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The genotoxic potential of two heavy metals in inbred lines of maize (*Zea mays* L.)

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Abstract: The genotoxic effects of 2 heavy metals (mercury chloride and cadmium chloride) on the gametic cells of 6 inbred lines of maize were tested in terms of cytological abnormalities. Meiosis was normal under control conditions. During the treatments with mercury and cadmium there was a concentration-dependent increase in meiotic abnormalities in all the inbred lines. A wide spectrum of chromosomal aberrations in the treated sets was stickiness, followed by laggards, bridges, scattering, precocious movement, fragments, etc. Maximum chromosomal anomalies were observed in inbred line CM-142 in both the treatment sets of heavy metals. Compared to $CdCl_2$, $HgCl_2$ induced more chromosomal damage in all the inbreds. Of the 6 inbreds examined during the present investigation, CM-138 was the most tolerant to both heavy metals, while CM-142 was the least resistant.

Key words: *Zea mays*, inbreds, mercury, cadmium, chromosomal anomalies

Introduction

Heavy metal contamination of soils has markedly increased in the past few decades. Many factors, such as metal-enriched parent materials, mining or industrial activities, non-point sources of metals, especially automotive emission, and use of metal-enriched materials, including chemical fertilizer, farm manures, sewage sludge, and wastewater irrigation, can contribute to this contamination (Freedman & Hutchinson, 1981; Herawati, 2000; Brun, 2001). Increased pollution due to the release of genotoxic chemicals and increased radiation levels have affected the ecosystem and the health of organisms, including humans (Houk, 1992). There is a need for quick and precise methods for the detection and evaluation of air, water, and soil contamination, and their effects on organisms (Sandhu et al., 1994).

Plants make up a large portion of our biosphere and constitute a vital link in the food chain. Due to the highly conserved structure of genetic material, it is possible to use a broad variety of species in genotoxicity tests. Among the heavy metals, mercury and cadmium are of particular concern because both are genotoxic (George, 2000; Herawati et al., 2000; Brun et al., 2001; Reichman, 2002; Matsumoto & Morales, 2004; Ivanova et al., 2005; Kumar & Srivastava, 2006; Kumar & Rai, 2007). They are reported to create a number of health hazards, even

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at low concentrations in foods. Heavy metal pollutants are stable in the environment, but highly toxic to biological organisms. High concentrations of heavy metals are chromotoxic and mutagenic to a large number of plant species. Characteristically, they inhibit root growth and cell division in such plants as *Allium cepa* L. (Liu et al., 1994), *Tradescantia* L., *Nicotiana tabacum* L. (Fojtova & Kovarik, 2000), *Zea mays* L. (Jiang & Liu, 2000), *Allium sativum* L. (Yi & Meng, 2003), *Helianthus annuus* L. (Kumar & Srivastava, 2006), *Lathyrus sativus* (Kumar & Tripathi, 2007). Mercury is one of the heavy metals released into the environment from a variety of industries, including dry battery, paper and pulp, plastic industries, etc. (Mitra, 1986; WHO, 1989). Mercury is genotoxic (Leonard et al., 1983; Kumar & Tripathi, 2003); its genotoxicity is usually attributed to the ability of the metal to bind with tubulin-SH, which impairs spindle function and results in genomic mutations or numerical chromosome aberrations. Mercury is also thought to induce DNA damage through oxidative mechanisms (Cantoni et al., 1984).

Mutagenic and toxic effects of metals have been studied in different test materials, but data are scant in higher plants. Analysis of the genotoxic potential of a substance by investigating the induction of chromosomal alterations represents an effective method for biomonitoring studies and for the analysis of the extent of pollution (Harden, 2001; Kumar & Rai, 2007). The present study investigated the cytological damage caused by 2 heavy metals ($HgCl_2$ and $CdCl_2$) in different inbred lines of maize (*Zea mays*).

Materials and methods

The seeds of 6 inbred lines of maize (CM-135, CM-136, CM-137, CM-138, CM-142, and CM-213) were obtained from the Division of Genetics, Indian Agricultural Research Institute (I.A.R.I.), New Delhi. The seeds were sown in soil and the plants thus raised were sprayed with aqueous solutions of $HgCl_2$ and $CdCl_2$ at different concentrations (25, 50, and 100 ppm) on the 10^{th}, 17^{th}, 24^{th}, and 31^{st} days of sowing. Plants in the control set were sprayed with distilled water only. Three replicates were maintained for each treatment concentration and then they were sown under natural conditions to raise the M_1 generation. At the time of flowering, young floral buds were fixed in 1:3 acetic acid and absolute alcohol solutions for 24 h, after which they were transferred to 70% alcohol and stored at 4 °C. For cytological analysis, slides were prepared using the chromosomal squash technique with 2% acetocarmine stain.

Observations

Meiosis was normal in all the control plants (n = 10) (Figures 1-3) and chromosomal anomalies were relatively negligible; however, in both treated sets ($HgCl_2$ and $CdCl_2$) a concentration-dependent increase in meiotic abnormalities was observed in all the inbred lines (Tables 1 and 2). The sets treated with $HgCl_2$ and $CdCl_2$ showed several effects of chromotoxicity. There was a highly significant difference in meiosis between both metal-treated sets and the control set, with respect to the arrangement and behaviour of chromosomes. Scattering (Figure 4), bridges, stickiness, and univalents were more

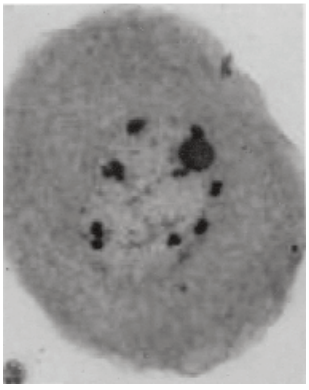

Figure 1. Normal diakinesis (n = 10).

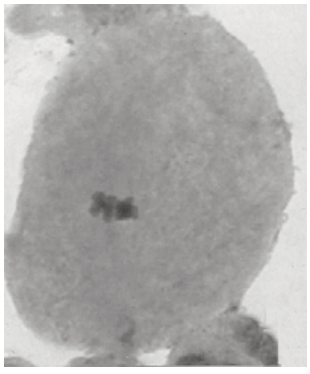

Figure 2. Normal metaphase I (n = 10).

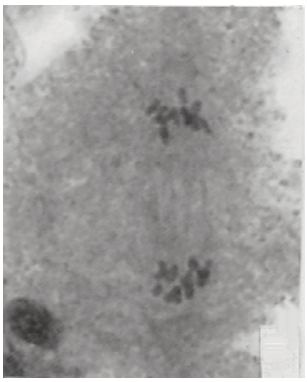

Figure 3. Normal anaphase I (10:10 separation).

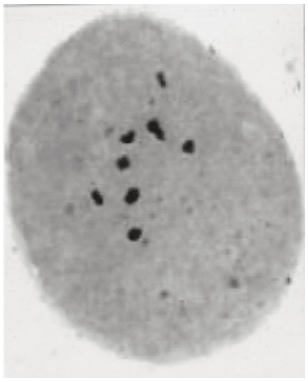

Figure 4. Scattering at metaphase I.

Table 1. The effects of $HgCl_2$ on 6 inbred lines of maize (*Zea mays*).

SN	I. L.	Conc.	No. of PMCs observed	Metaphasic abnormalities (%)					Anaphasic abnormalities (%)					T Ab (%)	Pollen fertility (%)
				St.	Sc.	PM	Dis.	Others*	L	B	St.	Mic.	Others**		Mean ± SE
1	CM-135	Cont.	2337	-	-	-	-	-	-	-	-	-	-	-	92.4 ± 0.12
		25	2156	0.62	0.31	-	-	0.17	-	0.59	0.31	-	-	2.29	90.3 ± 0.18
		50	1789	0.82	0.74	0.87	0.39	0.37	-	0.79	0.74	0.76	0.37	5.86	87.6 ± 0.41
		100	1432	3.88	2.16	0.86	-	0.29	0.43	0.86	1.29	1.86	0.43	12.17	83.1 ± 0.67
2	CM-136	Cont.	1998	-	-	-	-	-	-	-	-	-	-	-	90.3 ± 0.08
		25	1878	0.65	-	-	-	0.32	0.39	0.37	0.32	-	0.29	2.34	87.1 ± 0.17
		50	1654	1.82	1.45	0.36	0.36	0.71	-	0.86	0.73	0.36	0.36	7.01	83.2 ± 0.57
		100	1423	3.29	2.47	1.23	0.82	1.23	0.41	1.23	2.06	0.82	-	13.17	79.6 ± 0.63
3	CM-137	Cont.	2114	-	-	-	-	-	-	-	-	-	-	-	93.3 ± 0.23
		25	1867	0.39	-	0.39	-	0.23	0.39	-	0.39	-	0.34	2.13	91.7 ± 0.17
		50	1479	0.80	1.20	0.40	-	0.40	0.80	0.40	1.59	1.20	0.47	8.04	87.6 ± 0.38
		100	1287	3.23	2.30	0.92	0.92	1.92	0.38	0.92	0.84	1.46	0.92	13.82	80.1 ± 0.41
4	CM-138	Cont.	2378	-	-	-	-	-	-	-	-	-	-	-	94.7 ± 0.29
		25	1974	0.70	-	0.12	-	0.35	-	0.11	0.13	-	0.61	1.78	91.2 ± 0.31
		50	1768	1.95	0.78	0.39	-	0.39	0.78	0.39	1.17	-	0.39	6.22	89.7 ± 0.51
		100	1697	2.80	2.00	0.40	0.40	0.80	0.91	0.80	1.60	0.80	0.80	11.60	87.5 ± 0.63
5	CM-142	Cont.	2237	-	-	-	-	-	-	-	-	-	-	-	89.7 ± 0.27
		25	1987	0.36	0.36	-	-	0.43	-	0.39	0.57	-	0.83	2.94	83.2 ± 0.41
		50	1768	2.53	1.01	-	0.51	0.57	0.25	0.76	1.01	-	0.79	7.43	80.6 ± 0.53
		100	1567	4.97	2.86	1.62	-	0.69	0.62	2.62	1.73	0.24	0.93	16.28	78.1 ± 0.59
6	CM-213	Cont.	2341	-	-	-	-	-	-	-	-	-	-	-	91.3 ± 0.18
		25	2145	0.53	-	0.74	-	0.29	-	-	0.36	-	0.24	2.16	87.1 ± 0.31
		50	1765	2.03	1.20	-	-	0.41	0.81	0.41	1.20	0.81	0.31	7.18	81.2 ± 0.27
		100	1478	4.38	1.59	1.59	0.40	1.20	0.80	0.40	1.20	-	0.40	13.15	77.1 ± 0.33

I. L.: Inbred lines; PMCs: pollen mother cells; Tab: total abnormalities; St.: stickiness; B: bridges; L: laggards Dis.: disorientation; Sc: scattering; PM: precocious movement; Mic: micronuclei. Others: *Fragmentation, univalents; **Micronuclei, non-synchronous division, cytoplasmic connections; SE: standard error.

Table 2. The effects of CdCl2 on 6 inbred lines of maize (*Zea mays*).

SN	I. L.	Conc.	No. of PMCs observed	Metaphasic abnormalities (%)					Anaphasic abnormalities (%)					T Ab (%)	Pollen fertility (%)
				St.	Sc.	PM	Un.	Others*	L	B	St.	Mic.	Others**		Mean ± SE
1	CM-135	Cont.	2341	-	-	-	-	-	-	-	-	-	-	-	91.4 ± 0.10
		25	1975	0.35	-	-	0.35	0.35	-	-	-	0.35	0.37	1.78	87.2 ± 0.43
		50	1786	1.22	0.40	0.40	0.17	-	0.81	0.40	1.22	-	0.49	5.11	79.3 ± 0.59
		100	1368	2.69	1.34	0.89	0.44	1.34	0.64	0.79	1.79	1.24	-	11.16	73.1 ± 0.47
2	CM-136	Cont.	2010	-	-	-	-	-	-	-	-	-	-	-	90.1 ± 0.31
		25	1962	0.32	-	-	0.32	0.29	0.39	-	-	-	0.25	1.57	87.3 ± 0.16
		50	1787	0.76	0.53	0.76	1.38	0.38	0.38	0.38	0.76	0.38	0.38	6.15	81.1 ± 0.51
		100	1512	4.08	0.44	0.81	1.40	0.81	0.81	0.40	1.63	0.40	0.40	11.24	79.3 ± 0.61
3	CM-137	Cont.	2155	-	-	-	-	-	-	-	-	-	-	-	93.6 ± 0.21
		25	1908	0.32	-	0.12	-	0.47	0.32	-	0.37	-	0.19	1.79	89.7 ± 0.14
		50	1573	1.90	0.76	0.76	0.38	0.76	0.76	-	0.38	-	0.67	6.37	83.1 ± 0.31
		100	1306	3.41	0.47	0.98	-	1.71	0.91	0.51	1.47	-	1.98	11.44	79.6 ± 0.48
4	CM-138	Cont.	2304	-	-	-	-	-	-	-	-	-	-	-	95.7 ± 0.21
		25	2201	0.61	0.30	-	0.13	-	0.17	-	-	-	-	1.23	91.3 ± 0.16
		50	1829	1.56	0.78	0.39	-	0.39	-	0.58	1.56	-	-	5.26	87.1 ± 0.31
		100	1302	3.31	0.92	0.94	0.41	0.47	0.47	0.64	1.89	0.47	1.37	10.35	81.2 ± 0.48
5	CM-142	Cont.	2330	-	-	-	-	-	-	-	-	-	-	-	93.1 ± 0.19
		25	1975	0.64	0.32	-	-	0.27	-	-	0.32	0.29	-	1.84	87.2 ± 0.23
		50	1318	1.39	0.93	0.46	0.46	0.71	0.57	0.46	1.39	0.46	0.46	6.56	83.2 ± 0.31
		100	1011	4.09	1.16	1.75	0.16	1.75	2.16	0.87	0.58	-	0.58	12.81	79.3 ± 0.29
6	CM-213	Cont.	2011	-	-	-	-	-	-	-	-	-	-	-	94.3 ± 0.17
		25	1965	0.35	-	0.43	-	0.20	0.17	-	0.12	-	0.35	1.62	91.1 ± 0.31
		50	1693	1.62	-	0.67	0.40	0.81	0.40	-	0.81	0.39	0.73	5.83	87.7 ± 0.42
		100	1416	2.56	0.85	0.85	1.28	0.42	0.85	0.28	0.85	-	0.27	10.02	83.1 ± 0.67

I. L.: Inbred lines; PMCs: pollen mother cells; Tab: total abnormalities; St.: stickiness; B: bridges; L: laggards Dis.: disorientation; Sc: scattering; PM: precocious movement; Mic: micronuclei. Others: *Fragmentation, univalents; **Micronuclei, non-synchronous division, cytoplasmic connections; SE: standard error.

common in the Hg-treated sets. On the other hand, stickiness, precocious movement (Figure 5), and laggards (Figure 6) were more common in the Cd-treated sets. In addition, other abnormalities, such as non-synchronous division, forward movement (Figure 7), unequal separation, bridges (Figure 8), and cytoplasmic channels (Figure 9), were noted in the treated sets of all the inbred lines.

The most prominent abnormality induced by mercury and cadmium was chromosome stickiness. During stickiness, chromosomes formed a compact mass and the identity of individual chromosomes was lost. In inbred CM-142, stickiness was maximal at the 100 ppm concentration of both heavy metals, i.e. 4.97% in the $HgCl_2$ and 4.09% in the $CdCl_2$ treatment sets.

Scattering and bridge formation displayed a common tendency in the pollen mother cells of $HgCl_2$-treated sets, i.e. 2.86% and 2.62%, respectively, while there were comparatively very few in the $CdCl_2$-treated sets, i.e. 1.16% and 0.87%, respectively, even at the highest concentration in the same inbred line (CM-142).

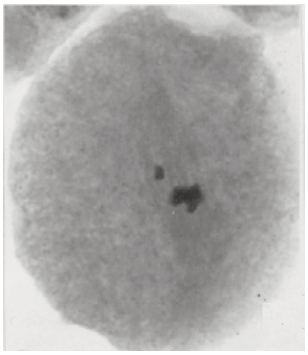

Figure 5. Precocious movement at metaphase I.

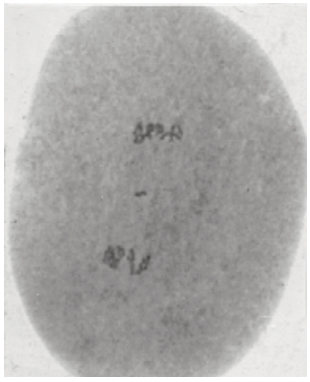

Figure 6. Laggard at anaphase I.

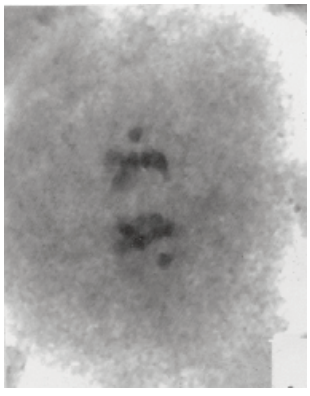

Figure 7. Forward movement at anaphase I.

Laggards and precocious movement were more frequent in the $CdCl_2$ treatment set than in the $HgCl_2$ treatment set (2.16% and 1.75%, respectively, at the 100-ppm concentration of $CdCl_2$ versus 0.62% and 1.62%, respectively at the 100-ppm concentration of $HgCl_2$) in inbred CM-142.

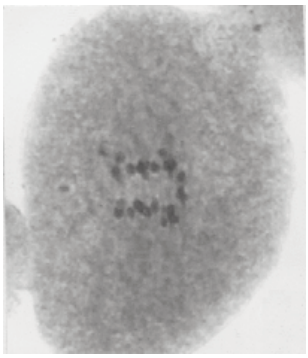

Figure 8. Lateral bridge at anaphase I.

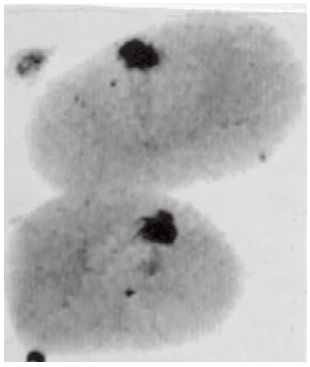

Figure 9. Cytoplasmic connections.

Maximum chromosomal damage was observed in CM-142 in both the $HgCl_2$ and $CdCl_2$ treatment sets, i.e. 16.81% and 12.81%, respectively, at the 100-ppm concentration, as compared to the other inbreds, while minimum chromosomal abnormality was observed in CM-138 at the 100-ppm concentration in both the $HgCl_2$ and $CdCl_2$ treatment sets, i.e. 11.60% and 10.35%, respectively, as compared to the other inbreds.

As a consequence of precocious migration of univalents, non-oriented bivalents and laggards, as well as some micronuclei were also observed. In the $HgCl_2$ treatment set the micronuclei rate was 1.86% at the 100-ppm concentration in inbred CM-135 versus 1.24% in the same inbred in the $CdCl_2$ treatment set. Laggards, abnormal prophase, and scattering were also observed. In addition, as compared to $CdCl_2$, $HgCl_2$ induced more chromosomal damage in all the inbreds (Tables 1 and 2). The test for pollen fertility showed a very low percentage of sterile pollen grains in the control sets. Pollen fertility was significantly correlated with meiotic irregularities. As meiotic

abnormalities increased along with the concentration of both heavy metals, the percentage of fertile pollen grains decreased, while the $HgCl_2$ treatment set had a greater decrease in pollen fertility than the $CdCl_2$ treatment set.

Among the 6 inbreds, CM-138 was the most tolerant to mutagenic treatment with heavy metals, while CM-142 was the least tolerant.

Discussion

In recent years there has been a rapid increase worldwide in the use of chemicals containing heavy metals. Such chemicals have been proven to be of immense value to mankind, but the deleterious effects they produce seriously threaten the genetic hygiene of living beings, including man. As maize is a crop of worldwide importance, the present investigation was an attempt to observe chromosomal damage during meiotic division in response to 2 heavy metals in this important crop.

Based on the results of the present investigation, it is quite clear that both tested metals ($HgCl_2$ and $CdCl_2$) are capable of inducing such cytological abnormalities as stickiness, scattering, laggards, bridges, precocious movements, fragments, etc. All the concentrations were capable of inducing different types of chromosomal abnormalities and the frequency of abnormalities increased, in most cases, as the concentration of the metals increased. This provides a case for comparison of the deleterious effects of these metals on the concerned plant. The induction of cytological disturbances in meiotic cells is of great value, as it results in genetic damage that is passed on to the next generation. There was a linear correlation between the concentration of metals and the percentage of abnormalities in the treated sets. Several researchers have performed similar studies on the genotoxic effects of different chemicals on different plant materials (Adam et al., 1990; Ahmad & Yasmin, 1991; Billani et al., 1991; Serpil et al., 2006; Kumar & Tripathi, 2007).

Chromosome stickiness leading to sticky metaphase and precocious separation of chromosomes is possibly due to chemicals breaking the protein moiety of the nucleoprotein backbone (Patnaik, 1984). Metal-induced chromosomal stickiness has also been reported (Kumar & Srivastava, 2006; Kumar et al., 2006; Kumar & Tripathi, 2007). Stickiness accompanied by pyknosis and chromatin degeneration has been reported in maize (Caetano-Pereira et al., 1995), *Allium cepa* (Kumar & Tripathi, 2003), *Helianthus annuus* (Kumar & Srivastava, 2006), and *Lens culinaris* (Kumar & Kesarwani, 2004). Disorientation and scattering, in which the chromosomes spread irregularly over the cell, may be due to disturbance of the spindle apparatus. The characteristic behaviour of laggard chromosomes is that they generally lead to micronucleus formation (Kumar & Rai 2007). The occurrence of micronuclei is regarded as a reliable parameter for the clastogenicity or mutagenicity of an agent (Auerbach, 1976; Kumar & Srivastava, 2006). Laggards and disturbed polarity might have appeared due to improper spindle functioning (Kumar & Singh, 2003; Kumar & Rai, 2007).

Fragments at metaphase may be due to the failure of broken chromosomes to recombine. Fragments might have arisen due to the stickiness of the chromosomes and the consequent failure of the arrival of chromatids at the poles. Fragments may also be acentric chromosomes formed as a result of inversion (Agarwal & Ansari, 2001).

Anaphasic bridges were formed due to unequal exchange or dicentric chromosomes. The breaks at the same locus and their lateral fusion might have led to the formation of dicentric chromosomes. The dicentric chromosome is pulled equally towards both poles at anaphase and a bridge is formed (Anis et al., 1998).

In many studies secondary associations, univalents, fragments, laggards, bridges, and micronuclei were observed due to the effects of heavy metals (George, 2000; Kumar & Tripathi, 2003; Kumar & Singh, 2003; Kumar & Gautam, 2004; Kumar & Srivastava, 2006; Serpil et al., 2007).

As more and more abnormalities accumulate, the process of gamete formation is affected and leads to non-viable gametes, which considerably reduces plant fertility. Studies on different plant species have shown that declines in seed production are correlated with meiotic irregularities (Kumar & Rai, 2007).

On the basis of these results it can be concluded that both of the studied heavy metals are capable of inducing chromosomal anomalies, but mercury chloride is much more genotoxic than cadmium chloride, as it induced more abnormalities. Of the 6 inbreds examined in the present study, CM-138 was the most tolerant to the 2 mercurials, while CM-142 was the least resistant.

Hence, the conservation of such economically important crops is the current priority. Increases in soil and water pollution can lead to certain irreversible cytogenetic effects in plants and higher organisms. Thus, mutagenic data from plant assays are very important for genetic research and for maintaining a stable ecosystem.

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