CAULOGENESIS APPROACHES APPLIED *IN VITRO* MICROPROPAGATION OF VARIETIES -CABEZA NEGRA 2, ARENA AND RED AMAGER- OF *BRASSICA OLERACEA VAR. CAPITATA RUBRA FORM*

Silvana Dănăilă - Guidea*, Ana Rosu*, Madalina Ionica*, Luminita Visan*, Ricuta Dobrinoiu*

*University of Agronomical Sciences and Veterinary Medicine Bucharest, Romania, Faculty of Biotechnologies
E-mail: silvana.danaila@yahoo.com

Abstract
In this paper, the authors have proposed a study on the variation in efficiency of micropropagation in vitro due to the influence of donor genotype and explant type used red cabbage head. In this test three commercial varieties of red cabbage seeds were tested for the ability of regeneration by organogenesis from hypocotyls, cotyledons, and stem segments. Our results have shown us that the intensity of red cabbage head regeneration depends on the genotype used but also the type of explant.
Hipocotil fragments taken from seedlings to "Cabeza Negra 2"had the greatest total capacity to produce shoots than the other two genotypes tested when the combination of phytohormones was used consisting of 2 mg / l 6-benzyl-aminopurine (BAP) and type auxine ß indolilacetic acid (IBA) and α naftilacetic acid (NAA) at concentrations of 0.2 to 0.4 mg /l.
The inoculated cotyledons explants also developed regenerative callus in about 6-8 weeks after initiation of cultures.

Keywords**:** *Brassica oleracea, red cabbage,* regeneration, caulogenesis.

1. INTRODUCTION

Vegetables biennial cabbage plants, valued for the content of valuable minerals, carbohydrates, protein substances, provitamin A and vitamins C, B1, B2. Red Cabbage is differentwhite cabbage in that it is red-purple, the heads smaller, stuffed, thin leaves, tender, with fine ribs. It is used less as cabbage, usually in salads and pickles. Red Cabbage has been shown to have an even greater nutritional value than fresh. It has more antioxidants vitamin C and a quantity of at least five times higher.

Micropropagation of plants using cultured cells and tissues in vitro is one of the methods most effective in obtaining copies of the plant donor. The success of the micropropagation process depends, however, a number of factors that influence the rate of plant regeneration and the maintenance of genetic stability of the biological material donor. Among these factors are mentioned: type of explant, sampling time, plant health of the donor plant genotype and perhaps most important.

Many efforts have been undertaken to establish a suitable *in vitro* regeneration protocol for *Brassica.* In 1991, Hachey and co-workers obtained a high frequency of shoot regeneration from some oilseed cultivars and Takasaki *et al.* (1996) got shoot from a few leafy vegetable cultivars of *Brassica* spp. It may be mentioned here that several attempts have been taken to establish *in vitro* regeneration protocol for *Brassica.* A high frequency of shoot regeneration was obtained from some oilseed cultivars (Hachey et al., 1991) and a few leafy vegetable cultivars (Takasaki et al., 1996) of *Brassica* spp. Narasimhulu and Chopra (1987, 1988) reported that *Brassica rapa* has the lowest frequency of regeneration from cotyledons among the three basic diploid species, *Brassica rapa, Brassica oleracea,* and *Brassica nigra* and their amphidiploids, *Brassica juncea, Brassica napus,* and *Brassica carinata.*

In Romania in vitro culture studies of *Brassicaceae* were also initiated in various aspects (Stoian et al., 1992; Roşu and Huazong,1997; Timofte et al., 1999, Cristea et al., 2005)

Therefore, present study was undertaken to establish a suitable and reproducible protocol for *in vitro* regeneration of ***Brassica oleracea var. capitata rubra form*** varieties. Also, the authors have proposed a study on the variation of efficiency of micropropagation in vitro due to the influence of donor genotype in red cabbage head.

2. MATERIAL AND METHOD

Biological material submitted micropropagării "in vitro" is represented by three genotypes of red cabbage for cabbage purchased from chain stores to market seeds.

Red Cabbage Black Head 2 is a variety extremely resistance to cold, easy to cultivate. Produces many large, round, compact heads of dark red color, foliage slightly wavy on the edges. It is a plant with a medium stalk. Red Cabbage RED Amager is a vegetation period: 110 days from planting to form round belly, weighing 2-3 kg. Shows a low and high temperature resistance and is suitable for storage.

The experiment was conducted at the tissue culture laboratory of the Department of Biotechnology, Faculty of Biotechnologies, Bucharest, during the period from October 2010 to April 2011.

The biological material used is represented by seeds germinated in controlled conditions, on Murashige-Skoog (1962) culture half strength solid medium without hormones (ph 5,8), containing 3% sucrose and 0,8 % Agar Noble (Figure 1.a) or on sterile filter paper (Figure 1.b). After seed germination, the resulting plants, seed-lobe are in phase, the source of explant donor, such as: meristenatic apex, hipocotyl fragment and cotyledons.

a.)  b.) 

Figure1. Red cabbage seeds germinated in controlled conditions:
a.) in vitro solid medium condition; b.) germination on sterile filter paper in petry dishes

Culture medium. Inoculation was achieved by placement of explants on the medium variant chosen for experimentation culture, distributed in sterile culture vessels, these operations are performed in a laminar flow hood. Basal medium Murashige - Skoog (1962), rich in nitrogen meets the nutritional requirements of the explants cultured in vitro with most species. This was the formula for optimal nutrition and adventitious shoots initiating cultures red cabbage with a hormonal supplement.

In these experiments series for initiated each red cabbage varieties we used the basal medium Murashige-Skoog (1962) supplemented by various concentrations and combinations of citokinins and auxins. For callus induction and shoot regeneration,two different combinations each containing MS (1962) culture with fitonormons: benzyl-aminopurine (BAP) and type auxine ß indolilacetic acid (IBA) and α naftilacetic acid (NAA) we use.

When the seedstalk was grown for 2 weeks after initial bolting in the plant box, the hipocotile were cut into pieces approximately 3-5 mm and were placed on 2 variants shoot inducing solid medium (ph 5,8),containing 0,4mg/l NAA + 2mg /l BAP (BR1) and 0,2mg/l IBA + 2mg / l BAP (BR2), 3% sucrose and 0,7% Agar Noble.

The surface sterilization of the explants: an aquaous solutions of sodium hiphocloride (0,5%) for 20 min. followed by 3 rinses with sterile distilled water.

The incubation conditions :photoperiod was 16 h of light provided by cool white fluorescent bulbs with 8 h in shadow. Temperature condition: 25 ± 2 °C in light period.
Multiplication phase. There were applied a few subcultures with a period of 3-4 weeks of culture medium variant used for initiation, because it has proven so effective in stimulating the development of multiple shoots and in terms of their elongation. The main goal was to increase the multiplication rate for this work material by increasing the cytokine quantity in the medium.

3. RESULTS AND DISCUSSIONS

Investigations of *in vitro* regeneration potentiality of these three genotypes were accomplished with callus induction, maintenance of calli, organogenesis, and finally plantlet regeneration and their establishment in field conditions. (Figure 2)

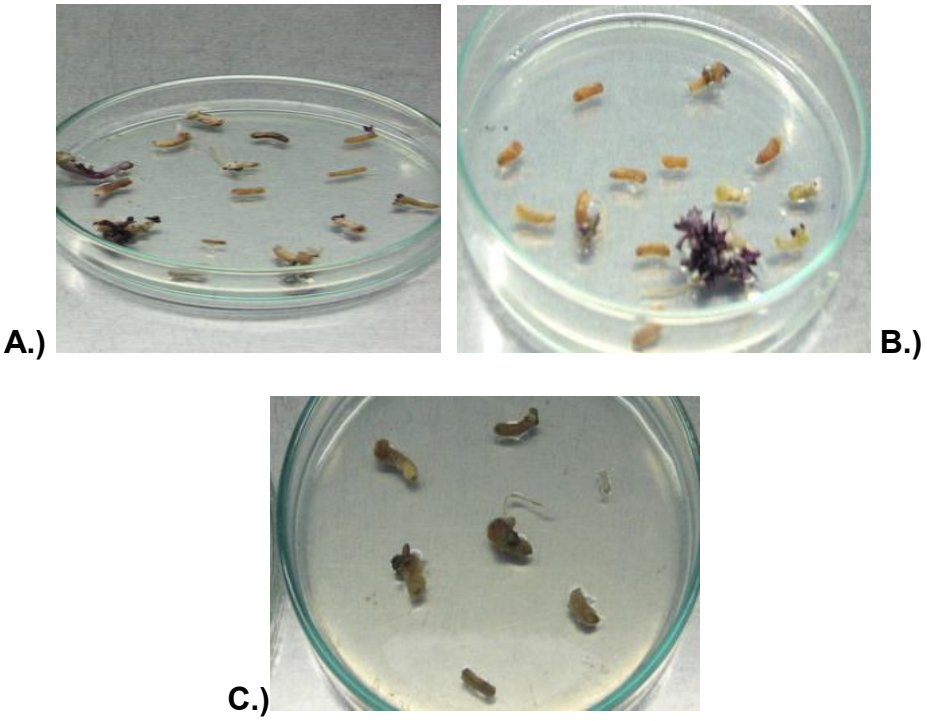


Figure 2. Different steps of in vitro regeneration of Brassica oleracea var. capitata rubra form genotypes via callus induction
(A) (B) Callus initiation of Black Head 2 genotype on MS+ 0,2mg/l IBA + 2mg / l BAP (BR2), 3% sucrose and 0,7% Agar Noble; (C) Callus initiation of Arena genotype on MS+ 0,2mg/l IBA + 2mg / l BAP (BR2), 3% sucrose and 0,7% Agar Noble.

Our observations on the effect of phytohormones on development of *Brassica oleracea var. capitata rubra form* varieties explants cultured in vitro have led to the conclusion as benzilaminopurine (BAP) at concentrations of 2 mg/l in the presence of low concentrations (0,2 mg/l) of auxine ß indolilacetic acid it is more optimal (IBA) than naphthyl acetic acid (NAA) in 0,4 mg /l hormonal supplement to stimulate shoot preformat the initial bud but also for the development of multiple shoots on long-term fit to be included in later phases of the micropropagation process, namely induction and acclimatization root development process. Number of shoots regenerated varied widely from one type of explant to another, especially from one genotype to another (Figure 3).

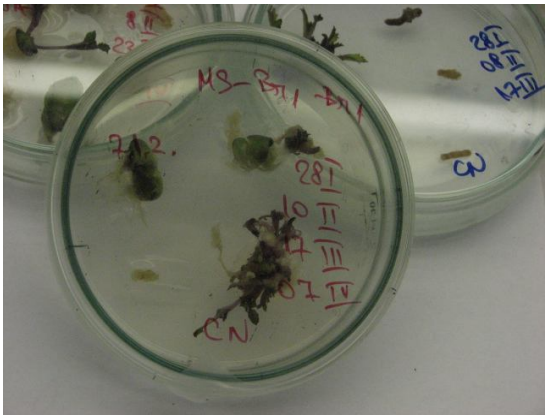
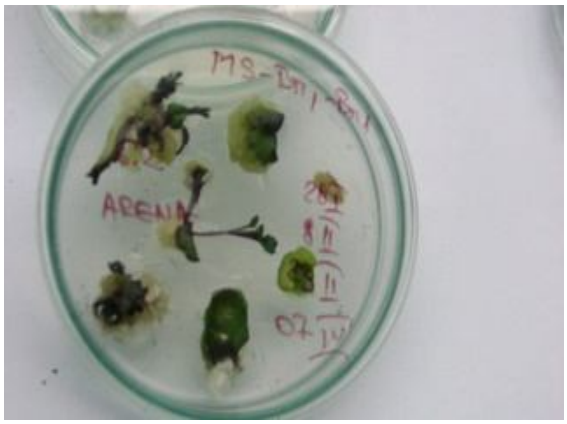
(A)  (B) 

Figure 3. In vitro shoot initiation of Brassica oleracea var. capitata rubra form genotypes from cotyledonary explant (A) Shoot initiation of Black Head 2 genotype on MS+ 0,4mg/l NAA + 2mg /l BAP (BR1); (B) Shoot initiation of Arena genotype on MS+ 0,4mg/l NAA + 2mg /l BAP (BR1)

Experimental results after 3 - 4 subcultures for each experiment shown that in all genotypes used with maximum efficiency explant hipocotile multiplier is that not only allows a large amount of plants but a small percentage of vitrified plants or other morphological changes (Figure 4).

Figure 4. **Adventitious shoots developed after3 -4 subcultures on MS+ 0,2mg/l IBA + 2mg / l BAP (BR2), 3% sucrose and 0,7% Agar Noble (genotype Cabeza Negra 2)**

4. CONCLUSIONS

- The process of improving plant brassicas can advantageously benefit from biotechnology vegetative multiplication in vitro.
- Meristematic apexes with 1 cm dimension not assure a significant percent of survival of adventitious shoots red cabbage explants (3-5 pieces/ explants) comparing the hipocotil cultures (25-30 pieces/ explants).
- The highest number of shoots were obtained for genotype Cabeza Negra 2 and Red Amager- (~ 300 shoots), while genotype most "recalcitrant"to these techniques was Arena who have won only 25 shoots from inoculated explants.In conclusion we can say that the efficiency of micropropagation in vitro for the cabbage red cabbage *Brassica oleracea* var. capitata rubra form varies widely, depending on the influence of strict donor genotype. ;
- Higher concentration of cytokine in the recipe BR2 (MS+30 g/l sucrose +7 g/l agar +2 mg/l BAP+0,2 mg/l IBA) determined the formation of a high percent of adventitious shoots (Figure 4).
- Based on these results we consider that the experimental model of multiplication by culture hipocotyl fragments and obtaining vitroplantelor red cabbage, established and optimized as a

result of our researches, is a method of multiplying the yield and reproductibility, which can be used with the efficiency practically.

5. REFERENCES

Cao J.S., X.L. Yu, A.J. Huang and S.Y. *Xu* (2000) Enhancement of plant regeneration frenquency of *in vitro* cultured Chinese cabbage. *Acta Horticulturae Sinica* 27: 452- 454.

Cristea T. O., Mihu G., Prisecaru M (2005) Variația randamentului de micropropagare "in vitro" determinată de influența genotipului la varza albă pentru căpățână – *brassica oleracea* l. var. *capitata*, forma *alba.* In: Lucrări Ştiinţifice, seria Horticultură, anul XLVIII (48), 2005, vol. 1 şi 2. ISSN 1454-7376 Editura "Ion Ionescu de la Brad", Iaşi.

Dhawan A. K., A. Jain and J. Singh (2000) An efficient plant regeneration protocol from seedling explants of *Brassica juncea* RH. 781, a freeze tolerant cultivar. *Crucferae Newslett.* 22: 21-22.

Du H., D.H. Zhuang and W.H. Hunang (2000) Stimulation effect of silver nitrate on shoot regeneration in cotyledon tissue culture of *Brassica campestris. J. Tropical and Subtropical Bot.* **8(2**): 109-112. FAO. 2001. Production Year Book for 1999. p. 118, FAO. UN. Rome, Italy.

Hachey J.E., K.K. Sharma and M.M. Moloney (1991) Efficient shoot regeneration of *Brassica campestris* using cotyledon explants cultured in vitro. *Plant Cell Rep* **9**: 549-554.

Khan M. M. A., Arif Hasan Khan Robin A. B. M., Nazim-UD-DOwla M.A.N., Talukder, S. K. and Hassan L. (2010) *In vitro* regeneration potentiality of *Brassica* genotypes in differential growth regulators. In: Bangladesh J. Agril. Res. **35(2)** : 189-199.

Murashige T. and F. Skoog (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol Plantarum* **15**: 473-497.

Narasimhulu S.B. and V.L. Chopra (1987) Plant regeneration from callus cultures of *Brassica carinata* A. Br: and its implication to improvement of oilseed *Brassicas. Plant Breeding* **99**: 49-55.

Narasimhulu S.B. and V.L. Chopra (1988) Species specific shoot regeneration response of cotyledonary explants of *Brassica. Plant Cell Rep* **7**: 104-106.

Roşu Ana, Huazong J. (1997) Experimentally induced plant chimeras by intervarietal and interspecific "in vitro" grafting in Brassica sp.In: Lucrari Stiintifice U.S.AM.V.B. Seria F –vol.II Biotehnologii 1997, p. 7-16, Bucuresti.

Stoian, L., Timofte, Valentina, Munteanu, N. (1992) Implicații ale tehnicilor "in vitro" în ameliorarea legumelor din grupa verzei. Analele I.C.L.F. Vidra, vol. XI, 10 pag.,

Takasaki T., K. Hatakeyama, K. Ojima, M. Watanabe, K. Toriyama and K Hinata, 1996. Effects of various factors (hormone combination, genotypes and antibiotics) on shoot regeneration from cotyledon explants in *Brassica rapa* L. *Plant Tissue Cult Lett* **13**: 177-180.

Timofte, V., Dorina Cachita –Cosma, Ghica Mihu, Lucian Stoian (1999) Integrarea microclonarii "in vitro" in procesul de producere a materialului initial de ameliorare, la Brassica oleracea L.In: Culturi in vitro la cormofite, Editori: D.Cachita-Cosma, Ardelean A., Craciun C., RISOPRINT, Cluj Napoca.

Wang J.X., Y. Sun, G.M. Cui, S.X. Liu, G.P. Wang, Y.J. Shang and H. Wang (2000) Effects of plant growth regulators and genotypes on the differentiation of *in vitro* cultured hypocotyls of rapeseed *(Brassica). Chinese J. Oil Crop Sci* **22**: 11-13.