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PHENYL MERCURY ACETATE INDUCED ALTERATIONS IN THE KARYOTYPE OF *PHLOX DRUMMONDII* HOOK

A. K. SRIVASTAVA AND R. GAUTAM

Department of Botany, C.C.S. University, Meerut 205 004

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ABSTRACT

The adverse effect of phenyl mercury acetate (PMA) solutions, 10^{-7} M, 10^{-6}M, 10^{-5}M, 10^{-4} M and 10^{-3} M, on the somatic karyotype of the M_1 seedlings (directly treated) and M_2 seedlings (untreated seedlings raised from seeds harvested from M_1 plants) of *Phlox drummondii* Hook was analysed. A concentration as low as 10^{-7}M of PMA could induce karyotypic changes confirming its genotoxic potentiality. Moreover, all the solutions produced alterations in the somatic karyotype of the M_1 seedlings most of which were also visible in M_2 seedlings. Although, M_1 treated sets were having both numerical as well as structural chromosome mosaicism, M_2 'treated sets' were showing only structural chromosome mosaicism. A major effect of PMA was change in the volume of chromosome and total length and volume of chromosome complements of the cells.

Key words : *Phlox drummondii*, Phenyl mercury acetate, karyotype, induced alteration.

The heavy metal, mercury can persist in numerous diverse physico- chemical forms having various environmental and biological behavior. The interconversion between these forms influences the environmental mobility of mercury and determines its biological enrichment and consequences. Further, it is an exceedingly toxic heavy metal whose concentration in the environment is progressing with an alarming rate due to its demand in numerous spheres of contemporary human society. With the first systematic evaluation of the cytological effect of mercury in *Allium* by Fahmy[1], information about their cytogenetic effects in several test systems have accumulated. However, most of these studies are related to its outcome on general cytology of somatic cells. The current article deals with the consequence of the treatment with an organomercurial, phenyl mercury acetate, on the somatic karyotype of *Phlox drummondii.*

MATERIALS AND METHODS

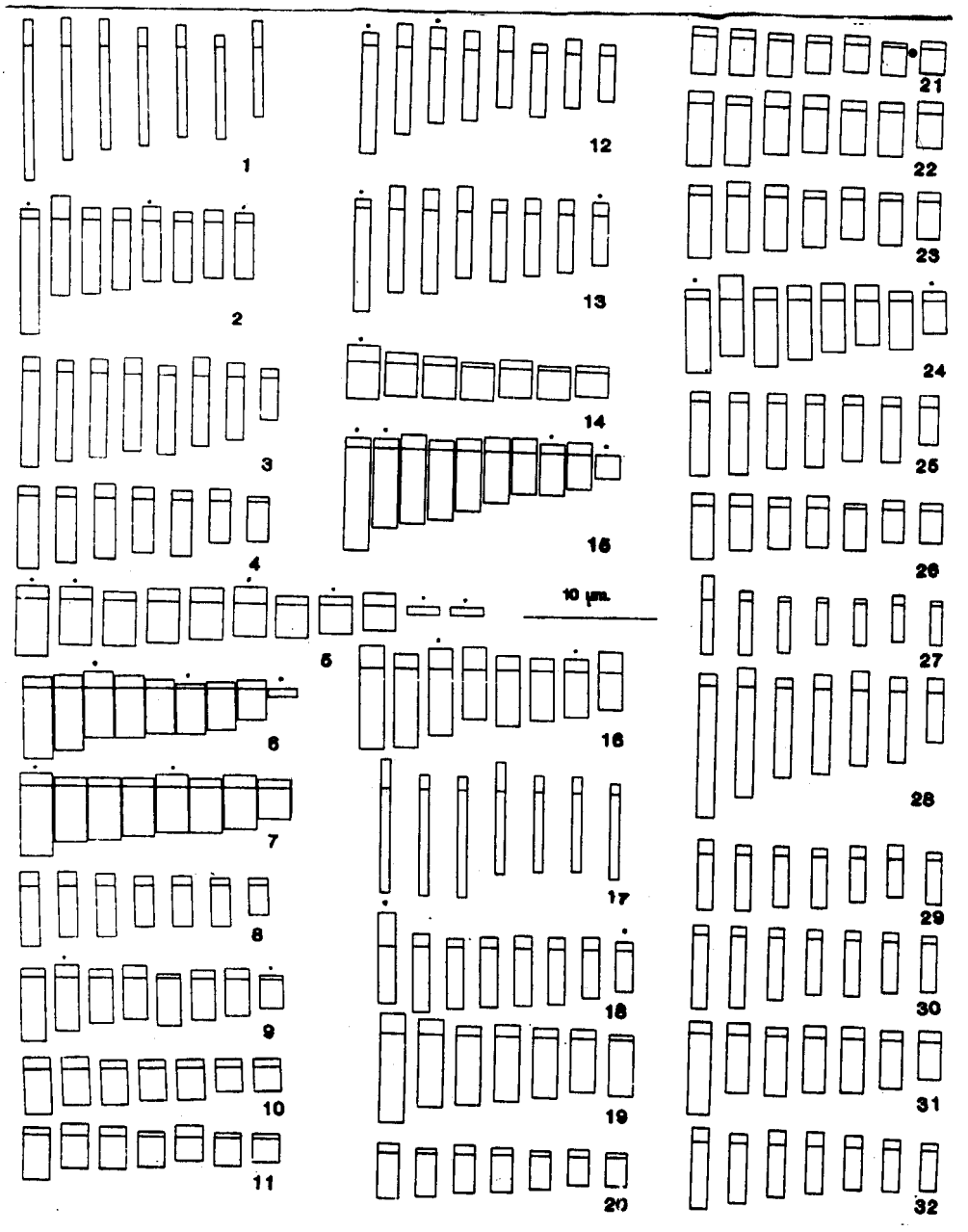
Phenyl mercury acetate (PMA) solutions of concentrations, 10^{-7}M, 10^{-6}M, 10^{-5}M, 10^{-4}M and 10^{-3}M, were used for treating the seeds/seedlings, for 48 hours, 96 hours

and 144 hours, of *Phlox drummondii* at about 20°C in total darkness. Following the treatments, the M_1 seedlings were washed carefully with distilled water and transferred to it for 24 hours. Control set was raised in distilled water. About twenty M_1 seedlings from each treatment and control set were grown in polythene bags having a mixture of soil manure, and irrigated with tap water, for raising M_1 plants. Each set of plants (control or treated) were grown in isolation at the time of flowering. Root tip samples of M_1 seedlings were collected and fixed in freshly prepared Carnoy's fluid I for about 48 hours and later stored in refrigerator in 70% ethanol. However, M_1 control root tips collected were pretreated with p-dichlorobenzene before fixation. The M_2 seeds including control were grown in distilled water and the root tips collected were pretreated and fixed in the said procedure. The karyotypes were analysed following Srivastava and Purnima[2]. The volume of chromosomes at C-metaphase was worked out by assuming each chromosome as of two cylinders, corresponding to two sister chromatids.

The chromosomes were climbed into categories on the basis of arm's ratio [3]. These were further subdivided into four types *i.e.* A-D, on the basis of total length of the chromosome (A = > 8.5 μm, B = 8.5-7.0 μm, C = 6.9-5.5 μm and D = < 5.5 μm). The chromosome complements, as a whole, were divided, into four types *i.e.* W-Z, on the basis of mean radius of the chromatids of a complement (W = > 0.45 μm, X = 0.45-0.30 μm, Y = 0.30-0.15 μm and Z = < 0.15 μm).

RESULTS AND DISCUSSION

PMA induced karyotypic changes were elucidated using the parameters like chromosome number, morphology of chromosomes (including length and volume), and total length and volume of chromosome complement of a cell. Data affiliated to chromosomes of the complements were used for constructing the haploid karyotype represented as ideograms in figures 1-32. The chromosome 'pairs' for constructing it were made on the basis of morphological equivalence between chromosomes of a set. Data on total length of short arms (TLSA), long arms (TLLA), chromosome complements (TLCC), average radius (AR) of a chromatid of a chromosome set and karyotype formulae for control and treated sets of M_1 and M_2 are given in tables 1 and 2 respectively. Graphical representation of total volume of chromosome complement (TVCC) is presented in figure 33. Data presented in table 1 are the mean data of the cells showing foremost type of deviation in the root tip issues of one set. The control sets of both M_1 as well as M_2 possessed 2n=14, with only 'A' categories of chromosomes bearing centromeres submedian, subterminal or nearly terminal in position. Although, the length of the chromosomes of the two controls (M_1 and M_2) differed slightly, the TLCC and TVCC were more or less the same.



Figs. 1-32. Effect of PMA on karyotype of *P. drummondii*; (for details please see tables 1 and 2) • = 'unpaired' chromosomes.

Table 1. PMA induced changes in the karyotype of M_1 seedlings

	2n	TLSA	TLLA	AR	KF	Fig.
Cont.	14	28.28	125.52	0.19	Y[10A(st)+2A(t)+2A(sm)]	1
48 h.						
10 M	12+3	14.40	77.33	0.35	X[1A(T)+2B(st)+7C(st)+4D(st)1D(T)]	2
10^{-6}M	11+3	15.16	85.10	0.35	X[2A(st)+4B(t)+5B(st)+2C(st)+1D(st)]	3
10^{-5}M	14	11.10	61.77	0.38	X(2C(t)+4C(st)+6D(st)+2D(t)]	4
10^{-4}M	10+6	14.42	48.84	0.66	W[1C(st)+8D(st)+5D(sm)+2D(T)]	5
10^{-3}M	12+3	12.19	62.09	0.65	Q[2B(st)+5C(st)+6D(st)+2D(T)]	6
96 h.						
10^{-7}M	12+2	11.84	59.93	0.71	Q[1B(st)+4C()st)+9D(st)]	7
10^{-6}M	14	11.45	50.69	0.35	X[4C(st)+10D(st)]	8
10^{-5}M	12+2	10.72	56.96	0.52	Q[2C(st)_+1C(st)+9D(st)+2D(T)]	9
10^{-4}M	14	11.84	42.18	0.56	W[8D(st)+6D(sm)]	10
10^{-3}M	14	8.49	34.02	0.48	Q[10D(st)4D(sm)]	11
144 h.						
10^{-7}M	12+2	20.69	83.61	0.32	X[4A(st)+2B(st)+2C(t)+2C(st)+2D(st)]	12
10^{-6}M	12+2	21.41	88.80	0.33	X[1A(T)+4A(st)+2B(sm)+2B(st)+5C(st)]	13
10^{-5}M	12+1	8.86	35.89	0.66	Q[1D(sm)+10D(st)+2D(t)]	14
10^{-4}M	12+4	13.30	66.96	0.48	Q[1A(T)+1B(t)+2B(st)+2C(t) 2C(st) +5D(st)+2D(sm)+1D(t)]	15
10^{-3}M	12+2	22.18	72.89	0.44	X[2A(st)+3B(st)+2C(sm)+4C(st)1D(st) +2D(sm)]	16

2n = 'Paired' + 'unpaired' chromosomes; TLSA = Total length of all long arms (μm); TLLA = Total length of all long arms (μm); AR-Average radius (μm) of one chromateid; KF = Karyotypic formula.

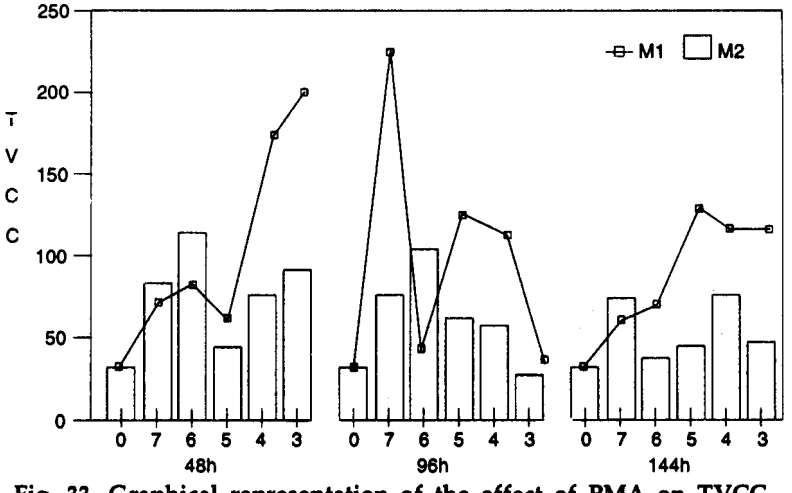


Fig. 33. Graphical representation of the effect of PMA on TVCC.

Table 2. PMA induced changes in the karyotype of M_2 seedlings.

	TLSA	TLLA	AR	KF	Fig.
Cont.	114.74	23.12	0.20	Y[6A(st)+6A(t)+2A(sm)]	17
48 h.					
10^{-7}M	15.72	71.58	0.38	X[1B(sm)+2B(st)+2C(t)+8C(st)1D(st)]	18
10^{-6}M	12.60	74.38	0.45	X[21A(st)+2B(st)+2C(t)+4C(st)2D(st)+2D(T)]	19
10^{-5}M	7.42	39.42	0.39	X[12SD(st)+2D(1)]	20
10^{-4}M	8.72	40.16	0.49	W[14D (st)]	21
10^{-3}M	12.42	53.82	0.47	W[2C(st) + 2D(t)+8D(st)+2D(sm)]	22
96 h.					
10^{-7}M	11.12	54.38	0.42	X[4C(st)+10D(st)]	23
10^{-6}M	15.74	61.07	0.46	W[1C(t)+2C(sm)+4C(st)+5D(st)+2D(sm)]	24
10^{-5}M	10.74	67.32	0.35	X[4C(t)+4C(st)+6D(st)]	25
10^{-4}M	10.40	45.48	0.41	X[12D(st)+2D(t)]	26
10^{-3}M	9.62	51.02	0.25	Y[2C(sm)+4D(st)+2D(st)+2D(T)+6D(t)]	27
144 h.					
10^{-7}M	15.92	96.20	0.33	X[2A(T)+2A(st)+2B(t)+4B(st)+2C(st)+2D(st)]	28
10^{-6}	11.68	54.20	0.29	Y[2C(st)+10D(st)+2D(sm)]	29
10^{-5}M	10.00	66.94	0.29	Y[4C(t)+2C(st)+8D(st)]	30
10^{-4}M	12.08	62.14	0.41	X[2B(st)+2C(st)+2D(t)+8D(st)]	31
10^{-3}M	12.60	54.76	0.34	X[2C(st)+12D(st)]	32

TLSA = Total length of all short arms(um); TLLA = Total length of all long arms(um)'
AR = Average radius (um) of one chromatid; KF = Karyotypic formula.

A concentration as low as 10^{-7}M of PMA could induce karyotypic changes confirming its genotoxic potentiality. PMA treatments accomplished chromosome mosaicism in all the treated samples of M_1 committing changes in number of chromosomes per cell, arms ratio, mean radius of chromatids and total length and value of the chromosome complements. The M_1 treated sets were having both numerical as well as structural chromosome mosaicism within root tips. However, the majority of cells possessed karyotype similar to that of control. The cells showing karyotypic deviations were differing from the control in number of chromosomes, morphology of chromosomes, AR of the chromatids, TLCC and TVCC. Numerical chromosome mosaicism resulted from the presence of telocentric fragments, in addition to fourteen chromosomes.

Numerical chromosome changes, present in some M_1 treated samples, were due to presence of telocentric fragments. Such fragments were also recorded by Baniskar and Srivastava[4] in *Pennisetum typhoides* after treatment with mercuric chloride. The existence of telocentric fragments indicate the competence of PMA to provoke

chromosome breakage and the susceptibility of centromeric region for breakage. The higher proneness of centromere for breakage than other portions of the chromosomes can also be strengthened by the earlier observations of Arora and Rao[5], Banerjee[6], Carrano and Wolff [7], Chakrabarti and Bhattachrya[8] and Hsu and Pathak [9]. Since the telocentric fragments were not found during M_2, it can be safely inferred that cells bearing them were eliminated in due course of development.

M_2 'treated sets' although were not showing numerical chromosomal mosaicism, these were showing structural chromosome mosaicism in many cells. The rest cells were similar in karyotype with the M_2 control.

In all the treated M_1 and M_2 sets, the reduction in total length of chromosome complements was associated with increase in mean radius of the chromatids, reasonably increasing the volume of the complement. The plausible comprehension for this change could be the alteration induced in packaging pattern of chromatin in chromosomes and/or increase in the amount of chromatin per cell.

The presence of chromosomal mosaicism and altered TLCC and TVCC in M_1 treated seedlings could be the direct effect of PMA on cytological set up of cells. But, their presence in M_2 'treated' seedlings which were raised in distilled water needs explanation. Whether these changes were due to mutation(s), can only be ascertained after proper genetic analysis which was not performed presently.

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