

THE EVALUATION OF GAMMA RADIATION AND MEDIUM SORT ON ANTHERS EMBRYOGENY IN THE SPECIES OF *BRASSICA* GENERA

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**ABSTRACT.** The influence of gamma radiation and medium composition on the production of embryos from anthers of four *Brassica napus* genotypes and one *B. carinata* genotype were investigated. Flower buds of 4.0-4.5 length were collected from the donor plants growing in the glass house. Collected buds were treated with 25 and 50 Gray doses of gamma rays. Isolated anthers were incubated on the basic B<sub>5</sub> medium and B<sub>5</sub> medium supplemented with 2.5 and 5.0 ppm of silver nitrate at 25°C continuous temperature and at 35°C during first 24 hours. Anthers from untreated buds and incubated on the basic B<sub>5</sub> medium at 25°C were treated as a control. Effectiveness of anthers culture was expressed by the percentage of embryogenic anthers and the mean number of embryos counted on 100 anthers. Results revealed different response to applied factors (dose of gamma radiation, addition of AgNO<sub>3</sub> and pretreatment with high temperature) depending on genotypes. There were no embryos observed in buds treated with 50 Gray dose of gamma rays.

**KEY WORDS:** haploids, gamma radiation, anther culture, *B. napus*, *B. carinata*

## INTRODUCTION

Haploid and dihaploid plants are valuable tools in plant breeding, because they enable shortening the period of receiving homozygous organisms from heterozygous initial plant material. Receiving haploids by *in vitro* culture comprise anther and microspore cultures (CHEN *et al.* 2001). In spite of this, that anther cultures are successfully used in plant breeding and there were received many new varieties using them, however widely using it is still limited by difficulties in androgenesis induction. It is commonly known that pretreatment of flower buds before anther culture or during anther culture (for example temperature shock, ethylene treatment) could modify the microspores embryogeny (WOJCIECHOWSKI 1998). Beginning from seventies of 20th century in researches concerning increasing the effectiveness of anther cultures, treatment of flower buds or anthers by different doses of gamma rays are observed (RAO *et al.* 1976, PECHAN and KELLER 1989, ZHANG *et al.* 1992, SHTEREVA *et al.* 1998, FARIS *et al.* 1999, CHEN *et al.* 2001). The results presented in these articles indicate different effectiveness of applied doses of gamma rays (2-300 Gy). For example according to the data given by FAO (REPORT... 1997) in potatoes 20 Gy dose efficiently helps in survival of plants, while 10 Gy dose was lethal for callus, similarly for the callus of garlic lethal was 8-10 Gy doses. According to FARRIS *et al.* (1999)

doses lower than 100 Gy were inefficient in induction haploids embryos, rising as an effect of pollination by irradiated pollen in cucumber. The most efficient were 100, 200 and 300 Gy doses and what was interesting, the biggest number of embryos was received after treatment by dose of 100 Gy, the number of regenerated plants was comparable in these three doses.

That is why the main aim of this research was to determinate how the gamma rays, incubation temperature and medium composition can influence the development of haploid embryos, and the same on increasing the effectiveness of anthers culture in several chosen *Brassica* species.

## MATERIAL AND METHODS

Two varieties ('Topas', 'White Flower'), one strain (Mah 1701) and F<sub>3</sub> hybrids (White Flower × Topas) of spring rapeseed (*B. napus* f. *annua* AACC = 38 chromosomes) and *B. carinata* (BBCC = 34) was the material used in this research.

Flower buds for anthers isolation were collected from plants growing in a glasshouse with controlled environmental conditions: 16 hours photoperiod and temperature 24/18 (day/night). After preliminary analyses it was concluded that the most embryogenic anthers there are in buds 4.0-4.5 mm length. Flower buds selected in



TABLE 2. Mean number of embryos obtained in anther culture of five *Brassica* genotypes (number/100 anthers)

Genotype	Gamma ray doses (Gy)	Incubation all time at 25°C			Incubation 24 h at 35°C			
		media			media			
		B <sub>5</sub>	B <sub>5</sub> + 2.5 Ag	B <sub>5</sub> + 5 Ag	B <sub>5</sub>	B <sub>5</sub> + 2.5 Ag	B <sub>5</sub> + 5 Ag	
<i>B. napus</i>	Topas	0	63	25	0	72	119	0
		25	5	2	0	0	0	0
		50	0	0	0	0	0	0
	White Flower	0	0	2	0	8	7	25
		25	2	2	0	21	0	0
		50	0	0	0	0	0	0
	1701	0	22	36	0	31	59	0
		25	0	0	0	3	11	0
		50	0	0	0	0	0	0
F <sub>3</sub>	0	0	2	0	71	13	0	
	25	0	0	0	0	0	0	
	50	0	0	0	0	0	0	
<i>B. carinata</i>	0	52	27	0	78	32	6	
	25	8	0	0	28	15	1	
	50	0	0	0	0	0	0	

temperature (24 h at 35°C) and incubated on B<sub>5</sub> medium with 5.00 ppm AgNO<sub>3</sub> (Table 2). B<sub>5</sub> medium supplemented with 2.5 ppm AgNO<sub>3</sub> did not cause increasing of embryo production compare to the basic medium. However, in the case of 'White Flower' it the stimulating influence of gamma irradiation at 25 Gy dose was observed. Irradiated anthers incubated at 35°C produced higher number of embryos (21) compared to unirradiated anthers (8).

The strain Mah 1701 showed similar response in anther culture as the variety 'White Flower'. Anthers of this genotype produced embryos in two combinations i.e. untreated with gamma rays and treated with 25 Gy dose. The biggest production of embryos (59) was observed on untreated anthers incubated on B<sub>5</sub> medium

with 2.5 ppm AgNO<sub>3</sub> at 35°C. For this genotype addition of 2.5 ppm AgNO<sub>3</sub> stimulated formation of embryos.

In unirradiated combinations it was noticed that supplement of 2.5 ppm AgNO<sub>3</sub> to the medium stimulated embryos formation both at 25°C and 35°C. While, anthers treated with 25 Gy showed better response but only in the case of pretreatment them with 35°C.

In our research concerning with influence the temperature, AgNO<sub>3</sub> and gamma rays doses on efficiency of anther culture, F<sub>3</sub> hybrid had the lowest embryogeny from all tested genotypes. Embryos were achieved only in three of nine combinations. The highest number of embryos (71) were found in anthers untreated with gamma rays and incubated on the basic medium at 35°C. Unirradiated anthers of F<sub>3</sub> hybrid showed similar reac-

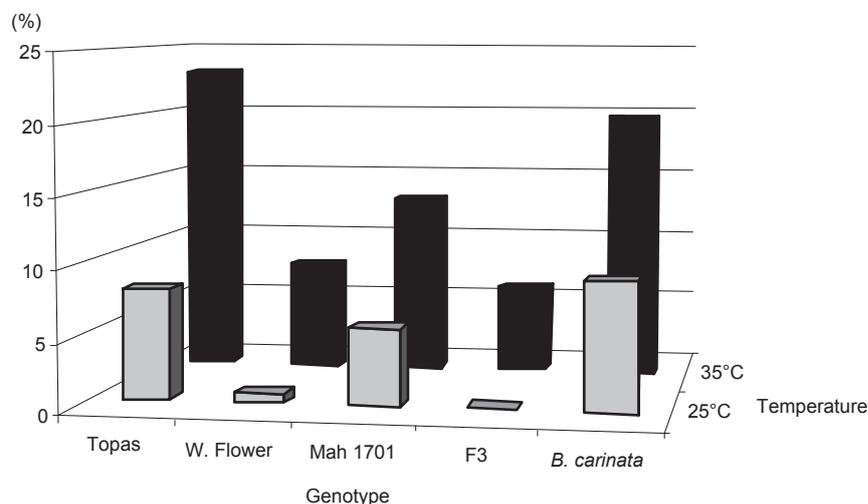


FIG. 1. Percentage of rooted plants of five genotypes *Brassica*, with and without temperature treatment

tion as anthers of Mah 1701 strain. In this case supplementation of 2.5 ppm AgNO<sub>3</sub> to the medium gave the best result at 35°C (13).

Reaction of *B. carinata* for the analysing factors was quite opposite to the anthers of genotype Mah 1701. Anthers of *B. carinata* showed the significant decrease of embryos production on the medium supplemented with 2.5 ppm AgNO<sub>3</sub> compare to the basic medium (Table 2). The same reaction was observed both for irradiated (25 Gy dose) and unirradiated anthers. The best response of *B. carinata* anthers was observed in the case of unirradiated anthers cultured on the basic medium.

In almost all tested genotypes the highest percentage of embryogenic anthers (Table 1) was always connected with the highest number of embryos produced by one anther. One exception was observed in the case of F<sub>3</sub> hybrid anther. In this combination in spite of the fact that there were embryogenic anthers (2.8%) there were no embryos counted on one anther.

Data on the Figure 1 show the percentage of rooted plants of five *Brassica* genotypes. The best rooting of regenerated plants was observed when they were originated from pretreated with high temperature 'Topas' anthers (23.14%). The least number of rooted plants were obtained for F<sub>3</sub> hybrid (6.48%).

None of tested genotypes did not produce any embryo from anthers treated with 50 Gy dose of gamma rays (Table 2).

In the experiment without high temperature pretreatment the highest percentage of rooted plants was achieved for *B. carinata* (9.25%), while in F<sub>3</sub> hybrids there were no rooted plants.

## DISCUSSION

Although, a large number of dihaploid plants have been achieved in several species of *Brassica* genera (CEGIELSKA 1994), from the practical plant breeding point of view the efficiency of *in vitro* culture and achieving dihaploid plants is still disappointing. Therefore, in this paper several factors, which can influence androgenesis in anther culture were checked.

The effectiveness of embryo production in anther culture is affected by numerous factors such as genotype, donor plant conditions, pretreatment of buds or anthers, culture medium composition and incubation conditions of the anthers. According to the data obtained by another authors (ARNISON et AL. 1990, ANANDARAJAH et AL. 1991, YANG et AL. 1992) a thermal shock treatment at the start of the culture period resulted in higher embryos production per 100 anthers. Based on the experience of over mentioned authors and some others, also for another species (KOLEVA-GUDEVA et AL. 2007). In our paper high temperature treatment at the start of the culture period (24 h at 35°C) stimulated the embryo formation in *in vitro* anther culture. The reaction of tested genotypes on high temperature treatment was almost the same. The obtained results indicate that, anthers incubation during the first 24 hours of cultures at 35°C temperature increases the number of embryos per 100 anthers, and finally the number of rooted plants. Anthers incubated all time of culture at

25°C produced lower number of embryos. Similar dependence was observed in KELLER and ARMSTRONG (1978) research, where highest temperatures than 25°C stimulated embryogenesis of *Brassica* anthers.

In the presented paper the influence of different doses of AgNO<sub>3</sub> added to the medium on achieving embryos from anther culture was examined as well. The effect of silver nitrate concentration varied from genotype to genotype. The best response showed anthers of 'Topas' variety. It was observed that androgenesis in anthers of untreated gamma rays 'Topas' variety was stimulated by the medium with 2.5 ppm AgNO<sub>3</sub>. However, increased dose of AgNO<sub>3</sub> to 5.0 ppm inhibited the androgenesis. WOJCIECHOWSKI (1998) in his work concerning 'Topas' variety anther culture has found much better embryos production on the medium with AgNO<sub>3</sub> compare to the basic medium. This author observed the highest percentage of embryogenic anthers on the B<sub>3</sub> medium with addition 3.0 ppm AgNO<sub>3</sub>. In the work of DIAS and MARTIN (1999) where effect of silver nitrate on embryo production of six *B. oleracea* subspecies was investigated, three different categories of response to the addition of silver nitrate were detected: (1) a small number of the morphotypes tested showed little or no response to the addition of silver nitrate at any concentration tested; (2) a few morphotypes were stimulated to form embryos by the addition of 5 mg·l<sup>-1</sup> AgNO<sub>3</sub>; and (3) the great majority of the morphotypes were stimulated to form embryos by the addition of 10 mg·l<sup>-1</sup> AgNO<sub>3</sub>. BEYER (1976) suggests that silver (Ag) inhibits ethylene action in plant tissue. This suggests that AgNO<sub>3</sub> promotes embryogenesis in *Brassica* genotypes by blocking the inhibitory effect of endogenous ethylene on embryo production. BIDDINGTON et AL. (1988) and OCKENDON and MCCLEANAGHAN (1993) also made similar observations and suggested that it was possible that these genotypes either produced lower levels of endogenous ethylene or were less sensitive to ethylene for embryo induction.

It is known that in many different plant species: e.g. watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), cucumber (*Cucumis sativus*), cabbage (*Brassica oleracea* var. *capitata* f. *alba*) haploid embryos could be induced by applying plant pollination with gamma rays treated pollen. There were no data concerning gamma rays used for inducing androgenic embryos. For that reason in this paper different doses of gamma rays (50, 100 Gy) were examined for induction androgenic embryos from anthers. It has been shown that the reaction of testing genotypes to applying doses of gamma rays was different. Gamma radiation in dose of 25 Gy was stimulating embryos and plants development in anthers culture of 'White Flower' (*B. napus*) and *Brassica carinata*. However, anthers treated with gamma rays at dose of 50 Gy did not create any embryos in any of the tested combination.

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