Chromosome Breakage Through Paradichlorobenzene Treatment

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Received February 8, 1956

Introduction

Paradichlorobenzene has been utilised for various cytological procedures by a number of workers. Its importance was first realized by Carey & McDonough (1943), who explored its polyploidizing property. Later on Meyer (1945) worked out its efficacy as a prefixing agent and advantageously employed the procedure in investigation of the chromosome counts of plants. With the realization of this important property of this chemical, it has been successfully applied for chromosome studies in varied groups of plant. Very recently, paradichlorobenzene has been found to be very suitable by Sharma & Mookerjee (1955) for chromosome counts of plants with high chromosome number, for which a special technique has been devised by them.

A number of chemicals has lately been nicely utilised by Levan and others (1948, 1949) for inducing chromosomal abnormalities in somatic cells of the plants. As is apparent, the potentialities of this line of investigation are immense. The present report deals with the properties of paradichlorobenzene in causing chromosome breakage in both Dicotyledonous and Monocotyledonous members of Angiosperms.

Material and methods

The following species of plants were the source of materials for this present investigation :

<i>Dicotyledons :</i>	1. <i>Limnanthemum cristatum</i> Griseb.
	2. <i>Lens esculenta</i> Moench.
	3. <i>Vicia sativa</i> Linn.
	4. <i>Lathyrus sativus</i> Linn.
	5. <i>Pisum sativum</i> Linn.
	6. <i>Cicer arietinum</i> Linn.
<i>Monocotyledons :</i>	7. <i>Allium cepa</i> Linn.
	8. <i>Zephyranthes mesochloa</i> Rafin.
	9. <i>Hemerocallis aurantiaca-major</i>
	10. <i>Hydrilla verticillata</i> Rich.
	11. <i>Crinum zeylanicum</i> Linn.

12. *Hymenocallis littoralis* Salisb.
13. *Hippeastrum rutilum* Herb.
14. *Hymenocallis rotata* Salisb.
15. *Haementhus Kalbreyeri* Baker.
16. *Hymenocallis harrisiana*

Most of the plants tried for the present experiment were collected from the experimental garden attached to the Botany Department of the University College of Science, Calcutta University, except the Leguminous plants. Seeds of the Leguminous plants were obtained from the store of the Botany Department and germinated in moist saw-dust in the Laboratory.

Healthy root-tips were collected for the experiment between 11 A.M. & 1 P.M. and treated in para-dichlorobenzene solution (saturated solution in distilled water without heating) for $2\frac{1}{2}$ – $4\frac{1}{2}$ hours, according to the requirement in different species (vide Table 1) at 10–14°C. Subsequent to the treatment, the root-tips were heated in orcein-hydrochloric acid mixture (2 % aceto-orcein and NHCl in the proportion of 9:1) for 5–6 seconds and then squashed in 1 % aceto-orcein solution.

Table 1. Period of treatment causing fragmentation

Plants examined		Hours of treatment 10–14°C
Dicotyledons:	1. <i>Limnanthemum cristatum</i>	$4\frac{1}{4}$ hours
	2. <i>Lathyrus sativus</i>	$4\frac{1}{4}$ „
	3. <i>Lens esculenta</i>	$4\frac{1}{2}$ „
	4. <i>Pisum sativum</i>	$4\frac{1}{2}$ „
	5. <i>Cicer arietinum</i>	$4\frac{1}{2}$ „
	6. <i>Vicia sativa</i>	$4\frac{1}{4}$ „
Monocotyledons:	7. <i>Allium cepa</i>	$3\frac{1}{2}$ „
	8. <i>Hymenocallis Littoralis</i>	4 „
	9. <i>Zephranthes mesochloa</i>	$3\frac{1}{4}$ „
	10. <i>Haementhus Kalbreyeri</i>	4 „
	11. <i>Hydrilla verticillata</i>	$3\frac{1}{2}$ „
	12. <i>Hemerocallis aurantiaca-major</i>	4 „
	13. <i>Crinum zeylanicum</i>	$3\frac{1}{2}$ „
	14. <i>Hippeastrum rutilum</i>	$1\frac{1}{2}$ „
	15. <i>Hymenocallis rotata</i>	$3\frac{1}{2}$ „
	16. <i>Hymenocallis harrisiana</i>	$3\frac{1}{2}$ „

Recovery experiments were also performed to note the fate of fragments (vide Table 2). For this purpose, plants with attached healthy roots were taken and their tips were treated with paradichlorobenzene solution for a period required to cause fragmentation of chromosomes (vide Table 1) at 10–14°C. Subsequent to the treatment, the roots were thoroughly washed in water and allowed to grow in Knop's culture solution. The root-tips were

Table 2. Recovery experiment in Knop's culture solution after paradichlorobenzene treatment

Plant examined	Data on the presence or absence of fragments following different periods of recovery				Remarks
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	
1. <i>Limnanthemum cristatum</i>	+	+	+	+	Frequency of fragments high after 24 and 48 hours, low after 72 and 96 hours.
2. <i>Lathyrus sativus</i>	+	+	+	+	Frequency of fragments comparatively high even after 96 hours.
3. <i>Lens esculenta</i>	+	+	+	+	Frequency of fragments high even after 96 hours.
4. <i>Pisum sativum</i>	+	+	+	+	Frequency of fragments high after 24, 48 and 72 hours, low after 96 hours.
5. <i>Cicer arietinum</i>	+	+	+	-	Frequency of fragments high after 24 and 48 hours, low after 72 hours. Absence of fragments after 96 hours indicates complete recovery.
6. <i>Vicia sativa</i>	+	+	+	+	Frequency of fragments high after 24, 48 and 72 hours, low after 96 hours.
7. <i>Allium cepa</i>	+	+	+	+	Frequency of fragments high even after 96 hours.
8. <i>Hymenocallis littoralis</i>	+	+	-	-	Frequency of fragment high after 24 hours, low after 48 hours. Absence of fragments indicates complete recovery after 72 hours.
9. <i>Zephyranthes mesochloa</i>	+	+	+	+	Frequency of fragments high even after 96 hours.
10. <i>Haemanthus kalbreyeri</i>	+	+	+	+	Frequency of fragments high after 24, 48 and 72 hours, low after 96 hours.
11. <i>Hydrilla verticillata</i>	+	+			Frequency of fragments high after 24 and 48 hours. Examination could not be continued after 48 hours as the plant did not survive.
12. <i>Hemerocallis aurantiaca-major</i>	+	+	+	-	Frequency of fragments high after 24 and 48 hours, low after 72 hours. Absence of fragments after 96 hours indicates complete recovery.
13. <i>Hymenocallis rotata</i>	+	+	+	+	Frequency of fragments high after 24 and 48 hours, low after 72 and 96 hours.
14. <i>Crinum zeylanicum</i>	+	+	+	+	Frequency of fragments high after 24 hours; low after 48, 72 and 96 hours.
15. <i>Hymenocallis harrisiana</i>	+	+	+	+	Frequency of fragments comparatively high even after 96 hours.
+ Indicates presence of fragments.					
- Indicates absence of fragments.					

cut from the mother plant after 24, 48, 72 and 96 hours of recovery. These were then treated in hot orcein-N/HCl mixture for a few seconds, and smeared in 1 % aceto-orcein following the usual procedure in order to examine the

fate of the fragments and fragment containing cells. Care was taken through-

Figs. 1-9. 1-5, somatic metaphase stage of different species showing various number of fragments. The fragments have been filled with ink. 1-2, *Hymenocallis harrisiana*. 3-4, *Allium cepa*. 5, *Zephyranthus mesochloa*. $\times$ ca.1330. 6-9, somatic metaphase stage of different species showing various number of fragments. The fragments have been filled with ink. 6-7, *Haemanthus kalbreyeri*. 8, *Hydrilla verticillata*. 9, *Hemerocallis aurantiaca-major*. $\times$ ca.1330.

Figs. 10-25. 10-19, somatic metaphase stage of different species showing various number of fragments. The fragments have been filled with ink. 10, *Hymenocallis rotata*. 11-12, *Crinum zeylanicum*. 13, *Hymenocallis littoralis*. 14-16, *Lathylus sativus*. 17-18, *Lens esculenta*. 19, *Vicia sativa*. $\times$ ca.1330. 20-25, somatic metaphase stage of different species showing various number of fragments. The fragments have been filled with ink. 20-21, *Limnanthemum cristatum*. 22-23, *Pisum sativum*. 24-25, *Cicer arietinum*. Fig. 20-21, $\times$ ca.1930, Figs. 22-25, $\times$ ca.1330.

out the recovery experiment to cover the tube containing the Knop's culture solution with black paper.

The majority of the figures were drawn at a table magnification of approximately $\times 2,000$ using a Olympus compensating eyepiece of $\times 12$ and a 1.3 apochromatic objective with an aplanatic condenser of 1.4 N.A. Few

Fig. 26. Microphotographs of somatic metaphase stage of different species showing various number of fragments. a, *Haementhus kalbreyeri*. b, *Zephyranthes mesochloa*. c, *Hemerocallis aurantiaca-major*. d, *Hymenocallis harrisiana*. e, *Hydrilla verticillata*. f, *Allium cepa*. g, *Limnanthemum cristatum*. h, *Lathyrus sativus*. i, *Lens esculenta*. j, *Vicia sativa*. k, *Pisum sativum*. $\times ca.780$.

figures were drawn using an eyepiece of $\times 20$ and a 1.3 apochromatic objective at a table magnification of approximately $\times 2900$.

Microphotographs were taken at a table magnification of $\times 900$ times using a Zeiss Winkel compensating eyepiece of $\times 20$ and a 1.3 apochromatic objective with a condenser of 1.4 N.A.

Discussion

Previous investigations (Sharma & Mookerjee, 1955) in paradichlorobenzene reveal that the chemical is effective for a clarification of chromosome structure, while applied in saturated concentration for 2-3 hours in plant tissues. The time requirement for treatment, however, varies with the different groups of plant and in certain cases the solution too, as our experience shows, need be diluted for bringing out the details of chromosome morphology. Emphasis has been laid on the present report for a cautious application of

the chemical, as a prolonged treatment may cause even fragmentation of chromosomes. It has been found that breakages can be caused by paradichlorobenzene if the treatment period is prolonged considerably over the period required for karyotype clarification. In case of Monocotyledonous members, as is evident from the table, in general more than three and half hours treatment is necessary for causing chromosome breakages. But there are also cases such as *Hippeastrum rutilum* or *Zephyranthes mesochloa* where one and half hours and three and one-fourth hours treatments are sufficient for causing breakages respectively. It may, however, be noted that in cases where such low treatment can cause fragmentation, application of the solution for an extremely low period can reveal the details of chromosome morphology without causing any injury to the complement (vide Figs. 1-26).

It is, therefore, clear that the duration of the treatment for causing fragmentation is much higher than the one needed for karyotype clarification. Therefore, the chemical may no doubt be advantageously employed for chromosome analysis, provided the exact time necessary for causing fragmentation is known.

In cases of Dicotyledonous members too, the results are to some extent different from the one obtained with Monocotyledons. In all such cases, more than four hours treatment is necessary in order to have chromosome breakages. Though a large number of Dicotyledons could not be tried, of the members on which attempts have been given, all yield more or less the same results. Therefore, with these groups of plant also, the chemical can be applied for chromosome analysis, if precautions are taken to avoid fragmentation.

As regards the property of chromosome breakages, it may be pointed out that the chemical is not much toxic to the plant even though it can cause fragmentation to a high degree. This particular issue had to be checked as recently it has been noted here that the tissue on verge of its death can also yield a large number of fragments as an effect of some of the chemicals. As is evident from the text, recovery experiments in Knop's culture solution were performed in order to have a correct understanding of the problem, and specially to note the fate of the fragments and the cells harbouring them. Simultaneously, control preparations were also made in every case for a check of the results. It has been revealed that the cells with fragments have been noted in a dividing condition even after ninety-six hours of recovery. The number of fragments per cell decreases with recovery. This may indicate that cases where heavy fragmentation occur, the cells may gradually lose their capacity of division, and as such the surviving cells which were noted under the microscope were mostly normal. But where the frequency of breakages does not reach a very high level, the fragments can persist through several cell generations. The persistence of fragment containing cells following the treatment may be taken as an indication of the potentiality of the chemical in mutagenic action.

Summary

1. Properties of paradichlorobenzene in causing fragmentation of chromosomes have been worked out after trials in different species of Monocotyledons as well as of Dicotyledons. Six species under Dicotyledons and ten species under Monocotyledons were used for the purpose.

2. Different species require different periods of treatment in the chemical for the breakage of their chromosomes. Dicotyledons, generally, require longer periods than the Monocotyledons.

3. The results of recovery experiments have been discussed and the potentiality of the chemical for mutagenic purposes has been pointed out.

4. In fixation schedules for chromosome analysis, a cautious application of the chemical is recommended, because if the period of treatment is prolonged over the one required, fragmentation of the chromosomes may result.

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