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MORPHOGENETIC STUDIES CONCERNING THE MAIN HAPLOID PATHWAYS "IN VITRO" AT *BRASSICA OLERACEA* var. *ITALICA* L.

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INTRODUCTION

Research programs that deals with plant haploidy start from the premise that a sporophyte can be produced, by biotechnological ways, with a gametic number of chromosomes, from a gametophyte without prior fertilization. This fundamental change in the life cycle of higher plants has strongly boosted the research activity in a large number of fundamental or applied botanical disciplines. Recent technological research, better understanding of the control mechanisms as well as the importance of the final applications have resulted in a progressive increase of the interest of the specialists in the development of haploids in the upper plants, boosting this activity in an alert rate of development.

The present paper targets to carry out an integrative study in order to establish the most efficient method of obtaining haploid plants in Brassica oleracea L. var. italica, (broccoli), using the in vitro culture. This study is focused on the culture of anthers, respectively androgenesis and the culture of unfertilized ovaries - gynogenesis, an alternative method of obtaining haploid plants, less mentioned in the specialized literature in Brassica, but with the help of which haploid plants were successfully obtained and isogenic lines in manyother species.

MATERIAL AND METHODS

The study was conducted at Vegetable Research and Development Station Bacau, in the Laboratory of Tissue Culture. The biological material used in the research is represented by five genotypes brocolli – BRO12, BRO18, BRO21, BRO35, BRO42.

The donor plants were grown in the growth room with a light intensity of 5000 lx and a 16-h photoperiod. The buds (3 - 4 mm in length) were collected when the microspores were at the mid uninucleate stage. Temperature pretreatment of flower buds was performed at 4°C for 72 h.

Sterilization of explants (about 2.5 mm in size) was performed by immersion in 0.1% HgCl 2 for 10 minutes, followed by repeated energy washing with sterile distilled water. For the induction of androgenesis and gynogenesis we utilized five media variants (tab. 1) with different hormonal formulas.

Table 1. Culture medium variants used for the "in vitro" culture of anthers and ovaries in brocolli

Component	V1	V_2	V_3	V_4	V_5
Macroelements	NLN	NLN	NLN	NLN	NLN
Microelements	NLN	NLN	NLN	NLN	NLN
Vitamins	MS	MS	MS	MS	MS
BAP	1mg/l	-	0,3 mg/l	0,2 mg/l	-
KIN	-	1 mg/l	-	-	0,1 mg/l
NAA	-	-	0,1 mg/l	0,1 mg/l	-
IAA	0,1 mg/l	-	-	-	-
2.4D	0,5 mg/l	1 mg/l	0,02 mg/l	-	0,5 mg/l
Zaharoză	30 g/l	30 g/l	30g/l	30g/l	30 g/l
Agar	8 g/l	8 g/l	8 g/l	8 g/l	8 g/l
pH	5,8	5,8	5,8	5,8	5,8

The anthers were incubated six days in the dark at 35^{O}C and 14 days at 23^{O}C. Calibrated anthers containing embryos were exposed to light during photoperiod 16 hours a day (at 3000 lx) and 8 hours in the dark, at a temperature of 25^{O}C.

Statistical analysis

The data were analyzed by ANOVA (analysis of variance) and the means were compared using the Duncan multiple comparison test at $P < 0.05$.

RESULTS AND DISCUSSIONS

The whole androgenetic and gynogenetic process performed in this study was similar with other results obtained by us on other *Brassica* species. After 2 weeks of culture, both the anthers – fig. 1 and the ovaries maintained under photoperiod conditions began to increase in volume on the V1 and V3 media variants and microscopic calli were visible on the surface of the explants. After about 3 weeks, in the friable calus formed, green meritematic centers began to develop which evolved into embryos and seedlings. Passed on fresh media and under photoperiod conditions, embryogenic structures and seedlings of androgenetic and gynogenetic origin have rapidly developed into fully rooted plants.

The main morphogenetic reaction recorded on kinetin culture media is the generation of callus. The callus is an unorganized, informed mass of proliferating parenchymal cells that, by cultivation, form clusters of meristematic cells, elements of conducting systems, pigmented cells, etc. Embryo development on this variant often stopped after several divisions or during the globular embryo to the heart-shaped embryo stage, followed by embryo death. The detailed results are presented in table 2 and fig. 1.

Table 2. The type of morphogenetic reaction recorded and the proliferation capacity of the cultures initiated on culture media

Variant	Recorded morphogenetic reaction
V1	calus (+++) very friable, light green, embryo (++), shoots (++)
V2	embryo (+), calus (++) compact, white green
V3	calus (++) semi-friable, yellowish-green colored, roots (+++)
V4	calus (+) compact, yellowish-green colored, in small quantities
V5	shoots (+), calus (++) semi-compact, white green and roots (+++)

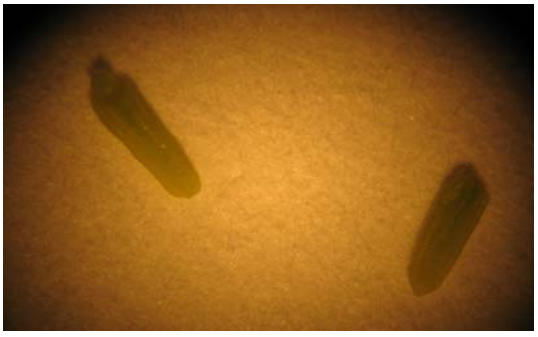

Fig. 1. Excised anthers – photo stereomicroscope

It has been observed that, in the case of the Brassica anthers, the induction of the callus formation and its proliferation is favored by the presence of auxin 2.4D. Also, the presence of auxin IAA in larger quantities favored the emergence and proliferation of the callus, whereas auxin NAA in combination with Kinetin predominantly causes the formation of adventitious roots either directly on the initial (respectively anterior) explant or in the calus mass.

Transfered on fresh media, the embryogenic callus - fig. 2 had a large proliferation capacity. Each callus from the anthers produced 10-15 embryos, while the gynogenetic callus generated 2-5 embryos that evolved into seedlings – figure 3.

Not all genotypes showed androgenetic and/or gynogenetic aptitude (tab. 3). Thus, out of 5 genotypes of brocolli only BRO12 and BRO18 responded in both the anthers and ovarian cultures.

Fig. 2. Embryogenic calus

Fig. 3. Emergence of new shoots

Table 3. Comparative analysis of the androgenetic and gynogenetic response in Brassica oleracea L.

Genotype	Morphogenetic reaction	
	Androgenesis	Gynogenesis
BRO12	+++	+
BRO18	+++	+
BRO21	+++	-
BRO35	-	-
BRO42	++	+

As can be seen from the table presented above, the best morphogenetic reaction was recorded in the case of the use of the anthers as initial material, gynogenesis being not, at least under the conditions tested by us, a reliable way of obtaining haploid plantlets at brocolli.

CONCLUSIONS

The study revealed the superiority of the anthers cultures in the initiation of the cultures destined to obtain haploid plants in Brassica oleracea L. The success of these cultures "in vitro" depends both on the genotype and on the culture conditions.

In the case of the Brassica anthers the induction of calus formation and its proliferation is favored by the presence of auxin 2.4D. Also, the presence of auxin IAA in larger quantities favored the emergence and proliferation of the callus, while the auxin NAA in combination with Kinetin predominantly causes the adventitious root formation

either directly on the initial (respectively anterior) explant or in the calusal mass.

ABSTRACT

The present paper aims at carrying out an integrative study to establish the most effective method of obtaining haploid plants in *Brassica oleracea* L. var. capitata, using "in vitro" culture, respectively androgenesis and non-fecunded ovary culture - ginogenesis, an alternative method of producing haploid plants, less mentioned in the literature at Brassica, but with which haploid plants and isogenic lines have been successfully obtained in other species.

The study revealed the superiority of anther cultures in the initiation of cultures dedicatet to the production of haploid plants in *Brassica oleracea* L. The success of these cultures "in vitro" depends both on genotype and on culture conditions.

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