# EFFECT OF „IN VITRO" PLANT GROWTH REGULATORS OVER THE MERISTEMATIC AND MITOTIC ACTIVITY AT DIFFERENT GENOTYPES OF *CAPSICUM ANUUM* L.

## EFECTELE INDUSE DE HORMONII DE CREŞTERE UTILIZAŢI „*IN VITRO*" ASUPRA ACTIVITĂŢII MITOTICE ŞI MERISTEMATICE LA DIFERITE GENOTIPURI DE *CAPSICUM ANUUM* L.

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**Abstract.** *The multiplication „in vitro" of pepper genotypes is a tool frequently utilized due to its viability when comparing with classical methods. This viability is due to the high efficiency expressed in the number of regenerated plants but especially due to the possibility of obtaining of plants with a low degree of somaclonal variability, strictly keeping the genetic inheritance of donor plants. The covering of a life stage "in vitro" is generally a stage in which the different environmental factors acts like stressing factors for plants. The effects induced by these factors with synergic activity are located at cellular level through the alteration of meristematic cycle, through the apparition of different types of chromosomal aberrations, through and increase or decrease of prophase index, metaphase index, etc. The present study concentrates on the determination of influence of hormonal formuli utilized in the multiplication of valuable genotypes, over the mitotic and meristematic activities at pepper. The selection of a hormonal formuli that should allow the limitation of this somaclonal variability to a level similar with the level in which the natural variability occur represents an important premise for the "in vitro" multiplication process. The study in root meristematic tips allows the selection in early stages of a hormonal variant that determines the lowest number of chromosomal aberration as well as that allows the support of mitotic activity of meristematic cells.*

**Rezumat.** *Multiplicarea „in vitro" a genotipurilor valoroase de ardei este o practică des utilizată datorită fiabilităţii acestei metode comparative cu metodele clasice. Această fiabilitate este dată pe de o parte de eficienţa exprimată în numărul de plante generate dar mai ales datorită posibilităţii obţinerii unor plante cu un grad de variabilitate genetică mic, păstrându-se nealterate zestrea genetică a plantelor donor. Parcurgerea unei etape de viaţă "in vitro" este în general o perioadă în care diferiţii factori de mediu acţionează ca factori de stress. Efectele induse de aceşti factori cu acţiune sinergică se regăsesc la nivel celular prin alterarea ciclului meristematic, prin apariţia diferitelor tipuri de aberaţii cromosomiale, prin creşterea sau scăderea indicelui profazic, metafazic etc. Prezentul studiu se concentrază pe determinarea influenţei formulei hormonale utilizate în procesul de multiplicare a genotipurilor valoroase asupra activităţii mitotice şi meristematice la ardei. Selecţionarea unei formule hormonale care să permită limitarea variabilităţii somaclonale la o valoare similară variabilităţii naturale reprezintă o premiză importantă pentru micropropagarea „in vitro". Studiul în meristemele*

*radiculare ale plantelor regenerate permite selecționarea încă din stadii timpurii a variantei hormonale care determină cel mai mic număr de alterații cromosomiale precum şi care permite susținerea activității mitotice a celulelor meristematice.*

# INTRODUCTION

The literature tackled the problem of chromosomal studies that regards the evolution of cells division of *Capsicum* but the studies focused especially toward the following aspects: the influence of different types of mutagen or non-mutagen substances like caffeine (Rosu et al., 2006), or cytogenetical study in inter-varietal crosses (Raghuvanshi, 1991, Mascone, 1992, 1993, 1999), chromosome numbers in wild and semi-domesticated varieties (Pozzobon, 2006), cytogenetic studies of F1 hybrids (Panda, 2004, Pickersgill, 1991, Bapa, 1992), etc and less on the influence of "in vitro" stage.

In what concern cytogenetics, karyotype aspects have been studied in wild and domesticated species (Pickersgill 1971, 1977, 1991, Limaye and Patil 1989, Moscone 1990, 1993, 1999, Bertão 1993, Moscone *et al.* 1993, 1995, 1996, Tong and Bosland 1997, 2003, Ferreira 1998, Park *et al.* 2000). Meiotic behavior evaluation has been performed in wild and domesticated species as well as in some hybrids, aiming at verifying genomic diversification during evolution as well as possible inter-specific phylogenetic relations (Otha 1961, Lippert *et al.* 1966, Shopova 1966a, 1966b, Carluccio and Saccardo 1977, Pickersgill 1971, 1977, 1991, Saccardo and Ramulu 1977, Egawa and Tanaka 1984, Mirkova and Molchova 1985, Kumar *et al.* 1987, Raghuvanshi and Saxena 1991, Moscone 1992, Bapa Rao *et al.* 1992, Lanteri and Pickersgill 1993, Tong and Bosland 1999, Panda *et al.* 2004).

The present study concentrates on the determination of influence of hormonal formuli utilized in the multiplication of valuable genotypes, over the mitotic and meristematic activities at pepper. The selection of a hormonal formuli that should allow the limitation of this somaclonal variability to a level similar with the level in which the natural variability occur represents an important premise for the "in vitro" multiplication process. The study in root meristematic tips allows the selection in early stages of a hormonal variant that determines the lowest number of chromosomal aberration as well as that allows the support of mitotic activity of meristematic cells.

## MATERIAL AND METHODS

The biological material is represented from 3 genotypes of pepper (*Capsicum anuum* L.) from Vegetable Research and Development Station Bacau, Romania. The seeds were utilized for the "in vitro" multiplication of these valuable genotypes and the meristematic root tips were excised from the "in vitro" plantlets regenerated on D1-D3 variants, characterized through the presence of BAP and Kinetin alone or in association with BAP- table1.

The control variant is represented by plants germinated "ex vitro" in Petri dishes. The cytogenetic studies were accomplished in meristematic root cells, stained in Carnoy fixing solution for 24 hours at $4^0$C then hydrolyzed with HCl for 7 minutes and colored with the basic coloring solution Carr. The root meristems were displayed using squash technique and for each genotype and variant 6000 cells were counted.

*Table 1*

**Experimental variants utilized in the cytogenetic studies at *Capsicum anuum* L.**

| Components | D0 | D1 | D2 | D3 |
|---|---|---|---|---|
| Macro elements | seeds germinated "ex vitro" | 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 ‰ |

Chromosome slides were then observed microscopically. Numbers of dividing cells at different levels of mitosis were recorded. Mitotic data were subjected to statistical analysis by calculating the mitotic index (% cells in division per total number of examined cells), prophasic index (% cells in prophases per total number of examined cells), metaphasic index (% cells in metaphases per total number of examined cells), anaphasic index (% cells in anaphase per total number of examined cells) and telophasic index (% cells in telophase per total number of examined cells).

## RESULTS AND DISCUSSIONS

The main indexes (mitotic index, prophasic index, metaphasic index, anaphasic index, telophasic index) calculated for each genotype in controls and on hormonal formuli are shown in table 2,3,4.

***Table 2***

**The values of the main cellular indexes monitorised in the root tips of BENDINGO F1genotype**

| Hormonal variant | Repartition of the main division indexes | | | | |
|---|---|---|---|---|---|
| | Mitotic index | Prophasic index | Metaphasic index | Anaphasic index | Telophasic index |
| **Control** | 29,98 | 56,01 | 29,90 | 7,18 | 6,89 |
| **D1** | 39,84 | 47,74 | 33,21 | 9,65 | 9,27 |
| **D2** | 32,27 | 54,76 | 54,76 | 9,07 | 5,07 |
| **D3** | 32,34 | 53,89 | 32,18 | 7,99 | 5,92 |

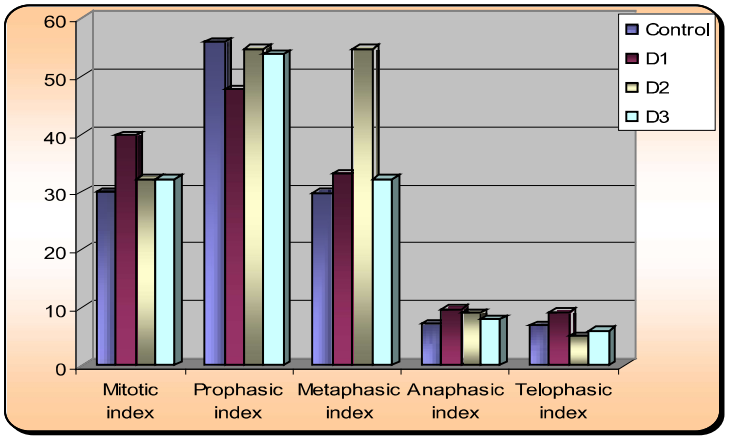


**Fig. 1 – Graphical representation of the main indexes observed in root meristematic cells – genotype Bendingo F1**

*Table 3*

**The values of the main cellular indexes monitorised in the root tips of FIESTA F1genotype**

| Hormonal variant | Repartition of the main division indexes | | | | |
|---|---|---|---|---|---|
| | Mitotic index | Prophasic index | Metaphasic index | Anaphasic index | Telophasic index |
| **Control** | 33,78 | 56,87 | 13,80 | 13,36 | 15,95 |
| **D1** | 40,87 | 47,00 | 26,18 | 15,17 | 11,64 |
| **D2** | 34,90 | 50,05 | 24,89 | 13,54 | 11,51 |
| **D3** | 40,16 | 50,04 | 27,21 | 10,74 | 11,99 |

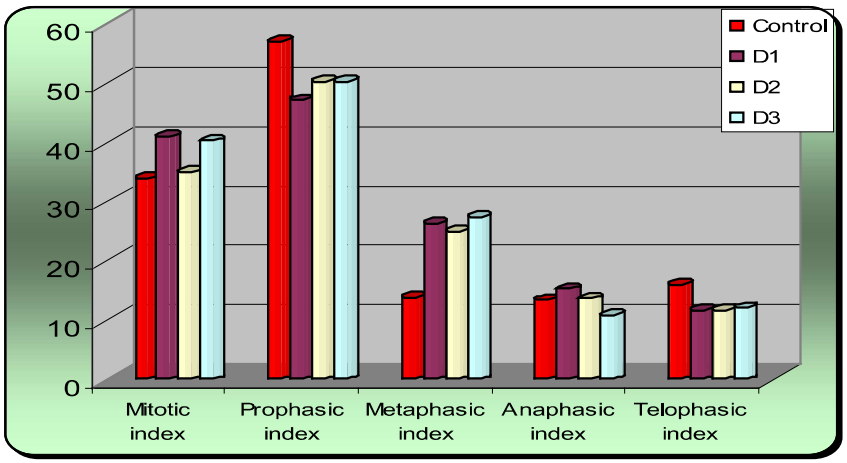


**Fig. 2 – Graphical representation of the main indexes observed in root meristematic cells – genotype Fiesta F1**

*Table 4*

**The values of the main cellular indexes in the root tips of CERES genotype**

| Hormonal variant | Repartition of the main division indexes | | | | |
|---|---|---|---|---|---|
| | Mitotic index | Prophasic index | Metaphasic index | Anaphasic index | Telophasic index |
| **Control** | 29,13 | 51,75 | 14,34 | 21,79 | 12,10 |
| **D1** | 32,09 | 52,73 | 14,87 | 21,44 | 10,94 |
| **D2** | 28,25 | 49,96 | 25,94 | 17,79 | 6,29 |
| **D3** | 31,88 | 55,66 | 16,10 | 16,28 | 11,94 |

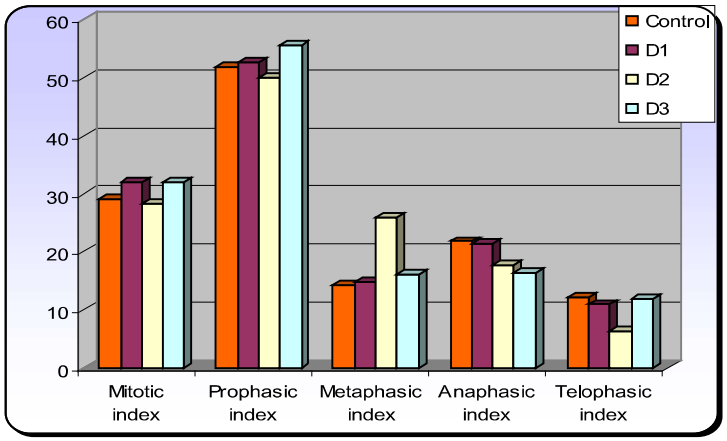


**Fig. 3– Graphical representation of the main indexes observed in root meristematic cells – genotype Ceres**

As shown in Fig. 1, 2, 3 the exposure to “in vitro” cultivation conditions determined positive modifications of the mitotic index. At all the genotypes utilized in the present study, when comparing with the control variant (seeds germinated “ex vitro”), the frequency of dividing cells is much superior to the control.

The cultivation of the excised pepper explants on different “in vitro” media did not affect the distribution of the cells in the mitosis cycle. At all three genotypes the main numbers of cells were in prophases, then in metaphases, anaphases and telophases. Only at Ceres genotype the anaphasic index was larger then the telophasic one. Due to the fact that this modification in the distribution of cells phases was observed also on the control variant, suggest that is not determined by the cultivation technique but by genetic or other physical factors.

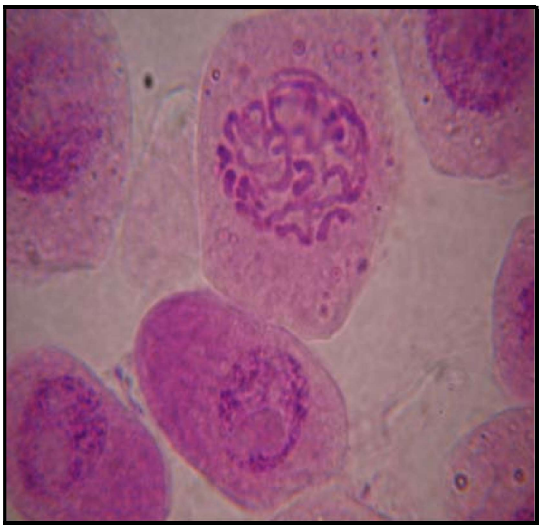

**Fig 1 – Cells in interphases and prophases**

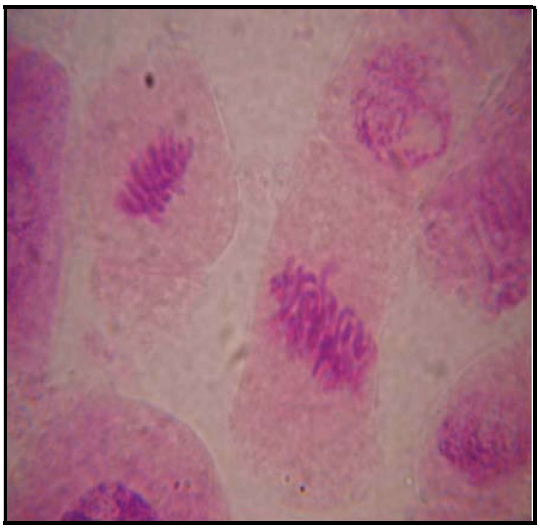

**Fig 2 – Cells in metaphases**

**Fig 3 – Cells in anaphase**

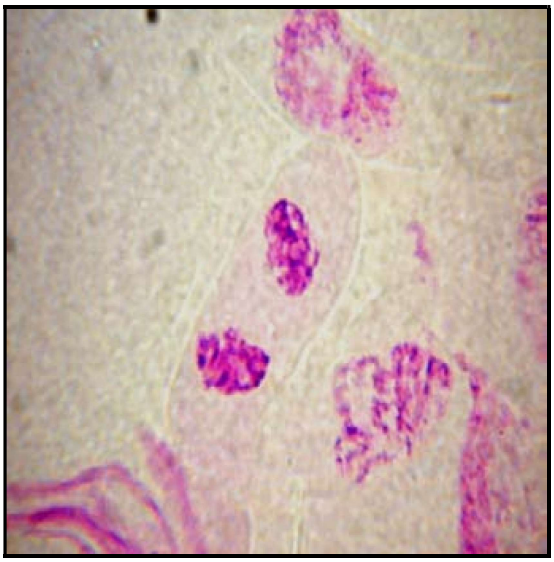

**Fig. 4 - Cells in telophase**

## CONCLUSIONS

The results obtained in our experiment proved that the exposure to "in vitro" cultivation conditions determined positive modifications of the mitotic index. At all the genotypes utilized in the present study, when comparing with the control variant (seeds germinated "ex vitro"), the frequency of dividing cells is much superior to the control.

The cultivation of pepper shoot tips on nutritive medium modified with Kinetin and BAP allows the regeneration of new plants with a stable genetic material that shows little modification in the spectrum of the cells distribution in mitosis. At all three genotypes the main numbers of cells were in prophases, then in metaphases, anaphases and telophases.

## REFERENCES


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