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Comparative Genotoxic Potential of Mercury and Cadmium in Soybean

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Abstract: Genotoxic effects of two heavy metals viz. mercury (Hg) and cadmium (Cd) on somatic and gametic cells of soybean were investigated. Seeds were treated with different doses of these heavy metals. Treatments with Hg and Cd not only reduced the frequency of dividing cells but a wide spectrum of chromosomal abnormalities were also recorded. Pollen fertility was found to be significantly correlated with meiotic irregularities found in the metal treatment sets. It was found that both of these heavy metals are capable of inducing chromosomal aberrations, but Hg is much more genotoxic than Cd, since it induces greater abnormalities.

Key Words: Soybean, cadmium, mercury, mitosis, meiosis, pollen fertility

Introduction

It is impossible to visualize an environment without trace levels of heavy metals. However, anthropogenic activities have concentrated some of these elements in certain areas up to dangerous levels for living organisms (1). Activities such as mining and agriculture have polluted extensive areas throughout the world (2,3). Among heavy metals, cadmium (Cd) and mercury (Hg) are of special concern because both are found to be genotoxic (4,5,6,7,8,9,10,11). They have been found to create a number of health hazards, even at lower concentrations, through food. Reporting on the carcinogenic activity of these heavy metals on the genetic system of living organisms is an important task of the biologists. Knowledge of the toxic effects of heavy metals on biochemical and physiological processes is potentially useful to establish an index of toxicity. Physical and chemical analysis data alone are probably not enough to evaluate the impact of pollutants on the environment. Furthermore, these measurements are almost always expensive and sometimes difficult to undertake (12). Analysis of the genotoxic potential of a substance through the investigation of the induction of chromosome alterations represents an effective method for bio-monitoring studies and for the analysis of the extent of pollution (13). For this reason, the assessment of chromosome damage is an efficient, reliable and economical criterion to measure genetic damage.

The purpose of the present investigation was to evaluate the influence of Cd and Hg concentrations on somatic as well as gametic cells of soybean, since the most pronounced effect of heavy metals on plant development is growth inhibition, which is inseparably connected with cell division.

Materials and Methods

For mitotic studies, germinated seeds of soybean were treated with $HgCl_2$ and $CdCl_2$ in aqueous solution for 3 hrs at different concentrations viz. 50, 100, 200, 300 and 400 ppm. Controls were maintained simultaneously by treating the seeds with distilled water only. The treated seeds were washed thoroughly and fixed in 1:3 acetic alcohol solution. Slides were prepared using chromosome squash technique with 2% acetocarmine.

For meiotic studies, normal seeds were sown in soil and the plants thus raised were sprayed with aqueous solution of $HgCl_2$ or $CdCl_2$ at different concentrations at the 10^{th}, 17^{th}, 24^{th} and 31^{st} day of sowing. Plants of control set were sprayed with distilled water only. Flower buds were fixed in 1:3 acetic alcohol solution and were analyzed cytologically using 2% acetocarmine stain. Pollen fertility was also calculated by using acetocarmine glycerine stainability test.

Observation

Mitosis

In soybean, the somatic complement consists of 40 chromosomes. Mitotic index was recorded to be 13.5% and no chromosomal aberrations were encountered in the control set. However, in root tips of the heavy metal-treated seeds, there was a gradual reduction in mitotic index (from 13.5% in controls to 4.86% at 400 ppm in the case of $HgCl_2$ and 8.12% at 400 ppm in the case of $CdCl_2$ treatment).

Treatments with all five concentrations of Hg and Cd not only reduced the frequency of dividing cells but a wide spectrum of chromosomal abnormalities was also recorded. The individual abnormalities and the total abnormal cells increased along with the increasing concentrations of $HgCl_2$ and $CdCl_2$. The maximum abnormality percentage was recorded in the 400 ppm $HgCl_2$ treatment set, which was found to be 29.70%. The most frequent chromosomal aberrations in the Hg-treated set were stickiness followed by fragmentation and bridges, while in Cd treatment, stickiness, non-orientation (disturbed orientation), and laggards were more frequent. Scattering, precocious movements, binucleate cells, and micronuclei were the other abnormalities observed during the present investigation (Table 1).

Meiosis

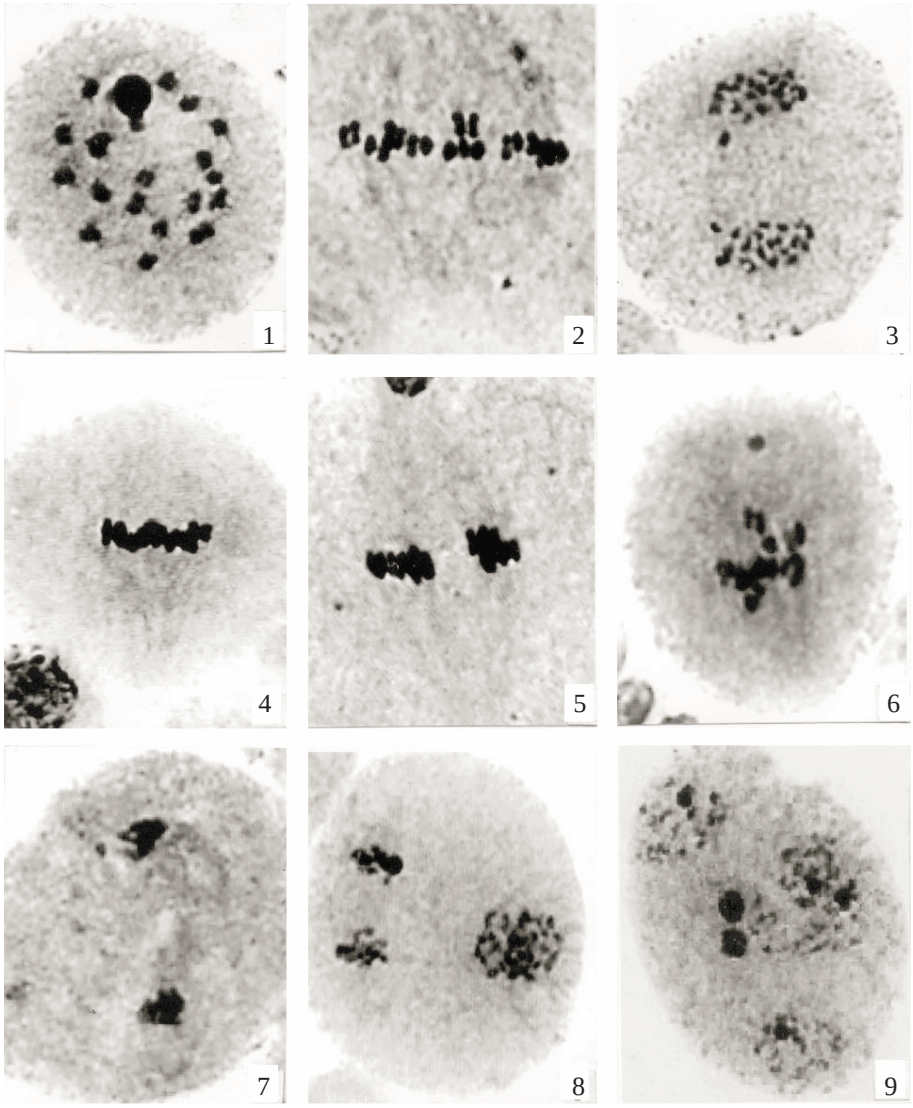
Meiosis was perfectly normal in the control plants with 20 bivalents at diakinesis (Figure 1) and at metaphase I (Figure 2) and 20:20 separation at anaphase I (Figure 3). However, the plants in both treatment sets displayed varying degrees of chromosomal abnormalities distributed in all phases of division. A dose-based increase in meiotic abnormalities was observed in both Hg and Cd treatment sets. Although a number of abnormalities were present in both treatment sets, stickiness (Figures 4, 7), secondary association (Figure 5), univalents and bridges were more common in Hg-treated sets. On the other hand, stickiness, precocious movements (Figure 6), secondary association and laggards were more common in Cd-treated sets. In addition, other abnormalities like unequal separation, scattering, non-synchronous division (Figure 8), micronuclei, and binucleate cells (Figure 9), etc. were also encountered. Table 2 gives a comparative account of various abnormalities observed under treatment with Hg and Cd.

The most prominent abnormality induced by both metals was stickiness at metaphase (I/II) and at anaphase (I/II). The phenomenon ranged from slight stickiness to an indistinct compact chromatin mass involving the entire complement. It was recorded to be highest (6.02%) at

Table 1. Effect of $HgCl_2$ and $CdCl_2$ on mitosis of soybean (*Glycine max*).

Treatment	Dose ppm	MI %	Metaphase abnormality %					Anaphase abnormality%			Ot		Tab %
			St	Non	Sc	Pr	Fr	Br	Lg	St	Mic	Bi	
	Con	13.5	-	-	-	-	-	-	-	-	-	-	-
$HgCl_2$	50	13.10	1.02	-	-	0.51	0.51	0.51	-	-	-	0.51	3.06
	100	12.55	1.56	0.52	-	0.52	-	1.04	-	1.04	-	-	4.68
	200	11.75	1.61	0.53	0.53	-	1.61	1.07	0.53	1.61	0.53	-	8.02
	300	8.73	3.08	1.23	0.61	1.23	1.85	2.46	1.23	2.46	1.85	0.61	16.61
	400	4.86	4.95	1.98	1.98	0.99	3.96	3.96	1.98	4.95	3.96	0.99	29.7
$CdCl_2$	50	13.46	0.45	0.45	0.45	-	-	-	-	0.45	0.45	-	2.25
	100	13.03	0.90	0.46	0.46	-	-	0.46	-	0.46	-	-	2.74
	200	12.85	1.01	1.01	-	0.50	0.50	-	0.50	1.51	-	-	5.03
	300	10.90	1.62	1.08	0.54	1.08	0.54	0.54	1.62	1.08	0.54	0.54	9.18
	400	8.12	3.33	2.00	1.33	1.33	1.33	2.00	1.33	2.66	2.00	0.66	17.97

MI - Mitotic index; Ot - Other abnormalities; Tab% - Total abnormality percentage;
Con – control; St – Stickiness; Non – Non-orientation; Sc – Scattering; Pr - Precocious movement;
Fr – Fragmentation; Br – Bridge; Lg – Laggard; Bi - Binucleate cells; Mic - Micronuclei.



Explanation of figures

1. Diakinesis with 20 bivalents.
2. Normal metaphase I with 20 bivalents arranged at the equatorial plate.
3. Normal anaphase I with 20:20 separation of bivalents.
4. Stickiness at metaphase I.
5. Secondary association at metaphase I.
6. Precocious movement at metaphase I.
7. Stickiness at anaphase I.
8. Nonsynchronous division at metaphase II.
9. Binucleate cell.

400 ppm $HgCl_2$ treatment, while it was 3.54% at anaphase (I/II) with the same treatment of $CdCl_2$. Secondary associations were also common in the $CdCl_2$ treated set, but the percentage was low (2.73% at 400 ppm) as compared to $HgCl_2$ treatment. Univalents and bridge formations were found to be a common tendency in the PMCs of Hg-treated sets (2.55% and 3.38% at 400 ppm, respectively), while they were comparatively very few in $CdCl_2$ (1.01% and 0.70%, respectively) even at the highest dose of treatment.

Table 2. Effect of $HgCl_2$ and $CdCl_2$ on meiosis and pollen fertility in soybean (*Glycine max*).

Treatment	Dose ppm	Metaphase (I/II) abnormality %					Anaphase (I/II) abnormality %						Tab %	% Pollen Fertility
		SA	Pr	Non	St	Uni	Ot*	Br	St	Lg	Mic	Ot**		
	Con	-	-	-	-	-	-	-	-	-	-	-	-	96.5
$HgCl_2$	50	-	0.16	-	1.38	-	0.27	-	1.01	-	0.20	0.26	3.28	94.1
	100	0.41	0.17	0.05	1.37	0.23	0.17	0.23	1.84	0.17	0.33	0.23	5.17	91.8
	200	1.21	0.40	0.16	2.51	0.73	0.48	1.29	3.16	0.97	0.48	0.47	11.88	87.0
	300	2.09	0.66	0.47	3.71	1.23	0.76	2.02	4.28	1.33	1.43	0.76	18.74	80.3
	400	5.20	1.98	2.39	4.62	2.55	2.06	3.38	6.02	2.31	1.51	0.80	32.82	65.6
$CdCl_2$	50	0.24	0.18	0.18	0.48	-	0.18	-	0.36	-	-	0.18	1.8	94.4
	100	0.45	0.51	0.32	0.71	-	0.58	0.12	0.51	0.12	0.26	0.12	3.7	92.3
	200	0.65	0.71	0.58	1.17	0.13	0.78	0.26	0.97	0.32	0.45	0.26	6.28	89.9
	300	1.83	1.60	1.30	2.21	0.68	1.30	0.38	2.44	0.84	1.38	0.68	14.64	86.4
	400	2.73	2.43	1.72	3.24	1.01	2.12	0.70	3.54	1.21	1.53	0.70	20.93	80.2

Tab% - Total abnormality percentage; Con - Control; Ot - Other abnormalities;
SA - Secondary association; Pr - Precocious movement; Non – Non-orientation; St - Stickiness;
Uni - Univalents; Lg - Laggard; Br - Bridge; Mic - Micronuclei; Ot* - Scattering, non-synchronous division, fragmentation;
Ot** - Unequal separation, multipolarity.

Laggards and precocious movements were found to be greater in $CdCl_2$ treatment as compared to $HgCl_2$ treatment (2.12% and 2.43%, respectively, at 400 ppm of $CdCl_2$ versus 2.06% and 1.98%, respectively, at 400 ppm of $HgCl_2$). Disturbed orientation or non-orientation of bivalents was found in both treatment sets, but in the case of $HgCl_2$ it was negligible at lower doses and then increased considerably (2.39%) at 400 ppm, while in the $CdCl_2$ set, it was registered at all concentrations of treatment. As a consequence of precocious migration of univalents, non-oriented bivalents and laggards, some micronuclei were also observed. In the Hg treatment set, the percentage of micronuclei reached as high as 1.51% at the highest dose from 0.20% at the 50 ppm dose. In the Cd treatment set, maximum percentage of micronuclei (1.53%) was also recorded at the 400 ppm dose.

The test for pollen fertility showed a very low percentage of sterile pollen grains in control sets. Pollen fertility was found to be significantly correlated with meiotic irregularities: as the meiotic abnormalities increased along with dose of metal treatment, the percentage of fertile pollen grains decreased. The Hg-treated set recorded a greater decrease in pollen fertility compared to the Cd-treated set.

Discussion

During the present investigation, both heavy metals, i.e. Hg and Cd, elicited similar types of chromosomal abnormalities, but the percentage of these abnormalities and the total abnormalities induced differed between the two treatments. This provides a case for comparison of deleterious effects of these metals on the concerned plant. The induction of cytological disturbances in the mitotic as well as meiotic cells is of great value, as it results in genetic damage that is handed over to the next generation. The results also showed that a close colinearity existed between the concentration of metal treatment and percentage of chromosomal aberrations.

The mutagen-induced chromosomal variations have been extensively investigated from the point of view of understanding the damaging effects of heavy metals viz. Hg and Cd on important biological systems like soybean. The spectrum of chromosomal abnormalities induced by both metals was broad, and included a comparatively higher proportion of stickiness both in mitosis as well as in meiosis. Several agents have been reported to cause chromosomal stickiness, including X-rays (14), gamma rays (15), temperature (16), herbicides (17) and some chemicals present in soil (18). However, the primary

cause and biological basis of chromosome stickiness are still unknown. Stickiness has also been reported to occur spontaneously due to known and unknown environmental factors in various plants like *Rosa* (19), *Pennisetum* (20), wheat (21), maize (18), etc.

Stickiness has been reported to be a result of partial dissociation of nucleoproteins and alteration in the pattern of organization of chromosomes (22) or due to disturbances in cytochemically balanced reactions (23). However, it seems most probable that the heavy metals may have caused some kind of gene mutation, which led to incorrect coding of some non-histone proteins involved in chromosome organization. When affected, these proteins lead to chromosome stickiness. It it also possible that the metal itself reacts with the histone proteins and brings about a change in the surface property of chromosomes due to improper folding of DNA, thereby causing them to be sticky. The sticky chromosomes may result from defective functioning of one or two types of specific non-histone proteins involved in chromosome organization that are needed for chromatid separation and segregation (24).

Scoring of univalents suggests that following chemical treatments, chromosomes display a weak pairing resulting in the complete or partial failure of synapsis between homologous chromosomes (25). The phenomenon of univalents was in conformity with some previous reports obtained by several investigators in several genera following chemical treatments (26,27). Univalent chromosomes may result from low chiasma frequency, precocious chiasma terminalization or by the presence of asynaptic or desynaptic genes in prophase I (28,29,30). Irrespective of their origin, the meiotic behavior is always the same, with univalents showing precocious movement at metaphase or remaining as laggards at anaphase I. In both cases, they may give rise to micronuclei at different stages of division. Precocious movements, laggards, non-orientation and scattering may also appear because of abnormal spindle activity (31). Precocious movements are possibly due to the effect of chemicals in breaking the protein moiety of the nucleoprotein backbone.

The occurrence of secondary association demonstrates that both metals have a tendency to induce structural alteration, which leads to the rearrangement of chromosomes. Stebbins (32) interpreted secondary associations to be a result of modified chromosome arrangement due to the duplication, interchanges or stickiness. Bridges observed seem to be due to non-separation of chiasma due to stickiness. Irregular chromosome segregation in meiosis I and II could be the result of the non-oriented bivalents formed due to spindle dysfunctioning or they could be due to the formation of univalents at diakinesis or metaphase I, which shows an inability to congregate on the equatorial plate resulting in the formation of micronuclei and abnormal pollen grains. Laggards and non-oriented bivalents may produce micronuclei if they fail to reach the poles in time to be included in the main nucleus (33).

As more and more abnormalities accumulate, the process of gamete formation is affected and it leads to non-viable gametes, which considerably reduce the plant fertility. Studies on different plant species have shown that the decline in seed production is correlated with the meiotic irregularities (34).

On the basis of these results, it can be concluded that both of the studied heavy metals are capable of inducing chromosomal aberrations, but Hg is much more genotoxic than Cd since it induces greater abnormalities.

Soybean has not been considered as a model system for cytogenetical studies. According to Singh and Hymowitz (35), this may explain why soybean cytogenetics has lagged behind genetic studies of maize, barley and tomato despite its importance in the biological system. Squash preparations of PMCs routinely employed for other species did not give good results. Despite these difficulties, cytogenetic studies on soybean should be initiated following its usefulness in biological systems. Plant assays are not a surrogate for mammalian assays. However, most of the higher plants cannot leave their growing sites and thus, may become victims of ecological disturbances directly due to pollution. Mutagenic data from plant assays are thus very important for genetic research and may serve as the basis of means for maintaining a stable ecosystem throughout the entire biological kingdom.

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