

THE INFLUENCE OF DIFFERENT GENETIC AND ENVIRONMENTAL FACTORS ON POLLEN SELF-COMPATIBILITY IN SUNFLOWER

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Summary

The genetic control of pollen self-compatibility in sunflower is very complex and greatly influenced by the environment. In F_1 , the effects of incomplete gene dominance are the most frequent. Additive effects were found in 28 percent of the cases.

The F_2 generation is characterized by large genetic variances, the heritability in the broad sense having values between 60.4-89.1%. The most important negative influence on self-compatibility and pollen quality was exerted by high temperature (35/24°C) and short light/dark sequences (6/18 h).

No significant differences were found between self-compatible and self-incompatible genotypes with respect to pollen size and density and pollen tube growth.

The study of correlations between self-compatibility levels and various morpho-physiological plant characteristics points out that, although it is possible to obtain self-compatible high yielding and high oil sunflower hybrids, breeding for self-compatibility should be done with priority only for those regions where the frequency of pollinating insects is very low or where crossed pollination is hindered by the unfavorable meteorological conditions occurring in floraison blooming time.

Introduction

Pollen self-compatibility is the main biological reaction of sunflower plants against self-fertilization, this species being allogamous and entomophile. Foreign pollen germinates on stigma much faster than the own pollen, it has nearly a double speed of penetration into the style and achieves fertilization in a shorter period (Putt, 1943; Ustinova, 1951; Habura, 1957).

The genetic and physiological nature of self-incompatibility or self-compatibility in sunflower is little known. Habura (1957) considers that the relationship between pollen and pistil is determined by two pairs of dominant alleles (S), one of them controlling the pollen traits, the other the pistil characteristics. Our data reveal a sporophytic influence on the genetic mechanism, which is rendered evident by differences in self-compatibility between direct and reciprocal hybrids (Vranceanu, 1974).

Before the genic or cytoplasmic male-sterility become available, attempts were made to use self-compatibility in hybrid seed production (Rudorf, 1958;

Stefansson, 1959; Putt, 1962; Schneider, 1965; Luciano et al, 1965; Vranceanu, 1967; Stoyanova, 1967; Vranceanu and Stoenescu, 1969). Now, when all sunflower hybrids are produced on the basis of cytoplasmic or genic male-sterility, the phenomenon of self-compatibility has become much more important than that of self-incompatibility, and it is desirable mostly in those regions in which the frequency of pollinating insects is very low or the unfavorable meteorological conditions, such as heavy rains and cloudy sky, make difficult their flight.

The present paper contains a series of research data obtained at Fundulea under field and phytotron conditions with respect to: correlations between the self-compatibility level and the main morpho-physiological characteristics of plants and pollen; heritability of self-compatibility in F_1 and F_2 ; the way this phenomenon is influenced by different major genes, by the degree of heterozygosity, by the pollination type and by day-length and temperature during flowering period.

Materials and Methods

In order to estimate the correlation coefficients, the following biological material was used: the early cultivars Issanka, Cherneanka 66 and Wielkopolski; the half-late cultivars Record, Peredovik and VNIIMK 8931; 38 early and 110 half-late hybrids obtained at Fundulea; 11 early and 16 half-late inbreds in an advanced stage of inbreeding.

The study of pollen self-compatibility in F_1 included 46 single hybrids resulted from crossing 19 different inbred lines. Heritability in the broad sense was calculated for the crosses AS-110 T x S-1358, S-1358 x RF-9 and S-1358 x RF-1, in which AS-110 T is a genic male-sterile line (ms_1), S-1358 is one of the most self-compatible line available at present, S-1358 AC is its cytoplasmic male-sterile analogue, and RF-1 and RF-9 are two restorers (Rf_1).

The seven isogenic lines of S-1358 contain the genes Pl_1 , ms_1 , T , Rf_1 , Rf_2 , as well as other restorer genes from two different strains (Table 3). The influence of day-length and temperature on pollen self-compatibility was studied using the early and half-early cultivars and hybrids included in F.A.O. trials in 1976-1977, along with eight Romanian inbred lines.

Pollen self-compatibility was evaluated as the percentage of the filled seeds, i.e., the ratio filled seeds/filled + unfilled seeds. Such a determination is quite objective, since female sterility has not been noticed in the investigated material. Sunflower heads were isolated with double gauze bags for preventing foreign pollination. Paper bags were not used because they maintain a stifling atmosphere with a higher temperature and humidity, which may affect the seed setting.

In order to establish the density and the size of pollen grains, the content of individual anthers was homogenized in one cm^3 of water. Samples, containing a drop from this suspension, were examined under microscope (ocular 7x with micrometer, objective 20x).

Simultaneously, pollen density was also determined by Thoma's method, which is currently used for blood-globule determination. Thoma's chamber has

a surface of 1 m², divided into 400 squares. Each sample was analyzed in 80 squares and the mean density calculated for 1 mm². In this paper only the results obtained by Thoma's method are presented, because the correlation coefficient between pollen density established by this method and that obtained by using the microscopic fields, estimated for 50 different samples, showed a high positive and distinctly significant value ($r = +0.91$).

The culture medium for pollen germination consisted of an aqueous solution with the following composition: 10% glucose, 1.5% agar-agar and 1% gelatine. After boiling and cooling, H₂BO₃ 0.001% and Ca (NO₃)₂ 0.003% were added. The nutritive drops with pollen grains were placed on microscopic slides and introduced into a moist chamber of Van Tieghen type. The growth intensity of pollen tubes was determined by their measurement after three hours.

The homogeneity of the correlation coefficients was estimated by chi-square test.

The heritability in the broad sense was calculated using Allard's (1960) formula:

$$H = \frac{F_2 - E}{F_2}, \text{ where } F_2 \text{ is the variance of the } F_2 \text{ generation and } E \text{ is the environmental variance, the last one being equal to the mean of the three nonsegregating generations } P_1, P_2 \text{ and } F_1,$$

$$F = \frac{P_1 + P_2 + F_1}{3}, \text{ where } P_1 \text{ and } P_2 \text{ are the variances of parents and } F_1 \text{ the variance of the } F_1 \text{ generation.}$$

With the view of establishing the significance of differences, the analysis of variance and Duncan's multiple range were calculated.

The effect of photoperiod and temperature on pollen self-compatibility was studied both in phytotron and in field experiments.

In one experiment, in phytotron, the treatment consisted of three different light/dark sequences: 6h/18h, 12h/12h, 18h/6h, maintaining during flowering a constant temperature of 26/19°C. In the second experiment, the following day/night temperature regimes were used in bud and flowering stages: 19°C/16°C, 26°C/19°C and 35°C/24°C, maintaining for the whole vegetation period and long day-light conditions of 18h/6h. In phytotron, plants were grown in pots of 10 l each, one plant per pot. Each treatment consisted of 4-6 randomized plants. The minimum intensity of the artificial light was 26.500 lux and the relative air moisture 65%.

In field experiments, the investigated material was set up in one randomized complete-block design with three replications and five Latin rectangles with five replications, each entry having 5-10 plants.

Results and Discussion

The small and insignificant correlation coefficients presented in Table 1 indicate the possibility of obtaining high yielding self-compatible genotypes.

TABLE 1. Correlation coefficients between self-compatibility and different morpho-physiological characteristics (Fundulea, field 1977)

Cultivars	No.	Correlation coefficient between self-compatibility level and:						
		Seed yield (q/ha)	% oil in dry seed	Plant height	Head diam.	Veget. period (days)	Pollen density no/mm ² (Thoma)	Pollen grain Size () (/3)
Open-pollinated cultivars	early (98-115 days)	3	-0.21	-0.25	-0.126	-0.42	+0.16	+0.08
	half-late (116-134 days)	3	-0.18	-0.08	-0.17	-0.28	+0.11	+0.15
Single hybrids	early (98-115 days)	38	-0.07	-0.33	-0.09	-0.51*	+0.26	+0.18
	half-late (116-134 days)	110	-0.20	-0.13	=0.18	-0.37*	+0.23	+0.15
Inbreds	early (90-110 days)	11	+0.14	-0.19	+0.10	-0.47	+0.12	+0.19
	half-late (111-124 days)	16	+0.03	+0.07	+0.02	-0.36	+0.27	+0.09
Ponderated means			-0.13	-0.19	-0.12	-0.43*	+0.23	+0.15

There were analyzed: for open-pollinated cultivars, 10 plants from each number and replication.
for hybrids inbreds, 5 plants from each number and replication.

* $P > 1\%$.

Hybrid combina- tions	P ₁		a		$\frac{P_1+P_2}{2}$		a		P ₂	
	No.	0	0	4	13	19	7	3		
	%	0	0	9	28	41	15	6		
				incomplete dominance	additivity	incomplete dominance	complete dominance	over- dominance		

ISD 1 % for self-compatibility level = 11.6 %; 2 a = 100 %

Fig.1. Pollen self-compatibility of 46 sunflower F₁ hybrids, in comparison with their parental inbreds (field, Fundulea 1977)

with high oil content and adequate height and maturity.

However, the negative values for the correlation coefficients between self-compatibility and seed yield and oil content support the previous findings (Vranceanu, 1974; Lialiuskin and Sarova, 1975) that the incompatible genotypes bring about in general a greater heterosis effect than the compatible ones. Also, the negative coefficients of correlation between compatibility and the head size suggest that it is more difficult to create self-compatible genotypes with big heads.

That is why breeding for self-compatibility should constitute a special objective only for those ecological zones in which the natural conditions do not permit a good crossed pollination.

Although the values for the correlation coefficients between self-compatibility and pollen density, pollen size and pollen tube growth are positive, the fact that they are insignificant proves that self-compatible forms do not produce necessarily pollen of a superior quality. Similar results had also been obtained by Obolenski (1958). Thus, we may assume that genes controlling self-compatibility in sunflowers have rather a physiological action in respect of pollen-pistil relationship than an effect on pollen structure.

In F_1 generation, the phenomenon of self-compatibility presents in most cases an incomplete dominance (Figure 1). The additive effect of genes was noticed to 28% of the investigated F_1 hybrids, the values for these hybrids being equal or close to the mean of the parents. This category of genotypes evidences the possibility of selecting self-compatible forms. In a small number of cases, hybrids with a degree of self-compatibility equal or even superior to that of the best parent could be obtained.

Data from Table 2 show that the heritability in the broad sense of pollen self-compatibility has very high values for all the three hybrid combinations, which implies that the genetic variance for this character is greater than the environmental variance.

Comparing the values of the F_1 generation with those of the parent means, one can see an incomplete dominance in the first cross, an overdominance in the second and a clear additivity in the third cross. These completely different results, obtained even in the case when the three crosses have a common parent (S-1358), point out the genetic complexity of pollen self-compatibility in sunflower. It appears therefore necessary to determine the self-compatibility degree directly for each hybrid, even if there is a common parent or a similarity in self-compatibility.

The self-compatible inbred S-1358 was used as male parent in the first cross and as female parent with cytoplasmic male-sterility in the other two crosses. The three F_1 generations are characterized by high self-compatibility levels (57.8-68.7%), which proves that the sterile cytoplasm has not an evident influence on this character.

The high uniformity of the nonsegregating generations P_1 , P_2 and F_1 is emphasized by the small values of their variances as compared to those calculated for the F_2 generations.

TABLE 2. Means and variances of pollen self-compatibility of parents and hybrid generations derived from three single crosses.

Generation	AS-110 T x S-1358			S-1358 AC x RF-9			S-1358 AC x RF-1		
	No. of Indivi- duals	Mean	Variance	No. of Indivi- duals	Mean	Variance	No. of Indivi- duals	Mean	Variance
P ₁ (♀)	8	13.0	34.8	8	62.8	158.2	8	62.8	158.2
P ₂ (♂)	8	62.8	158.2	8	37.8	44.6	8	68.7	251.9
$\frac{P_1 + P_2}{2}$		37.9			50.3			63.6	
F ₁	8	57.8	251.8	8	68.7	153.5	8	64.4	141.3
E			148.3			118.8			183.8
F ₂	79	39.4	832.4	121	44.6	1088.0	109	57.1	464.1
Heritability									
$(H = \frac{F_2 - E}{F_2})$		82.2%			89.1%			60.4%	

TABLE 3. The influence of some major genes on pollen self-compatibility in sunflower inbred S-1358 (Fundulea, field 1977)

Isogenic inbreds	Back- cross genera- tions	Genes and their origin	% filled seeds (natural self- pollination)
S-1358 (initial inbred)			
S-1358 Pl_1	6	Pl_1 , downy mildew resistant (inbred AD-66)	62.8 abc*
S-1358 AST	8	ms_1 , genic male-sterility (VNIIMK 8931 T anthocyan (Fuxinka)	58.1 bcd
S-1358 Rf_1	6	Rf_1 , restoration (inbred T-66006-B-2-1)	53.1 de
S-1358 Rf_2 (SL)	7	Rf_2 , restoration (cultivar Slovenska siva)	54.2 cd
S-1358 Rf_2 (DV)	6	Rf_2 , restoration (cultivar Discovolante)	70.4 a
S-1358 Rf (S-11)	6	Rf , restoration (Sint. popul. No. 11)	66.0 ab
S-1358 Rf (OG)	7	Rf , restoration (German orn. sunfl.)	62.9 abc
			44.8 a

* Means followed by the same letter do not differ significantly at the 1% level.

In the case of the cross between two self-compatible lines (S-1358 AC x RF-1) it has been expected to find a more reduced variance of the F₂ generation as compared to that encountered in self-compatible x self-incompatible crosses.

Assuming that the seven isogenic lines differ from the initial inbred S-1358 only by the major gene specified in Table 3, we may consider that the gene for male-sterility ms₁, or eventually the gene I for the anthocyanic color, exert a negative influence on self-compatibility genes.

Among the genes for pollen fertility restoration, those coming from the German ornamental forms (Vranceanu and Stoenescu, 1976) reduce significantly the self-compatibility level of the inbred line S-1358. The gene Rf₂ influences positively this character, but not significantly.

Table 4 contains data regarding self-compatibility, pollen density, size of pollen grains and pollen tube growth for four cultivars, eight hybrids and five inbred lines subjected to seven environmental variations. The best behavior was noticed in field experiments (Fundulea, 1977). Similar results were obtained in phytotron with 18 h daylight and 26°/19°C temperature during flowering. The short daylight (6 h) affected seriously the level of self-compatibility and the growth of pollen tube.

The high temperature (35°/24°C) is yet the factor which influenced the most significantly the level of self-compatibility, diminishing drastically the pollen density and the pollen tube growth. The pollen quality was 2-3 times lower and stigmas suffered a pronounced desiccation. The abortion of the fertilized ovules was probably frequent. Thus, high temperatures in blossoming time appear to be the main cause of the high percentage of unfilled seeds, even in the case of the self-compatible genotypes.

The low temperature (19°/16°C) affected particularly only the self-compatibility degree, delaying the opening of the tube flowers and the anther appearance and in some cases, especially in inbreds, favoring the phenomenon of protogeny.

The analysis of variances show that the fluctuations of self-compatibility under different light and temperature conditions are very high, but nevertheless they do not succeed in overlaying the differences due to the genetic variants (entries). Therefore, the genes for self-compatibility seem to have a more pronounced phenotypical stability than it has been considered by now. The combined actions "entries x photoperiodicity" and "entries x temperature" have high and significant values, indicating a specific response of each entry to photoperiodicity and temperature. Temperature is the factor which influences the most sunflower self-compatibility and pollen quality. Photoperiodicity has a similar influence only in the case of pollen tube growth.

In Table 5, the entries were divided into two groups: self-compatible genotypes, with a self-compatibility degree in the field higher than 30% and self-incompatible genotypes with a self-incompatibility degree lower than 30%. In comparison with the natural self-pollination, the artificial self-pollination and the sib pollination increase substantially the percentage of the fertilized and normal filled seeds. The artificial pollination is very efficient in the case of the self-incompatible genotypes.

TABLE 4. Variation of self-compatibility and pollen quality under field and phytotron conditions (17 sunflower genotypes)

Factors	Means				
	% filled Seeds	Pollen Density No/mm ² (Thoma)	Pollen Grain Size ()	Pollen Tube Growth (/3h)	
Field (Fundulea, 1977)	29.8	10.7	25.4	48.3	
Photoperiodicity					
6 h	11.4	6.6	22.6	24.1	
12 h	17.3	7.8	25.1	35.0	
18 h	21.8	8.9	24.9	43.7	
Temperature in blooming time					
19°/16°c	9.2	7.1	26.3	39.3	
26°/19°c	21.8	8.9	24.9	43.7	
35°/24°c	0.1	4.8	22.3	29.8	
Variances					
Source of variation	GL				
Entries (E)	16				
Photoperiodicity (Ph)	2				
Temperature (T)	2				
E X Ph	32				
E X T	32				
Error	64				
	12,253*	6.3*	5.2	202*	
	1,199*	52.3*	16.1*	1,236*	
	7,214*	156.9*	32.7*	1,066*	
	717*	34.7*	14.2*	815*	
	1,098*	106.2*	23.5*	723*	
	130	1.2	2.7	7	

*P > 1%

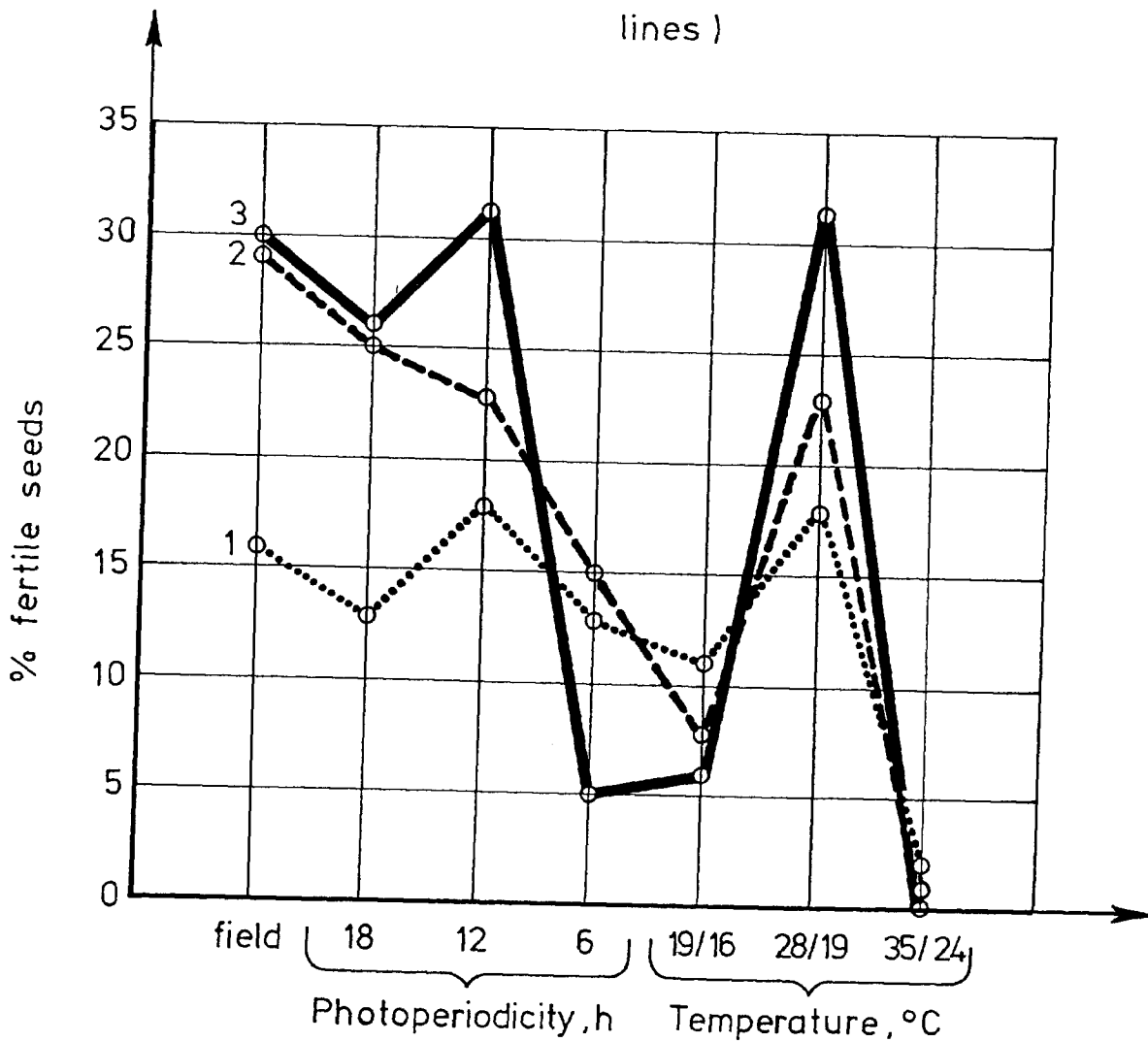
TABLE 5. Mean percentage of filled seeds obtained by natural, artificial and sib pollination, under different environmental conditions.

Factors	Self-compatible genotypes				Self-incompatible genotypes			
	Artifi- cial		Artifi- cial		Artifi- cial		Artifi- cial	
	No. of entries	Natural self- pollination	Artifi- cial selfing	sib polli- nation	No. of tries	Natural self- pollination	Artifi- cial selfing	sib polli- nation
Field (Fundulea, 1977)	22	58.3*	71.1 b	88.8 a	37	12.8 c	31.9 b	81.4 a
Photoperiodicity:	6 h light	27.5 b	25.6 b	38.2 a	13	6.4 c	18.9 b	40.1 a
	12 h "	43.4 c	54.2 b	70.8 a	26	9.3 c	44.9 b	68.3 a
	18 h "	55.1 b	60.3 b	84.3 a	26	11.5 c	47.8 b	80.0 a
Temperature in blooming time:	19°/16°C	22.3 b	17.5 b	34.6 a	24	4.8 c	13.7 b	29.9 a
	26°/19°C	43.4 c	54.2 b	70.8 a	26	9.3 c	44.9 b	68.3 a
	34°/24°C	0.6 b	1.9 b	6.3 a	26	0.0 b	0.6 b	7.8 a
Ponderated means %		41.7	49.0	65.1		8.2	30.0	56.7
		100.0	118.0			100.0	366.0	692.0

* Means followed by the same letter are not significantly different at the 1% level, within a particular measurement and experiment.

The variation of self-compatibility differs also according to the genetic background of the biological material (Figure 1). So, the single hybrids and especially the inbred lines, have much higher correlation coefficients than the open-pollinated cultivars. Consequently, the environmental conditions influence the expression of genes for self-compatibility to a greater extent when they are present in genotypes with a more reduced degree of heterozygosity.

Fig.2. Pollen selfcompatibility variation depending on photoperiodicity and temperature (average data for 4 open-pollinated varieties, 21 single hybrids and 8 inbred lines)



1 - open-pollinated varieties ; cv=46%. 2 - single hybrids ; cv = 60 % . 3 - inbred lines ; cv = 75 %

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