x M-082	230	27.5	29.5	23.5	8.5	80.3	3.5	31.0	2.3	69	6.1	2.7	49	6.5	1.9
Wild <i>Helianthus</i> species															
<i>H. divaricatus</i>	132	11.1	3.5	0.5	10	60	0.8	2.4	0.73	38	1.6	0.3	15	1.8	0.6
<i>H. mollis</i>	100	12.0	10.6	0	16	40.7	0.7	3.9	0.7	35	1.6	0.5	17	1.9	0.9
Cultivated sunflower - <i>Helianthus annuus</i>															
line 2607	119	20.4	19.8	11.4	0	0	2.1	17.4	1.8	74	4.4	2.3	48	3.8	1.5
line 1234	113	28.4	24.8	15.6	0	0	2.2	19.6	2.4	74	6.8	3.4	55	8.0	1.7
line 3064	96	19.6	19.2	11.2	0	0	2.1	17.8	1.9	59	6.2	2.9	45	6.9	2.0
line HA 300	114	20.6	19.8	11.2	0	0	2.6	24.2	2.6	77	6.4	3.8	60	7.4	2.2
line HA 821	137	23.8	24.8	12.3	0	0	1.8	18.0	2.1	77	4.3	2.5	55	7.2	2.6
Peredovik cv.	183	25	25.4	17.2	0	0	2.0	18.4	2.5	76	6.3	2.8	54	8.0	2.2

* - Plants with the perennial growth habit

to the wild species. Another wild trait, the anthocyanin colour, could also be seen in several combinations, both in the petiole and the inflorescence.

Fourteen of the F_1 plants obtained in 1995 had the perennial growth habit. As we said before and as could be seen from Table 4, the heterosis here was very well expressed. These plants resembled the wild parent, but still differences existed. The type of branching for example. The main branching of the hybrids started from the basal point of the stem. Secondary branching could be found in the later stages of development in the upper parts of the main branches near the inflorescence.

A great diversity of phenotypes was produced in the F_2 and BC_1 generations of these crosses (Tables 6 and 7). The plants were from very short, 140 cm, to very tall, 270 cm; from unbranched to densely branched (0 - 21); with different type of branching; with different head diameter (18 - 33 cm), leaf size and inflorescence. Some of them were anthocyanin coloured, some were yellow. They differed very much in flowering period too. The great segregation in these generations could allow to select forms on the basis of different characters and to produce lines varying in genotype and phenotype.

Helianthus divaricatus* x *Helianthus annuus

Only two plants were obtained from the cross combinations between *H.divaricatus* and the different inbred sunflower lines. The data from the morphological observations are presented in Table 5. The plants resembled the wild parent and had a perennial growth habit, but were shorter and earlier in flowering, and with a larger inflorescence. They had basal main branching with additional secondary branching. The seeds were larger than those of the wild parent.

Helianthus annuus* x *Helianthus mollis

Two different types of F_1 plants were produced when *H.mollis* was used as the pollinator, annual (I) and perennial (II). The results are summarized in Table 4. The hybrid character of the material was proved again by heterosis expressed in the phenotype.

I. Almost all annual hybrid plants were intermediate, with basal and axillary branching and slight anthocyanin colouring, becoming darker in F_2 and BC_1 generation. The type of branching was preserved to some extent in F_2 , but great diversity of phenotypes was obtained in BC_1 , where at least 3 types of branching were observed. The plants varied in plant height, 225 cm to 275 cm; in the presence of anthocyanin; in flowering period.

II. Two perennial F_1 hybrid plants were grown from the cross 1234A x *H.mollis*. They had a phenotype closer to that of the wild parent, but taller plant height, larger leaves, a short petiole, 1 cm; much larger inflorescence; slight

Table 5: Morphology of some characters of the F₁ perennial plants from crosses of wild species with the cultivated sunflower

Material	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	No. of I class branches	Length of the longest branch (cm)	Stem thick ness (cm)	Head diameter (cm)	Head thick ness (cm)	No. of bract leaves	Length of bract leaves (cm)	Width of bract leaves (cm)	No. of ray floret	Length of ray florets (cm)	Width of ray florets (cm)
	Hybrid combinations														
F1(M-082 x 2607)	174	17.2	9.6	0	15	48.8	1.2	2.8	1.1	45	1.8	0.3	26	4.3	1.6
F1(M-082 x 2607)	156	17.0	4.1	0	27	60.4	0.9	2.0	0.8	50	2.3	0.2	19	4.0	1.0
F1(M-082 x 1234)	170	11.3	6.1	0	20	49.6	0.7	2.6	0.8	42	1.6	0.3	21	3.6	1.1
F1(M-082 x HA-300)	122	10.1	8.8	0	19	34.7	0.9	3.7	0.8	42	1.1	0.4	33	3.0	1.2
F1(M-015 x HA-300)	121	18.0	6.7	1.4	9.6	66.1	1.0	3.4	1.3	30	2.4	0.5	15	5.7	1.8
	Wild Helianthus species														
<i>H. divaricatus</i>	132	11.1	3.5	0.5	10	60	0.8	2.4	0.73	38	1.6	0.3	15	1.8	0.6
<i>H. mollis</i>	100	12.0	10.6	0	16	40.7	0.7	3.9	0.7	35	1.6	0.5	17	1.9	0.9
	Cultivated sunflower - <i>Helianthus annuus</i>														
line 2607	119	20.4	19.8	11.4	0	0	2.1	17.4	1.8	74	4.4	2.3	48	3.8	1.5
line 1234	113	28.4	24.8	15.6	0	0	2.2	19.6	2.4	74	6.8	3.4	55	8.0	1.7
line HA 300	114	20.6	19.8	11.2	0	0	2.6	24.2	2.6	77	6.4	3.8	60	7.4	2.2

Table 6: Morphology of some characters of the F₂ plants (1995)

Material	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	No. of I class branches	Length of the longest branch (cm)	Stem thickness (cm)	Head diameter (cm)	Head thickness (cm)	No. of bract leaves	Length of bract leaves (cm)	Width of bract leaves (cm)	No. of ray floret	Length of ray florets (cm)	Width of ray florets (cm)
F2(2607 x M-082)	150	14.0	10.0	9.0	14	112.0	2.8	11.0	1.3	55	4.5	1.5	37	3.5	1.2
	150	14.0	12.0	1.0	20	118.0	2.9	11.0	1.4	81	5.2	1.5	57	6.7	1.5
	270	18.0	7.0	16.0	0	0	2.2	18.0	1.3	62	5.4	2.7	25	7.5	2.4
F2(HA-300 x M-015)	270	22.0	22.5	11.0	0	0	2.5	27.0	1.3	62	5.3	2.8	56	6.7	1.9
	255	25.0	24.0	18.5	0	0	2.5	29.0	1.5	55	4.5	2.0	33	6.3	2.0
	250	22.0	9.0	19.0	0	0	2.8	19.0	1.3	53	5.1	3.0	35	7.0	1.5
F2(HA-821 x M-015)	220	25.0	30	14	0	0	3.8	29.0	1.9	64	5.3	3.4	47	8.0	2.1
	170	23.0	10.0	9.0	0	0	3.3	31.0	1.4	82	7.5	4.9	56	7.6	2.1
	140	29.0	19.0	6.0	9	109	4.7	22.0	1.4	83	5.0	3.4	43	5.8	1.8
	150	19.0	21.0	9.0	0	0	3.1	31.0	1.7	85	6.2	4.5	50	7.0	1.9
	180	26.0	28.0	12.0	0	0	3.4	33.0	2.0	100	6.0	3.8	70	7.7	2.0
	180	32.0	26.0	11.0	1	46	3.4	33.0	1.7	83	6.1	4.2	46	7.7	2.2

Table 7: Morphology of some characters of BC₁ plants (1995)

Material	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	No. of I class branches	Length of the longest branch (cm)	Stem thickness (cm)	Head diameter (cm)	Head thickness (cm)	No. of bract leaves	Length of bract leaves (cm)	Width of bract leaves cm)	No. of ray floret	Length of ray florets (cm)	Width of ray florets (cm)
(1234xM-082) xHA300	275	24.0	19.0	14.0	11	38	2.6	12.0	1.3	40	3.8	2.1	25	5.0	1.5
	260	24.0	22.0	15.0	12	54	2.8	12.0	1.3	56	4.0	2.0	39	5.1	1.0
	250	27.0	19.0	15.0	11	32	2.5	13.0	1.4	64	4.3	1.8	35	7.2	2.1
	225	26.0	20.0	16.0	7	53	2.7	15.0	1.3	69	4.3	2.5	50	6.4	1.5
	270	25.0	24.5	18.0	13	39	3.1	17.0	1.7	70	4.9	2.0	58	7.7	2.0
(P-k x M-015) xHA300	250	19.0	17.0	15.0	0	0	2.4	15.0	1.3	53	4.0	1.6	43	7.3	1.7
	171	20.0	17.0	6.0	19	172	3.6	18.0	1.6	35	4.1	2.5	17	6.0	1.6
	240	29.0	30.0	15.0	21	96	3.9	22.0	2.0	61	5.6	2.5	29	6.9	2.4
	265	23.0	25.0	16.0	13	64	3.7	18.0	1.8	64	3.7	2.6	44	5.1	2.1
	230	29.0	29.0	18.0	11	62	3.2	22.0	1.9	78	5.8	2.9	41	7.2	1.6

anthocyanin peduncle; basal branching and secondary branching. Self pollination was done in order to produce the F₂ progeny.

Helianthus mollis* x *Helianthus annuus

Four F₁ hybrid plants were grown from crosses *H.mollis* x *H.annuus* in 1994 and one in 1995. They all had the main features of the wild parent and were perennial in habit, but all of them differed from the other. The characteristics of the plants are presented in Table 5. Even the two F₁ plants, coming from one and the same combination, *H.mollis* x 2607, had absolutely different phenotype. The main differences observed between the hybrid plants were in plant height, leaf size and shape, branching type and days to anthesis initiation. There were, however, two common features typical for all of them, the lack of petiole and the heterosis present.

Self pollination and backcrossing were the main methods applied in the study with the hybrid material, obtained with the participation of the two diploid perennial species.

CONCLUSIONS

The results of the hybridizations of *Helianthus divaricatus* L. and *Helianthus mollis* Lambert with the cultivated sunflower (*Helianthus annuus* L.) showed that hybrid material was produced with both perennial diploid wild species. The study of the plant morphology and their reproductive abilities confirmed the hybrid character of the material obtained. The application of self pollination and backcrossing rendered seeds allowing us to work further with the hybrid material.

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**INVESTIGACIONES PRELIMINARES OBRE HÍBRIDOS
INTERSPECÍFICOS ENTRE *Helianthus annuus* L. Y
ESPECIES PERENNES DE *Helianthus***

RESUMEN

La hibridación interspecífica tuvo lugar entre girasol cultivado, *Helianthus annuus* L. y dos especies diploides perennes, *H. divaricatus* L. y *H. mollis* Lambert. Se hicieron cruces recíprocos. Solo métodos de mejora convencional fueron utilizados. Seis líneas puras y una variedad, Peredovik, fueron incluidas en la hibridación. Las plantas híbridas fueron producidas en ambas direcciones.

Las plantas híbridas originadas a partir de cruces entre las especies perennes y el girasol cultivado tuvieron un hábito perenne. Tales plantas fueron también producidas a partir de cruces recíprocos: línea 1234 A x *Helianthus mollis* y línea 3064 A x *Helianthus divaricatus*. Las generaciones F₂ y BC₁ fueron producidas a partir del material F₁ mostrando una gran diversidad de formas.

**ETUDES PRÉLIMINAIRES D'HYBRIDES
INTERSPÉCIFIQUES ENTRE *Helianthus annuus* L. ET
ESPÈCES PERÈNNES D'*Helianthus* L.**

RÉSUMÉ

L'hybridation interspécifique a été réalisée entre le tournesol cultivé *Helianthus annuus* L. et deux espèces pérennes diploïdes -*Helianthus divaricatus* L. et *Helianthus mollis* Lambert. Des croisements réciproques ont été effectués et seules les méthodes conventionnelles utilisées. Six lignées fixées et une variété -Peredovick- ont servi à l'hybridation. Les plantes hybrides sont produites dans les deux sens.

Les plantes hybrides issues de croisements entre les espèces pérennes et le tournesol cultivé sont de type pérenne. De telles plantes ont été aussi obtenues à partir de croisements réciproques: lignée 1234 A x *Helianthus mollis* et lignée 3064 A x *Helianthus divaricatus*. A partir des hybrides F₁ des hybrides doubles et des générations BC₁ ont été obtenus, montrant une grande diversité de formes.