CYTOGENETIC STUDY OF DIFFERENT SOURCES OF CMS IN SUNFLOWER

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Abstract

The cms sources PET 1 (LECLERCQ, 1969), PET 2 (WHELAN, 1980), MAX 1 (WHELAN ET AL., 1980), GIG 1 (WHELAN, 1981) and ANN 6 (ŠKORIĆ, 1987) were incorporated by backcrossing into the line HA 89, while PET 1, PET 2, ANN 5 (MARINKOVIĆ AND MILLER, 1995), ANN 44 and ANN 164 (MARINKOVIĆ, unpublished) were introduced into the inbred lines L-1, L-98, L-74 and L-22.

The material was analysed for microsporogenesis by the acetocarmine method (GEORGIEVA-TODOROVA, 1976) and pollen viability determined by the method of ALEXANDER (1969).

In the line HA 89 with GIG 1 and the lines L-1, L-98, L-78 and L-22 with PET 2, anthers and sporogenous tissue were developed normally. Some of the lines had two meiotic stages, others only the tetrad one. With GIG 1, pollen viability was 10,42 %, whereas with PET 2, it ranged from 1 to 63,43 %. The other cms sources differed as to the anther development, sporogenous tissue and the number of PMCs in the preparation. In the lines HA 89, L-1, L-98, L-74 and L-22, there were no meiotic stages with PET 1, while with the other sources some were detected such as diakinesis and metaphase I, but most frequently the tetrad one. Microspores of irregular shape were registered in the line L-22 with the cms sources ANN 44 and ANN 164. Normal, viable pollen grains were not found in HA 89, L-1, L-98, L-74 and L-22 with PET 1, HA 89 with MAX 1 and ANN 6 and, finally, L-1, L-98, L-74 and L-22 with ANN 5, ANN 44 and ANN 164.

Key words: Sunflower, cms sources, meiosis, microsporogenesis, pollen viability.

Introduction

Male sterility in higher plants is most often defined as the failure to produce functional pollen. With sunflower, the discovery of cytoplasmic male sterility was preceded by that of genetic (chromosomal) one.

The first cms source (LECLERCQ, 1969) and genes for the restoration of fertility (KINMAN, 1970; FICK, 1974 etc) made possible the development of hybrids and the use of heterosis with sunflower.

30 new cms sources have been registered since (SERIEYS, 1991), most of them cases of alloplasmic male sterility from interspecific crosses.

Cytogenetic researches with regard to creating and stabilizing new cms sources are connected with analysing abnormalities in the meiosis and microsporogenesis in the early generations of interspecific crosses (up to BC_4) (WHELAN, 1978, 1980; WHELAN AND DORRELL, 1980)

Examining Leclercq's cms source (PET 1) in four inbred lines, PAUN (1974) observed the complete absence of meiosis in two of them (sporogenous tissue had degenerated as early as the pre-meiotic stage). In the other two, meiosis was almost normal (with a small number of meiocites in preparations relation to the fertile analogs). Microsporogenesis here ceased subsequent to the tetrad stage. Sunflower cms is explained by WLČKOVA (1981) as the occurence of degenerative changes in PMCs induced by changes in the tapetum.

In the last decade, new sunflower cms sources have at the molecular level been investigated rather extensively, in terms of establishing the differences between the mitochondrial and plastid DNA with sterile and fertile analogs. At the same time, there has been little classical cytogenetic research with regard to these new sources.

Given the significance of the new cms sources, as well as the efforts to realize the possibility of their application in sunflower breeding (the problem of CMS stability), a series of cytogenetic studies was conducted analysing meiosis and microsporogenesis in various cms sources.

Materials and methods

The study included the classical cms source PET 1 (LECLERCQ, 1969) and seven new ones: PET 2 (WHELAN, 1981), MAX 1 (WHELAN AND DORRELL, 1980), ANN 5 (MARINKOVIĆ AND MILLER, 1995) ANN 6 (ŠKORIĆ, 1987) and ANN 44 and ANN 164 (MARINKOVIĆ, unpublished). By means of backcrosses, the sources PET 1 and PET 2 were introduced into the inbred lines HA 89, L-1, L-74, L-98 and L-22, the sources GIG 1, MAX 1 and ANN 6 into the line HA 89 and the sources ANN 5, ANN 44 and ANN 164 into the lines L-1, L-98, L-74 and L-22 (these four have been bred at the Institute of Field and Vegetable Crops).

The stage in the development of the stamen and anther and the presence of pollen were determined under the microscope at flowering. Pollen vitality was established by means of the stain method (ALEXANDER, 1969) and the analysis of meiosis and microsporogenesis by the acetocarmine one (GEORGIEVA-TODOROVA, 1976).

Anthers were fixed at the budding stage and subjected to the procedure of preparing preparations for meiosis. The occurence of meiocites (the

development of archesporial tissue) was registered, as well as the stages of meiosis and postmeiotic cycle. In order for us to inspect as much anthers as possible (50-265), a large number of preparations was prepared.

Results and discission

Anthers were present in the disc flowers of all of the inbred lines, regardless of the sterility source, and their development varied from normal (PET 2, GIG 1) to near-rudimentary (Fig.1).

Figure 1. Rudimentary anthers (PET 1)

Normal pollen grains were found in all of the inbred lines containing the cms sources PET 2 and GIG 1 (Fig.2), whereas those containing the sources PET 1, MAX 1, ANN 5, ANN 6, ANN 44 and ANN 164 contained no pollen, save for a few individual lines whose pollen grains were deformed and lacking exines.

Pollen mother cells (meiocites) were observed in most of the preparations (Table 1), but they were small, of irregular shape and had no visible chromatinic material. None of the meiotic stages was registered in the lines incorporating PET 1 and ANN 5, whereas those with ANN 6, ANN 44 and ANN 164 had some. These were most often the tetrad stages, with tetrads of irregular shape (Fig.3). HA 89 with ANN 6 and L-22 with ANN 44 and ANN 164 also had the stage of microspores, which were of irregular shape as well. No pollen grains were observed in these lines. The source MAX 1 in the line

HA 89 had most of the meiotic and microsporogenetic stages, including the pollen grain one, with grains that were small, deformed and lacking exines.

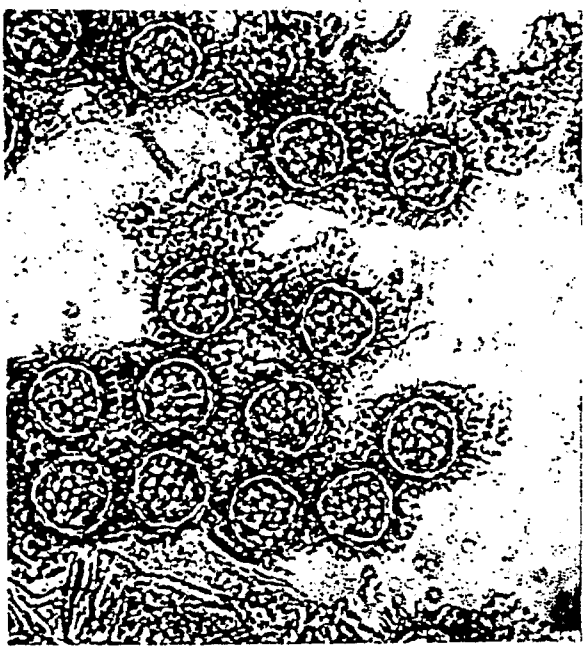

Figure 2. Normal pollen grains (PET 2)

The line HA 89 containing the source GIG 1 and all the lines containing PET 2 (except HA 89) had all the meiotic stages, tetrads, microspores and pollen grains. With PET 2, pollen viability varied from 1 to 63, 43 %, depending on the line into which it had been incorporated, whereas in the line HA 89 with GIG 1 it amounted to 10,42 %.

The findings with regard to the level of anther development (sporogenous tissue) with the investigated sources are similar to those reported by VRANCEANU ET AL (1986), namely that there are several levels sterility can be expressed at: the presence of normally developed anthers, anther rudiments with some pollen, anther rudiments with no pollen and a complete anther degeneration.

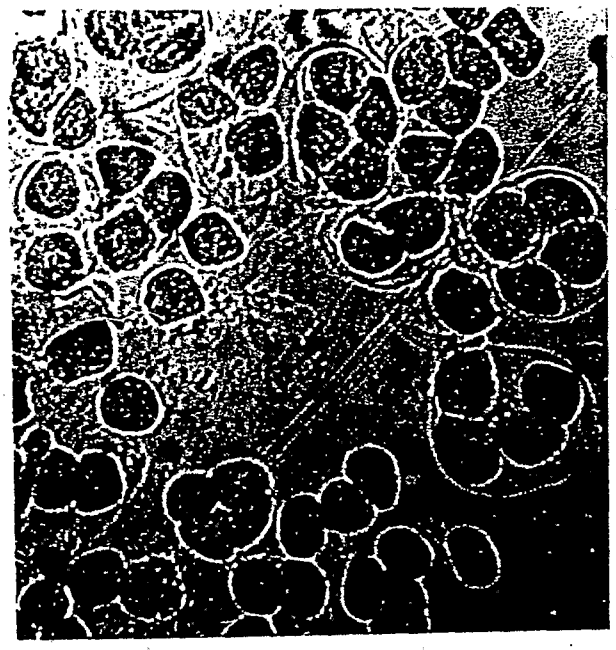

Figure 3. Tetrads (ANN6)

The absence of meiosis and microsporogenesis in the lines containing PET 1 supports the findings of PAUN (1974). and the presence of normally developed anthers with functional pollen in the lines L-1, L-98, L-74 and L-22 with PET 2 and the line HA 89 with GIG 1 those of VRANCEANU ET AL. (1986).

Table 1. Meiosis - microsporogenesis of different CMS sources

CMS sources	Inbred line	Pollen mother cells (PMC)	Meiotic stage	Micro spore	pollen grain	Pollen viability
	HA-89	+	-	-	-	-
	L-1	+	-	-	-	-
PET-1	L-98	+	-	-	-	-
	L-74	+	-	-	-	-
	L-22	+	-	-	-	-
	HA-89	+	diak. met.I ana.I tetrad.	+	small with out exine	0
	L-1	+	diak. tetrad.	+	+	62.08
PET-2	L-98	small irr. shape	tetrad.	+	+	63.43
	L-74	small irr. shape	tetrad.	+	+	54.42
	L-2	small irr. shape	tetrad.	+	+	1
GIG-1	HA-89	+	all	+	+	10.42
MAX-1	HA-89	+	diak. met. I ana. I Tetrad.	irr.	small with out exine	0
	L-1	-	-	-	-	-
ANN-5	L-98	-	-	-	-	-
	L-74	-	-	-	-	-
	L-22	-	-	-	-	-
ANN-6	HA-89	+	irr. tetrad.	irr.	-	-
	L-1	+	irr. tetrad.	-	-	-
ANN-44	L-98	+	-	-	-	-
	L-22	+	diak. met. I tetrad.	irr.	-	-
	L-1	+	-	-	-	-
ANN-164	L-98	+	met. I tetrad.	-	-	-
	L-74	+	tetrad.	-	-	-
	L-22	+	tetrad.	irr.	-	-

In the line HA 89 with MAX 1, all the stages of meiosis and microsporogenesis were present. However, the pollen was not functional, which is not in congruence with the findings by MILLER AND WOLF (1991). They found this source to be unstable, since they discovered anthers with little pollen and a low percentage of fertilization (5%). The incongruity of the

findings results from the fact that the studied sources were incorporated into different inbred lines, and a change of genetic environment affects the stability of cms.

There are apparently certain interactions between the nucleus and cytoplasm that have not yet been sufficiently studied.

Conclusions

Cytogenetic analyses of various cms sources show PET 2 and GIG 1 to be unstable. The other sources are stable, but differ with regard to the point at which meiosis and microsporogenesis cease. PET 1 and ANN 5 are characterized by the total absence of meiosis, which is in contrast to most of the other cms sources, with which the meiotic stages up to that of tetrads are detectable and followed by a break at a further meiotic stage which causes sterility.

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