STUDIES REGARDING THE MITOTIC DIVISION AND CHROMOSOMAL ABERRATIONS AT *ECHINACEA ANGUSTIFOLIA* D.C.

STUDII PRIVIND DIVIZIUNEA MITOTICĂ ŞI ABERAŢIILE CROMOSOMIALE LA *ECHINACEA ANGUSTIFOLIA* D.C.

CRISTEA Tina Oana[1], FALTICEANU Marcela[1], PRISECARU Maria[2]
[1]Research and Development Station for Vegetable Growing Bacau, Romania
[2]University of Bacau, Faculty of Biology, Romania

Abstract. *Native to North America, Echinacea species have an important place in herbal medicine. Echinacea has long been used by Native Americans to treat many conditions, including venomous bites, rabies, cold, headache, and stomach cramps (Foster, 1991; Kindscher, 1989; Li, 1998). Its non-selective, immune-enhancing properties have promoted the use of and demand for Echinacea products in recent years and, consequently, its field production. Understanding the cytogenetics of the plant has a key role for controlling the „in vitro" behavior of different explants by a better understanding of the influence of these peculiar conditions over the growth processes. Echinacea angustifolia plants are diploids with a somatic chromosome number of 2n = 22. The study focused toward the determination of the main cellular indexes (mitotic index, prophase index, metaphase index, telophase index and anaphase index) as indicators of growth and development processes speed. In order to have more accurate results, the studies were accomplished in the root meristematic tips on three different plants, originated from Vegetable Research and Development Station Bacău, Romania.*

Key words: anaphase, telophase, metaphase, genotype, development**.**

Rezumat. *Native în America de Nord, speciile de Echinacea au o importanţă majoră în medicina naturistă. Utilizarea plantelor de Echinacea are rădăcini adânci, fiind folosită prima dată de indieni pentru tratarea diferitelor boli, inclusiv muşcăturile veninoase, rabie, răceală, dureri de cap şi crampe stomacale (Foster, 1991; Kindscher, 1989; Li, 1998). Proprietăţile lor de stimulare a imunităţii au determinat creşterea popularităţii acestor plante, cererea de produse pe bază de Echinacea fiind din ce în ce mai crescută, ceea ce a condus în mod firesc la creşterea suprafeţelor destinate producerii lor. Determinarea aspectelor citogenetice ale unei plante joacă un rol crucial într-o mai bună înţelegere a principiilor care stau la baza dezvoltării lor, precum şi a modului în care condiţiile specifice de cultivare „in vitro" afectează aceste procese de creştere şi dezvoltare. După cum este bine cunoscut, plantele de Echinacea angustifolia D.C. sunt plante diploide, cu un număr de cromosomi somatici 2n=22. Prezentul studiu se referă la determinarea principalilor indici celulari (indicele mitotic, indicele profazic, metafazic, anafazic şi telofazic), ca indicatori ai vitezei proceselor de creştere şi dezvoltare. Pentru o mai mare acuratețe a rezultatelor testele au fost realizate în meristemele radiculare aparţinînd la trei plante diferite provenite de la Staţiunea de Cercetare Dezvoltare pentru Legumicultură Bacău, Romania.*

Cuvinte cheie: anafază, telofază, metafază, genotip, dezvoltare**.**

INTRODUCTION

Cultivation of *Echinacea* has gained a great importance at international level and it is more and more cultivated in many countries, including the United States, Canada, Germany, Norway, Romania, Finland, Australia, Poland, Russia, New Zealand, Egypt, and China (Dou et al., 2001). The importance of the plant is due to its multiple therapeutic utilities: including venomous bites, rabies, cold, headache, and stomach cramps. But, even most important is its utility in enhancing the immunity of the organisms.

In addition to its medicinal uses, *Echinacea* has enormous ornamental potential. *E. purpurea*, the only species for which ornamental cultivars have been bred, is both productive and profitable as a field grown specialty cut flower (Starman et al. 1995). In fact, *E. purpurea* is the only species of the genus which has been domesticated thus far. It is interesting to note that the cultivars of *E. purpurea* that are now in production as source materials for herbal extracts were actually developed for ornamental purposes. Commercial field plantings of the other species in the genus have been sown from generally unimproved, wild seed. Plant breeders have an important task and a number of challenges before them.

Echinacea species are hardy, herbaceous perennials with either simple or branched stems. The terminal single flowering heads have fertile disc florets that terminate in spines (paleae). These are surrounded by infertile drooping or spreading ray flowers that have 2 or 3 teeth at each end. The leaf shape varies from lanceolate to ovate, its margin may be dentate and the leaf may be pubescent or smooth. Roots are either single taproot or fibrous in form (6–11).

Since the increasing development of genetic methods, the chromosomal data has become a valuable tool both for cytogenetic specialists and for breeders. These studies focused on chromosomes are often employed for suggesting taxonomic and phylogenetic relationships (Stuessy, 1990). Until now, at *Echinaceae* species, few information are available regarding the main cellular indexes like: mitotic index, prophase, metaphase, anaphase, telophase index, the incidence of abnormalities in normal cells, chromosome features and behaviour, phylogenetic of the cultivars, etc. The rate of cell division has been depicted to reflect the rate of increase in size and weight. In addition, good mitotic indexing will generate information available for a better characterization of *Echinacea angustifolia* germplasm collections. In generally, good knowledge of the Echinacea genomes is a quite important goal for the establishment of a sound approach to its improvement.

MATERIAL AND METHODS

Root tips from germinating seeds were used for chromosome preparation. *Echinacea angustifolia* var. *angustifolia* seeds were provided by Vegetable Research and Development Station Bacau, Romania.

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 colored with the basic coloring solution Carr. The root meristems were displayed using squash technique and for each genotype and variant 2000 cells were counted.

Fig. 1. The biological material utilized in our experiments (seeds – before and after the germination)

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). In the same time we monitored the incidence of abnormalities in ana-telophasic stage.

RESULTS AND DISCUSSIONS

The main indexes (mitotic index, prophasic index, metaphasic index, anaphasic index, telophasic index) calculated for each plant are shown in table 1, 2.

Table 1

The number of cells identified in different phases of mitotic cycle at *Echinacea angustifolia* D.C. plants

Variant	Total no of cells analyzed	Interphase	No. of cells in active division	Repartition of cells in different division phases			
				P*	M*	A*	T*
Plant 1	2010	1623	387	209	108	43	27
Plant 2	1980	1589	391	206	104	47	34
Plant 3	2045	1629	416	221	116	45	34
Media	*2012*	*1614*	*398*	*212*	*109*	*45*	*32*

*** P – prophase, M – metaphase, A – anaphase, T - telophase**

Table 2

The values of the main indexes registered in the meristematic cells of *Echinacea angustifolia* D.C. plants

Variant	IM	Repartition of cells percentage/phases of division			
		%P	%M	%A	%T
Plant 1	19,25	54,00	27,90	11,11	6,97
Plant 2	19,75	52,68	26,59	12,02	8,69
Plant 3	20,33	53,12	27,88	10,81	8,17
Media	***19,78***	***53,27***	***27,46***	***11,31***	***7,94***

At all the three plants utilised in our research the values obtained are similar, which denotes the fact the values of media calculated for each type of index are the correct one that represents the characteristic of the repartition of cell phases in the experimental condition tested in the present study.

As it is shown also in table 1 and 2 most of the cells are in prophase (53.27%), followed by metaphase (27.46%), anaphase (11.31%) and telophase (7.94%) - fig. 3-8. The value of the mitotic index was 19.78, which denotes that the plant was in a phase of active growth.

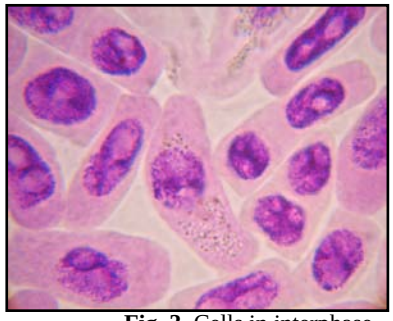

Fig. 3. Cells in interphase

Fig. 4. Cells în prophase

Fig. 5. Cells in methaphase

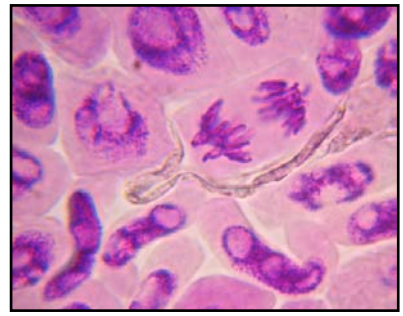

Fig. 6. Cells in anaphase

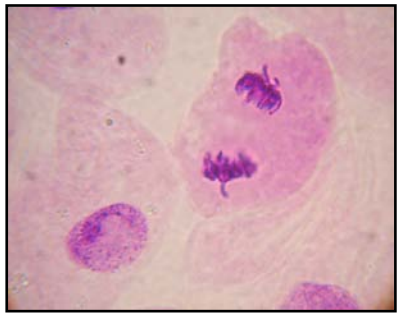

Fig. 7. Cells in telophase

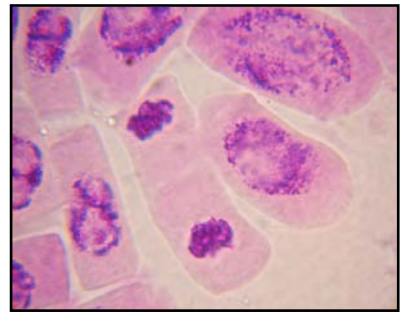

Fig. 8. Cells in late telophase

In what concern the main types of abnormalities observed in the root cells of *Echinacea*, most of them were ana-telophases with simple or multiple bridges

and ana-telophases with fragments, but also expelled or late chromosomes and multipolar ana-telophases – table, 3 and figure 9 - 12.

Table 3

The frequency of cells with chromosomal aberrations and their spectrum identified in the ana-telophase of *Echinacea angustifolia* D.C. plants

Variant	Total A-T studied	A-T aberrance %	$\bar{x} \pm s_{\bar{x}}$ %	from which	
				A-T with bridges%	A-T with fragments%
Plant 1	158	4,43	4,96±0,07	54,2	36,8
Plant 2	192	2,60	2,84±0,06	48,1	51,8
Plant 3	153	1,57	1,57±0,05	50,3	49,5

All the three plants had the same cytogenetic behaviour, the frequency of chromosomal aberrations in ana-telophase being relatively low. The main types of abnormalities were A-T with bridges.

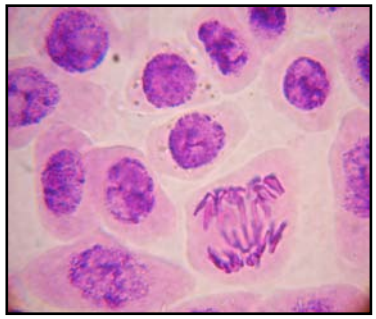

Fig. 9. A-T with multiple bridges

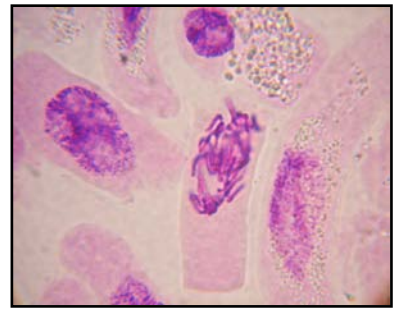

Fig. 10. Disorganised ana-telophase

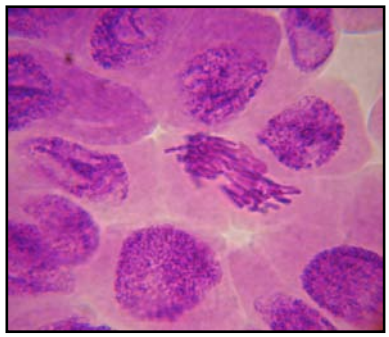

Fig. 11. A-T with multiple bridges

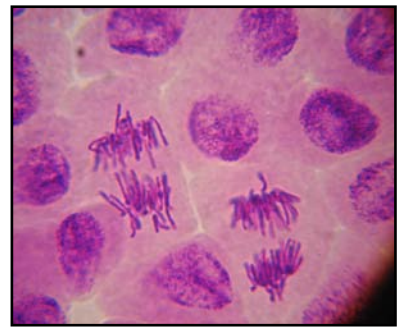

Fig. 12. A-T with delayed chromosomes

But we also observed metaphases with lagging chromosomes, expelled chromosomes or ring chromosomes, multipolar ana-telophases, as well as binucleate cells and interphases with micro-nucleuses – fig. 13 and 14.

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

Fig. 13. C-metaphase

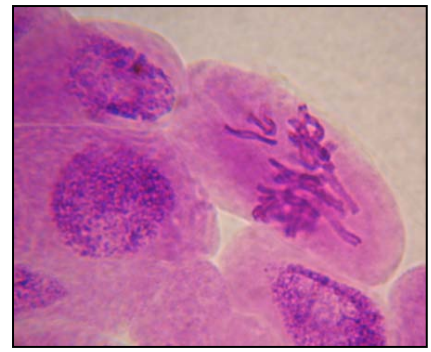

Fig. 14. Metaphase with expelled chromosomes

CONCLUSIONS

The study focused toward the determination of the main cellular indexes (mitotic index, prophase index, metaphase index, telophase index and anaphase index) as indicators of growth and development processes speed. In order to have more accurate results, the studies were accomplished in the root meristematic tips on three different plants, originated from Vegetable Research and Development Station Bacău, Romania.

The results obtained in our study showed that most of the cells are in prophase (53.27%), followed by metaphase (27.46%), anaphase (11.31%) and telophase (7.94%) - fig. 3-8. The value of the mitotic index was 19.78, which denotes that the plant was in a phase of active growth.

In what concern the main types of abnormalities observed in the root cells of Echinacea, most of them were ana-telophases with simple or multiple bridges and ana-telophases with fragments, but also expelled or late chromosomes and multipolar ana-telophases. But we also observed metaphases with lagging chromosomes, expelled chromosomes or ring chromosomes, multipolar ana-telophases, as well as binucleate cells and interphases with micro-nucleuses. In a smaller number we detected prophases that presented different types of chromosomal aberrations like late prophases, with ring chromosomes, expelled chromosomes etc.

REFERENCES

1. **Kindscher K., 1989** - *Ethnobotany of purple coneflower (Echinacea angustifolia, Asteraceae) and other Echinacea species*. Econ. Bot.;43:498–507.
2. **Li M., M. Zhang, 1991** - *Technology for plant chromosome research.* Northwest Forest Univ. Press, Shenyiang, China. p. 31–39.
3. **Tuna M., Gill K.S, Vogel K.P., 2001** - *Karyotype and C-banding patterns of mitotic chromosomes in diploid bromegrass (Bromus riparius Rehm).* Crop Sci.;41:831–834.