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Micronuclei, as Indicator of Mutagenecity In Cultivated *Allium*

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Abstract

This study was performed to determine the relationship between chromosome aberrations in PMCs and micronuclei formation as well as chiasma frequency in cultivated *Allium cepa* collected from two sites. One site adjacent to the high way which is characterize by heavy traffic and the plants grown exposed to the different components of ignition exhaust in air as well as in soil, The other site away from the traffic pollution as control samples. Different types of chromosomal aberrations observed in first and second meiotic cells were: bridge, stickiness, chromosome aggregates in the form of a ring, vaculated nucleus, misorientation and multinucleated cells. Micronuclei formation represented the most dominant type of mieiotic abnormalities, The results indicated that aberration percentages of meiosis I were more frequent than those of meiosis II. Also a decrease in chiasma frequency in site I over site II was observed.

Introduction

Micronuclei as nuclear bodies have been induced in several pecies of plants by treatment with mutagenic substances. El-Bayoumi *et al*. (1979) found micronuclei in root tip cells of *Allium cepa* after treatment of seeds with papaverin hydrochloride. Micronuclei also occurred following treatment with other chemical agents on plants e.g. *Vicia faba* (Das *et al*., 1968; Raj and Reddy, 1971; Amer and Farah, 1979). Also, micronuclei can be attained by application of gamma rays (Fayed *et al*. 1988). Several authors (Das *et al*., 1968; Bennett and Jellings, 1975; El-Bayoumi *et al*., 1979; Hesemann and Fayed, 1982) found micronuclei in untreated controls of various plant species. Although the production of micronuclei in root meristem-cells is well established (Soran *et al*. 1981; Degrassi and Rizzoni, 1982; Fayed and Hesemann, 1983 and Tang *et al*. (1984), similar studies in meiotic divisions are relatively few. Das *et al*. 1968; Raj and Reddy, 1971 and Amer and Mikhael, 1972 observed micronuclei in PMC's of *Vicia faba* originated from seeds treatment with mutagenic agent. These authors considered micronuclei as a feature of meiotic abnormality.

In this study, the frequency of micronuclei and chromosome abnormalities as well as chiasma frequency in PMC's of cultivated *Allium* cepa collected from two sites. One site has been chosen adjacent to the high way which is characterized by heavy traffic and the plants grown exposed to the different components of ignition exhaust in air as well as in soil. The other site away from the traffic solution as control samples to study.

Materials and Methods

Plant materials used in this study were collected from two different sites in Dakahlia District in the Nile Delta of Egypt: Site I located along Mansoura - Gamasa high way which is characterized by heavy traffic.

Site II located apart from the high way by a distance of about 200 m. Young flower buds of *Allium* cepa were collected between 10-12 a.m., fixed immediately in the field in a fresh mixture of absolute alcohol, chloroform and glacial acetic acid (6 : 3 : 1 v/v/v).

The fixed buds were stained in alcoholic hydrochloric carmine (Snow, 1963) for 5 days, washed in 70 percent ethyl alcohol.

Anthers were dissected out in 45 percent acetic acid and squashed. Well spread cells were photographed.

The frequency of bivalents and chromosomal abnormalities were recorded from about 500 pollen mother cells in each division (I and II) as well as in each site. Chiasma frequency was calculated from cells at diakinesis.

The differences in meiotic abnormalities between the two sites were statistically tested using two-tail t-test (Snedecor and Cochran, 1968 and Dougherty, 1990) to measure the degree of significance of chromosome abnormalities in the two sites.

Results

Different types of chromosomal aberrations were observed in first and second meiotic cells: bridge, stickiness, chromosome aggregates in the form of a ring at one pole or at both poles, vaculated nucleus, micronuclei, laggards, asynchrony, misorientation and multinucleated cells (Fig. 1).

The most frequent form of chromosome aberrations was micronuclei. Fig. 2 shows micronuclei in different stages of first meiotic division and persisting to the end of the second meiotic division.

To test significancy of different chromosome aberrations including the formation of micronuclei in the two sites two-tail t-test was used. Results are shown in Table 1.

Among site measures (Table 1), the normal cells numbers varied significantly in both 1st and 2nd meiotic divisions ($p < 0.01$ and $p < 0.001$ respectively). Micronuclei is the only significant measure of chromosome abnormalities between sites, their number showed high significant variation in the second division ($p < 0.01$) compared with the first division ($p < 0.05$). The average values of bridges, stickiness, laggards, ... etc. did not differ significantly between sites (Table 1).

Concerning chiasma frequency, decrease in chiasma was more in site I than II (Table 2).

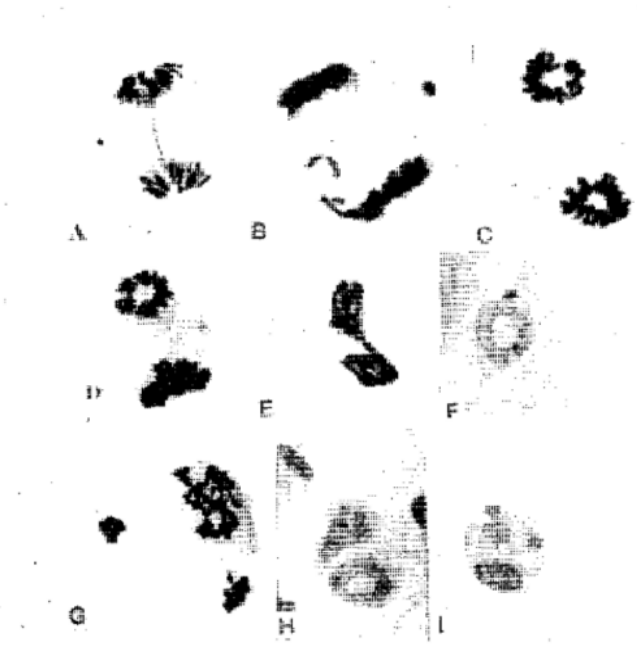

Fig.1: Shows different types of chromosomal abnormalities in first and second meiotic cells:
A = bridge and laggard, B = laggard and micronuclei, C = chromosome aggregate in the form of ring at both poles, D = chromosome aggregate in the form of ring at one pole, E = stickiness, F and H = vaculated nucleus, G = asynchrony, I = multinucleated cell. (X = 1700)

Fig.2: Shows micronuclei in different stages of first meiotic division (A-C) and persisting till the end of the second meiotic division (D-G). (X = 1700)

Table 1: Mean number of cells with chromosomal abnormalities in *Allium cepa* plants growing in two sites of different exposure to road traffic pollution. Total number of cells examined in each site and each division = 500

	A - First Division				B - Second Division			
Parameters	Site I	Site II	Two tail t-test	p level	Site I	Site II	Two tail t-test	p level
Normal cells	318.2	465.4	-6.8	**	301.8	483.4	-36.5	***
Bridge	11.4	4.0	2.2	ns	19.6	2.2	1.7	ns
Stickiness	8.6	4.0	1.2	ns	10.8	2.2	1.6	ns
Chromosome ring	18.6	3.4	1.4	ns	27.2	2.2	1.9	ns
Vaculated nucleus	34.6	1.8	2.3	ns	20.8	1.2	1.8	ns
Micronuclei	93.2	14.0	2.8	*	93.6	3.0	4.4	**
Laggard		15.4	7.4	1.4	ns			
Asynchrony					2.6	1.2	0.4	ns
Misorientation					8.2	2.2	0.7	ns
Multinucleated cells					15.4	2.4	1.4	ns

* = Significant at $p<0.05$, ** = Significant at $p<0.01$, *** = Significant at $p<0.001$, ns = Non significant

Table 2: Chiasma frequency from cells collected from both sites

	Total No. of cells examined	Bivalent configurations observed	No. of cells	No. of chiasmata
Site I	60	2 ring + 6 rod	10	10
		3 ring + 5 rod	50	11
Site II	50	4 ring + 4 rod	40	12
		5 ring + 3 rod	10	13

Discussion

The different types of chromosomal abnormalities noticed in meiotic cells were bridge, stickiness, chromosome aggregate in the form of a ring, micronuclei, laggard, unequal distributions of chromosomes, mis-orientation, asynchrony and multinucleated cells. The most frequent form of chromosome aberrations was the occurrence of micronuclei in the first meiosis and persisting to meiosis two.

Occurrence of a micronucleus implies for the cell a loss of DNA material in the nucleus. Fayed (1981) and also Hesemann and Fayed (1982), assumed that the production of micronuclei is based on the effect of automutagenic substances.

in the present study, aberration percentages of meiosis I were more frequent than those of meiosis II. This result was in contrast with that of El-Ashry (1986) who found that *Vicia faba* plants fumigated with different concentrations of SO_2 produced PMC's with equal aberration percentages in meiosis one and two.

Accordingly, the induction of aberration in the present study could be due to the immediate effect of the different components of ignition exhaust in air on the dividing PMC's or due to translocated exhaust pollution from soil or both. The same results have been reported by El-Said (1989) on studying the effect of traffic pollution on wheat (*Triticum aestivum*) and maize (*Zea mays*) where aberration percentage of meiosis one were more frequent than those of meiosis two in wheat and maize grown at different sites from the high way.

Chiasma frequency is a sensitive and demonstrable parameter carrying a wide range of implications (Rees and Thompson, 1956 and Dowrick, 1957). The process of chiasma formation is known to be under the control of both major genes and polygenic system (Shaw, 1974 and Parker, 1975). Sharma and Singh (1968) observed a significant decrease in chiasma frequency over control after treatment with Actinomycin-D in Vicia faba. They hypothesized that the decrease in chiasma frequency can be attributed to inactivation of gene or genes responsible for crossing over. Moreover, they demonstrated that the failure of pairing genes may result in the inhibition of specific protein necessary for a normal meiosis.

Also, it was published that the application of radiation, heat treatment or metabolic inhibitors can affect chromosome pairing and alter the number and distribution of crossing over and/or chiasmata (Henderson, 1970 and Stern *et al*. 1975). It is thus possible to suggest that traffic pollution may reduce the degree of chromosome pairing which in turn would lower chiasma frequency in *Allium* in the present study.

Concluding the above results it seems that chromosomal aberrations, micronuclei formation and chiasma frequency are three cytological phenomena of strong inter-relation at the level of chromosomal DNA replication. Considering traffic pollution as a damaging agent to the natural discipline of a chromosomal complement in a plant species, their influence is preserved through the various developmental processes between the seedling and flowering stages.

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