CYTOGENETIC STUDY OF CMS IN RAPESEED GENOTYPES AT THE NOVI SAD BREEDING CENTER

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ABSTRACT

Given the commercial importance of developing rapeseed hybrids, we have in our breeding program began working on the detection of CMS sources and the incorporation of this trait into genotypes with desirable genes for other agronomically important traits. The expression of CMS into a new genetic environment was accompanied by visual assessment of male sterility in plants grown in the field. Further, microscopic inspection of the stamen was carried out to assess the anther development level and presence of pollen in male-fertile and male-sterile flowers. Pollen viability was assessed by the staining method, while the progression of meiosis/microsporogenesis was assessed by the acetocarmine method (a modified version used in sunflower). When assessing male sterility in plants that are potential CMS sources, it is necessary to examine the stamens microscopically, because visual assessment in the field is inaccurate. In male-sterile flowers, anthers are most often poorly developed, with no pollen, although cases have been reported in which small amounts of non-viable pollen were found. Meiosis progressed normally. The following phases were observed: pachyten, diakinesis, metaphase I, anaphase I, anaphase II, telophase II. Differences in the advancement of microsporogenesis between the male-fertile and male-sterile flowers were observed in the phases following the tetrads. In the male-sterile flowers, mononuclear microspores were of normal of shape and size, while irregularities (such as deformed microspores or nonviable pollen grains of irregular shape and size) were observed after this phase.

Key words: rapeseed, CMS, cytogenetics, meiosis, pollen viability.

INTRODUCTION

The male sterility and fertility restoration system is used in most open-pollinated crop species when positive effects of heterosis for agronomically important traits need to be exploited. Male sterility, although generally defined as the condition when viable pollen is not produced, is variable in expression and can range from the complete absence of stamens to the failure of anther dehiscence and release of normal viable pollen (Vipen, et al. 1999). The inheritance patterns of male sterility are also variable. Genetic male sterility (GMS) is controlled by nuclear genes and cytoplasmic male sterility (CMS) by specific male sterility–inducing cytoplasm know as the sterile (S) cytoplasm. Gene – cytoplasmic male sterility (GCMS) which results from a combined effect of male sterile nuclear genes (fr) and sterile (s) cytoplasm, is also known (Kaul, 1988). In most cases, the CMS trait is a result of of interspecific or intergeneris hybridization (aloplasmatic sterility). In rapeseed the Ogura–CMS system (INRA – France) and the MSL–system (German Lembeke Institute) are most commonly used. According to Yang et al (1999), pol CMS (Fu, 1981) and ogu CMS (Ogura, 1968) are still the important CMS systems for rapeseed hybrid breeding in the world. The main disadvantage of pol CMS is its unstable male sterility. The male sterile lines could become partially fertile at relatively lower or higher temperature situations (Yang and Fu, 1987, according to Yang et al., 1999). The main problem for ogu CMS is its fertility restoring gene linked tightly to the genes with a high content of glucosinonates (Renard et al., 1997 po Yang et al., 1999).

The discovery of new CMS lines and study of their stability requires cytogenetic research. This is confirmed by Prokash and Chopra (1999) and their review of cytogenetic research in rapeseed in the last 80 years, where an entire period was labelled "Synthesis of alloplasmic of crop species for expression of male sterility based on very diverse cytoplasm".

MATERIALS AND METHODS

In the CMS trial with winter rapeseed (*B. napus*) carried out at R. Šančevi near Novi Sad, genotypes obtained as self-fertilization progeny of plants of the hybrid Artus (MSL system) were grown. Genotypes having male-fertile and male-sterile forms (CMS-24 and CMS-25) were selected and visual assessment of male sterility was performed in the field. Microscopic examination of stamens/anthers was also carried out whereby the level of anther development and the presence or

absence of pollen were determined. The male-fertile plants were sampled for analysis of pollen viability by staining (Alexander, 1969). Samples were also taken from male-fertile and sterile plants for analysis of meiosis by the acetocarmine method (a modified version used in sunflower, Georgieva-Todorova, 1990). An analysis of meiosis/microsporogenesis in various stages was carried out.

RESULTS

Upon visual inspection of flowers in the field, male-fertile and male-sterile CMS-24 and CMS-25 plants were observed. The male-fertile plants had a large production of pollen whose viability was high (>90%) (Tab.1.).

In the male-sterile plants, the stamens were underdeveloped and anthers rudimentary and empty. In some of the anthers minor amounts of nonviable pollen were found (Tab. 1).

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	Telofphasell	Tetrads	Microspores	Pollen grains	Pollen viability
CMS-24♂ CMS-25♂	normal	yes	normal (19 ^{II})	normal	normal	normal	normal	normal	normal	>90 %
CMS-24♀ CMS-25♀	rudimentary	no	normal	normal	normal	normal	normal	iregular shape	small without exine	-

Analysis of meiosis revealed the following phases in the male-fertile plants: pachyten, diploten, diakinesis, metaphase I, anaphase I, and telophase II. The post-meiotic cycle included five phases: tetrads, mononuclear microspores, microspores, post-mitotic division microspores and pollen grains (Tab.1.). Analysis of meiosis in the male-sterile flowers showed phases of meiosis I identical to those of the male-fertile flowers. Differences appeared after the tetrad or mononuclear microspore stages. In the male-sterile flowers, that is when microsporogenesis stopped and microspores were aborted occurred. In only several instances small, deformed microspores were observed in the microscopic preparations. In one male-sterile plant small, deformed, nonviable pollen grains were observed after staining.

DISCUSSION AND CONCLUSION

Looking at the morphological characteristics and anther development level of male-sterile rapeseed flowers, it becomes clear that the CMS system in this crop functions the same as in other crop species. Vranceanu et al. (1986) describes different degrees of sterility in sunflower: the presence of normally developed anthers, anther rudiments with some pollen, anther rudiments with no pollen and a complete anther degeneration. The present paper's cytogenetic findings indicate that this MS system works on the basis of interrupted meiotic cycle at the tetrad, or microspore, stage. In the present MSL system, there were no interruptions in the premeiotic stage, such as those reported for higher plants by Kaul (1988) and some CMS sunflower sources by Atlagić et al. (1996). From the point of view of methodology, it is important to note that the methods most commonly used to assess pollen vitality and meiosis in rapeseed (as described in Choudhary and Joshi (1999)) can be successfully replaced with those used in sunflower. Staining (Alexander, 1969) is used successfully not only in sunflower but in many other species as well. It gives better results, as it provides better separation of viable and nonviable pollen grains. The method most often used for analysis of meiosis in sunflower involves fixation by the Carnoy I fixative (1:3 Glacial acetic acid: Apsolute ethanol), a pre-treatment with 4% iron ammonium sulphate, and staining by 4% acetocarmine. Use of this method in rapeseed

makes the phases clearly distinguishable, the chromosomes are well stained, and chromosome configurations can be studied at diakinesis as a result. It is definitely clear that the detection of new CMS sources and development of CMS lines (and their increase) and hybrids (complete or partial restoration) has to involve microscopic stamen/anther inspection and analysis of meiosis/microsporogenesis in addition to visual assessment in the field. Such studies would contribute to the description of various CMS systems and the mode of expression of CMS traits in new genetic environments.

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