Cytogenetic studies of cytoplasmatic male sterility in rapeseed

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Abstract

The focus of current rapeseed breeding programs is the creation of hybrids. That implies finding new sources of CMS and their transfer to genotypes that have the genes for other agronomically important traits. Different types of CMS were used in this study and they were transferred to inbred lines by backcrossing. CMS traits were analysed by evaluating the phase of stamens – anthers development, presence of pollen in anthers, pollen viability and analysis of meiosis – microsporogenesis. Anthers were frequently poorly developed and without pollen in male sterile flowers. Meiosis was regular (pachyten, diakinesis, metaphase I, anaphase I, anaphase II, telophase II). The difference in CMS types can be made by the phase in which microsporogenesis is interrupted. Most often, the interruption happened after the phase of tetrads. Microspores were found in some cases and even pollen grains, but they were deformed and sterile.

Key words: Rapeseed, CMS, cytogenetics, meiosis, pollen viability

Introduction

The focus of current rapeseed breeding programs is the creation of hybrids. That implies finding new sources of CMS and their transfer to genotypes that have the genes for other agronomically important traits. In general, male sterility defines abnormal vital pollen grain development in plants. The expression of this trait is variable in expression and can range from complete absence to poorly developed anthers. In the aspect of pollen, it can vary from absence or deformed pollen grains to normally developed pollen grains in non-dehiscenced anthers (Vipen et al., 1999).

According to Yang et al., (1999), pol (Fu, 1981) and ogu (Ogura, 1968) CMS systems are still the most important CMS systems for rapeseed hybrid breeding in the world. The CMS trait is unstable, because partial fertility can be found under high temperatures. That presents a serious problem when different types of CMS are used. The main problem with Ogura type of CMS is that the fertility-restoring gene is tightly linked to the genes that control high glucosinolates content (Renard et al., 1997, according to Yang et al., 1999).

The objectives of this work were to use cytogenetic methods to analyse the stability of CMS trait after the introduction in to the rapeseed inbred lines and to identify the differences between various types of CMS.

Materials and Methods

The material used in this study consisted of sterile inbred lines (H-314, H-321, H-325, H-343, H-405, H-441, H-312, CMS 61, CMS 62, CMS 57 and CMS 15) followed by the progenies of backcrossing between selected sterile plants and the maintainer varieties. Nine genotypes were used from variety B 009, 3 genotypes from E 301, 3 genotypes from E 305 and 2 genotypes that were obtained through hybridisation with restorers, AK 21 and AK 22). The third group of the analysed material was made of inbred lines with different types of introduced CMS from the Novi Sad breeding program, and restorer lines (Ku 27 Draghon, Ku 58 Draghon, Ku 85 Topas and R 2000 INRA).

The samples of flowers were taken at flowering time from the mentioned material and microscopic examination was used to determine the level of anther development, presence of pollen, morphology and viability of pollen grains.

Samples were also taken from plants with different types of CMS and the restorer lines for the analysis of meiosis – microsporogenesis (on flower buds). Acetocarmine method was used for the analysis of meiosis (Georgieva-Todorova, 1990) and the pollen viability was determined by staining (Alexander, 1969).

Results

Microscopic examination of inbred lines H-314, H-321, H-325, H-343, H-405, H-441, H-312, CMS 61, CMS 62, CMS 57 and CMS 15 showed that they are all male sterile. The differences between those lines considering the expression of CMS trait, were mainly at the level of anther development (Fig. 2) in compare to the male fertile flowers (Fig. 1b). Pollen was occasionally found in anthers, but it was sterile. The results of CMS trait stability testing in the progenies of backcrosses (13 genotypes from 3 different sources) showed that most of the plants were completely male sterile. Some progenies were marked as male fertile after the field inspection, but microscopic analysis showed that it was a matter of low pollen production with low viability (less than 10%) or the pollen was completely sterile. The genotypes AK 21 and AK 22 exhibited complete fertility restoration and the pollen viability was 96.8%.

Fig. 1. Rapeseed flower – a) male sterile; b) male sterile

Fig. 2. Male sterile rapeseed flower-a) well developed anthers; b) rudimentary developed anthers

Table 1. Morphological and cytogenetic characteristics of male-fertile and male-sterile flowers in rapeseed

Genotype	Characteristics									
	Morphological		Cytogenetic: stages of meiosis - microsporogenesis							
	Anther development level	Presence of pollen	Diakinesis	Metaphase I	Anaphase I	Telophase II	Tetrads	Microspores	Pollen grain	Pollen viabillity
R 2000 INRA	normal	+	19^{II}	normal	normal	normal	normal	normal	normal	>90%
Ku 27 Draghon	rudimentary	-	19^{II}	normal	normal	normal	normal	irregular shape	small without exine	-
Ku 58 Draghon	rudimentary	-	19^{II}	normal	normal	normal	normal	irregular shape	small without exine	-
Ku 85 Topas	rudimentary	-	19^{II}	normal	normal	normal	irregular shape	irregular shape	small without exine	-

The analysis of meiosis in R 2000 INRA revealed normal meiotic (diakinesis with 19^{II}) and post meiotic phases that ended with pollzen formation (Table 1., Fig. 3.). Normal phases of meiosis were found in Ku 27 Draghon, Ku 58 Draghon and Ku 85 Topas, but their frequency in the studied microscopic samples was low. The samples for the analysis of meiosis were male sterile flowers in which the archesporial anther tissue is degenerated so that the number of pollen mother cells is considerably lower in compare to the fertile flower sample.

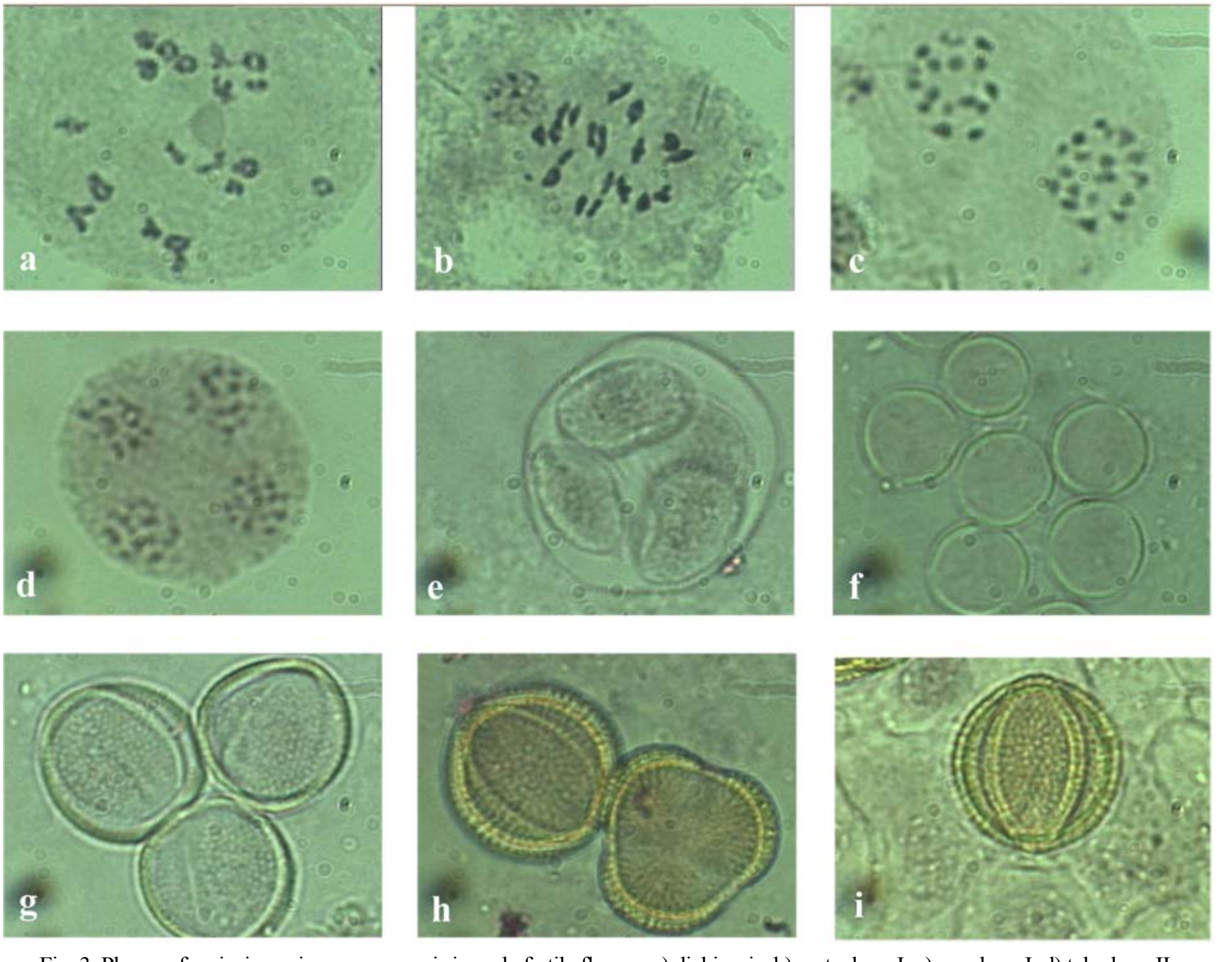

Fig. 3. Phases of meiosis – microsporogenesis in male fertile flowers a) diakinesis: b) metaphase I; c) anaphase I: d) telophase II; e-i) phases of postmeiotic division

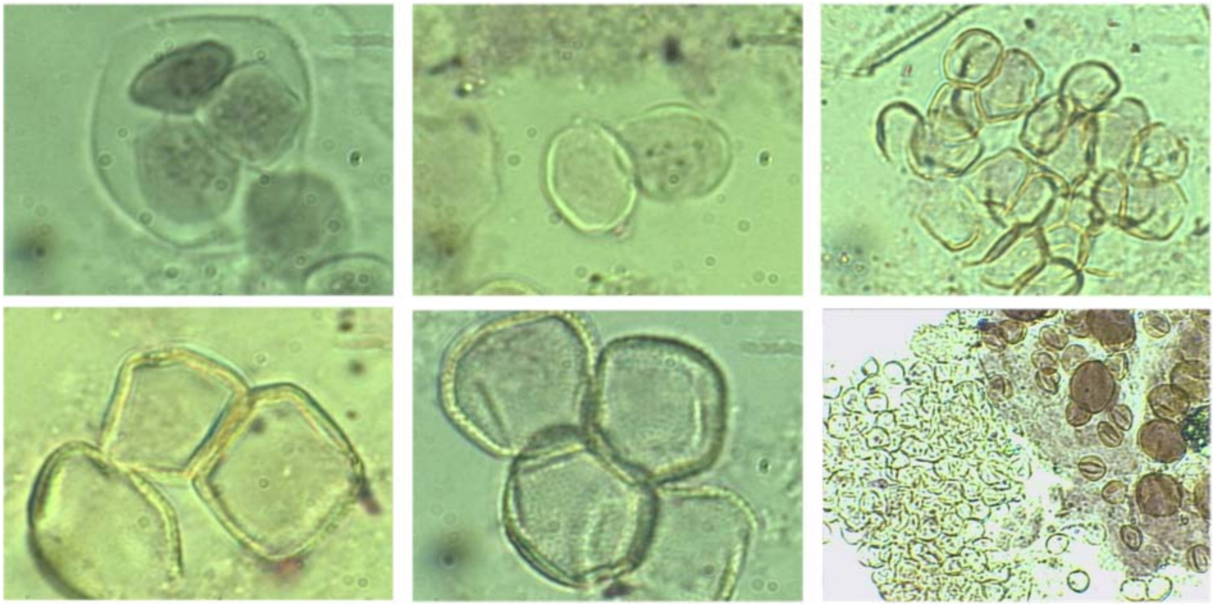

Fig. 4. Phases of postmeiotic division in male sterile flowers of rapeseed

The interruption in post meiotic phases starts after the phase of tetrads and deformed microspores are frequently found in the sample preparation (Table 1., Fig. 4.). The analysis of sterility source Ku 85 Topas revealed degenerated tetrads which implied even earlier interruption of microsporogenesis than in other analysed CMS sources like Ku 27 Draghon and Ku 58 Draghon (Table 1.).

Discussion

The results of CMS stability analysis showed in this paper, confirm the results of Atlagić et al. (2003), where the unreliability of visual field inspection was pointed out and the necessity for the use of microscopic analysis to determine the level of anther development and the presence of pollen. The findings at the molecular level showed that the interruption of pollen development is coded in the mitochondrial DNA. That is confirmed by the research of Dieterich et al. (2003), who identified regions of the mitochondrial genome potentially involved in the expression of alloplasmic »Tournefortii-Stiewe« cytoplasmic male sterility (CMS) in *Brassica napus*, after he analyzed transcripts of 25 mitochondrial genes in fertile and near isogenic male-sterile plants (BC_8 generations). On the other hand, after the introduction of CMS trait in to the new genetic environment, the interaction between nucleus and cytoplasm is possible and it can result in male fertility restoration. Schnabel & Wise (1998) confirmed such interaction and moreover concluded that CMS provides an excellent model for the investigation of pollen development and cytoplasm-nucleus interaction.

The analysis of meiosis in majority of rapeseed CMS types, showed that the phase of tetrads is when the interruption of microsporogenesis occurs. Contrary to those results, the research in higher plants (Kaul, 1988) and in some types of CMS in sunflower (Atlagić et al., 1996) show that the interruption occurs in the earlier phases of meiosis.

Conclusion

All analyzed inbred lines were male sterile with different level of anther development. The back cross progenies showed that all three CMS sources are stable and restorers were found for two genotypes. The analyzed types of CMS all had normal meiotic phases, but the post meiotic division showed irregularities that led to the interruption of microsporogenesis, most frequently at the phase of tetrads.

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