

SOME CYTOGENETIC EFFECTS OF SODIUM AZIDE TREATMENTS IN CARAWAY ROOT MERISTEMS

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Abstract: Sodium azide (NaN₃) still remains a popular plant mutagen. In the present investigation, its effects on the cytogenetic changes were studied in root tip cells of *Carum carvi* L., an important economical and medicinal crop plant. The study revealed that sodium azide decreased mitotic index, and caused increase of chromosomal aberrations. Altogether, sodium azide treated root tip cells exhibited an increased incidence of bridges, lagging and/or expelled chromosomes and C-metaphases.

INTRODUCTION

Cytological analysis with respect to mitotic behavior is considered to be one of the most dependable indicator for estimate the potency of mutagen. It also provides a considerable clue to assess sensitivity of plants to different mutagens (Bhat et al., 2007). Many researches have compared the mutagenic efficiencies of different agents on different crops. Their results seem to be entirely specific for particular species and even varieties. While many of these like Rao and Rao (1983), Kumar and Dubey (1998), Kumar and Singh (2003), Dhanayanth and Reddy (2000) and Bhat et al. (2005) found chemical mutagens to be more effective than physical ones, others like Tarar and Dnyansager (1980), Zeerak (1980) and Singh (2003) found the reverse case.

Sodium azide (NaN₃) is a major environmental mutagen as it is used in medicine, agriculture etc. (Kleinhofs and Smith, 1976) and it causes cytotoxicity in several animal and plant test systems, by inhibiting the protein synthesis and replicative DNA synthesis at low dosages (Grant and Salamone, 1994). It acts directly on resting nucleic acids (Butnaru, 1985) and it is known as strongly mutagen in cereals and legumes, as inhibitor of respiration, catalase and peroxidase activity (Kleinhofs et al., 1974). In *Triticum aestivum* this substance induces mutants with different colors of glumelles (Crowley and Jones, 1989), but in barley, sodium azide stimulates the germination (Lenoir et al., 1986), and induces chromosomal aberrations such as break, gap, iso-chromatid break and exchange in *Allium cepa* root tip cells (Ragunathan and Panneerselvam, 2007).

Data concerning sodium azide effect on medicinal plant are enough few. Recently, Siddiqui et al., 2007, using concentrations varying between 0.1-0.5% sodium azide, show that mutagen decreased the percentage of seed germination, radicle length (at higher dose), mitotic index, and caused an increase in the chromosomal aberrations in a dose-dependent manner.

Our studies are focused by estimation of genotoxic effect on radicular meristem cells of *Carum carvi* L. treated with sodium azide.

MATERIALS AND METHODS

Biological material: caraway seed, harvest from 2007. Germination was ensured in Petri dishes covered with distilled water moisturized filter paper, in thermostat at 22°C. At 5-10mm root length it was applied the following treatment:

- the control dish – little embryonic roots were kept, for three hours, in distilled water, at room temperature;
- the variants of treatment in which were used three dilutions of sodium azide (10⁻⁷ M/L; 10⁻⁵ M/L; 10⁻³ M/L) the germinated seeds were maintained for three hours, at room temperature.

After this step all variants are kept in distilled water, for two hours. As fixative, the mixture absolute ethyl alcohol : glacial acetic acid, was used for 12 hours. For storage use 70% ethyl alcohol solution and the vials were placed in refrigerator conditions.

The microscopic preparations were obtained by squash method (Cîmpeanu et al., 2002) and analyzed by used light microscope 40x objective. The photos were realized at 100x in immersion with Cool Pix Nikon digital camera, 1600x1200 dpi resolution. Five preparations per variant were scored and ten microscopic fields per slide to estimate mitotic index and chromosome aberrations.

RESULTS AND DISCUSSIONS

The main analyzed parameters were: mitotic index, mitotic phase frequency, type and percent of chromosome aberrations.

1. Mitotic index

Sodium azide effects on cell division in root tips of caraway varying with dilution. Total frequency of division cells is lower in treatment variants than control sample. Mitoinhibitory effect of sodium azide increase proportionally with its concentration, the highest is at 10^{-3} M/l where mitotic index is 3.00 percent lower than control probe (Fig. 1)

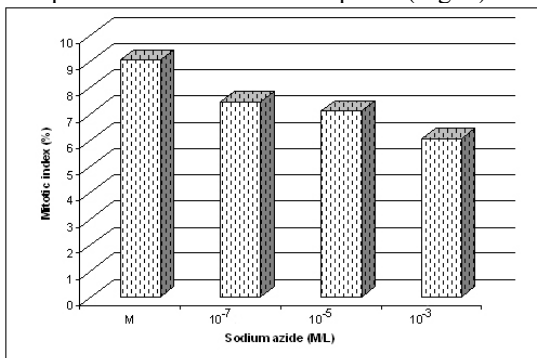


Fig. 1. Mitotic index in *Carum carvi* L. root tips after sodium azide treatment

2. Cells division frequency

The frequency of occurrence of each phase of mitosis is known to be proportional to its duration. Consequently, with an increase or decrease in the number of mitosis in each phase in the experimental sample compared with their number in the control, it would be valid to conclude that there is a corresponding increase or decrease in the duration of that phase and of mitosis as a whole (Dobrokhotov and Valvas, 1981).

In all variants the prophase percentage is the highest followed by metaphases, telophases and anaphases. Obviously modification concerning prophases who are diminishing proportionally with increase sodium azide concentration (Fig. 2). This behavior is an argument that this agent acts in early phases of cellular cycle and DNA macromolecule have maximum of despiralization being more permissive to the xenobiotic agents.

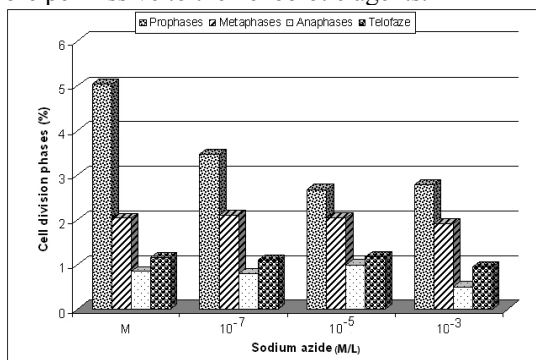


Fig. 2. Mitotic phase frequency in *Carum carvi* L. root tips after sodium azide treatment

3. Chromosomal aberration frequency

Sodium azide treatment induced negatively repercussions in mitotic division promoting. The chromosomal aberration frequency in radicular apex cells increase proportionally with chemical agent concentration. For example, at 10^{-3} concentration their number was 3.86 times higher than control (Fig. 3).

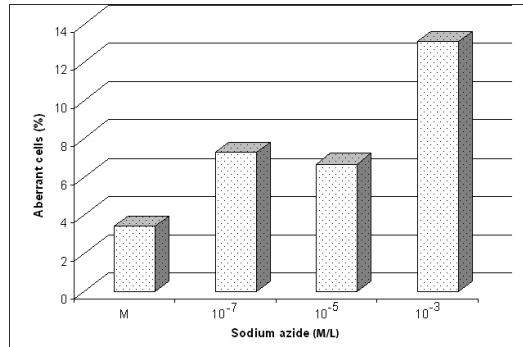


Fig. 3. Chromosomal aberration frequency in *Carum carvi* L. root tips after sodium azide treatment

The most frequent types of aberrations were evidenced in ana-telophase and, in less, in metaphases. Among ana-telophases with aberration were simple and multiple bridges, lagging and/or expelled chromosomes and in metaphase both C-metaphases and expelled chromosomes (Fig. 4-9). This fact denotes that agent acts directly at nuclear spindle and kinetochores function delaying migration of chromatides to the poles or total blocking of those when all chromosomes are spread in the cytoplasmic mass (C-metaphase).

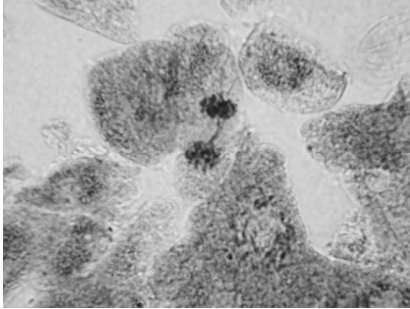


Fig. 4. Ana-telophase with simple bridge

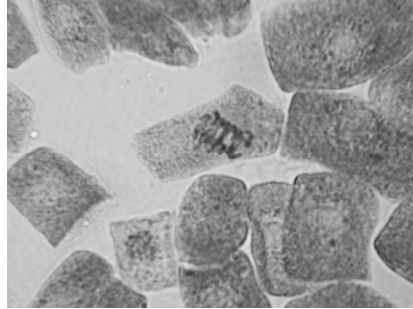


Fig. 5. Ana-telophase with multiple bridges

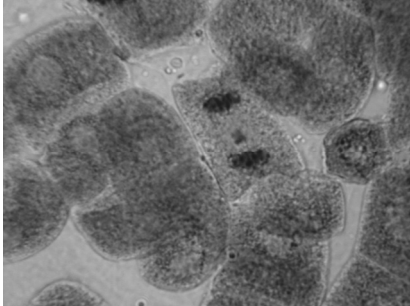


Fig. 6. Lagging chromosome

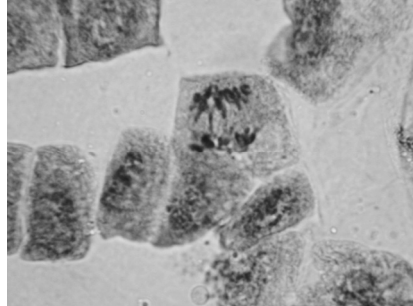


Fig. 7. Multipolar anaphase with double bridge

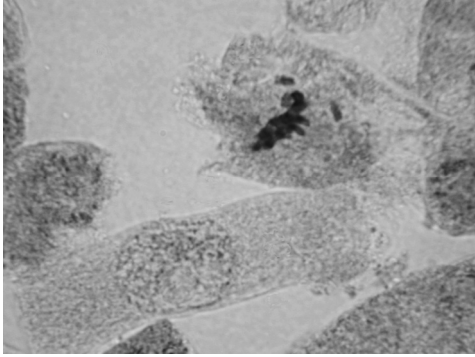


Fig. 8. Metaphase with expelled chromosomes

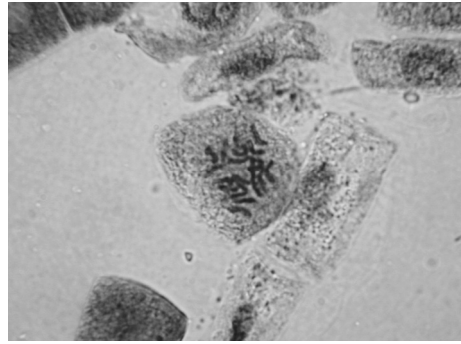


Fig. 9. C-metaphase

CONCLUSIONS

Sodium azide treatment induced diminishing of mitotic index in apex radicular cells, proportionally with increase of concentration.

Significantly modification of mitotic cells division is in prophase, their percentage is lower in all treatment variants.

The numbers and types of aberration cells increase comparatively to control. The highest frequency of them is at 10^{-3} M/L sodium azide treatment (10.3%).

Chromosomal aberration were identified both ana-telopase and metaphase.

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