STUDIES REGARDING THE INCIDENCE OF CHROMOSOMAL ABERRATIONS AT PLANT REGENERATED „IN VITRO" VERSUS PLANTS OBTAINED FROM SEEDS AT *BRASSICA OLERACEA* L.

STUDII PRIVIND INCIDENŢA ABERAŢIILOR CROMOSOMIALE LA PLANTELE REGENERATE „IN VITRO" VERSUS PLANTE OBŢINUTE DIN SEMINŢE LA *BRASSICA OLERACEA* L.

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Abstract. *Brassica oleracea contains many important vegetable crops, such as cabbage, cauliflower, broccoli and brussels sprouts. Among them, one of the most popular crop is white cabbage (Brassica oleracea var. capitata, forma alba). Currently, the breeding techniques involve the utilization of tissue culture for the regeneration of plants. But, during the "in vitro" culture, due to the medium culture composition, alteration of chromosomes morphology may occur According with the literature, chromosome aberrations have been used as a measure of reproductive success in plants for many years and have been correlated with morphological changes, fertility-sterility relationships, mutations, etc. The main objective of the present paper is screening of some aspects regarding the type and frequency of chromosomes aberrations that appeared at cabbage plants regenerated from "in vitro" culture versus seed-born plants. The main types of aberrations identified at regenerated plants and seed-born plants are: contraction, stickiness, fragmentation, inter-chromatin bridges, ring chromosomes, C-mitosis.*

Key words: cabbage, genetic, analyses, vitro, plant

Rezumat. *Brassica oleracea cuprinde numeroase plante legumicole cum ar fi varza, conopida, broccoli şi varza de Bruxelles. Dintre acestea, cea ma mare popularitate o înregistrează varza albă pentru căpăţână (Brassica oleracea var. capitata, forma alba). Astăzi, tehnicile moderne de ameliorare implică utilizarea culturilor de ţesuturi pentru regenerarea de plante. Dar, pe parcursul cultivării „in vitro", datorită compoziţiei mediului de cultură, se pot genera alterări ale morfologiei cromosomilor. Conform literaturii, aberaţiile cromosomiale au fost utilizate curent pentru determinarea succesului regenerării „in vitro", fiind corelate cu schimbări ale morfologiei, relaţiilor fertilitate-sterilitate, mutaţii. Principalul obiectiv al studiului de faţă este realizarea unui screening privind tipul şi frecvenţa aberaţiilor cromosomiale ce pot apărea la plantele de varză cultivate „in vitro" versus plante obţinute pe cale clasică din seminţe. Principalele tipuri de aberaţii identificate la plantele de varză regenerate „in vitro" şi la plantele obţinute din seminţe sunt: contractare, stickness, fragmentare, punţi, C-mitoze, cromosomi în inel.*

Cuvinte cheie: varza, genetic, analize, vitro, plante

INTRODUCTION

Brassica oleracea contains many important vegetable crops, such as cabbage, cauliflower, broccoli and brussels sprouts. In the literature are numerous reports regarding the plant regeneration from explants in several *Brassica* species including *B. napus* (Glimelius, 1984; Zhao et al., 1995), *B. oleracea* (Jourdan and Earle, 1989; Hansen and Earle, 1994), *B. campestris* (Zhao et al., 1994), *B. juncea* (Kirti and Chopra, 1990; Bonfils et al., 1992), *B. carinata* (Jaiswal et al., 1990) and *B. nigra* (Narasimhulu et al., 1993). Still there are few studies regarding the chromosomes aberrations that may appear during the cultivation of explants "in vitro". Chromosome aberrations have been used as a measure of reproductive success in plants for many years and have been correlated with morphological changes, fertility-sterility relationships, mutations etc.

The cultivation of different explants on nutritive media "in vitro" is often related with an increase in the frequency of structural chromosomal alterations as well as an increase in the frequency of gene mutations. How these factors are related to one another and how they cause changes in the chromosome and gene mutation rates are not well understood. However, the fact that all these external agents cause similar changes and indicate a broad fundamental process may be a primary cause of mutations.

Somaclonal variation (that may affect the "in vitro" regenerated plants) can pose a severe threat to the genomic integrity of regenerated plants, which is particularly required during the genetic transformation experiments. For the multiplication techniques, one important goal is to achieve genetic uniformity of the propagules and to maintain with fidelity the genetic structure of mother plants. Somaclonal variation can either bring the changes at the DNA level or it may induce changes in chromosome numbers. For most of the micropropagated crops only 5 % somaclonal variation is permitted (Leela *et al.* 2003). Although reports are available for propagation of cabbage *via* tissue culture, relatively few results are available on the type and frequency of chromosomal aberration that may occur during the cultivation of plant tissues "in vitro".

Understanding the cytogenetic of the plant has a key role for controlling the „in vitro" behaviour of different explants by a better understanding of the influence of these peculiar conditions over the growth processes. Subsequent studies at different plant species have shown that plant chromosomes exhibit many different types of aberration, as a result of different types of chemicals used for the preparation of "in vitro" culture medium.

MATERIAL AND METHOD

The experiments were performed in the Laboratory of Tissue Culture at Vegetable Research and Development Station Bacau, Romania.

The biological material is represented by seeds belonging to two genotypes of cabbage (*Brassica oleracea* L.IS 21 and IS 57 provided by Vegetable Research and Development Station Bacau, Romania.

The seeds were subjected to experiments regarding the "in vitro" multiplication of these valuable genotypes and the meristematic root tips were excised from the "in

vitro" plantlets regenerated on V2-V4 variants, characterized through the presence of BAP and Kinetin alone or in association with IAA - table1.

The seed born plants were obtained through the classic germination technique, in sterile Petri dishes (variant V1).

Table 1

Experimental variants utilized in the cytogenetic studies at *Brassica oleracea* L.

Components	V1	V2	V3	V4
Macro elements	seed born plants	MS, 1962		
Microelements		MS, 1962		
Vitamins		B_5		
BAP		2,0 mg/l	-	1,5 mg/l
Kinetin		-	2 mg/l	-
IAA		-	-	0,5 mg/l
Sucrose		3%	3%	3%
Agar		8 ‰	8 ‰	8 ‰

The cytogenetic studies were accomplished in meristematic root cells, stained in Carnoy fixing solution for 24 hours at 4^0C then hydrolyzed with HCl for 7 minutes and coloured with the basic colouring solution Carr.

The root meristems were displayed using squash technique and for each genotype and variant an average number of 5000 cells were counted. Dates regarding the type and frequency of chromosomal aberrations for each phase of divisions were recorded and subjected to statistical analyses, both for plants regenerated „in vitro" and for seed born plants.

RESULTS AND DISCUSSIONS

The results of the present study show that there are no significant differences between the seed-grown and tissue-cultured plants. In the root meristems of both type of plants we identified similar chromosomal aberration, the incidence of these aberrations in tissue-cultured plants does not exceed the values recorded for seed born plants.

The results obtained for each genotype and variant (V1 – V4) are presented in table 2 and 3, as media of cells with aberrations and illustrated in fig. 1 and 2.

Table 2

Types and frequency of chromosomal aberrations observed in root meristematic cells - genotype IS 21

Variant	Total no of cells	% in prophases	% in metaphases	% in A+T	$\bar{X} \pm s\bar{X}$ %
V1	5312	1.11	1.59	1.82	1.50±0,07
V2	5430	1.02	1.24	1.54	1.26±0,06
V3	5390	1.23	1.71	1.95	1.63±0,05
V4	5294	1.26	1.43	1.69	1.46±0,05

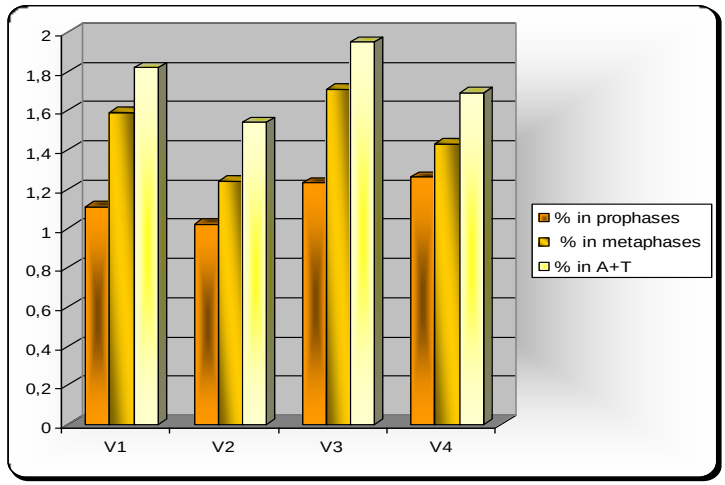

Fig. 1. Types and frequency of chromosomal aberrations observed in root meristematic cells – genotype IS 21

Table 3

Types and frequency of chromosomal aberrations observed in root meristematic cells – genotype IS 57

Variant	Total no of cells	% in prophases	% in metaphases	% in A+T	$\bar{X} \pm s\bar{X}$ %
V1	5129	1.23	1.62	1.07	1.30±0,04
V2	5085	0.88	1.39	1.69	1.32±0,02
V3	5226	1.11	1.20	1.92	1.41±0,05
V4	5112	1.16	1.51	1.32	1.33±0,02

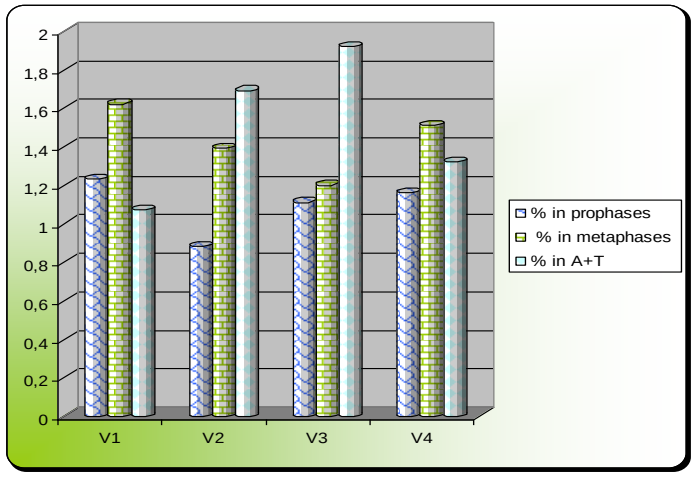

Fig. 2. Types and frequency of chromosomal aberrations observed in root meristematic cells – genotype IS 57

The cytogenetical studies accomplished in the present study demonstrate that the cultivation of cabbage shoot tips on nutritive medium modified with

Kinetin and BAP allows the regeneration of new plants with a stable genetic material that shows little genetic variability. This variability manifested at cellular level through the different types of chromosomal abnormalities does not exceed the natural variability present also on plants germinated in natural conditions.

Both at plants generated "in vitro" and at plants from seeds, the main types of aberrations identified are chromosome clumping, contraction, stickiness, paling, fragmentation, dissolution, chromosome and chromatid bridges, C-mitosis and endoploidy.

The highest incidence of aberrations was observed in ana-telophases. The most common abnormalities were ana-telophases with simple or multiple bridges, expelled or late chromosomes and multipolar ana-telophases (figure 3). The average percentage of cells in ana-telophase that presented these type of aberrations ranged between 1.07-1.92 at IS 57 genotype and 1.54-1.82 at IS 21 genotype.

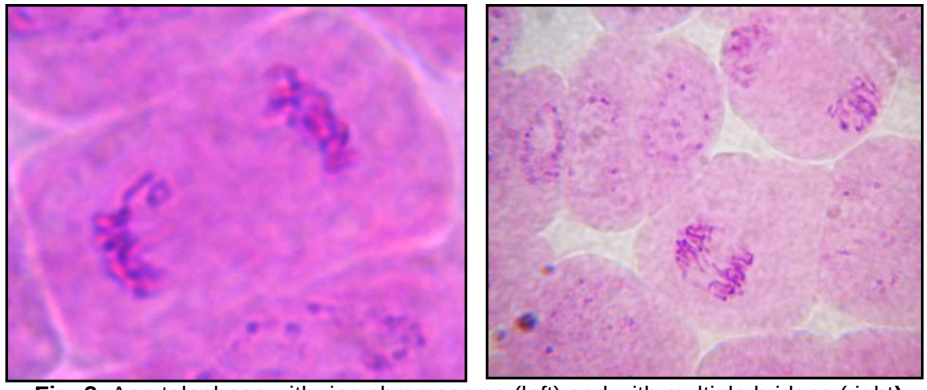

Fig. 3. Ana-telophase with ring chromosome (left) and with multiple bridges (right**)**

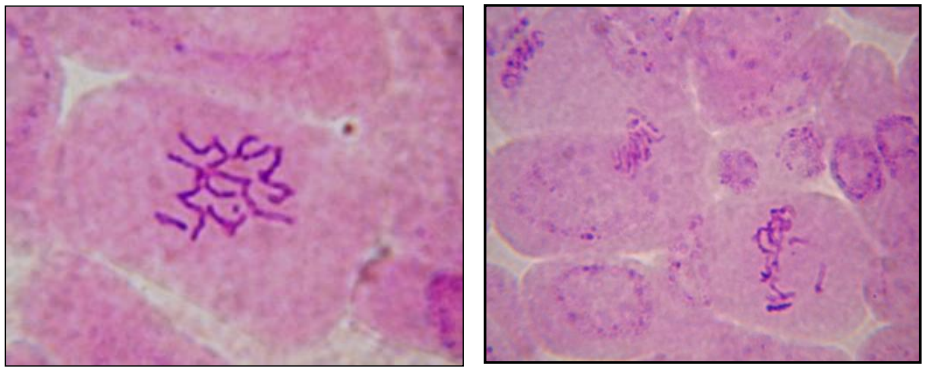

Fig. 4. C-metaphase (left) and metaphase with late chromosomes

The values registered for tissue cultured plants were similar with seed born plants, slightly higher, the difference between them being not significant. The highest number of aberrations were detected at plants regenerated on culture media supplemented with kinetin, which suggests that BAP is a much proper

growth regulator. We also detected abnormalities is metaphases that were abnormally organized, with ring chromosomes, minutes, expelled chromosomes, fragment, C-metaphases etc (figure 4).

In a smaller number we detected prophases that presented different types of chromosomal aberrations like late prophases, with ring chromosomes, expelled chromosomes etc.

Both IS 21 and IS57 genotypes had the same cytogenetic behavior, the plants regenerated from "in vitro" culture presenting abnormalities in similar percentages as plants obtained from seeds.

CONCLUSIONS

1. Among the plants regenerated "in vitro", the highest number of aberrations were detected at plants regenerated on culture media supplemented with kinetin, which suggests that BAP is a much proper growth regulator. The cultivation of cabbage shoot tips on nutritive medium modified with BAP allows the regeneration of new plants with a stable genetic material that shows little genetic variability.

2. Both IS 21 and IS57 genotypes had the same cytogenetic behavior, the plants regenerated from "in vitro" culture presenting abnormalities in similar percentages as plants obtained from seeds.

3. No significant differences were observed between tissue cultured plants and seed born plants suggesting that genetic fidelity of tissue cultured plants can be maintained if appropriate plant growth regulators are used with less number of subcultures in the multiplication stage.

4. The main types of aberrations identified at regenerated plants and seed-born plants are: contraction, stickiness, fragmentation, inter-chromatin bridges, ring chromosomes, C-mitosis.

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