

Effect of 5-Aminouracil on mitotic cell division in *Allium cepa*

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ABSTRACT. Cytological preparations of *Allium cepa* meristematic cells were examined under a light microscope to evaluate the effect of prolonged exposure (at least two-cell cycle) to 5-aminouracil (5-AU) on mitotic cell division in the roots. The results are a cytological demonstration that a lower concentration of 5-AU than usually employed for synchronizing proliferating cells in *A. cepa* root meristems induces chromosomal abnormalities and formation of micronucleus but does not fully block the cell flow into mitosis, and that micronucleated cells can proceed in mitosis and contribute to the enhancement of root length values reported by other authors. *A. cepa* root tip cells seems to support a certain damage level in DNA (around 10% of micronucleated cells in mitosis) without having any negative effect on cellular proliferation and growth of the roots. This genetic flexibility of vegetable genomes may permit an attenuation of the adaptative metabolic-response to sustained adverse environmental conditions.

Key words: 5-AU, onion, micronucleus, cell proliferation.

RESUMO. Efeito de 5-Aminouracil na divisão mitótica de células de *Allium cepa*. A exposição de células meristemáticas de *Allium cepa* por dois ciclos de divisões (48 horas), em soluções contendo concentrações mais baixas de 5-aminouracil (50, 100 e 150 μ M) do que as usualmente empregadas para proceder à sincronização da proliferação celular, mostrou que as concentrações usadas causam anormalidades cromossômicas e formação de micronúcleos, mas não bloqueiam a entrada destas células em mitose. As células em mitose e com micronúcleos podem prosseguir em divisão e assim contribuir para o crescimento das raízes de *A. cepa* expostas às concentrações de 5-aminouracil estudadas. As células meristemáticas da raiz de *A. cepa* parecem suportar um certo nível de danos não reparados no DNA, os quais não impedem a divisão celular e o conseqüente crescimento dos tecidos. Esta flexibilidade genética do genoma de vegetais deve se constituir numa resposta metabólica adaptativa para sustentar condições ambientais adversas.

Palavras-chave: 5-AU, cebola, micronúcleo, proliferação celular.

It is known that blocking DNA replication with inhibitors of DNA synthesis such as hydroxyurea or 5-aminouracil (5-AU) prevents somatic cells from progressing through the S phase into the G2 and M phases. Thus, a common feature in the cell cycle of probably all somatic eukaryotic cells is that completion of DNA replication is a prerequisite for cell division, and probably for tissue growth (Alberts *et al.*, 1994; Lewin, 1994).

The 5-AU is a structural analogue of thymine that inhibits DNA synthesis at concentrations as low as 0.5 μ M after 14 hours of treatment, producing a block in the middle of the S period and a reduced rate of cell proliferation in *Allium cepa* root tip cells

(Navarrete *et al.*, 1984). Low concentrations of 5-AU offered advantages for synchronizing proliferating cells and since their action was readily reversible by removing the drug, the cytotoxic effects were avoided (Clowers, 1965). However, their effect on proliferating cells elicited the typical response induced by DNA-damaging agents, such as mitotic delay, and a potentiation of chromosome damage by caffeine post-treatment (Gonzalez-Fernandez *et al.*, 1985; Pepper *et al.*, 1988).

On the other hand, a continuous and prolonged exposure at ten times lower 5-AU concentrations (50 μ M) produced an unexpected growth-stimulating effect on *Cereus peruvianus* callus tissue

culture and induced the root growth of *Allium cepa* (Mangolin and Machado, 1997). A higher length was observed in roots cultured in solutions containing different concentrations of 5-AU, compared to root growth in water without 5-AU.

Since there is few information that cover the direct effects of 5-AU on mitotic cells (López-Sáez *et al.*, 1988), the present study was undertaken to evaluate the effect of 5-AU after a prolonged time of exposure (at least two-cell cycles) on mitotic cell division in *A. cepa* roots. The cytological preparations were examined under a light microscope to score possible mitotic and chromosomal aberrations and cells with micronuclei.

Material and Methods

Equal-sized bulbs were chosen from a population of a commercial variety of *A. cepa* and were grown in the dark at a temperature of $26 \pm 1^\circ\text{C}$ in a cylindrical glass container, in tap water which was renewed every 24 hours and continuously aerated by bubbling air. When the roots growing in tap water reached 10–20mm in length the onion bulbs were transferred to 0, 50, 100 and $150\mu\text{M}$ 5-AU (Sigma) solution in tap water according to the procedure previously reported by Mangolin and Machado (1997).

Three bulbs were exposed to each 5-AU concentration and at the end of 48 hours three roots were collected from each bulb, and the root length was measured (Mangolin and Machado, 1997). The root tips were prepared for light microscopic examination by squashing and staining by Feulgen method. One thousand cells per root were analyzed to determine the number of cells in metaphase and anaphase, the frequency of these meta-anaphase with chromosomal abnormalities, as well as the frequency of cells with micronuclei.

Results

The cytological analysis of root meristems revealed that 5-AU induced mitotic alterations and micronuclei, whereas no alterations were found in

the water-treated cells (control cells). Table 1 shows the incidence of chromosomal alterations and formation of micronuclei in *A. cepa* root tip cells after exposure to three 5-AU concentrations.

The mitotic alterations recorded in metaphase and anaphase cells were abnormal figures compared to that typical metaphase and anaphase, metaphase and anaphase with lagging chromosome(s), cells with chromosome fragments, and chromosome bridges in anaphase. All of them can be considered as mitoses with abnormal spindle function (Figure 1). Relatively large and small micronuclei were observed in interphase, or differentiating cells, and mitotic cells. The scored chromosomal alterations were expressed as percentages in Figure 2. The frequencies of chromosomal abnormalities and micronuclei were increased with higher 5-AU concentrations (Figure 2).

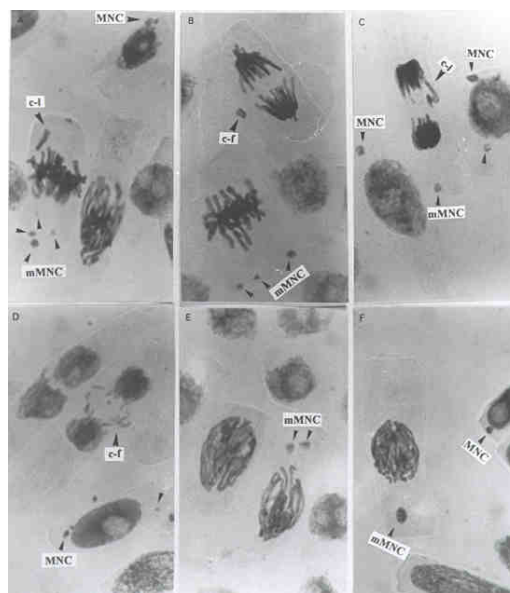


Figure 1. Interphase and mitotic nuclei of *Allium cepa* root tip cells showing mitosis with chromosomal abnormalities. Metaphase and anaphase with lagging chromosomes (l-c) in A and C, and chromosome fragments (f-c) in B and D. A-F show cells with micronuclei in interphase (MNC) or in mitosis (mMNC) after exposure to 50, 100 and $150\mu\text{M}$ 5-AU concentrations. [Obj. 100x/Axioskop-Zeiss]

Table 1. Effect of 0, 50, 100 and $150\mu\text{M}$ 5-aminouracil (5-AU) on frequency of mitotic phases (metaphases: m, and anaphases: a) with chromosomal abnormality (l-c: lagging chromosome; a-maf: abnormal figures of metaphase and anaphase; c-f: chromosome fragments; b-c: chromosome bridges in anaphase), and on frequencies of meristematic cells of *Allium cepa* with micronucleus (MNC/1.000 cells) and mitotic cells with micronucleus (mMNC)

Concentration 5-AU (μM)	Analysed cells		Total	Chromosomal abnormality				Total	%	Micronucleus (%)	
	m	a		c-l	maf-a	c-f	b-c			MNC	mMNC
0	100	63	163	0	0	0	0	0	0.0	0	0
50	83	29	112	5	0	5	0	10	8.9	5.6	5.3
100	118	55	173	14	1	3	2	20	11.5	9.2	3.6
150	135	56	191	6	1	16	1	24	12.6	10.8	3.6

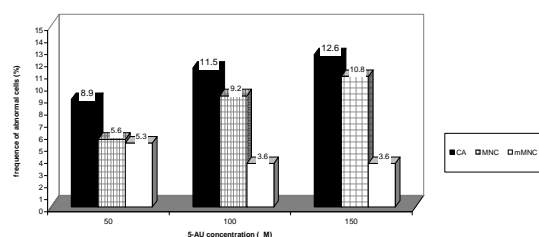


Figure 2. Frequencies of chromosomal alterations (CA) and of micronucleated cells (MNC) after exposure to 50, 100 and 150 μ M 5-AU concentrations in *Allium cepa* root tip cells. Micronuclei were also recorded in mitotic cells (mMNC)

Discussion

Among the several cytological studies in which root meristems of *Allium cepa* were exposed to chemical agents (for a review see Ma *et al.*, 1995), the descriptions of mitotic abnormalities, in general, are associated with or followed by inhibition of root tip growth (Panda *et al.*, 1992; Rank *et al.*, 1993). However, different results were observed in the present study with exposure of *A. cepa* root tip cells to different 5-AU concentrations. A 5-AU concentration-response effect was apparent with respect to root growth, frequency of mitosis with abnormalities and cells with micronuclei. The mean-root length of *A. cepa* growing in zero, 50, 100 and 150 μ M of 5-AU aqueous solution for 48 hours was 10.3, 28.0, 25.3 and 21.2 mm, respectively (Mangolin and Machado, 1997)

A possible explanation for the higher root length values found by Mangolin and Machado (1997) is that interphase cells double their mass but if they have no capacity for DNA replication, they can pause in their progress along the cycle and enter Go or are moved upward to lengthen the root structure where they can remain for an indefinite time. Thus, a cell population with double volume should contribute to the greater root extension. Furthermore, in the present study a relative proportion of the micronucleated cells proceeded in mitosis despite their damaged chromosomes. Micronucleated cells proceed in mitosis with damaged chromosomes and should also contribute to the greater root extension. It is shown that the checkpoint to prevent cells with damaged DNA from entering mitosis until the damage has been repaired (Ferreira *et al.*, 1994) is absent in *A. cepa* root tip cells exposed to 50, 100, 150 μ M of 5-AU concentrations.

The small micronuclei observed indicate that 5-AU acted as a clastogenic agent. The *Allium* micronucleus assay has been used as a standard

genotoxicity assay (Dash *et al.*, 1988; Panda *et al.*, 1989; Panda *et al.*, 1995). Micronuclei are the result of acentric chromosome fragments as well as of changed spindle function (Degrassi and Rizzoni, 1982; Schmid, 1982). A large micronucleus indicates that the agent interferes in some way with the mitotic spindle (Yamamoto and Kikuchi, 1980; Hogstedt and Karlsson, 1985) or with some other structures or processes involved in chromosome distribution (Brinkley *et al.*, 1985; Onfelt, 1986), while the small micronuclei indicate that chemical agents act as clastogenic agents (Von Ledeber and Schmid, 1973; Heddle and Carraro, 1977).

Chromosome break could be interpreted as due to the absence of chromatin proteins or alterations of the DNA replication processes (Brinkley *et al.*, 1985; Vig, 1987; Vig and Paweletz, 1988; Basic-Zaninovic *et al.*, 1991). Atypical metaphase and anaphase, as well as lagging chromosomes observed in *A. cepa* root meristems indicate that 5-AU interferes with the proteins of the mitotic spindle. This interference may represent an interference on the protein synthesis and so extend to chromatin proteins to produce chromosomal breaks.

Thus, the results of the present study show that 5-AU does not always cause the same effects on proliferating cells as described by González-Fernández *et al.* (1985) and Pepper *et al.* (1988). The lower concentration of 5-AU than usually employed for synchronizing proliferating cells acted in a rather complex way, causing a variety of different genetic damages to *A. cepa* roots which showed a higher growth rate (Mangolin and Machado, 1997) and unchanged morphology as well as unchanged development of leaves (results not shown). Our data show that the micronucleated cells can proceed in mitosis and contribute to a greater root extension in *A. cepa* bulbs. Therefore, it seems that root-tip cells of *A. cepa* can support a certain damage level in DNA (around 10% of micronucleated cells in mitosis) without having any negative effect on cellular proliferation and an growth of the roots. This genetic flexibility of vegetable genomes may permit an attenuation of the adaptative metabolic-response to sustained adverse environmental conditions.

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