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Full Length Research Paper

Comparative effects of colchicine, 8-hydroxyquinoline and paradichlorobenzene on arm ratio of mitotic chromosomes of *Allium cepa* L.

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Studies were carried out to assess the comparative effects of aqueous solution of colchicine (0.5%), 0.004M 8-hydroxyquinoline and saturated solution of paradichlorobenzene on the arm ratios of mitotic chromosomes using *Allium cepa* L. as the test organism. Samples were collected separately and fixed in acetic alcohol (1:3 v/v) for 24 h at room temperature. Chromosome characteristics in *A. cepa* were determined. Karyotypic analysis of the onion root tips showed a preponderance of median chromosomes. Analysis of variance for chromosome arm ratios showed that there are no significant differences ($p<0.05$) among the treatments in arm ratio. This research work therefore suggests that though the three pre-treatment chemicals are all effective in inhibiting the mitotic spindle and arresting the chromosomes at metaphase, each chemical elicits a differential contraction of the chromosomes.

Key words: Arm ratio, mitotic chromosomes, idiograms, haploid complement, centromeric position.

INTRODUCTION

Much of the studies of chromosomes are conducted on their number, size and visual appearance during cell division of all plant materials employed in the studies of somatic cell chromosomes. Naturally, chromosomes have a tendency to resist spreading which renders chromosome number counts, size determination and structural studies difficult at best and most often impossible (Storey et al., 1968).

A number of substances have been used to enhance the spread of mitotic chromosomes during squashing. These microtubule inhibiting pre-treatment substances include among others colchicine, 8-hydroxyquinoline, paradichlorobenzene, ice cold water and ά-bromonaphthalene (Osalou et al., 2013). Though these chemicals have been observed to produce desirable effects as it pertains to chromosome condensation and spread, and though the roles of these chemicals in spindle fibre inhibition have been elucidated by many authors (Burley, 1964; Amer, 1960), there is a dearth of reports on their effects on the arm ratio of the chromosomes.

Leme and Marin-Morales (2008) noted that onion is an outstanding test organism because of its sensitivity to xenobiotics as well as its suitable chromosome features: almost all *Allium* species possess symmetrical median to submedian centromeric chromosomes with little deviation in size although a few telocentric chromosomes are present in a number of species (Ved-Brat, 1965; Hiroya et al., 2002). In his study on the karyotype of the sitka spruce, *Picea sitchensis*, Burley (1964) observed that at five hours of pretreatment, the total haploid complement of the chromosome had a 41.6% contraction due to the effect of 1% aqueous solution of colchicine but at twenty four hours in 0.002M 8-hydroxyquinoline, the total haploid compliment showed only a 37.2% contraction. Mergen

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and Burley (1963) however showed that the contraction of chromosomes by 0.002M 8-hydroxyquinoline was intermediate between those of 1% and 0.5% aqueous solution of colchicine.

It is clear that the effect of the three pretreatment chemicals on chromosome metrical characteristics have not been completely elucidated. It was therefore considered that the study of the comparative effects of colchicine, 8-hydroxyquinoline and paradichlorobenzene could lead to a better understanding and use of the pretreatments during cytological studies.

MATERIALS AND METHODS

Onion bulbs used in this study were obtained from the Uyo Main Market, Uyo, Akwa Ibom State, Nigeria. The plants were identified in the Herbarium Unit of the Department of Botany and Ecological Studies, University of Uyo, where the voucher specimens have been deposited. The onion bulbs selected where those that had wintered and budded. The dried external leaves and roots were removed before the onion bulbs were floated off in sterile distilled water with the stem disc just touching water, until the roots sprouted.

The root tips were harvested at 10 am and immediately transferred to four glass flasks containing:

(a) Distilled water;
(b) 0.5% aqueous solution of colchicines;
(c) 0.004M 8-hydroxyquinoline;
(d) Saturated solution of paradichlorobenzene.

A preliminary study showed a preponderance of the metaphase stage between 9 am and 10 am at Uyo lying between Latitude 5.0232°N and Longitude 7.92338892°E. The flask containing the root tips were then stored in a refrigerator maintained at 15°C for 4 h. Samples were collected separately and fixed in acetic alcohol (1:3 v/v) for 24 h at room temperature. The root tips were thereafter hydrolyzed in 1N hydrochloric acid at 60°C for 6 min. The hydrolyzed root tips were rinsed in three changes of distilled water and excess fluid was removed with filter paper. About 1.5 mm of the root tip was cut off and placed in a drop of aceto-orcein stain on a clean microscope slide and examined under the light microscope. The number of chromosomes per cell was counted. The length of the long and short arms and the total lengths of the chromosomes were measured with the aid of an ocular micrometer. The mean chromosome lengths were calculated from six cells per treatment. Chromosome arm ratio was determined as length of the long arm divided by the length of short arm. The sixteen chromosomes in each cell were arranged in eight homologous pairs in descending order by the inspection of individual arm lengths, total chromosome lengths and arm ratios. The means of the chromosome lengths and arm ratios due to the three chemicals were used to calculate a mean haploid compliment for each treatment. The means were subjected to analysis of variance and significantly different means were separated using the Least Significant Difference (LSD) test. Idiograms of the haploid compliments of *A. cepa* pre-treated with the three chemicals were prepared.

RESULTS AND DISCUSSION

The idiograms of the *A. cepa* chromosomes pretreated with the three chemicals, namely colchicine, 8-Hydroxyquinoline and paradichlorobenzene are shown in Figure 1. The lengths of the corresponding chromosomes for each pre-treatment varied slightly. The arm ratios were closely similar and the idiograms were symmetrical with predominantly submetacentric chromosomes. These results agree with those of the works of Ved-Brat (1965) and Hiroya et al. (2002), who showed that there is little variability among the chromosomes of *A. cepa*, and that the species displays a symmetrical karyotype with median chromosomes.

Tables 1-3 show the mean arm ratio of the *A. cepa* chromosomes pretreated with the three chemicals. The chromosomes pre-treated with paradichlorobenzene showed a higher arm ratio than those pre-treated with colchicine and 8-Hydroxyquinoline. For the haploid complement of chromosomes treated with colchicine, there was a generally decreasing value of short arm and long arm for each of the eight chromosomes. However for the haploid complement of chromosomes treated with 8-hydroxyquinoline, chromosomes 1 and 6 showed shorter long arm than the trend value while chromosome 2 showed shorter short arm than the trend value. For the haploid complement for paradichlorobenzene, chromosome 4 showed shorter long arm than the trend value while the trend value for all long arms showed decreasing value. These however did not show significant difference when subjected to analysis of variance.

This result strongly suggests that each pre-treatment chemical has a differential contracting effect on the chromosomes resulting in the observed differences among the chromosomes. This fact is more obvious when the mean haploid karyotypes due to three treatments are compared as shown in Figures 1 and 2. This is in line with findings of Mergen and Burley (1963) which showed that the contraction of the chromosomes by 8-Hydroxyquinoline was intermediate between those of 1% and 0.5% aqueous solutions of colchicine.

Conclusions

This research work suggests that the three pre-treatment chemicals: colchicine, 8-Hydroxyquinoline and paradichlorobenzene are all effective in inhibiting the mitotic spindle and arresting the chromosomes at metaphase. However, each chemical elicits a differential

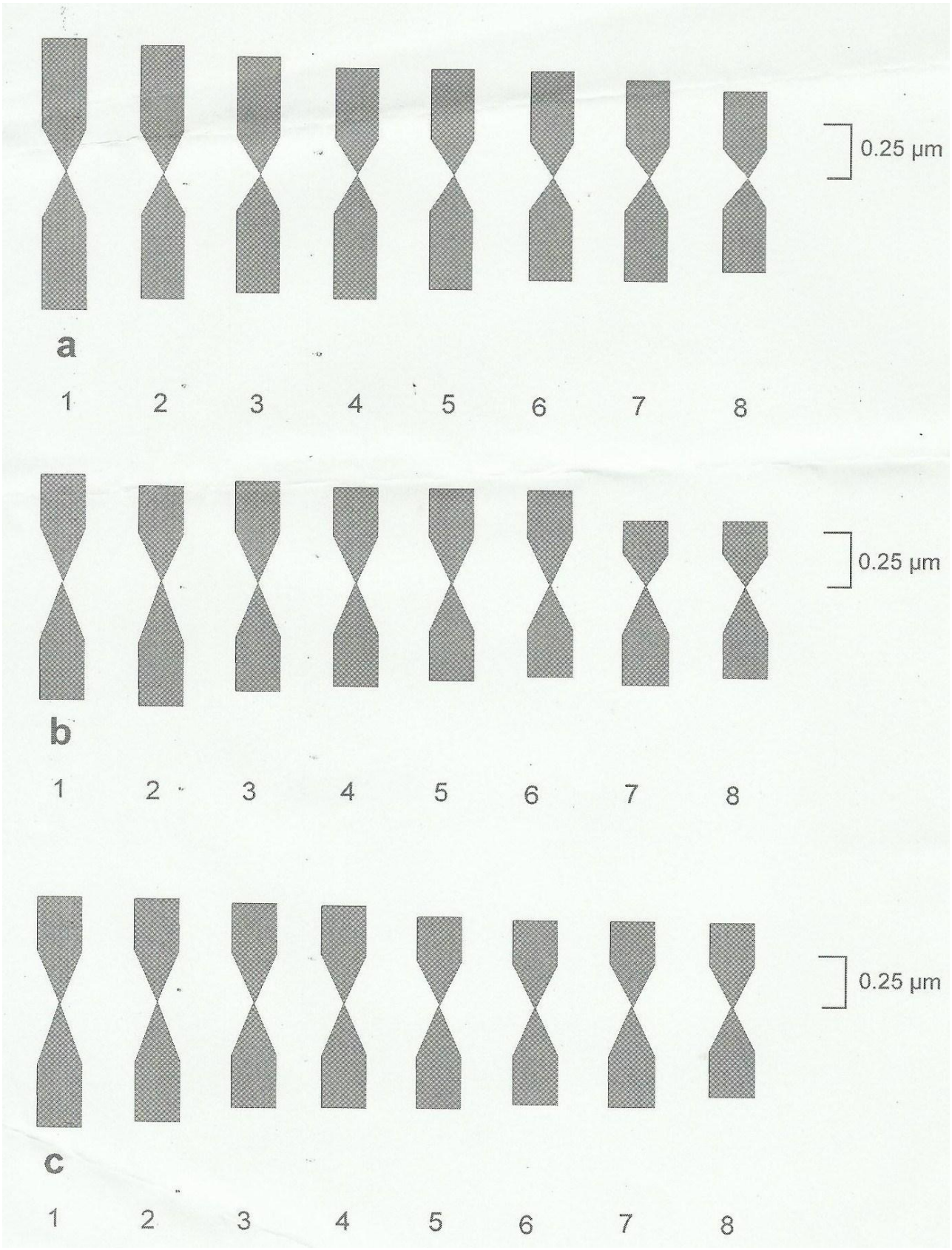

Figure 1. Idiogram of the haploid complement of *A. cepa* pre-treated with (a) colchicine, (b) 8-hydroxyquinoline, and (c) paradichlorobenzene.

Table 1. Mean (± SE) metrical chromosome characteristics in *Allium cepa* L. root tip cells treated with colchicine.

Chromosome number	Long arms (µm)	Short arms (µm)	Arm ratio	Centromeric position	Chromosome type
1	0.743±0.038	0.645±0.025	1.152±0.025	m	A
2	0.675±0.029	0.575±0.024	1.174±0.040	m	A
3	0.654±0.042	0.508±0.033	1.287±0.033	m	A
4	0.596±0.046	0.496±0.020	1.202±0.022	m	A
5	0.554±0.036	0.479±0.022	1.157±0.020	m	A
6	0.504±0.059	0.429±0.033	1.175±0.033	m	A
7	0.488±0.063	0.368±0.040	1.326±0.024	m	A
8	0.400±0.057	0.334±0.025	1.198±0.025	m	A
Total	4.614	3.834	9.671		
Mean	0.577	0.479	1.209		

Table 2. Mean (± SE) metrical chromosome characteristics in *Allium cepa* L. root tip cells treated with 8-hydroxyquinoline.

Chromosome number	Long arms (µm)	Short arms (µm)	Arm ratio	Centromeric position	Chromosome type
1	0.588±0.038	0.497±0.017	1.183±0.181	m	A
2	0.608±0.029	0.425±0.021	1.430±0.787	m	A
3	0.537±0.042	0.442±0.021	1.215±0.083	m	A
4	0.529±0.046	0.425±0.204	1.245±0.021	m	A
5	0.475±0.036	0.408±0.833	1.164±0.043	m	A
6	0.421±0.059	0.400±0.233	1.053±0.043	m	A
7	0.446±0.063	0.296±0.164	1.507±0.037	m	A
8	0.375±0.057	0.296±0.021	1.267±0.111	m	A
Total	3.979	3.189	9.981		
Mean	0.497	0.399	1.248		

Table 3. Mean (±SE) metrical chromosome characteristics in *Allium cepa* L. root tip cells treated with paradichlorobenzene.

Chromosome number	Long arms (µm)	Short arms (µm)	Arm ratio	Centromeric position	Chromosome type
1	0.633±0.047	0.533±0.031	1.188±0.040	m	A
2	0.592±0.047	0.483±0.028	1.226±0.043	m	A
3	0.528±0.055	0.455±0.018	1.160±0.064	m	A
4	0.492±0.050	0.408±0.019	1.206±0.021	m	A
5	0.504±0.035	0.350±0.025	1.440±0.088	m	A
6	0.454±0.034	0.346±0.012	1.312±0.070	m	A
7	0.442±0.043	0.308±0.014	1.435±0.111	m	A
8	0.358±0.028	0.296±0.029	1.209±0.056	m	A
Total	4.003	3.179	10.176		
Mean	0.500	0.399	1.279		

*A = represent type of chromosome with the same centromeric positions and no secondary constriction. *m = represents metacentric chromosomes. ±SE = standard error.

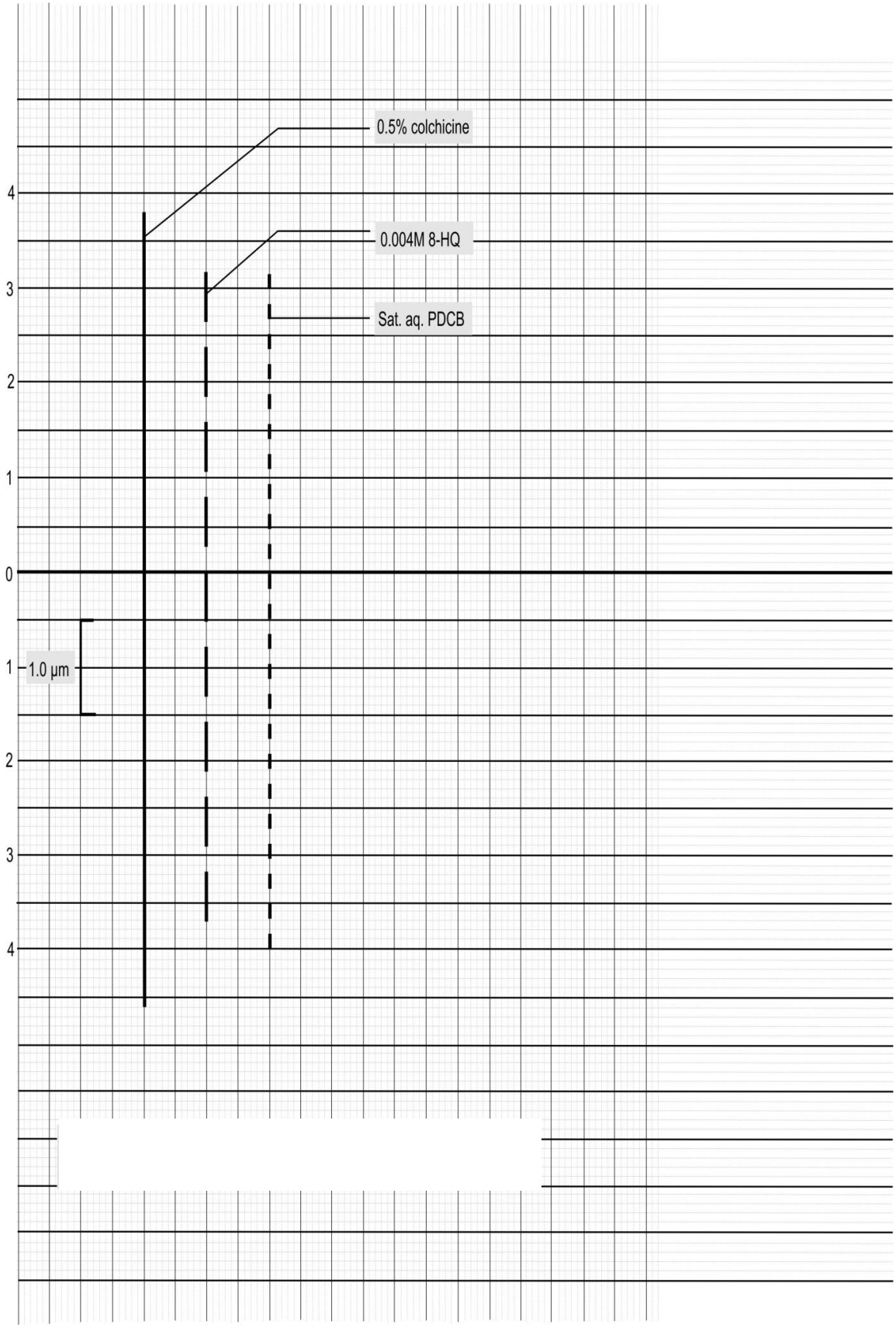

Figure 2. Mean haploid karyotypes due to the three treatments based on six cells per treatment.

contraction of the chromosomes.

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