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CHEMICAL MUTAGENESIS IN HEAD CABBAGE (*Brassica oleracea var. capitata* L.). III EFFECT OF ETHYL METHANE SULPHONATE ON MYCOSPOROGENESIS AND POLLEN FERTILITY

NIKOLOVA VESSELINA, ANTONOVA GALINA, TOMLEKOVA NASIA, PETKOVA VALENTINA[1]

ABSTRACT: The aim of present investigation was to study the effect of different ethyl methane sulfonate (EMS) concentrations (0.5, 0.6 and 0.7 %) on microsporogenesis and on the expression of pollen fertility in M_1 plants, obtained from treated cabbage seeds (variety "Ditmarsko"). The investigation was conducted to identify particular stages of male sterility expression at cytological level.

It was established that the increase of EMS concentration resulted in decrease of pollen fertility values and in increase of the percentage of plants with low level of this trait. Cytological analysis in plants with 10.0 % pollen fertility showed, that EMS has a negative effect on the homologous chromosome pairing (early stages of PMC meiosis) and on chromosomes correct division and migration to the spindle poles (second metaphase - anaphase). As a result of this the microsporogenesis runs with disturbances and formation of non - reduced and non - balanced gametes.

Key words: *microsporogenesis, pollen fertility and sterility, ethyl methane sulfonate, Brassica olerasea var. capitata L.*

INTRODUCTION

Various hybrid varieties of *Brassica olerasea var. capitata* L. have been obtained by crossing of self-incompatible inbred lines. The incompatibility is not complete and depends on the developmental stage of the flowers and on the external factors (Murabaa, 1957). Better results in hybrid programs could be received if one of the two inbred lines is male sterile.

Male sterile plants could be obtained by mutagenic induction. The aim of this study was to be made an attempt to induce complete or partial sterile mutants through treatment of seeds from *Brassica olerasea var. capitata* L. (variety "*Ditmarsko*") with ethyl methane sulfonate. This investigation was conducted to identify expression of male sterility in M_1 plants and also to determine particular stages of this expression at cytological level.

Materials and methods

Pollen fertility was determined in *Brassica oleracea var. capitata* L. plants variety "*Ditmarsko*", obtained after seed treatment with ethyl methane sulfonate (EMS) in concentration 0.5, 0.6 and 0.7 %. The effect of the chemical application was evaluated. The frequency of forms with different level of male fertility in M_1 were examined, in comparison to control plants, received from untreated seeds.

Pollen fertility was established by staining with 4 % acetocarmine and glycerol (1:1). Cytological investigations were performed on buds with different size, fixed in acetic alcohol (1:3). The anthers were squashed in 4 % acetocarmine.

Results and discussion

The M_1 plants were evaluated in respect to the pollen fertility. Depending on this charac-

[1] NIKOLOVA VESSELINA, ANTONOVA GALINA, TOMLEKOVA NASIA, PETKOVA VALENTINA, Maritsa Vegetable Crops Research Institute, 4003 Plovdiv, Bulgaria

ter they were divided into six classes: with 0 % fertility - infertile plants; from 0.1 to 20 % - forms with low fertility; from 20 to 40 and from 40 to 60 % - plants with medial fertility and from 60 to 80 and 80 to 100 % - plants with high level of fertility.

The data from Table 1 show, that in treatment of seeds from variety "*Ditmarsko*" with EMS (concentration 0.5, 0.6 and 0.7 %) completely sterile forms were not obtained. From all studied plants 14.2 to 16.1 % were with partial sterility and produced very little amount of normal pollen (0.1 to 20 %), resulting in complete failure of selffertilization. It was established, that the increase of EMS concentration reflected in decrease of pollen fertility and in grow of the percentage of plants with low values of this trait.

Tab. 1. Pollen fertility in M_1 plants

EMS concentration%	Plant number	Plants (%) with pollen fertility					
		0.0 %	From 0.1 to 20 %	From 20 to 40 %	From 40 to 60 %	From 60 to 80 %	From 80 to 100 %
EMS - 0.5 %	19	0.0	15.8	31.6	21.0	31.6	0.0
EMS - 0.6 %	31	0.0	16.1	45.2	35.5	3.2	0.0
EMS - 0.7 %	14	0.0	14.2	50.0	35.7	0.0	0.0
Control plant	9	0.0	0.0	0.0	11.1	55.5	33.3

Tab. 2. Number and percentage of PMC with various chromosome associations in B. oleracea var. capitata plants with partial pollen sterility

Chromosome associations	EMS 0.6% - plant № 36		EMS 0.7% - plant № 18	
	PMC number	PMC %	PMC number	PMC %
9II	66	86.8	40	59.7
8II + 2I	1	1.3	4	6.0
7II + 4I	2	2.6	5	7.5
6II + 6I	1	1.3	2	3.0
5II + 8I	0	0.0	2	3.0
4II + 10I	0	0.0	1	1.5
3II + 12I	1	1.3	3	4.5
7II + 1IV	2	2.6	5	7.5
6II + 1IV + 2I	0	0.0	1	1.5
5II + 2IV	1	1.3	1	1.5
4II + 2IV + 2I	0	0.0	1	1.4
4II + 1IV + 6I	1	1.3	0	0.0
3II+ 3IV	0	0.0	2	3.0
1II + 1IV+ 12I	1	1.3	0	0.0
Total number of PMC	76		67	

Tab. 3. Mean frequency of various chromosome configurations per cell at diakinesis and metaphase I in B.oleracea var. capitata plants with partial pollen sterility

Plant №	Number of cells	II	I	IV
EMS 0.6 % - plant № 36	76	8.55	0.60	0.08

The average fertility from all studied plants were 40.2 % (EMS concentration 0.5 %), 27.8 and 28.6 % (EMS concentration 0.6 and 0.7 %), respectively. In the control plants average 74.9 % from pollen grains were viable and stained in acetocarmine.

The negative influence of the mutagen factor resulted in loss of forms with high pollen

fertility (from 80 to 100 %) and reducing the per cent of plants from class number 5 (from 60 to 80 % fertility).

Tab. 4 Percentage of different sporads in B.oleracea var. capitata plants with partial pollen sterility

Plant №	Tetrads (%)	Dyads (%)	Triads (%)	Polyads (%)	Pollen sterility (%)
EMS 0.6 % - plant № 36	47.8	31.9	20.4	0.0	89.9

Cytological investigations were performed with two partial male sterile plants (№ 36 and №18), received from treated seeds - EMS concentrations 0.6 and 0.7 %. The results presented in Table 2 show that in these two plants the chromosomes paired regularly and during diakinesis and metaphase one (MI) were formed 9 bivalents (II) in 86.8 and respectively 59.7 % of pollen mother cells (PMC). In the other cells the chromosomes paired partial and remained as univalents (I) (from 2 to 12I in 6.5 and 25.5 % of PMC in plant № 36 and 18, respectively). It was observed associations of 7II + 1 quadrivalent (IV) (Fig.1) and 5II + 2IV and associations of bivalents plus univalents plus 1 to 3 quadrivalents.

Fig. 1. Pollen mother cells with 7II + 1IV

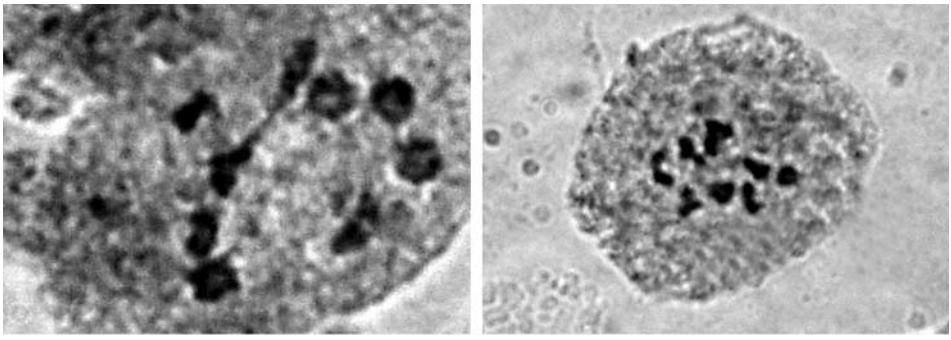
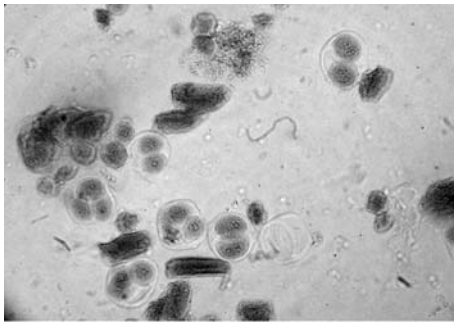


Fig. 2. Different type of sporads



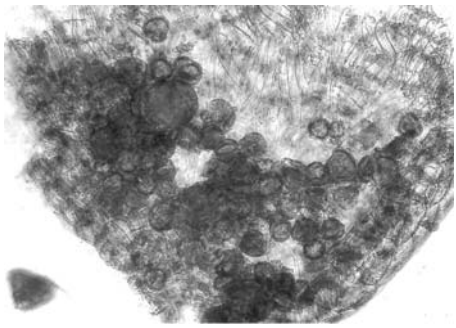
The various frequencies of univalents, bivalents and quadrivalents per cell are present in Table 3. These results demonstrated that the higher EMS concentration (0.7 %) indicated more disturbances in the chromosome pairing and the value of bivalent frequency was less in plant № 18 (7.73II per cell) in comparison to the plant № 36 (EMS concentration 0.6 %) (8.55II per cell). The negative influence of 0.7 % EMS concentration in plant № 18 was confirmed also with higher average frequency of univalents and quadrivalents (1.5I and 0.24IV, respectively) per cell. In the other plant № 36 it was registered 0.60I and 0.08IV per cell.

Meiotic differences in the PMC of the studied two plants were observed during AI, MII and AII.

The chromosome behavior during AI and AII in the plant № 18 was correct and we established regular division and distribution of chromosomes to the spindle poles, which resulted in formation of tetrads of mikrospores in 96.4 % of the cells. On the basis of these results good pollen fertility could be supposed, but only 9.0 % from pollen grains were viable

and stained in acetocarmine (Fig.3). The performed investigation indicated, that the sterilizing effect of EMS mutagen in plant № 18 was manifested during early stages of meiotic phases (partial pairing of chromosomes, remained as univalents and formation of quadrivalents in diakinesis and MI in about 40.3 % of PMC).

Fig. 3. Anther tissue with sterile pollen grains



The univalents in PMC of the other plant (№ 36) were orientated regular to the poles during AI in 92.4 % of the cells. In the 7.6 % of PMS we observed unbalanced distribution of chromosomes in type n - 3 and n + 3. Meiotic nonreduction and formation of unreduced male gametes with 18 chromosomes were observed during second division (MII - AII) in 31.9 % of cells. In these PMC the chromosomes divided during MII, but did not move to the opposite poles and formed dyads of microspores (Fig.2). The mutagen influenced unfavorable on chromosome behavior in a part of the cells of plant № 36, which reflected in three poles orientation of chromosomes in 20 - 25 % from the studied PMC. As a result triads of microspores were established. Probably the meiotic disturbances during later meiotic phases (MII - AII) in plant № 36 indicated formation of very little amount of normal pollen (10.1%).

On the basis of meiotic differences in the studied two plants, could be supposed, that the genotype influenced on the chromosome behavior in PMC in forms, received from treated with EMS mutagen seeds and that the disturbances during different meiotic stages could be reason for breakdown of significant part of microspores.

According Krouleva, 1978 information about 99 ms genes in 48 plant species manifested their sterilizing effect during the final stages of meiosis (between second anaphase and pollen formation). Kumar et all., 2001 also concluded, that in Capsicum annuum L. sterile plants, meiosis was irregular, especially at telophase two (TII). Shifriss, 1997 described, that the breakdown of microspores had been observed after tetrad formation. McNaughton (according Ellerstr m and Zagorcheva, 1977) explained that the extremely low fertility was more likely to be the result of genetic unbalance, than to cytological anomalies.

We supposed that all stages during microsporogenesis were very precise and disturbances of some of them from EMS mutagen could break the regular formation of the viable male gametes.

Conclusion

In treatment of seeds from *Brassica oleracea var. capitata* L., variety "*Ditmarsko*" with EMS (concentration 0.5, 0.6 and 0.7 %) completely sterile plants were not obtained.

The increase of EMS concentration reflected in decrease of pollen fertility and in grow of the percentage of plants with low values of this character.

The genotype influenced on the chromosome behavior in pollen mother cells in plants, received from treated with EMS mutagen seeds.

The disturbances during different meiotic stages (early stages - diakinesis and metaphase one in plant № 18 and later phases - second metaphase - anaphase in plant № 36) could be a reason for breakdown of significant part of microspores.

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SUMMARY

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In treatment of seeds from *Brassica oleracea var. capitata* L., variety "*Ditmarsko*" with EMS (concentration 0.5, 0.6 and 0.7 %) completely sterile plants were not obtained.The increase of EMS concentration reflected in decrease of pollen fertility and in grow of the percentage of plants with low values of this character (Table 1). The cytological investigation indicated, that the genotype influenced on the chromosome behavior during mikrosporogenesisin in plants, received from treated with EMS mutagen seeds (Table 2 and 3).

The disturbances during different meiotic stages (early stages - diakinesis and metaphase one in plant № 18 (Fig.1) and later phases - second metaphase - anaphase in plant № 36) could be a reason for breakdown of significant part of microspores (89.9 and 91.0 % sterile pollen grains in plants № 36 and 18) (Fig.3).