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POLLEN FERTILITY IN SOME *Helianthus L.* SPECIES AND THEIR F_1 HYBRIDS WITH THE CULTIVATED SUNFLOWER

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SUMMARY

Pollen fertility is an important biological trait which is gratifying to study in F_1 interspecific hybrids. Eight wild species of different ploidy levels, six inbred lines and 22 F_1 hybrid combinations were screened for pollen fertility by the method of Alexander (1969). The percentage of pollen fertility was high (above 90%) in all inbred lines as well as in most wild species (only two of them were below 90%). The F_1 hybrids had lower pollen fertility than their parents, the percentages ranging from 1.9% to 70.0%.

INTRODUCTION

Helianthus genus is polymorphous and large - it contains 50 different species. High genetic variability of the genus is exploited in sunflower breeding. Genetic distance between the wild species and the cultivated sunflower causes problems in the course of hybridization (pre-zygous incompatibility), embryo abortiveness (post-zygous incompatibility), reduced fertility or complete sterility of F_1, BC_1F_1 plants, etc.

Pollen fertility is an important biological trait which is gratifying to study in wild sunflower species, especially in interspecific hybrids.

Pollen fertility is differently referred to in the literature: "vitality" , "functionality", "stainability", etc. Walden and Ewereit (1961) (according to Pausheva, 1980), make a distinction between pollen vitality and fertility. Pollen vitality is defined as a capacity of the male gametophyte to grow in its own tube while pollen fertility is defined as a capacity to fertilize ("zygous potential"). According to these authors, pollen vitality and fertility should be determined in *in vivo* conditions. Since these conditions are difficult to provide, methods of relative assessment of fertility, based on the reaction to staining (acetocarmine, iodine, TTC methods), are most frequently used. Alexsander (1969) designed a method suitable for staining all kinds of pollen grains (of different plant species), in which sterile fertile polen grains are clearly distinguishable.

Prokopenko (1975) studied the fertility and size of pollen grains in diploid and auto-tetraploid sunflowers. His results showed that pollen fertility becomes lower as the ploidy level increases. However, pollen size increases too.

Variability for pollen fertility has been observed in *Helianthus* genus by Heiser (1962), Whelan (1978, 1980, 1981), Chandler (1979), Georgieva (1976), Bohorova (1977) etc. All of these authors have found that interspecific hybridization tends to reduce pollen fertility or causes complete sterility in F_1 interspecific hybrids.

Chandler (1979) explains the occurrence of reduced fertility and complete sterility by differences in the number and structure of chromosomes (genetic diversity) in different species within the genus. Since pollen is a direct product of meiosis - microsporogenesis, all changes and irregularities occurring in the course of meiosis bear effect on pollen fertility.

This paper reviews an investigation on pollen fertility in several *Helianthus* species of different ploidy levels and their F_1 hybrids with the cultivated sunflower.

MATERIAL AND METHOD

We studied wild sunflower species, inbred lines of the cultivated sunflower and their F_1 hybrids.

Wild species. Two diploid (2n = 34) annual species, two populations each, and six perennial species, 1 - 2 populations each, were used. Of the perennial species, two were diploid (2n = 34), two tetraploid (2n = 68) and two were hexaploid (2n = 102). The annual species were *H. neglectus* (1361, 1539) and *H. debilis* (1295, 1569); the perennial species were *H. maximiliani* (33001), *H. nuttallii* (290-F), *H. hirsutus* (1536), *H. laevigatus* (1618, 1920), *H. rigidus* (72272) and *H. tuberosus* (NS-2).

Cultivated sunflower. We used male and female lines developed at the Institute of Field and Vegetable Crops in Novi Sad - cms 9, L-9, H-26, L-1, L-3 and cms ANN 22. The females were either cytoplasmically male sterile lines or castrated B analogues. Restorer lines or fertile analogues of the cms lines were used as pollinators. All lines of the cultivated sunflower were dipoid (2n = 34).

F_1 hybrids. Twenty-two hybrid combinations between the wild species and the lines of the cultivated sunflower.

Pollen fertility was scored by the method of Alexander (1969), based on differences in the staining of fertile and sterile pollen grains (fertile - red, sterile - green). The stain contained 10 ml 95% ethyl alcohol, 10 mg Malachite green (1 ml of 1% solution in 90% alcohol), 50 ml distilled water, 25 ml glycerol, 5 g phenol, 5 g chlor hydrate, 50 mg fuchsic acid (5 ml of 1% solution in water), 65 mg Orange G (0.5 ml of 1% solution water), 1-3 ml glacial acetic acid.

Pollen was sampled in the field (between 9 AM and 11 AM), either from flowers of single plants or bulked from several plants of the same species. Three to five preparations were made from each sample and observed under a microscope. Fertile and sterile pollen grains were stained in ten vision fields of each preparation. Pollen fertility was expressed in percents, as:

$$\frac{\text{number of fertile pollen grains}}{\text{total number of pollen grains}} (\text{x } 100)$$

RESULTS AND DISCUSSION

Before going into a discussion on pollen fertility, it is important to mention that pollen fertility was scored on fertile plants only; hybrid combinations which rendered sterile plants were excluded from the experiment. Sterility did occur in F_1 hybrids between the cultivated sunflower and wild perennialis, as shown in Table 1.

Table 1 - Sterility in F_1 hybrids between wild perennial species and lines of the cultivated sunflower

Species	No. of hybrid plants	Sterile plants	Fertile plants	% of sterile F_1 hybrids
H. hirsutus	66	24	42	36.4
H. laeviguatus	51	12	39	23.5
H. rigidus	105	39	66	37.1
H. tuberosus	90	23	67	25.6

Sterility in F_1 hybrids was registered in the crosses between tetraploid (*H. hirsutus, H. laevigatus*) and hexaploid species (*H. rigidus* and *H. tuberosus*) on one side and the cultivated sunflower on the other. Full sterility was not registratered in the crosses between perennial and annual diploid species. The highest percentage of sterility (37.1%) occurred in the hybrid combinations with *H. rigidus*, the lowest with *H. laevigatus* (23.5%).

The percentage of pollen fertility was high in the diploid annual wild species *H. neglectus*, ranging from 89.0% in population 1361 to 98.2% in population 1539. The percentage of pollen fertility was also high in the cultivated line H-26 (98.4%) (Table 2).

Table 2 - Pollen fertility in the species *H. neglectus* (n=17) and its F_1 hybrids with lines of the cultivated sunflower

Parents and combinations		% of pollen fertility
H. neglectus 1361	P	89.0
H. neglectus 1361 x H-26	F_1	5.1
H-26	P	98.4
H. neglectus 1539	P	98.2
H. neglectus 1539 x H-26	F_1	6.3
H-26	P	98.4

The F_1 hybrids had exceptionally low pollen fertility which ranged from 5.1% (*H. neglectus* 1361 x H-26) to 6.3% (*H. neglectus* 1539 x H-26).

Two subspecies represented the annual diploid wild species *H. debilis*. Pollen fertility in the subspecies *ssp. vestitus* 1569 was 97.0%. The lines of the cultivated sunflower which were crossed to them also had high pollen fertility, from 97.4% to 98.4% (Table 3).

Table 3 - Pollen fertility in the species *H. debilis* (n=17) and its F_1 hybrids with lines of the cultivated sunflower

Parents and combinations		% of pollen fertility
H. debilis ssp. cucumerifolius 1295	P	94.1
cms 9 x *ssp. cucumerifolius* 1295	F_1	3.1
cms 9	P	97.4
H. debilis ssp. vestitus 1569	P	97.0
H. debilis ssp. vestitus 1569 x H-26	F_1	1.9
H-26	P	98.4

Pollen fertility of the F_1 hybrids was exceedingly low, ranging from 1.9% in the combination *H. debilis ssp. vestitus* 1569 x H-26 to 3.1% in the combination cms 9 x *H. debilis ssp. cucumerifolius 1295 (Table 3).*

H. maximiliani is a perennial diploid species which had high pollen fertility (95.9% and 98.5%). The cultivated lines crossed to it also had high pollen fertility, from 95.4% to 97.4% (Table 4).

Table 4 - Pollen fertility in the species *H. maximiliani* (n=17) and its F_1 hybrids with lines of the cultivated sunflower

Parents and combinations		% of pollen fertilty
H. maximiliani 33001	P	95.9
cms 9 x *H. maximiliani* 33001	F_1	29.5
cms 9	P	97.4
H. maximiliani M	P	98.5
L-3 x *H. maximiliani* M	F_1	6.1
L-3	P	95.4

The obtained F_1 hybrids had low values of pollen fertility as compared with the parents, from 6.1% in the combination L-3 x *H. maximiliani* n to 29.5% in the combination cms 9 x *H.maximiliani* M to 29.5% in the combination cms 9 x *H. maximiliani* 33001.

The wild species *H. nuttallii 292-F* had a high percentage of pollen fertility, 86.0% as well as the cultivated line cms 9, 97.4% (Table 5).

Table 5 - Pollen fertility in the species *H. nuttallii* (n=17) and its F_1 hybrids with lines of the cultivated sunflower

Parents and combinations		% of pollen fertility
H. nuttallii 292 - F	P	86.0
cms 9 x *H. nuttallii* 292 - F	F_1	40.4
cms 9	P	97.4

The hybrid combination cms 9 x *H. nuttallii* 292-F had lower pollen fertility than its parents, 40.4%.

The tetraploid wild species *H. hirsutus* 1536 which had high pollen fertility (99.0%) was crossed to four cultivated lines whic also had high pollen fertility (Table 6).

Table 6 - Pollen fertility in the species *H. hirsutus* (n=34) and its F_1 hybrids with lines of the cultivated sunflower

Parents and combinations		% of pollen fertility
H.hirsutus 1536	P	99.0
H.hirsutus 1536 x L - 1	F_1	19.9
L - 1 x *H.hirsutus* 1536	F_1	92.8
L - 1	P	92.8
cms ANN 22 x *H.hirsutus* 1536	F_1	18.4
cms ANN 22	P	95.6
H.hirsutus 1536 x H - 26	F_1	16.3
H - 26	P	98.4
H.hirsutus 1536 x L - 9	F_1	16.4
L - 9	P	97.9

The F_1 combinations had low pollen fertility, from 16.3% in *H. hirsutus* 1536 x H-26 to 19% in *H. hirsutus* 1536 x L-1.

The tetraploid wild species *H. laevigatus* was represented by two populations. Both of them had high pollen fertility - 98.8% in *H. laevigatus* 1618 and 98.3% in *H. laevigatus* 1620. The cultivated lines crossed to them also had high pollen fertility, from 92.8% in L-1 to 97.4% in cms-9 (Table 7).

Table 7 - Pollen fertility in the species *H. laevigatus* (n=34) and its F_1 hybrids with lines of the cultivated sunflower

Parents and combinations		% of pollen fertility
H. laevigatus 1618	P	98.8
cms 9 x *H. laevigatus* 1618	F_1	63.5
cms 9	P	97.4
H. laevigatus 1618 x L - 1	F_1	61.1
L - 1	P	92.8
H. laevigatus 1620	P	98.3
cms 9 x *H. laevigatus* 1620	F_1	63.9
cms 9	P	97.4
L - 3 x *H. laevigatus* 1620	F_1	70.0
L - 3	P	93.4

The percentages of pollen fertility in the F_1 hybrids were lower with respect to those in the parent plants. They ranged from 61% in the combination *H. laevigatus* 1618 x L-1 to 70.0% in the combination L-3 x *H. laevigatus* 1620.

The hexaploid wild species *H. rigidus* was represented by two populations, *H. rigidus* 72272 which had high pollen fertility (98.8) and *H. rigidus* 1640 with much lower fertility (32.2%). The cultivated lines crossed to them had high pollen fertility, 92.8% in L-1 and 97.4% in cms 9 (Table 8).

Table 8 - Pollen fertility in the species *H. rigidus* (n=51) and its F_1 hybrids with lines of the cultivated sunflower

Parents and combinations		% of pollen fertility
H. rigidus 72272	P	98.8
H. rigidus 72272 x L-1	F_1	46.1
L-1	P	92.8
cms 9 x *H. rigidus* 72272	F_1	55.5
cms 9	P	97.4
H. rigidus 1640	P	32.2
cms 9 x *H. rigidus* 1640	F_1	47.7
cms 9	P	97.4

Pollen fertility in the F_1 hybrids ranged from 46.1% in *H. rigidus* 72272 x L-1 to 55.5% in cms 9 x *H. rigidus* 1640.

The hexaploid wild species *H. tuberosus* NS-2 had high pollen fertility (99.1%). The cultivated lines crossed to it also had high pollen fertility, from 97.4% in cms 9 to 98.4% in H-26 (Table 9).

Table 9 - Pollen fertility in the species *H. tuberosus* (n=51) and its F_1 hybrids with lines of the cultivated sunflower

Parents and combinations		% of pollen fertility
H. tuberosus NS-2	P	99.1
H. tuberosus NS-2 x H-26	F_1	37.9
H-26	P	98.4
L-9 x H. *tuberosus* NS-2	F_1	26.6
L-9	P	97.9
cms 9 x *H. tuberosus* NS-2	F_1	58.3
cms 9	P	97.4

The F_1 hybrids had lower pollen fertility than the parents but not exceedingly low. The lowest fertility was recorded in the combination L-9 x *H. tuberosus* NS-2, the highest in cms 9 x *H. tuberosus* NS-2 - 26.6% and 58.3%, recpectively (Table 9).

The investigation of pollen fertility in eight wild species showed that there existed variability for the examined trait although most of the tested populations (9) had the percentages of pollen fertility above 90%. There were no significant differences within the species, between subspecies and between populations.

Studying pollen fertility and vitality in diploid and autotetraploid sunflowers, Prokopenko (1975) found that polyploidy causes enlargement of pollen vitality and fertility. Unlike him, we found some diploid species (e. g., *H. neglectus, H. nutallii*) to have lower pollen fertility than tetraploid species *H. hirsutus* and *H. laevigatus* or hexaploid species *H. rigidus* and *H. tuberosus*.

The examined F_1 interspecific hybrids had reduced pollen fertility with respect to their parent components. The extent of reduction was variable. It should be pointed out that the largest reductions occurred in the crosses with the annual diploid species (*H. debilis* and *H. neglectus*), from 1.9% to 6.3%, while pollen fertility remained relatively high (slightly lower than in the parents) in the crosses with the hexaploid and the tetraploid species. These results contradict earlier findings (Georgieva, 1976) which stated that the wild annual species are genetically close to the cultivated sunflower.

Effect of interspecific hybridization on pollen fertility has been studied by many researchers (Heiser, 1962; Georgieva, 1976; Bohorova, 1977; Whelan, 1978, 1980; Chandler, 1979). Pollen fertility reduction in the F_1 generation has been invariably reported. Chalender (1979) explains the occurence of sterility and reduced fertility in F_1 interspecific hybrids by irregularities in meiosis (unhomologity of cromosome, genetic distance between species).

CONCLUSION

Pollen fertility in the studied wild sunflower species ranged between 86.0% and 99.1%.

The subspecies and populations of the same species did not differ significantly in pollen fertility.

The cultivated lines used for crossing had high percentages of pollen fertility, from 92.8% to 98.4%.

All F_1 hybrid combinations had lower pollen fertility than their parent components.

Pollen fertility in the F_1 hybrids ranged from 1.9% to 70.0%.

The largest reductions in pollen fertility were recorded in the crosses with the diploid annual wild species (*H. debilis* and *H. neglectus*), the lowest in the crosses with the tetraploid wild species *H. laevigatus*.

REFERENCES

Alexander, M. P., 1969: Differential staining of aborted and nonaboted pollen. Stain Technology, Vol. 11, No. 3, 117-123.

Bohorova, N., 1977: Izučavane rodstvenie vzaimootnošenija meždu različno ploidni vidove *Helianthus* i citogenetična harakteristika na hibridite v Fl. Avtoreferat, Sofia.

handler, J. M., 1979: Chromosome Structural Differentiation Among the Annual *Helianthus* Species. Dissertation, University of California, Davis.

Georgieva, J., Lakova, M., Bohorova, N., 1979: Hybridization of Diploid Sunflower, *Helianthus annuus* (2n=34) with some Tetraploid *Helianthus* Species. Z. Pflanzenzüchtg. 83, 340-349, Berlin i Hamburg

Heiser, Ch. B., Martin, W. C., Smith, D. M., 1962: Species Crosses in *Helianthus*: T. Diploid Species, Rep. from Britania, Vol. 14, No. 2, 137-148.

Pauševa, Z. P., 1980: Praktikum po citologii rastenia, Kolos, Moskva.

Prokopenko, A. J., 1975: Sravniteljnoe izučenie piljci diploidnoi i avtotetraploidnoi form podsolnečnika. Voprosi fiziologii masličnih rasteni v svrzi s zadačami selekcii i agrotehniki, Krasnodar.

Whelan, E. D., 1978: Hybridization between Annual and Perennial Diploid Species of *Helianthus*. Can. J. Genet. Cytol. 20, 523-530.

Whelan, E. D., Dorrell, D. G., 1980: Interspecific Hybrids between *Helianthus maximiliani* Schred. and *H.annuus* L.: Effect of Backtics. Crop Science, Vol. 20, 29-35.

Whelan, E. D., 1981: Cytoplamic Male Sterility in *H.gigantus L.* x *H.annuus L.* interspecific hybrids. Crop. Science, Vol. 21, 855-859.

FERTILITE DU POLLEN DE QUELQUES ESPECES DE *Helianthus* ET CHEZ LEURS HYBRIDES F-1 OBTENU PAR CROISEMENT AVEC LE TOURNESOL CULTIVE

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La fertilité du pollen est un caractère biologique important, dont l'étude chez les hybrides interspécifiques (F-1), est déterminante. Huit espéces sauvages de différents niveau de ploidie, six lignées pures, et vingt deux combinaisons hybrides (F-1) ont été testés pour la fertilité de leur pollen d'aprés la méthode d'ALEXANDER (1969). Le pourcentage de fertilité du pollen etait élevé (environ 90%) pour toutes les lignées pures, ainsi que pour la plupart des espéces sauvages (seules deux d'entre elles avaient un niveau inferieur à 90%). Les hybrides F-1 ont presenté un niveau de fertilité inférieur à celui de leurs parents, le pourcentage variant de 1,9 a 70,0%.

FERTILIDAD DEL POLEN EN ALGUNAS ESPECIES DE *Helianthus L* Y SUS HIBRIDOS F_1 CON EL GIRASOL CULTIVADO.

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La fertilidad del polen es un caracter biológico importante para ser estudiado en híbridos interspecífico. Ocho especies silvestres de diferentes niveles de ploidia, seis líneas puras y 22 combinaciones / híbridas F_1 fueron examinadas para fertilidad del polen por el método Alexander (1969). El porcentaje de fertilidad del polen fué alto (más del 90%) en todas las líneas puras así como en la mayoría de las especies silvestres (solo dos de ellas estuvieron por bajo del 90%). Los / híbridos F_1 tuvieron mas baja fertilidad del polen que sus padres, variando el porcentaje desde 1.9 a 70.0%.