PRELIMINARY STUDIES REGARDING THE ANDROGENETIC RESPONSE OF WHITE CABBAGE (*BRASSICA OLERACEA* L.) ANTHERS UNDER THE INFLUENCE OF BASAL MEDIUM COMPOSITION

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Abstract

The present study targeted toward the accomplishment of a screening regarding the androgenetic response of white cabbage (Brassica olerace L.) anthers under the influence of basal medium composition. The biologic material is represented through unopened flower buds collected from mother plants belonging to a variety – DL20 developed and maintained by Vegetable Research and Development Station Bacau. The buds contained anthers with microspores at late uninucleate to binucleate stage. We tested three different variants: variant M1- MS (Murashige Skoog, 1962), variant M2 - Gamborg B5, 1968, variant M3 - NLN (Lichter, 1982). In the experimental condition tested in our study and previously presented, the anthers reacted through direct organogenesis and embryogenesis but mainly through the formation of callus (indirect embryogenesis and organogenesis). The best results were obtained on variant M3, the standardized basal medium NLN, established by Lichter, 1982, which also seemed to support more the development of embryos directly on the anthers.

Key words: callusogenesis, embryogenesis, organogenesis, flower, buds

The genus Brassica comprises many commercially important food and oilseed crop plants displaying a vast range of morphological forms. *B. oleracea* contains many important vegetable crops, such as cabbage, cauliflower, broccoli and brussels sprouts. All these vegetable crops are important for human nutrition due to their content in vitamins and minerals, which explains their wide spread cultivation.

For decades, plant biotechnology has been used as a tool for plant breeding programs improvement. Among the many techniques employed, anther culture is designed for generating haploid plants which through different diploididization methods can be transformed in homozygous dihaploids, utilizable as parental lines for F1 hybrids. If traditionally, plant breeders usually achieve homozygosity of the cross products through self-fertilization - which usually needs 8-10 year, by anther culture, homozygous plant can be produced within a year.

In the literature are numerous reports regarding the anther culture in several *Brassica* species including *B. napus*, *B oleracea*, *B. campestris*, *B. juncea*, *B. carinata* and *B. nigra.* Still, due to the recalcitrant nature of Brassica tissues and to genotypic peculiarities, the researches regarding the establishment of viable protocols still draws the attention of many specialists all over the world. The main factors that orientate the morphogenetic reaction of anthers cultivated *in vitro* are: genotype, culture media, physiological status of donor plant, anther wall factor, stage of pollen development, temperature and light. Few studies were accomplished regarding the influence of mineral nutrients over the morphogenesis of anthers cultivated *in vitro*, which is somehow surprising giving the fact that the mineral composition is the main component of any tissue culture medium. Thus, the researches presented in this study focus toward the screening of morphogenetic reaction of white cabbage anthers cultivated on different basal medium formula.

MATERIAL AND METHOD

The biologic material is represented through unopened flower buds collected from mother plants belonging to the variety – DL20 developed and maintained at Vegetable Research and Development Station Bacau. Donor plants were grown in controlled conditions, in greenhouses, with a proper regime of watering, fertilization and pest control. The unopened flower buds measured 3.0 – 3.4 mm in length (fig. 1). At this length the buds contained anthers with microspores at late uninucleate to binucleate stage (observed using 1% aceto-carmine under microscope).

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Figure 1 **The unopened flower buds utilised as source of explants**

After being excised, the explants were washed thoroughly under running tap water for 30 min and treated with a surfactant, Tween 20 (10 drops per 100ml of sterilized distilled water). Later these explants were surface sterilized with 0.1% mercuric chloride (w/v) for 15 min and repeatedly washed using sterilized distilled water. Under aseptic conditions, anthers were removed from the sterilized buds using a fine Tweezers (forceps) and inoculated on sterile tubes with culture media (fig. 2) containing different mineral composition formula – as illustrated in table 1.

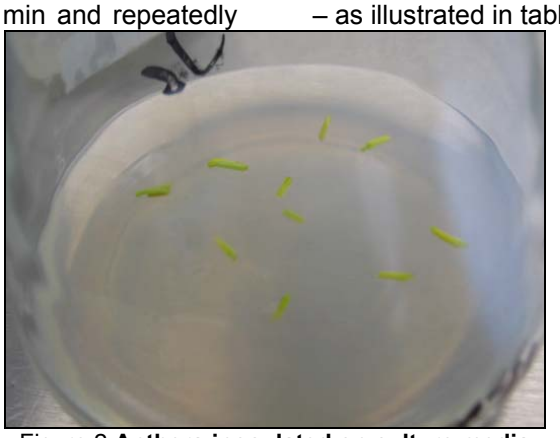

Figure 2 **Anthers inoculated on culture media**

Table 1

Variants of tissue culture media utilised in our experiment

Variant M1	Variant M2	Variant M3
Murashige Skoog, 1962	Gamborg B5, 1968	NLN (Lichter, 1982)

Figure 3 **Cultures in incubators**

The cultures were incubated at 33°C temperature for one week in complete dark (fig. 3).

After that the cultures were transfer in culture chambers with controlled light, humidity and temperature control at 25^0C, a 16-h photoperiod, and 5000 lx light intensity. Fifty anthers of each genotype were inoculated into each treatment.

Four to five weeks after inoculation of anthers, they were removed aseptically from the culture tubes on a sterilized glass plate inside the laminar airflow cabinet and were placed again on freshly prepared sterilized medium with the same formula. The sub cultured culture tubes were then incubated at 25°C with 16 hrs photoperiod for 5-7 days. Repeated sub cultures were done at an interval of 15 days and incubated under the same temperature as mentioned previously. The culture vessels showing signs of contamination were discarded. Day to day observation was carried out to note the responses.

During the development of experiments we emphasize over the number of anthers producing callus, embryoids and organogenic meristematic centers. The frequency of direct and indirect organogenesis and embryogenesis reaction and the frequency of reacted anthers were calculated in percentage to the *in vitro* initial explants.

RESULTS AND DISCUSSIONS

In the experimental condition tested in our study and previously presented, the anthers reacted through direct organogenesis and embryogenesis but mainly through the formation of callus (indirect embryogenesis and organogenesis). The percentage of reactive anthers vary among the tested variant, 20.8% from anthers gave positive response on M1, 11.2% on variant M2 and 23.6% on M3.

Immediately after inoculation, anthers started to increase in volume and gradually to be swallowed by callus structures. The induction of callus was the main morphogenetic reaction, 13.1% from the reactive anthers determined the formation of callus on variant M1, 8.2% on variant M2, while at variant M3 the percent was 12.9%.

Figure 4 **Friable, cream coloured callus developed from anthers**

We identified different types of callus, the consistence and colour being the most distinguished features that also allowed us to separate the embryogenic and organogenic callus by the non-regenerative ones. The main types of callus obtained from the white cabbage anthers cultivated *in vitro* were: calluses with friable consistency, white or cream coloured (fig. 4), calluses with a granular aspect, mainly coloured in green-yellowish or light green (fig. 5) and a more compact, hard green callus covered with patches of buds and leafy structures that gradually developed into elongated shoots.

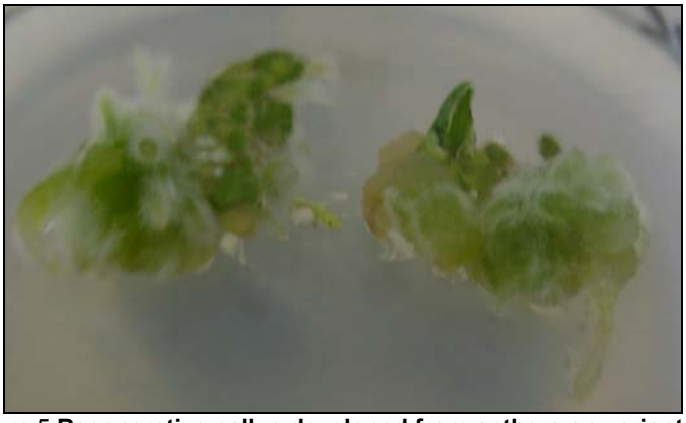

Figure 5 **Regenerative callus developed from anthers on variant M1**

This last type of callus was the most regenerative one and it was identified on all 3 variants tested, the difference between them being related to the procent of anthers producing callus and intensity of callus proliferation.

The granular callus showed a very good callus proliferation, especially after transferring it on fresh media, but the regenerative structures (buds, embryoids) were scarce.

Figure 6 **Meristematic centers developed from anther – photo taken under binocular microscope**

Another morphogenetic reaction identified among the anthers cultivated *in vitro* was the direct organogenesis and embryogenesis. Thus, on the surface of globular-shaped anthers started to appear small meristematic centers. The transfer on fresh media allowed their gradually development toward embryogenesis and organogenesis (fig. 6).

The results obtained are synthesized and graphically presented in figure 7.

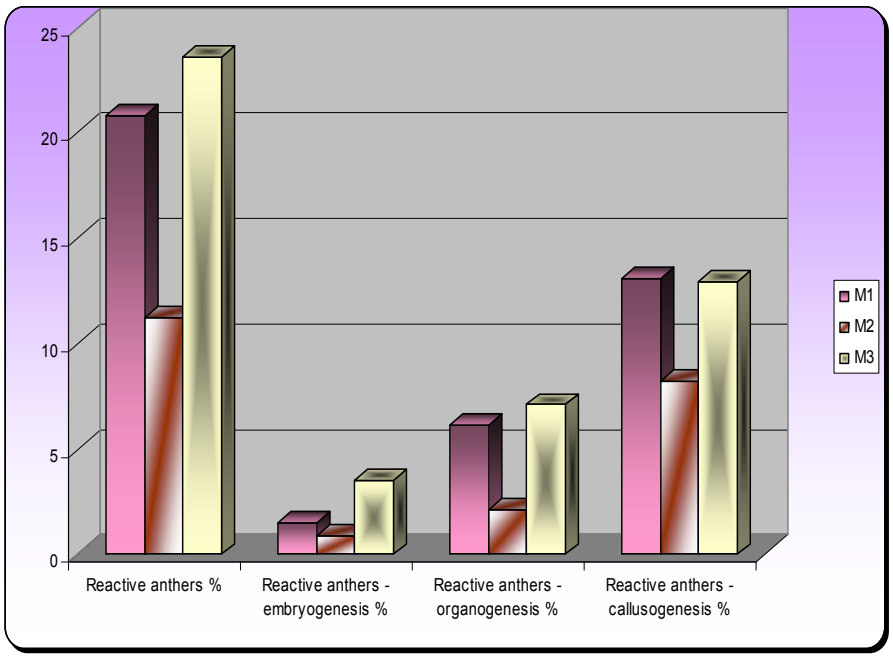

Figure 7 **Graphical representation of morphogenetic reaction of anthers**

Synthesizing, the results obtained until now demonstrate that anthers reacted on all the variants tested, the type and intensity of reaction ranging from callusogenesis to direct organogenesis and embryogenesis. The main type of reaction was callus formation, while organogenesis and embryogenesis was registered in smaller percentages. The best results were obtained on variant M3 and M1. Comparatively with the rest of the basal media utilized in our experiment, the variant M3, represented through the standardized basal medium established by Lichter, 1982, seemed to support more the development of embryos directly on the anthers. The results obtained until now, allows us to support the promotion of NLN (Lichter, 1982) medium for utilisation as mineral source of nutrition for cabbage anthers cultivated on solid media *in vitro.*

CONCLUSIONS

The results highlighted in the present study show that on all tested variants we managed to obtain morphogenetic reaction, the genotype DL20, being a responsive genotype for cultivation on solid medium *in vitro*. The percentage of reactive anthers vary among the tested variant, 20.8% from anthers gave positive response on M1, 11.2% on variant M2 and 23.6% on M3.

The androgenetic response of cabbage anthers cultivated on the three variants of basal medium composition was mainly oriented toward the obtaining of callus, but, even if in smaller amount, we also identified anthers with organogenetic and embryogenetic ability. The best results were obtained on variant M3, the standardized basal medium NLN, established by Lichter, 1982, which also seemed to support more the development of embryos directly on the anthers.

The continuation of the experimentations will allow us to verify and certify our theory. The results obtained will be correlated with other studies, regarding other factors that influence the androgenetic response of anthers cultivated *in vitro* in order to establish a viable protocol for cabbage anthers cultivation *in vitro*.

ACKNOWLEDGMENT

This work was cofinanced from the European Social Fund through Sectorial Operational Programme Human Resources Development 2007-2013, project number POSDRU/I.89/1.5/S62371 ,,Postdoctoral Schole in Agriculture and Veterinary Medicine area.

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