T1974GEN01

THE CYTOLOGIC MECHANISM OF MALE STERILITY IN SUNFLOWER

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The genetic delimitation in sunflower male sterility types was established according to the location of the genetic factors that induce pollen degeneration and stamen atrophy (Vrânceanu, 1967)

Gene male sterility and cytoplasmic male sterility in sunflower are distinct phenomena not only on account of the place where the genetic factors are located, but also on account of the specific cytologic activating mechanism of these factors that determine pollen degeneration.

In literature no comparative study is known differenriating from a cytologic point of view the pollen abortion mechanism in sunflower with gene male sterility and cytoplasmic male sterility. The findings of Riabota in his investigations on microsporogenesis, carried out in 1969 on sterile and fertile plants originating from sunflower inbred lines as well as those of Ilisulu of 1968 on a male sterile plant have been issued.

A series of studies were undertaken in other crop plants in which the male sterility phenomenon had been discovered, following up the microsporogenesis process as well as the process of pollen abortion, and we quote here some of the most important: Dubei and Singh studied in 1965 the mechanism of pollen abortion in flax plants with male sterility. Singh and Hadley in 1961 and Erichsen and Ross in 1963 undertook similar investigations in sorghum. Zenkteler undertook similar studies in 1962 in carrots and Artschwager carried them out on sugar beet in 1947.

In 1956, Jones et al. carried out investigations on the mechanism of pollen abortion in male sterile plants in maize. Microsporogenesis and the mechanism of pollen abortion were also studied by numerous researchers in male sterile plants of small grain. Among these researchers we quote: Chanhan and Singh who investigated in 1966 on wheat, Belousov and Klinciki in 1970 on rye, Kaul and Singh in 1966 on barley, et.

In the present paper we give the results and conclusions of our study on the microsporogenesis process in two gene male sterile lines developed in the Laboratory for Oilseed Plants-Fundulea by V r â n c e a n u and colab. in 1969, as well as in the cytoplasmic male sterile lines, so as to contribute to detect the specific cytologic mechanism in both types of male sterility.

MATERIALS AND METHODS

The investigations were carried out during two successive years (1972—1973), in the AS 110 and AS 116 lines with male sterility induced by the *ms 1* gene whose dominant allele is linked to the *T* gene (anthocyan presence). The microsporogenesis process was studied by comparing the green male sterile plants ($ms_1\ ms_1$) to the red male fertile plants ($Ms_1\ ms_1\ Tt$).

The 1A ; 2A ; 4A and 5A male sterile lines formed the material for the study of the cytologic mechanism specific for the plants with cytoplasmic male sterility ; they came from a source developed by L e c l e r c q in 1966 by hybridation of the *H. petiolaris/H. annuus* species. The investigations were made in comparison to the fertile analogouses : 1B ; 2B ; 4B and 5B respectively.

In order to study microsporogenesis in the plants with gene male sterility, 10 inflorescences were taken from green sterile plants and from red fertile plants of the same inbred line, while for the plants with cytoplasmic male sterility the same system was used, taking 10 heads from the respective lines.

The investigation of the succesive microsporogenesis stages was followed up on the same inflorescence by cutting and fixing fragments of the head. In each fragment 40—60 tube flowers were found, at different development stages.

The anthers were fixed in a 3 : 1 acetic acid solution and stained in acetic carmine 4%.

Pollen fertility was studied by 2% acetic carmine staining and pollen diameter in the plants with gene male sterility was measured at 7×20×power, expressed by visual micrometer units and estimated by applying a variance analysis calculation.

RESULTS AND DISCUSSIONS

The green male sterile plants show, at the end of the first prophase, in diakinesis, cells that clearly exhibit 1—2 open bivalent chromosomes or 2—4 univalent chromosomes, that may appear by desynapsis or synapsis.

Thus, from the total cells analysed in diakinesis in line AS 110 during 1973—1974, 34% and 62% respectively exhibited 2 univalent chromosomes, while 63% and 30% exhibited 4 univalent chromosomes.

The unequal disjunction of the univalent chromosomes as well as their characteristic behaviour will determine the different anomalies observed in the subsequent meiosis stages. The anomalies observed in

the meiosis stages in the plants with gene male sterility are a consequence of the univalent chromosomes and suggest a correlation of cause to effect. Asynapsis and desynapsis of the chromosomes are controlled by a certain genetic mechanism, in our case gene ms_1 which probably induces chiasma formation.

Owing to the fact that we cannot yet situate the respective genetic factors, we are not prone to ascribe to this alone the presence of univalent chromosomes as well as the aberrant behaviour in the post-meiosis stages, thus explaining pollen sterility in the lines studied by us.

The types of anomaly most frequently encountered by us are: retarded chromosomes, chromatic fragments and bodies, chromosomal and chromatic bridges, micronuclei and microcytes (fig. 1, 2, 3).

When analysing the percentage of existent anomalies in meiosis in the two years of study in the AS 110 and AS 116 lines, it was found that both in the first and in the second division the percentage of anomalous cells is different from one stage to the other, ranging between narrow limits from one year to the other. Thus in M I the cells bearing anomalies are 50 and 55% in the AS 110 line and 35 and 60% respectively in the AS 116 line.

In anaphase I the anomalies in line AS 116 are 64 and 63%.

In telophase I the anomalies in line AS 110 are 52 and 59% and in line AS 116 they are 57 and 50% respectively (fig. 10 A).

In the homotypical division, cells with anomalies are 44% in M II (1972) in the AS 116 line and in telophase II they are 56 and 55% in line AS 110 and 47 and 57% respectively in line AS 116.

Abnormal tetrads were 52 and 57% in AS 110 and 48 and 58% in AS 116 (fig. 10 B).

By separately studying each stage of the reductional division, we found in M I a 63% predominance of cells with 3 univalents in the AS 110 line (1972) and 53% cells with bivalents in 1973. During the second year of study, cells with chromatic agglutinations and fragmentations were encountered in a 31% percentage. The AS 116 line showed in M I the highest percentage — 61% — in cells with 2 univalents. Investigations in anaphase I revealed in line AS 116 a predominance of cells with various anomalies: agglutinations, chromatic bodies and fragments, 76% bridges, while the rest of the cells showed a number of 1, 2, 3 retarded chromosomes. In the second year of study, the percentage of anomalies is practically identical. The cells affected by strong anomalies — such as agglutination or fragmentation of the chromatic material, will usually not develop subsequent division stages.

In telophase I, the AS 110 line exhibits the highest percentage (38%) and (34%) with 4 retarded chromosomes and 2 retarded chromosomes respectively (in the first investigation year) and 51% cells with bridges in the second year. The remaining cells exhibit 2, 3 and 4 retarded chromosomes.

The AS 116 line exhibits in the first investigtion year only in 16% of the cells 2 retarded chromosomes, 31% with 3 retarded chro-

mosomes and 31 and 24% cells respectively with other types of aberrations, i.e.: agglutinations, fragmentations and chromatic bridges. In the second year of study the picture of anomalies is similar, with a predominance (51%) of cells with bridges, 15% with agglutinations and fragmentations, while the rest of 25% and 9% consists in cells with retarded chromosomes (fig. 10 C).

The homotypical division also shows differences in what regards the percentage and type of anomalies for each phase. In telophase II, the AS 110 line held 34% of the cells with 2 retarded chromosomes and 28% of the cells with 4 retarded chromosomes. The highest cell percentage (38%) was affected by anomalies such as agglutinations and chromatic fragmentations. In the second year of study the highest percentage of cells (55%) exhibited chromatic bridges.

In 1972, the AS 116 line had 31% of the cells with 2 retarded chromosomes, 16% with 3 retarded chromosomes and 18% with 4. Chromatic bridges and micronuclei were encountered in 11% of the cells and in 14% respectively. In the second year of study the percentage of the various encountered and investigated anomalies had approximately similar distribution or very close to it, except the cells with chromatic bridges which held a higher (45%) percentage and those with micronuclei which represented 25% (fig. 10 D).

In the tetrad stage, during the first year of investigation, the highest percentage of anomalies was found in line AS 110, with 50% polyads. An unequal distribution of the chromatic material affected 31% of the cells, and 19% of the cells showed other anomalies such as: fragmentation of the chromatic material, merging of the nuclei or their linking by bridges. During the second year, the frequency of anomalous cells in the tetrad is represented by very close values, ranging from 20% cells with unequal distribution to 29% tetrads with micronuclei (fig. 10 E).

The analysis of pollen fertility at full maturity shows a percentage of apparently fertile pollen of 4 to 6%, when stained with carmine acid, but the pollen lacks the usual echinulates. Diameter of this pollen is very reduced and variable as compared to the normal fertile one, presenting values of 5.65 units at the visual micrometer in line AS 110 and of 5.81 in line AS 116, with a variability coefficient of 24.07 and

Table 1

Pollen diameter in the gene male sterile and male fertile plants

Crt. no.	Name of the plant	$\bar{X} \pm S\bar{x}$	S %	P
1	A.F.R, 110	7.79 ± 0.04	3.97	0.51
2	A.F.R, 116	8.19 ± 0.03	2.93	0.36
3	A.S.V, 110	5.81 ± 0.19	21.51	3.27
4	A.S.V, 116	5.65 ± 0.20	24.7	3.53

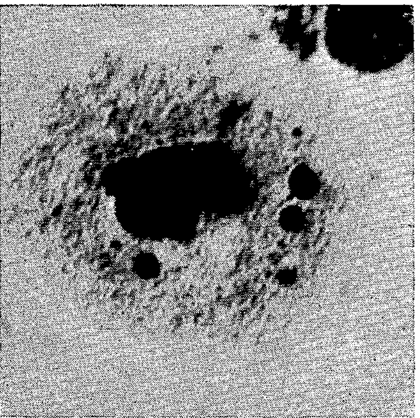

Fig. 1 — Metaphase I with chromatic eliminations.

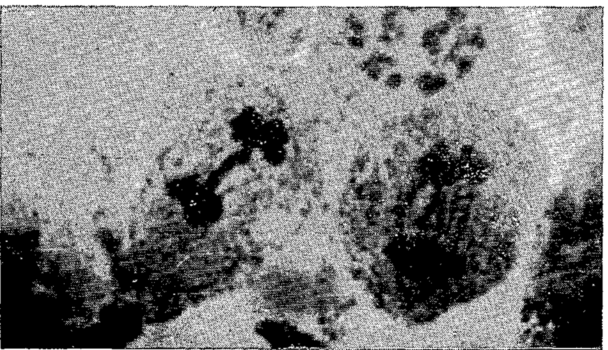

Fig. 2 — Telophase I with chromosomal bridges.

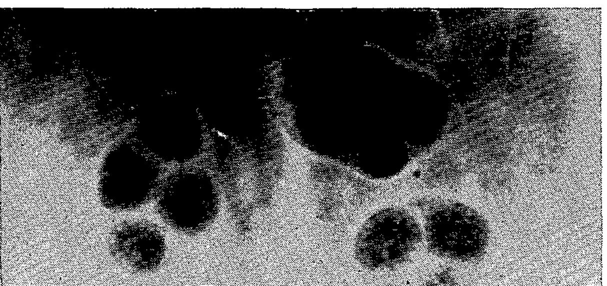

Fig. 3 — Polyads.

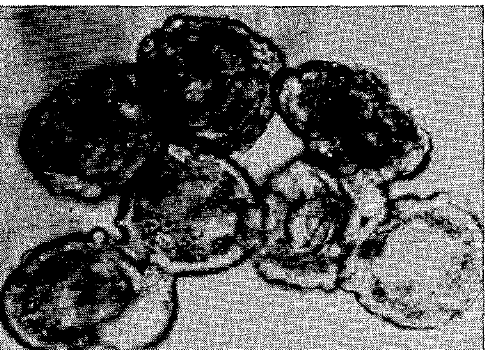

Fig. 4 — Sterile pollen.

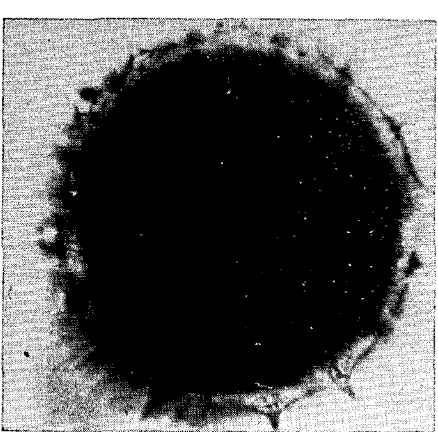

Fig. 5 — Normal fertile pollen.

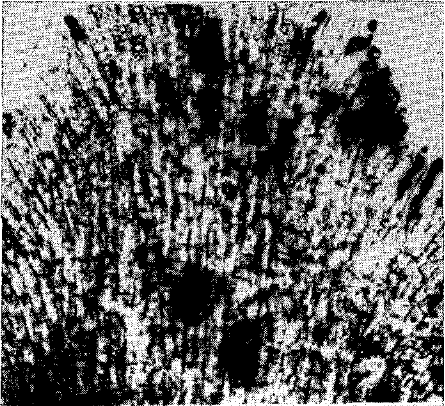

Fig. 6 — The aspect of anthers in cytoplasmic male sterile lines 1A and 5A.

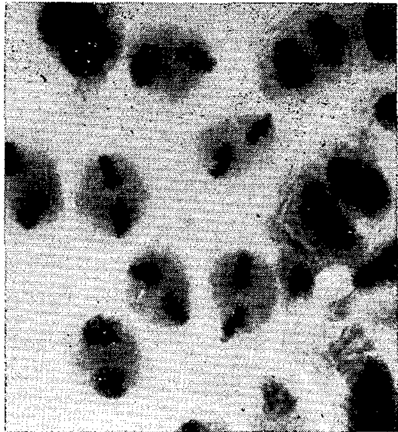

Fig. 7 — The normal aspect of the heterotype and homotype division in cytoplasmic male sterile lines 2A and 4 A.

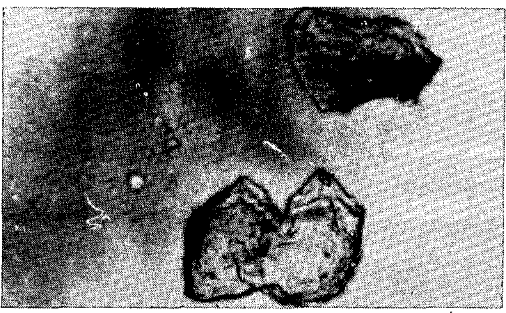

Fig. 8 — Sterile pollen in cytoplasmic male sterile lines 2A and 4A.

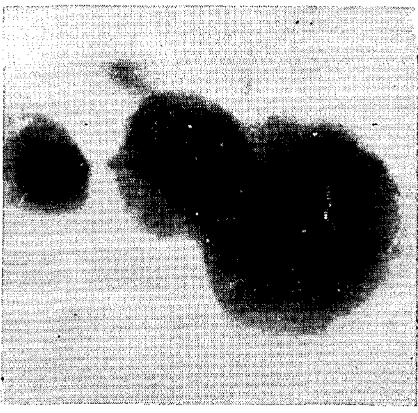

Fig. 8 — Degeneration of the microspore released from the tetrad.

21.51 respectively, as compared to 8.15 and 7.79 units at the visual micrometer and a 3.97 and 2.93 variability coefficient respectively, as held by normal fertile pollen (table 1).

The practically 100% sterile pollen has, when mature, a distinct — completely plasmolized — aspect and stays uncoloured (fig. 4). The anomalies detected in 40 to 70% in meiosis as well as the unequal disjunctions of the chromosomes resulted of the ms_1 gene activity determines the formation of hypo-and hyperploid gametes that will degenerate in the development stage after release from the tetrad.

Bearing in mind what we stated before, we can consider gene ms_1 as a gene with ameiotic action, determining in the last analysis a complete sterility of the pollen. The pollen, although apparently 4 to 6% fertile, also results from strong unequal disjunctions, and this will determine in the end its elimination at haplontic level where it will have to compete the normal fertile pollen. All these karyiologic findings clearly certify that the plants with gene male sterility can be successfully employed in sunflower hybrid production.

The microsporogenesis of the red fertile plants developed normally without deviations and the pollen was 92—100% fertile (fig. 5).

THE MECHANISM OF POLLEN ABORTION IN THE PLANTS WITH CYTOPLASMIC MALE STERILITY

The various hypotheses trying to explain the mechanism of pollen abortion in the plants with cytoplasmic male sterility did not yet completely succeed in explaining this phenomenon.

The data gathered by us during the investigation of plants with cytoplasmic male sterility confirm certain hypotheses that tried to explain the pollen degeneration phenomenon in other plant species.

Investigations of the anthers at different development stages in line 1A and 5A reveal the total absence of meiosis. The tapetum cells, the mechanic layer and the epiderm persist, so that the anther preserves its outline at maturity, but its dimensions are much more reduced. The sporogenous cells completely degenerate in the pre-meiotic stage and induce a total absence of the meiosis. In an ultimate stage, the anther gets a characteristic aspect due to its oblong epithelial cells — devoid of nucleo-cytoplasm content — this indicating the anther's death (fig. 6). These observations agree to a great extent with those of Singh and Hadley in 1961, as well as with those of Erichsen and Ross in 1963 for sorghum and with those of Zenkteler in 1962 for carrots.

Certains researchers i.e.: Pointer and Cooper 1952, Singh and Hadley 1961 revealed, at hand of the carried out observations, a biochemical correlation between the tapetum that persists and the degeneration of the sporogenous cells, induced by perturbations of a nutrition deficient in carbohydrates.

In contrast to the other lines, lines 2A and 4A exhibit a development of the anthers and of microsporogenesis approximately similar

to normal, fertile plants but with a much lower content in sporogenous cells entering activity.

The end of the heterotype pro-phase shows normal diakineses with 17 II, while the other phases of the heterotype and homotype division develop normally, without any aberrations (fig. 7).

It was however observed that most of the sporogenous cells degenerate, only a very reduced number entering division. A comparative study on 100 stamens in a normal male fertile plant shows that the number of cells at various stages of division ranges at least from 155 to 400, while in the cytoplasmic male sterile plant the number of dividing cells ranges from 9 to 15 at most. After the second meiotic division, all the 4 cells appear simultaneously, by the formation of the membrane from the outside towards the core with cytoplasm individually round each of the 4 nuclei. It is the normal type of simultaneous microspore formation with tetraedric location in the tetrad.

Pollen degeneration occurs after release from the tetrad and shows three distinct types :

1) Degeneration begins with changes occurring in the exine and intine, followed by plasmolization of the nucleo-cytoplasmatic content, so that in the end the pollen is completely sterile and formless (Fig. 8).

2) In the second case degeneration is preceded by a hypertrophy of the microspore, followed by plasmolization of the nucleo-cytoplasmatic content and then by membrane vanishing.

3) In the third and most frequantly encountered case, degeneration has a completely different aspect. The microspore released from the tetrad enters a direct division process, similar to burgeoning, a phenomenon that is synchronic with the degradation of the nucleo-plasmatic content.

This completely unusual microspore degeneration created the assumption of a chromatic elimination already existent in telophase II and becoming outlined at pollen maturation stage. But a rigurous investigation of the division process finally advocates for a direct division of the microspore and not for a chromatic individualization preexistent during division, this case having not been encountered even exceptionally (fig. 9).

The microsporogenesis of the fertile analogouses of all the investigated lines occurred normally, without anomalies, and the pollen was 96—100% fertile. While admitting the hypotheses that explain the degeneration of sporogenous cells or of microspores by the occurrence of nutritive perturbances or by the maladjustment of an enzymatic activity, we however consider that pollen degeneration can be induced by the interaction of the nuclear factors that control pollen maturation and become inactive in a changed cytoplasmatic environment.

Subsequent cyto-physio-biochemical investigations will probably give a definite answer concerning the nature of the cytoplasmatic factors and their specific way of actioning in cytoplasmic male sterility.

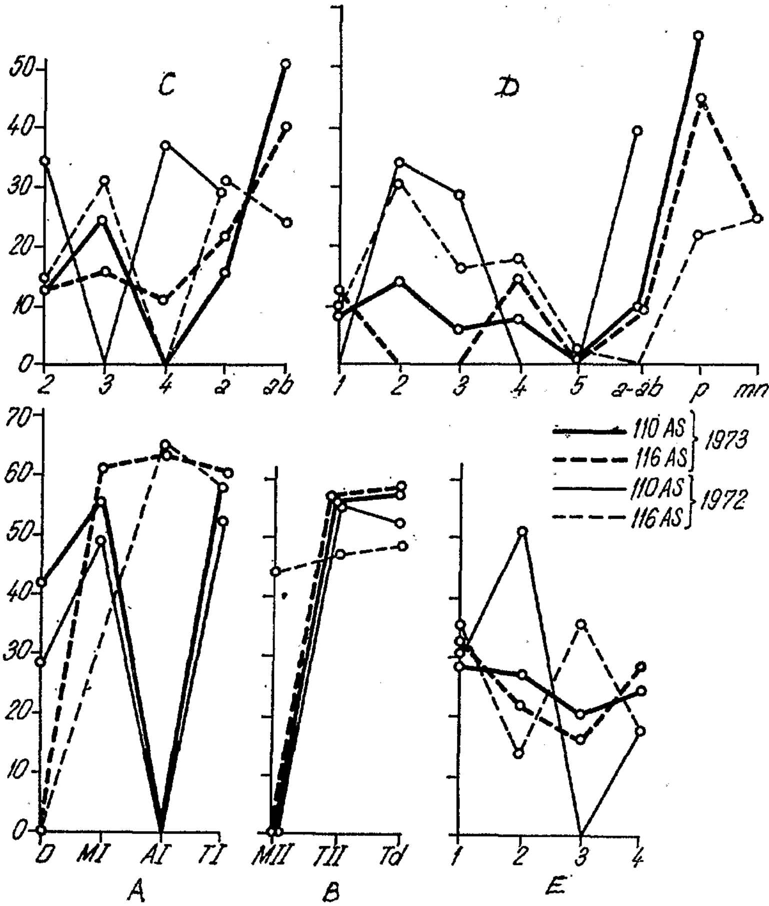

Fig. 10 — *A*. Heterotypical division (anomaly percentage by division phases); *B*—Homtypical division (anomaly percentage by division phases); *C* — Telophase I. 2, 3, 4 = retarted chromosomes; a = agglutinations; ab = other types of aberrations (chromatic fragments and bridges); *D* — Telophase II. 1, 2, 3, 4, 5 = retarded chromosomes; ab = aberrations (fragmentations and agglutinations); p = chromatic bridges; mn = micronuclei; *E* — Tetrad; 1 = micronuclei; 2 = polyads; 3 = unequal distributions; 4 = other aberrations.

CONCLUSIONS

1. Gene male sterility in lines AS 110 and AS 116 is determined by the existence of anomalies detected in meiosis, as a consequence of the activity of gene ms_1, which activates as an ameiotic gene, determining pollen complete sterility.

The 4—6% apparently fertile pollen will be competitively eliminated at maturity by the fertile pollen at fertilization, on account of its high degree of aneuploidy.

2. The total absence of meiotic division in lines 1A and 5A with cytoplasmic male sterility is induced by the degeneration of the sporogenous cells in the pre-meiotic stage. At maturity the stamen is completely devoid of pollen, the tapetum cells, the mechanic layer and the epiderm persist and show certain modifications proving that the anther is dead.

3. In contrast to the former lines, lines 2A and 4A show an evolution of the anthers and of microsporogenesis aproximately similar to the normal fertile plants, but they have a more reduced number of sporogenous cells entering division. Complete degeneration of the pollen, showing various aspects, occurs after release from the tetrad. Microspores direct division, followed by the degradation of the nucleo-cytoplasmatic content, leads to the complete vanishing of the pollen. When degeneration occurs by alteration of the exine or intine, followed by plasmolization, the microspores finally exhibit a membranous, shapeless aspect.

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