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Full Length Research Paper

Comparative study of the sensitivities of onion and broad bean root tip meristematic cells to genotoxins

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Chemicals which cause chromosomal aberration (CA) in plant cells frequently produce identical CA in cultured animal cells. Plant species however, differ in sensitivities. Onion and broad bean (BB) root meristem cells were compared for sensitivity to chlorpyrifos (CPF), mercury chloride ($HgCl_2$), ethyl methanesulphonate (EMS) and garden ripcord (GR). Seeds were germinated on moist filter paper until radicles appeared and exposed to three doses of each chemical for 24 h. About 1 - 2 cm length of root tip was cut, fixed, washed and hydrolyzed in 1 N HCl. Root tips were transferred to microscope slides, cut 2 mm from the growing tip, stained, covered with cover slip and squashed. Cytotoxicity was inferred when the mitotic index (MI) of treated cells was ≤ ½ of control. All chemicals were toxic to onion cells but only EMS and $HgCl_2$ were toxic to BB. Genotoxicity was determined by analyzing 100 anaphase and telophase cells for chromosome fragments, bridges, vagrant chromosome, c-anaphase, multipolarity and stick chromosomes and aberrations at each dose compared with the control using the chi-squared test. All chemicals were genotoxic ($P < 0.05$) to onion but only CPF, $HgCl_2$ and EMS were genotoxic to BB. Onion was more sensitive to 10 of 13 genotoxicity indices used.

Key words: Onion, broad bean, root meristem, genotoxicity.

INRODUCTION

Genotoxicity tests are *in vitro* and *in vivo tests* designed to detect compounds which induce genetic damage directly or indirectly by various mechanisms. The tests should enable hazard identification with respect to DNA damage and fixation (ICH, 1998). Genotoxicity tests have been used mainly for the prediction of carcinogenicity (ICH, 1998) and genotoxicity (Jena et al., 2002).

Over the past decade, issues of animal use and care in toxicology research and testing have become one of the fundamental concerns for both science and ethics. Emphasis has been given to the use of alternatives to mammals in testing research and education (Mukhopadhyay et al., 2004). Plants can be valid as alternatives because chemicals which cause chromosomal aberration (CA) in plant cells also produce CA in cultured animal cells and most frequently the aberrations are identical (Grant, 1978).

Many chromosome aberration assay systems using meristematic mitotic cells of plants have been developed and are now in use, such as onion (*Allium cepa*) (Ma, 1982; Rank and Nielsen, 1994), broad bean (*Vicia faba*) (Ma, 1982; Rank and Nielsen, 1994), barley (*Hordeum vulgare*) (Constantin, 1982) and tomato (*Solanum lycopersicum*) (Grant and Owens, 2002). However, it has been found that plant species differ in sensitivities and species like *Tradescantia* have been found to be less susceptible to chromosome breakage than *V. faba* (Ahmed and Grant, 1972) and barley is also less sensitive than *V. faba* (Grant, 1967) and the *A. cepa* root micronucleus assay is a more efficient test system than the *V. faba* test system (Ma et al., 1995).

Chlorpyrifos (CPF) is an emulsifiable broad-spectrum organophosphate contact and stomach insecticide (EPA, 1984) and a mutagen (Marquis, 1986). Mercury (II) chloride or mercuric chloride ($HgCl_2$) is a white crystalline

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Abbreviations: CA, Chromosomal aberration; **BB,** broad bean; **CPF,** chlorpyrifos; **$HgCl_2$,** mercury chloride, **EMS,** ethyl methanesulphonate; **GR,** garden ripcord; **MI,** mitotic index.

laboratory reagent. It is highly toxic to both eukariotic and prokaryotic cells; its effects depend on the route of exposure and the nature of the mercury compounds (Lee et al., 1997). Human and rat cells have been reported to have DNA fragmentation after *in vitro* and *in vivo* treatment with methylmercury chloride and dimethylmercury (Betti et al., 1993). Mercury chloride ($HgCl_2$) is toxic (Rozgaj et al., 2005). Garden ripcord (GR) is an emulsifiable concentrated insecticide with low toxicity towards mammals. The active ingredient in GR is cypermethrin (20 g/L), a pyrethroid (WHO, 1989). Ethyl methanesulfonate (EMS) is an alkylating agent used experimentally as a mutagen, teratogen, brain carcinogen and as a research chemical (IARC, 1974; Merck, 1989).

The present study was therefore aimed at comparing the sensitivity of the cells of the root meristems of onion and broad bean at detecting genotoxins by exposing them to CPF, $HgCl_2$, GR and EMS. Onion (*A. cepa*) with 2n = 16 (Ma, 1982; Rank et al., 1994) has a total length of the 2n complement of chromosomes as 14.4 µm (Ma et al., 1995) while broad bean (*V. faba*) with 2n = 12 (Hessayon, 2003) has a total length of the 2n complement of chromosomes as 9.32 µm (Ma et al., 1995).

MATERIALS AND METHODS

Test organisms *Onion seeds*

Variety of Texas Grano 502 PRR, a product of Sakata seeds Lanseria 1748, Republic of South Africa, was purchased from Maseru garden centre, Lesotho, Southern Africa.

Broad bean seeds

Aquadulce packed Mayford seeds, a product of Sakata seeds Lanseria 1748 South Africa, was purchased from Maseru garden centre, Lesotho, Southern Africa.

Test chemicals

Chlorpyrifos (480 g/L) and garden ripcord (20 g/L cypermethrin) used were products of Agro-serve (Pty) Ltd, trading as Efecto, Republic of South Africa. Mercuric chloride used was a product of Merck Laboratories supplies (Pty) Ltd Republic of South Africa while Ethyl methane-sulphonate was a product of Sigma-Aldrich Co. St Louis USA.

Reagents

Ethanol (absolute) was gotten from the Associated Chemical Enterprises (PTY) LTD of the Republic of South Africa; Hydrochloric acid and Glacial acetic acid were products of UNILAB of the Republic of South Africa; Aceto-carmine stain from Carolina Biological Supply Company, USA.

Preliminary seed dose selection experiments

Preliminary dose selection experiments were conducted for each chemical versus each plant with concentration ranges derived from the literature (% solution in water). However, in cases where no inhibition of germination was observed, other concentrations were tested. For each test, 100 onion seeds were spread on a filter paper moistened with a specific concentration of the test compound in a Petri dish and kept for 3 days at room temperature to germinate. For broad bean the number was 50 seeds per Petri dish and kept for 7 days. The number of seeds that produced a radicle were recorded and compared to the number of seeds that germinated in the concurrent water treated negative control to derive the percentage germinating at each concentration of test compound. The EC_{50} for each compound in each plant was determined from the curve of percentage that germinated against dose.

Genotoxicity assay

A discontinuous treatment protocol, similar to the method of Matsumoto et al. (2006) was employed. Seeds were spread on water moistened filter paper in Petri dishes until they germinated and the radicles reached a length of about 5 cm. Germinated seeds were transferred onto filter paper kept moistened in a Petri dish with specific concentration (EC_{50}, ½ EC_{50} or ¼ EC_{50}) of test compound, determined in the preliminary dose selection experiment and incubated for 24 h at room temperature. For the concurrent negative control, the seeds were placed on water moistened filter paper in Petri dish.

Root harvest and slide preparation

At the end of the 24 h exposure, two root tips from two seeds per dose were collected at random and assessed. Root tips 1 - 2 cm long were cut from each treated seed and placed in a small glass specimen bottle and fixed in acetic alcohol (ethanol: glacial acetic acid in 3:1 ratio) for 24 h in a fridge at 4 - 6°C. The root tips were washed twice with ice cold water for 10 minutes each and allowed to dry in a watch glass. A solution of 1 N HCl pre-heated to 60°C was added to the root tips in the watch glass for 10 min and the HCl was discarded. The HCl treatment was repeated a second time. The root tips were transferred to clean microscope slides and cut 2 mm from the growing tip. The tips were kept and the rest was discarded. Aceto-carmine stain was added to each slide to cover the root tip for about 10 min. A glass cover slip was placed on the root tip and tapped gently with a pencil eraser to spread the cells evenly to form a monolayer in order to facilitate the scoring process for normal and aberrant cells in the different stages of the cell cycle.

Scoring of slides and data analysis

Mitotic index (MI) determination

The slides were viewed under the light microscope (Olympus CX 21) using the 100X objective lens with oil immersion. On one slide for each treatment, a total of 2000 cells were scored and recorded as interphase or dividing (prophase, metaphase, anaphase and telophase) cell to determine the MI. MI was expressed as the number of dividing cells per 1000 cells scored.

Cytotoxicity: For onion or broad bean, the mitotic indices of the treated cells at each dose of each test compound were compared with that of the negative control group. A dose of test compound was adjudged cytotoxic if the mitotic index of treated cells was ≤ ½ of the mitotic index of the concurrent water treated cells. **Genotoxicity:** A total of 100 anaphase and telophase cells from one or two slides per dose of each test compound were examined

Table 1. Determination of the mitotic index among 2000 cells scored following 24 h exposure of onion root tip cells to three concentrations each of the different chemicals.

Compound	Conc. (% solution)	Interphase	Cells in division stages per 2000 cells scored					MI	MI as % of control
			Proph.	Metaph.	Anaph.	Teloph.	Total		
H_2O		1883	60	20	16	21	117	59	100
CPF	0.0194	1918	43	2	19	18	82	41	69
	0.0388	1939	75	10	5	9	99	49.5	83.9
	0.0775	1968	23	4	5	0	32	16	27*
$HgCl_2$	0.0246	1912	67	3	1	17	88	44	75
	0.0493	1919	44	5	4	22	81	40.5	69
	0.0985	1957	20	13	5	5	43	21.5	36*
EMS	0.02148	1948	36	5	4	7	52	26	44*
	0.043	1931	42	10	7	11	70	35	59
	0.08593	1972	28	0	0	0	28	14	24*
GR	0.1875	1522	327	50	45	56	478	239	405
	0.375	1644	276	22	22	26	346	173	293
	0.75	2000	0	0	0	0	0	0	0*

MI = Mitotic index (number of cells in division stages out of 1000 cells); CPF = chlorpyrifos; $HgCl_2$ = mercury chloride; EMS = ethyl methane sulphonate; GR = garden ripcord; H_2O = water; * = toxic.

for chromosome fragment, bridge, bridge + fragment, vagrant, c-anaphase, multipolarity and stick chromosomes. The percentage of anaphase-telophase cells with aberrations at each dose of each test compound was compared with that of the negative control using the chi-squared test (SPSS 10.0 for Windows statistical package). A dose of test compound was considered to be genotoxic if the chi-squared test was significant at P = 0.05. The most representative of each aberrant type was photographed using a Zeiss Primo Star microscope fitted with Canon digital camera model Power Shot A640.

Determination of CA/mole at 50% MI of control

Molarities were calculated using the formula:

Molarity = [(Conc. (that is, % of solution)/100) × 1000]/FW, for EMS and $HgCl_2$

Molarity = [(Conc. (that is, % of solution/100) × grams in formulation/1000]/FW, for CPF and GR.

The CA/mole at 50% MI of control was obtained from the trend line of the curve of CA/Mole against MI for each combination of chemical and plant seed. The point at which the vertical line from the 50% MI on the X-axis cut the trend line gave the CA/mole at 50% MI of control.

RESULTS

Cytotoxicity of the test compounds

The results of the cytotoxicity determination are presented in Tables 1 and 2 for onion root tips and broad bean root tips, respectively.

Onion test

In Table 1, all four test compounds were toxic to the onion root tip cells at the highest concentration and in the case of EMS at the lowest concentration as well. The first two concentrations of GR stimulated cell division while the highest concentration completely inhibited cell division.

Broad bean test

In Table 2, CPF was not toxic; the highest concentration of $HgCl_2$ was toxic. All three concentrations of EMS were toxic and the first two concentrations of GR stimulated division.

Genotoxicity of the test compounds

The result of the determination of the genotoxic effects of the test compounds are presented in Tables 3 and 4 for onion root tips and broad bean root tips, respectively.

Onion test

In Table 3, all four compounds were genotoxic to the onion root tip cells at two concentrations or more. At the highest concentrations of $HgCl_2$, EMS and GR the toxicity masked the genotoxicity.

Broad bean test

The results of the genotoxicity test of the chemicals to broad bean root tip cells are presented (Table 4). CPF, $HgCl_2$ and EMS treatment induced significant ($P < 0.05$) levels of genotoxic effect in a dose dependent manner at

Table 2. Determination of the mitotic index among 2000 cells scored following 24 h exposure of broad bean root tip cells to three concentrations each of the different chemicals.

Compound	Conc. (% solution)	Interphase	Cells in division stages per 2000 cells scored					MI	MI as % of control
			Proph.	Metaph.	Anaph.	Teloph.	Total		
H_2O		1793	112	44	27	24	207	104	100
CPF	0.1134	1897	65	17	16	14	112	56	54
	0.2268	1857	89	25	19	10	143	71.5	69
	0.4537	1880	90	22	6	2	120	60	58
$HgCl_2$	0.0318	1860	68	45	12	15	140	70	67
	0.0636	1885	56	36	9	14	115	57.5	55
	0.1273	1918	55	13	7	7	82	41	39*
EMS	0.0273	1951	38	7	2	2	49	24.5	24*
	0.0547	1988	6	3	2	1	12	6	6*
	0.1094	1980	20	0	0	0	0	20	10*
GR	0.0542	1560	194	106	71	69	440	220	212
	0.108	1573	245	89	38	55	427	213.5	205
	0.2167	1880	69	30	10	11	120	60	58

MI = Mitotic index (number of cells in division stages out of 1000 cells); CPF = chlorpyrifos; $HgCl_2$ = mercury chloride; EMS = ethyl methane sulphonate; GR = garden ripcord; H_2O = water; * = toxic.

Table 5. Genotoxicity sensitivity indices following treatment of onion or broad bean root tips for 24 h with four different chemicals.

TC	Test organism							
	Onion				Broad bean			
	CAMI	EC_{50} (moles)	MPC	GS	CAMI	EC_{50} (moles)	MPC	GS
CPF	153000	0.00106	0.00106	Yes	8500	0.00621	0.00621	Yes
$HgCl_2$	7000	0.00363	0.000908	Yes	17500	0.00469	0.00469	Yes
EMS	12500	0.00692	0.00173	Yes	1600	0.00881	0.00881	Yes
GR	160000	0.00036	0.00018	Yes	30000	0.000104	0.000104	No

Conc. = Molarity; MI = mitotic index (%MI of control); CA % = chromosomal aberrations as % of A-T cells scored; GR = garden ripcord (cypermethrin at 20 g/L); $HgCl_2$ = mercury chloride; EMS = ethyl methane sulphonate; CPF = chlorpyrifos; TC = test compound; CAMI = CA/mole at 50% MI of control; MPC = conc. that induced highest CA (most potent conc. in moles); GS = genotoxicity susceptibility.

two or three doses compared to the negative control. GR however did not induce significant levels of genotoxic effect at any of the concentrations tested, compared to the negative control.

All four chemicals induced significant ($P < 0.05$ and/or 0.01) level of genotoxic effect at one or more doses to onion root tip cells. CPF, $HgCl_2$ and EMS were genotoxic to broad bean root tip cells at one or more of the doses tested. GR however, did not induce significant ($P > 0.05$) level of genotoxic effect to broad bean root tips at any of the doses tested.

Comparison of the sensitivity of the plants to the genotoxins

In Table 5, the sensitivities of onion root tips and broad bean root tips to the four chemicals were compared using 4 sensitivity indices. These were EC_{50} (moles), CA/mole at 50% MI of control, concentration that induced highest CA (moles) that is, the most potent dose (MPC) and genotoxicity susceptibility. Onion was more sensitive to CPF and EMS when all three parameters were considered, to $HgCl_2$, when EC_{50} and MPC were considered and to GR when CA/mole was considered. Onion root tip cells detected the geonotoxic activities of all four chemicals. Broad bean was more sensitive to GR when EC50 and MPC were considered and to $HgCl_2$ when CA/mole was considered. Broad bean root tip cells could not detect the genotoxic activities of GR. Thus, of the 13 comparisons made (4 chemicals × 3 indices plus number of chemicals sensitive to), onion was more sensitive to 10 while broad bean was more sensitive to 3. Onion root tip cells were therefore more sensitive. The greater sensitivity of the *A.* roots is probably due to the greater total length of the diploid complement and the higher number of metacentric chromosomes. In Figure 1, the photographs of the most representative of each aberrant type are presented.

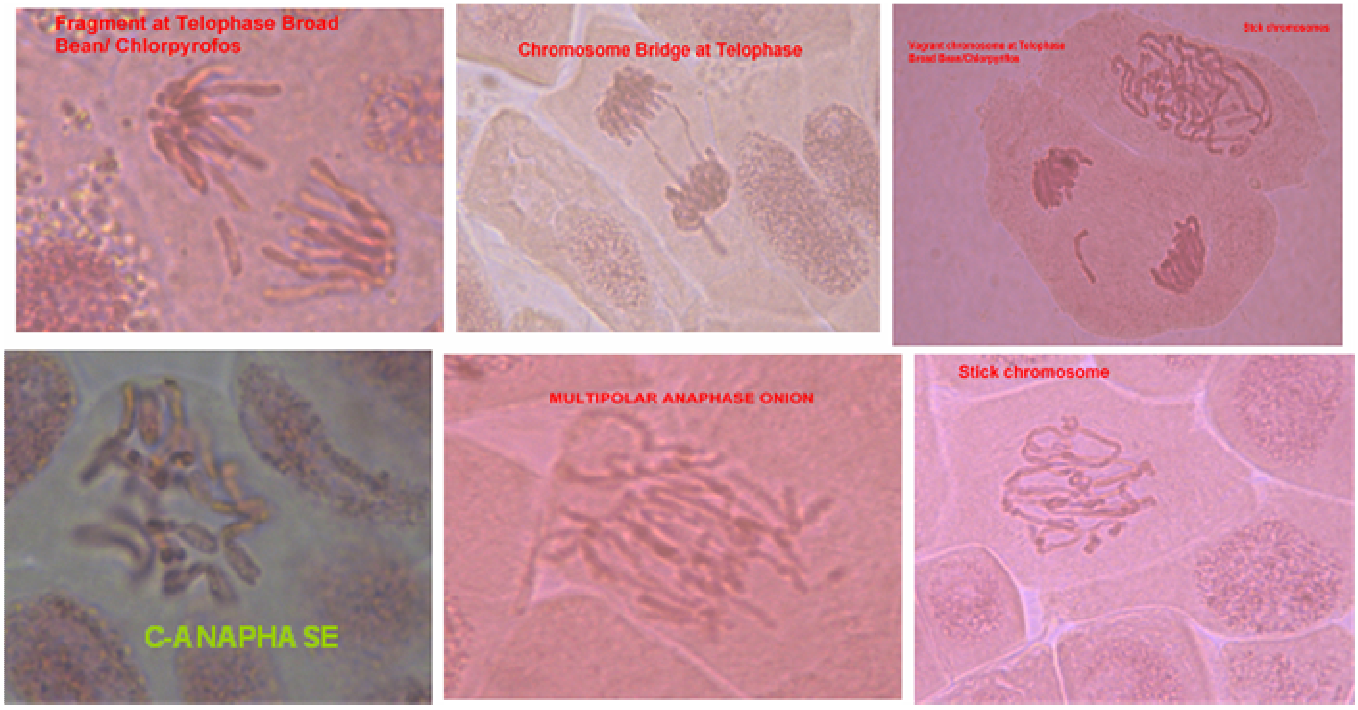

Figure 1. The aberrations observed when onion or broad mean root tip cells were exposed to the chemicals.

DISCUSSION

Since the use of animals in toxicology research and testing is a concern for both science and ethics, it is important to consider plants as alternatives to mammals. Plants can be valid as alternatives because chemicals which cause CA in plant cells also produce CA in cultured animal cells and most frequently the aberrations are identical (Grant, 1978). However, plant species differ in their sensitivities and species like *Tradescantia* have been found to be less susceptible to chromosome breakage than *V. faba* (Ahmed and Grant, 1972) and barley is also less sensitive than *V. faba* (Grant, 1967).

The present study compared the susceptibility of onion and broad bean root tips to the toxic and genotoxic effects of CPF, $HgCl_2$, EMS and GR. All four chemicals were toxic to onion root tip cells at one or more of the doses tested and GR also stimulated cell division at low concentrations. EMS and $HgCl_2$ were toxic to broad bean at one or more doses tested, none of the doses of CPF or GR tested was toxic to broad bean. In addition, GR stimulated cell division at low concentrations. All four chemicals induced significant ($P < 0.05$ and/or 0.01) level of genotoxic effect at one or more doses to onion root tip cells. CPF, $HgCl_2$ and EMS were genotoxic to broad bean root tip cells at one or more of the doses tested. GR however, did not induce significant ($P > 0.05$) level of genotoxic effect to broad bean root tips at any of the doses tested. CPF (Marquis, 1986; Asita and Makhalemele, 2008), mercury and/or mercury compounds (Grant, 1978; Rossmann, 1995), EMS (IARC, 1974; Merck, 1989) and cypermethrin and/or formulations containing cypermethrin such as GR (NPTN, 1998; Amer et al., 1993) have been shown to induce genotoxicity and/or toxicity in different test systems *in vitro* and/or *in vivo* involving the use of animals, animal cells or microorganisms.

When the sensitivities of onion and broad bean were compared using the EC_{50} (in moles), CA/mole at 50% MI of control, the concentration that induced the highest CA and the number of chemicals sensitive to, onion was more sensitive to 10 and broad bean to 3 out of the 13 comparisons. In a study that was designed to compare the *Allium* and the *Vicia* root micronucleus (MCN) assays on the basis of chromosome length, peak sensitivity of the mitotic cells, and the regions of the root tip where the MCN are formed, the *Allium* root MCN was found to be a more efficient test system (Ma et al., 1995). Though the indices used and the test agents (X-ray and chemicals) were different from the chemicals and indices used in the present study, both studies have found the onion root tip assay to be a more efficient test system. The greater sensitivity of the *Allium* roots was considered to be due, probably to the greater total length of the diploid complement and the higher number of metacentric chromosomes of onion cells (Ma et al., 1995).

Conclusion

The present study compared the susceptibility of onion and broad bean root tip cells to the toxic and genotoxic

effects of CPF, $HgCl_2$, EMS and GR. Onion was more sensitive to 10 and broad bean to 3 out of the 13 indices of genotoxic effects. In addition, onion root tip cells were sensitive to the toxic effects (MI of treated cells ≤ ½ of control) of all four chemicals. Only EMS and $HgCl_2$ were toxic to broad bean at one or more doses tested and none of the doses of CPF or GR tested was toxic to broad bean. In addition, GR stimulated cell division at low concentrations.

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