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POSSIBILITIES AND PROBLEMS IN THE HYBRIDIZATION OF CULTIVATED SUNFLOWER WITH SPECIES OF THE GENUS Helianthus L.

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SUMMARY

Results of hybridization between cultivated sunflower and 30 species of the genus Helianthus are presented in the paper. It was conducted by conventional methods. Some problems of the methodological and practical nature are clarified and also possibilities for producing interspecific hybrids using wild species of *Helianthus* from the collection of the Wheat and Sunflower Institute "Dobroudja", General Toshevo, Bulgaria, are investigated..

Key words: Sunflower, hybridization, cultivated form, wild species

INTRODUCTION

Possibilities of interspecific hybridization in sunflower are considerable, but the hybridization and transfer of interesting genetic material from *Helianthus* species into cultivated sunflower are limited by a series of diffuculties (Pustovoit, 1975; Georgieva–Todorova, 1976; Laferriere, 1986; Škorić, 1988; Seiler, 1988; Christov, 1990). This is why the clarification of the reasons and the improvement or detection of new methods for solving problems of non–crossability between cultivated sunflower and the Helianthus species and the sterility of interspecific hybrids produced is an indispensable condition for using these species in sunflower breeding.

The aim of this study was to clarity the possibilities for producing interspecific hybrids using different wild species and cultivated sunflower by hybridization according to conventional methods.

MATERIALS AND METHOD

The annual species *H. annuus* /cultivated and wild forms/ (2n = 34), *H. argophyluus* (2n = 34), *H. debilis* (2n = 34), *H. negluctus* (2n = 34), *H. petiolaris* (2n = 34) and *H. praecox* (2n = 34), and the perennial ones, *H. divaricatus* (2n = 34) *H. giganteus* (2n = 34), *H. glaucophyllus* (2n = 34), *H. grosserratus* (2n = 34), *H. maximiliani* (2n = 34), H. *microcephalus* (2n = 34), *H. mollis* (2n = 34), *H. nuttallii* (2n = 34), *H. occidentalis* (2n = 34), *H. salicifolius* (2n = 34), *H. silphioides* (2n = 34), *H. smithii* (2n = 34), *H. decapetalus* (2n = 68), *H. hirsutus* (2n = 68), *H. laevigatus* (2n = 68), *H. scaberimus* (2n = 68), *H. tomentosus* (2n = 68), *H. ciliaris* (2n = 102), *H. eggertii* (2n = 102), *H. resinousus* (2n = 102), *H. rigidus* (2n = 102), *H. strumosus* (2n = 102), *H. tuberosus* (2n = 102), and *H. x. laetiflorus* (2n = 102) were included in the study.

For clarification of generic relations between cultivated sunflower and the wild species of *Helianthus* the following techniques were applied: reciprocal crossing; the method of mixed pollen; backcrossing; and other methods and procedures. Inflorescences of the mother plants were castrated and isolated. Pollen was first placed on the stigmas between 13 and 17 h every other day. The hybrid materials produced were grown under field conditions. Their female fertility was determinated by the quantity of seeds produced in open pollination.

RESULTS AND DISCUSSION

Reciprocal crossing increases the crossability of cultivated sunflower with the *Helianthus* species. In some cases the percentage of seeds and F_1 plants produced is also increasing (Table 1). A higher percentage of viable hybrid plants is obtained by the two direction crossing from the species *H. annuus* (w.f.), *H. argophyllus, H. praecox, H. eggertii, H. laevigatus and H. tuberosus*. Viable hybrid plants were produced with the species *H. divaricatus, H. glaucophyllus, H. nuttallii, H. smithii and H. ciliaris* only in the cases when cultivated sunflower was used as the female parent. In the hybridization with the species *H. eggertii, H. hirsutus* and others, and the use of cultivated sunflower as the mother line, it is possible to produce a higher percentage of hybrid plants from seeds of one pollinated plant.

In some cases the hybridization was more successful when wild species, like *H. rigidus* in this study, were used as the female parent.

Simultaneous flowering of cultivated sunflower and the wild species used provided a possibility for the pollination of the inflorescences also in two crossing directions with fresh viable pollen. Thus, interspecific hybrids were produced with *H. eggertii, H. laevigatus, H. smithii, H. grosseserratus and H. microcephalus* which flower later. Also, other species such as H. debilis and H. petiolaris, with a longer period of flowering, can be used for hybridization later than normal for cultivated sunflower.

The castration of female plants was necessery because all the species expressed a certain degree of autofertility (Christov, 1990). The removal of stamens and natural pollen from the inflorescences of the wild mother plant gave a better chance for the pollen of cultivated sunflower (especially, if it is a mixture from several varieties and lines), to facilitate and provide contact with the stigma at the moment of its placing.

It was found that the pollination of noncastrated inflorescences of *H. rigidus, H. mollis* and other species with pollen from cultivated sunflower produced more seeds and wild plants than self–pollination. This indicates that cultivated sunflower's pollen appears as a stimulant for pollination.

Good results of hybridization were obtained when cultivated sunflower, used as female plant, was sterile and pollen from the wild parent, cut before pollination, was placed on it. This inflorescence was preliminarily isolated with a paper isolator (it is recommended that 2–3 inflorescences are isolated at least) or the whole male plant was isolated with a plastic net, to prevent the access of pollen carriers. Pollen is applied by rubbing the male inflorescence tenderly against the female one.

Table 1. Hybridization between cultivated sunflower and some species of the genus *Helianthus (1983–1989)*

Cross	Pollinated inflorescensces		Seed produced No	Hybrid plants produced	
	Total number	With seeds		No.	(%)
01. *H.annuus (W.f.) x H.annuus*	259	179	14680	5402	36,8
02. *H.annuus x H.annuus (W.f.)*	78	50	3934	1750	44,5
03. *H.argophyllus x H.annuus*	281	231	10964	4747	43,3
04. *H.annuus x H.argophyllus*	42	23	1859	1423	76,6
05. *H.debilis x H.annuus*	469	306	6493	419	6,5
06. *H.annuus x H.debilis*	37	15	147	111	75,5
07. *H.neglectus x H.annuus*	14	2	2	0	0
08. *H.annuus x H.neglactus*	4	3	37	29	78,4
09. *H.petiolaris x H.annuus*	304	230	2500	96	3,8
10. *H.annuus x H.petiolaris*	56	9	209	69	33,0
11. *H.praecox x H.annuus*	488	347	13172	3724	28,3
12. *H.annuus x H.praecox*	59	26	1890	1283	67,9
13. *H.divaricatus x H.annuus*	87	15	27	3^	11,1
14. *H.annuus x H.divaricatus*	15	2	10	5	50,0
15. *H.giganteus x H.annuus*	121	21	38	1^	2,6
16. *H.annuus x H.giganteus*	5	1	3	3	100
17. *H.glaucophyllus x H.annuus*	95	10	17	1^	5,9
18. *H.annuus x H.glaucophyllus*	9	3	55	50	90,9
19. *H.grosseserratus x H.annuus*	51	7	75	0	0
20. *H.annuus x H.grosseserratus*	14	14	142	68	47,9
21. *H.maximiliani x H.annuus*	30	2	3	0	0
22. *H.annuus x H.maximiliani*	9	0	0	0	0
23. *H.microcephalus x H.annuus*	5	0	0	0	0
24. *H.annuus x H.microcephalus*	13	11	29	4	13,8
25. *H.mollis x H.annuus*	462	126	567	5	0,9
26. *H.annuus x H.mollis*	45	6	61	32	52,5
27. *H.nuttallii x H.annuus*	100	36	77	0	0
28. *H.annuus x H.nuttallii*	18	1	1	1	100
29. *H.occidentalis x H.annuus*	92	27	588	2	0,3
30. *H.annuus x H.occidentalis*	2	0	0	0	0
31. *H.salicifolius x H.annuus*	200	45	112	4	3,6
32. *H.annuus x H.salicifoliu*	19	6	153	59	38,6
33. *Hsilphioides x H.annuus*	8	0	0	0	0
34. *H.annuus x H.silphioides*	2	0	0	0	0
35. *H.smithii x H.annuus*	53	1	1	0	0
36. *H.annuus x H.smithii*	9	5	302	168	55,6
37. *H.decapetalus x H.annuus*	193	99	453	9	2,0
38. *H.annuus x H.decapetalus*	9	0	0	0	0
39. *H.hirsutus x H.annuus*	154	96	1239	78	6,2
40. *H.annuus x H.hirsutus*	37	15	283	221	78,1
41. *H.laevigatus x H.annuus*	87	55	461	121	26,3
42. *H.annuus x H.laevigatus*	6	4	41	19	46,3
43. *H.scaberimus x H.annuus*	161	120	716	76	10,6
44. *H.annuus x H.scaberimus*	17	3	3	1	33,3

Table 1. Hybridization between cultivated sunflower and some species of the genus *Helianthus* (1983–1989) (continued)

Cross	Pollinated inflorescensces		Seed produced No	Hybrid plants produced	
	Total number	With seeds		No.	(%)
45. *H.tomentosus x H.annuus*	51	8	16	0	0
46. *H.annuus x H.tomentosus*	4	1	2	2	100
47. *H.ciliaris x H.annuus*	16	3	45	1*	2,2
48. *H.annuus x H.ciliaris*	1	1	5	3	60,0
49. *H.eggertii x H.annuus*	109	58	453	102	22,5
50. *H.annuus x H.eggertii*	13	10	450	223	49,6
51. *H.resinosus x H.annuus*	22	4	8	2	25,0
52. *H.annuus x H.resinosus*	6	3	43	30	69,8
53. *H.rigidus x H.annuus*	316	182	3896	690	17,7
54. *H.annuus x H.rigidus*	38	9	414	21	5,1
55. *H.strumosus x H.annuus*	159	34	95	15	15,8
56. *H.annuus x H.strumosus*	5	1	5	0	0
57. *H.tuberosus x H.annuus*	195	82	507	160	31,6
58. *H.annuus x H.tuberosus*	25	8	153	40	26,1
59. *H.x.laetiflorus x H.annuus*	61	33	185	23	12,4
60. *H.annuus x H.x.laetiflourus*	41	6	56	6	10,7

* The plants which died before reaching the budding stage

It was interesting that the quantity of hybrid plants produced (expressed in %) was different for single samples from the same wild species. Such a difference was found for the hybrids produced with the species *H. annuus* (w.f.), *H. argophyllus, H. petiolaris, H. salicifolius, H. hirsutus and H. tuberosus*. Similar results were obtained with the hybrids developed from different subspecies of *H. debilis, H. praecox, and H. rigidus.*

In clarifying the question of overcoming the interspecific hybrids' sterility it was found that a great part of the F_1 plants produced from the annual species yielded sufficient amounts of seed via sib pollination between hybrid plants and through backcrossing, and in some cases through self–pollination. These F_1 plants can be pollinated also by a pollen taken only from one variety or a line producing a sufficient number of seeds and plants for the next generation. Thus there is a possibility for a more rapid production of forms which combine the useful qualities of the two parents. Also, alloplasmic lines of cultivated sunflower could be developed more quickly by including the annual species.

It was difficult and in some cases impossible to overcome sterility of the F_1 plants developed with the perennial species. The application of self–pollination was useless. Seeds were not produced in the large part of the F_1 plants both in backcrossing and in open pollination. It was of great importance for the F_1 plants developed with *H. hirsutus, H. decapetalus, H. scaberimus* and some samples of *H. tuberosus* used as the female parent. The data presented in Table 2 give an idea about it.

The reasons for the sterility of the hybrids were probably due to anomalies which arise in meiosis owing to the chromosome nonhomologuos to the genomes of the species included in hybridization. The presence of different degrees of sterility and insufficient fertility requires the application of some methods to overcome the sterility. The assumption that in the interspecific hybrids the viability of the female gametes is higher

compared with the male ones, was confirmed by a successful application of backcrossing to the cultivated parent. Pollen from all F_1 plants was added to that collected from a group of varieties and lines. Single inflorescences were pollinated with this mixture and put under isolator. Seeds and plants of the next generation were produced in this way. More seeds were obtained by pollinating inflorescences with pollen mixture without subsequent isolation. This was due to a lack of negative effect of the isolator and the access of bees carrying extra pollen or inducing by their activity on the floret a response by the stigma which resulted in the secretion of the exudate which facilitated pollen sprouting and ovule fertilization.

Table 2. Produced seeds and BC_1 plants from backcrossing with cultivated sunflower /1984–1990/

Crosses	Polinated inflorescensces		Seed produced No.	BC_1 plants produced	
	Total number	With seeds		No.	(%)
01. *(H.annuus x H.glaucophyllus) x H.annuus*	4	3	59	58	98,3
02. *(H.mollis x H.annuus) x H.annuus*	4	2	46	18	39,1
03. *(H.salicifolius x H.annuus) x H.annuus*	34	16	486	58	11,9
04. *(H.annuus x H.smithii) x H.annuus*	61	9	81	20	24,7
05. *(H.occidentalis x H.annuus) x H.annuus*	12	1	5	4	80,0
06. *(H.hirsutus x H.annuus) x H.annuus*	196	5	26	9	34,6
07. *(H.annuus x H.hirsutus) x H.annuus*	6	3	53	41	77,4
08. *(H.decapetalus x H.annuus) x H.annuus*	68	2	5	3	60,0
09. *(H.annuus x H.decapetalus) x H.annuus*	1	1	56	10	17,9
10. *(H.laevigatus x H.annuus) x H.annuus*	98	38	195	110	56,0
11. *(H.annuus x H.laevigatus) x H.annuus*	3	1	24	16	66,7
12. *(H.scaberimus x H.annuus) x H.annuus*	66	1	1	0	0
13. *(H.eggertii x H.annuus) x H.annuus*	101	37	301	150	49,8
14. *(H.annuus x H.eggertii) x H.annuus*	17	13	364	86	23,6
15. *(H.x.laetiflorus x H.annuus) x H.annuus*	14	4	384	24	6,3
16. *(H.annuus x H.x.laetiflorus) x H.annuus*	3	1	45	13	28,9
17. *(H.annuus x H.resinosus) x H.annuus*	6	5	142	38	26,3
18. *(H.rigidus x H.annuus) x H.annuus*	375	149	1693	552	32,5
19. *(H.annuus x H.rigidus) x H.annuus*	33	4	15	0	0
20. *(H.strumosus x H.annuus) x H.annuus*	15	4	14	8	57,1
21. *(H.tuberosus x H.annuus) x H.annuus*	264	16	103	43	41,7
22. *(H.annuus x H.tuberosus) x H.annuus*	6	1	13	5	38,5

It was found for the F_1 and BC_1 hybrids produced with the perennial species that most seeds were obtained from branched plants and especially from poor by developed ones. The branched plants yield seeds mainly from the inflorescences on the branches. In most cases seeds were produced only from one inflorescence and rarely from two, three or more. This fact indicates that gametes which differ in physiological activity can be produced at different time and at different parts of the plants, and the gametes with normal activity are few which affects the normal seed formation. The enviromental conditions probably influence this process. A great number of inflorescences from a single hybrid plant which flower at different period have to be used for backcrossing.

An attempt was made to apply amphidiploidy, using the method of Jan et al. (1983). Partial success was obtained only with the hybrids from *H. eggertii, H.laetiflorus and H.laevigatus*. For better results, the method should be further improved, i.e., the concentration of the solution should be adjusted to hybrid type and the time of keeping the plant apex in the solution should be determined.

CONCLUSION

On the whole, the results of the study show that it is possible to produce hybrids between cultivated sunflower and most Helianthus species by conventional methods, but there are cases when interspecific hybridization is difficult or impossible. The difficulties may be overcome by more suitable methods, for example in vitro methods.

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POSIBILIDADES Y PROBLEMAS EN LA HIBRIDIZACION DE GIRASOL CULTIVADO CON ESPECIES DEL GENERO *Helanthus L.*

RESUMEN

Se presentan en esta publicación los resultados de la hibridación entre el girasol cultivado y 30 especies del genero *Helianthus*. Se llevó a cabo aplicando métodos convencionales. Se clarifican algunos problemas prácticos y metodológicos así como las posibilidades para producir híbridos interspecíficos utilizando especies silestres de *Helianthus* de la colección del Instituto Dobroudja de trigo y girasol, General Toshevo, Bulgaria.

PERSPECTIVES ET PROBLÉMES DE L'HYBRIDISATION DU TOURNESOL CULTIVÉ AVEC DES ESPÈCES APPARTENANT AU GENRE *Helianthus*.

RÉSUMÉ:

Les résultats d'hybridation entre le tournesol cultivé et trente espèces appartenant au genre *Helianthus* sont présentés dans cette publication. Ces hybridisations ont été réalisées suivant les méthodes classiques. Certains problémes d'ordre méthodologique et pratique sont clarifiés. Nous exposons les possibilités de produire des hybrides interspécifiques utilisant les espéces sauvages d' *Helianthus* présentes dans la collection de l'Institut du Blé et du Tournesol "Dobroudja", général Toshevo.