RADIOMIMETIC EFFICACIES OF SYNTHETIC BIOREGULANTS ON CHROMOSOMES OF INDIAN CHAROPHYTA. 1. — MORPHACTIN : CHLORFLURENOL

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ABSTRACT. — *Chara contraria, Nitella mirabilis* and *Tolypella prolifera* were subjected to the treatment of chlorflurenol and 23 types of nuclear aberrations were observed viz. mitotic inhibition, scattered metaphase, chromosome forward, chromosome condensation, clumping, chromatid separation, laggards, chromosome rings, chromatin bridge, polyploidy, spindle shifting, binucleate cells, polarity abolition, heterogenous staining, micronuclei, slit formation, chromosome breakage, precocious separation, fragmented chromatin material, dumb-bell shaped cells, nuclear erosion, clarity of chromatin material and pycnotic nuclei.

Implementation of chlorflurenol as polyploid inducing agent, mutagen and in chromosome analysis in advisable at lower doses. Corticated forms of *Chara* are more resistant than the ecorticated forms of *Nitella* and *Tolypella* and produce more aberrations. Besides this, the independent tribal status of *Tolypella* has been retained in-between *Chareae* and *Nitelleae* with comparatively closer affinities of *Tolypella* and *Nitelleae* as suggested earlier by BHATNAGAR (1983).

RÉSUMÉ. — Les auteurs étudient les effets d'une morphactine, le chlorflurenol sur de jeunes plantes fertiles de *Chara contraria, Nitella mirabilis, Tolypella prolifera*. Vingt-trois types d'anomalies nucléaires ont été observés : inhibition mitotique, métaphase dispersée, chromosome «en avant», chromosome traînard, chromosomes agglutinés, séparation des chromatides, anneau chromosomique, pont de chromatine, polyploïdie, déplacement du fuseau, cellules binucléées, suppression de la polarité, coloration hétérogène, micronoyaux, formation de fente, cassure de chromosomes, séparation précoce, matériel chromatique fragmenté, cellules en haltère, détérioration nucléaire, noyaux pycnotiques, etc. Le chlorflurenol peut être conseillé comme agent induisant la polyploïdie, comme mutagène ou pour l'analyse chromosomique à très faibles doses. Les formes corticquées de *Chara* sont plus résistantes que les formes acorticquées de *Nitella* et de *Tolypella*. Elles produisent plus d'anomalies. (traduit par la rédaction).

KEY WORDS : synthetic bioregulant, morphactin (chlorflurenol), nuclear aberrations, *Chara contraria, Nitella mirabilis, Tolypella prolifera*.

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INTRODUCTION

Chlorflurenol (methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate) has been found to exhibit diverse influences on plant growth, development and morphogenesis (BOPP, 1972; SANKHLA, 1971; SANKHLA et al., 1975; SCHNEIDER, 1965, 1969, 1970, 1972) but its radiomimetic properties could be explored by a few workers (DENFFER et al., 1969; RINGE and DENFFER, 1967; SCHNEIDER, 1970, 1972; TREICHEL, 1971; ZIEGLER, 1970) in higher plants. Pronounced effects of chlorflurenol were mitotic inhibition, polarity abolition and disturbed polarity. Recently, BISARIA and BISARIA Jr. (1977) elucidated the mutagenic efficacies of chlorflurenol in *Allium cepa* and observed eight types of cytological aberrations. BHATNAGAR (1981) observed eight types of chromosomal and nuclear changes in *Chara delicatula* and *Nitella mirabilis* establishing ever first report on Charophytes.

Chlorflurenol belongs to a novel group of synthetic bioregulants called morphactins. Looking into the negligible reports on Charophytes, present investigations have been concluded in three Charophytes belonging to three genera namely *Chara*, *Nitella* and *Tolypella*. The taxa are *C. contraria* (n = 28), *Nitella mirabilis* (n = 6) and *Tolypella prolifera* (n = 11), all collected from Rohilkhand division of U. P., India. These studies have been aimed to find the phylogenetic and evolutionary relationships between three major Charophyte genera on the basis of their chromosome behaviour towards chemicals and degree of susceptibility.

MATERIALS AND METHODS

Chlorflurenol was obtained from Sigma Laboratories, U.K. with the concentration of 12.5 g per 100 ml. Experimental plants, collected from Laal Phatak locality in Bareilly (U.P.), India during January-February, 1984, were maintained in soil-water culture medium at + 25°C. Soil and water from the original pond were sterilized before use and were supplemented with Chu-10 medium for enhanced growth of the plants. Young fertile plants were treated with 2 ppm (parts per million), 5 ppm, 10 ppm and 15 ppm solutions of chlorflurenol which were prepared in sterilized (soil-water) and filtered solvent. Plants immersed in distilled water were considered as control. Durations of treatments were 30 minutes, 60 minutes, 90 minutes and 120 minutes in each concentration. Growing fertile tips were then fixed in modified Carnoy's fluid (1:2 acetic alcohol) for 24 hr and were transferred to 70 % alcohol for preservation. GODWARD's (1948) iron alum acetocarmine method and Feulgen stain techniques were employed for smearing the antheridial filaments to study the chromosomal aberrations. Microphotographs were taken from the temporary slides which were made permanent by using tertiary butyl alcohol and euparal schedule. Hundred cells were taken into consideration for each treatment to analyse the cytological changes. Frequency of aberrations was observed by calculating them in one hundred cell each.

FIGURE I.

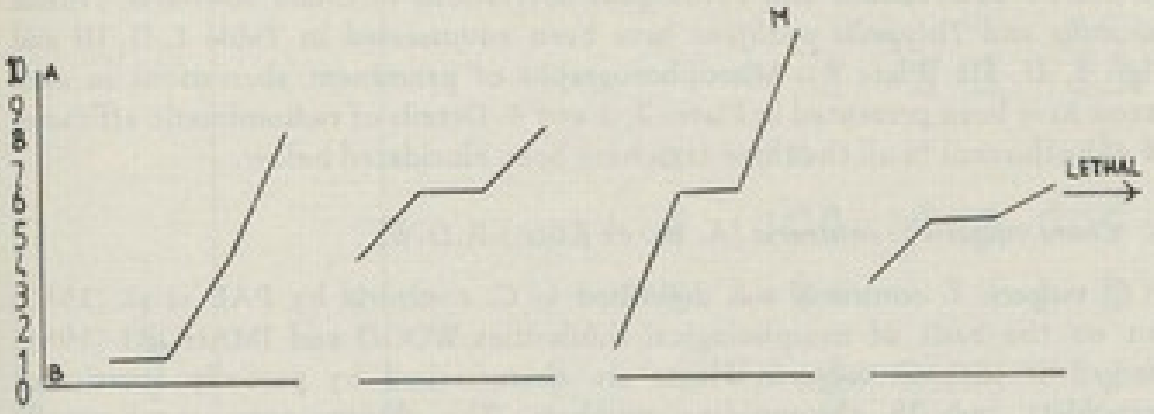

FIGURE II.

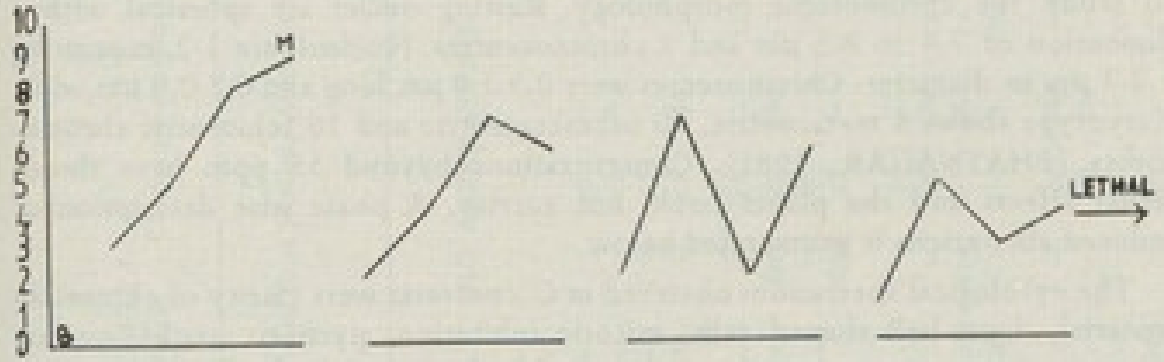

FIGURE III.

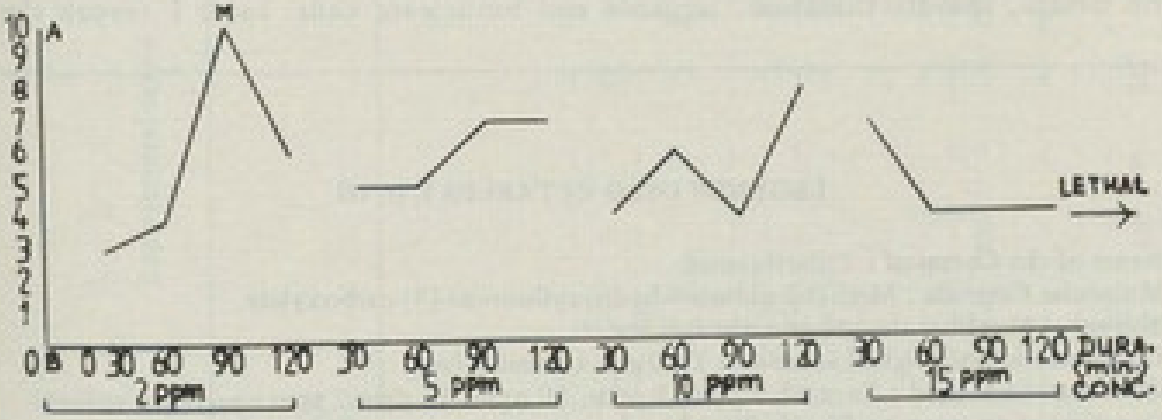

Plate 1. — Chlorflurenol : graphical presentation of induced aberrations.

Fig. I : *Chara vulgaris* f. *contraria*;
Fig. II : *Nitella mirabilis*;
Fig. III : *Tolypella intricata* f. *prolifera*.
A-B axis : number of aberrations; m : maximum aberrations.

OBSERVATIONS

Overall observations and cytological aberrations in *Chara contraria*, *Nitella mirabilis* and *Tolypella prolifera* have been enumerated in Table I, II, III and Figs. I, II, III (Plate 1). Microphotographs of prominent aberrations in each taxon have been presented in Plates 2, 3 and 4. Details of radiomimetic efficacies of chlorflurenol in all the three taxa have been elucidated below.

A. *Chara vulgaris* f. *contraria* (A. Br. ex Kütz.) R.D.W.

C. vulgaris f. *contraria* was described as *C. contraria* by PAL et al. (1962) but on the basis of morphological similarities WOOD and IMAHORI (1965) merged it with *C. vulgaris*. Plants are characterised by partially ecorticated branchlets and 28 chromosome numbers. The chromosomes are normally sticky and remain adhered even at metaphase. Pretreatment is always required to study the chromosome morphology. Resting nuclei are spherical with a dimension of 7.4 to 8.5 µm and 2 chromocentres. Nucleoli are 1-2, measuring 1.2-2 µm in diameter. Chromosomes were 0.9-1.8 µm long and 0.7-0.9 µm wide. Karyotype shows 8 metacentric, 10 submetacentric and 10 telocentric chromosomes (BHATNAGAR, 1981). Concentrations beyond 15 ppm have shown lethal effects and the plants could not survive. A phase wise description of induced aberrations is enumerated below.

The cytological aberrations observed in *C. contraria* were clarity of chromatin material, dumb-bell shaped cells, mitotic inhibition, pycnotic nuclei, nuclear erosion, chromosome clumping, micronuclei, fragmented chromatin material, scattered metaphase, chromosome forward, chromosome condensation, chromatid separation, ring formation, heterogenous staining of chromosomes, chromatin bridge, spindle furcation, laggards and binucleate cells. Table I reveals the

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LEGENDS USED IN TABLES I, II, III

Name of the Chemical : Chlorflurenol.
Molecular Formula : Methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate.
Solvent : Absolute alcohol and distilled water.
Concentration of original solution : 12.5 g in 100 ml solvent.
Concentrations used : Control, 2 ppm, 5 ppm, 10 ppm, 15 ppm. (ppm : parts per million)
Duration of treatment : 30, 60, 90, 120 minutes.

Abbreviations :

Bn : Binucleate cells; Cb : Chromatin bridge; CB : Chromosome breakage; cc : Chromosome condensation; cf : Chromosome forward; ccm : Clarity of chromatin material; cs : Chromatid separation; cl : Clumping of chromosomes; Db : Dumb-bell shaped cells; Er : Erosion; fcm : Fragmented chromatin material; HS : Heterogenous staining; Lag : Laggards; Mn : Micronuclei; MI : Mitotic inhibition; Pa : Polarity abolition; Poly : Polyploidy; PS : Precocious separation; Pn : Pycnotic nuclei; Ri : Rings; SM : Scatered Metaphase; SL : Slit Formation; Sf : Spindle Furcation.

TABLE I.

Experimental taxon : *Chara contraria*. — Chromosome number : n = 28

Concentrations (ppm)	Duration of treatment (minutes)	Induced Nuclear Aberrations																	Number of Aberrations
CONTROL	30 60 90 120	NO ABERRATIONS																	
2 ppm	30	–	–	–	–	–	–	–	–	cf	–	–	–	–	–	–	–	–	01
	60	–	–	–	–	–	–	–	–	cf	–	–	–	–	–	–	–	–	01
	90	ccm	–	–	–	–	cl	–	fcm	cf	–	–	–	–	–	–	–	–	04
	120	–	–	–	–	Er	cl	Mn	–	cf	SM	–	–	–	HS	Cb	Sf	–	–	08
5 ppm	30	–	–	–	–	–	cl	–	–	cf	–	cc	–	Ri	–	–	–	–	–	04
	60	ccm	–	–	–	–	–	–	fcm	cf	SM	cc	–	Ri	–	–	–	–	–	06
	90	ccm	–	–	–	–	–	–	–	cf	–	cc	–	–	HS	Cb	Sf	–	–	06
	120	–	–	–	–	Er	cl	–	fcm	cf	–	–	cs	Ri	–	Cb	–	Lag	–	08
10 ppm	30	–	–	–	–	–	–	–	–	cf	–	–	–	–	–	–	–	–	–	01
	60	–	–	–	–	–	–	–	–	cf	SM	cc	–	Ri	–	Cb	–	Lag	–	06
	90	–	–	–	–	–	–	–	fcm	cf	SM	cc	cs	–	–	Cb	–	–	–	06
	120	ccm	Db	–	–	Er	cl	Mn	–	–	–	cc	cs	Ri	–	Cb	–	Lag	–	10
15 ppm	30	–	–	–	–	–	–	–	–	cf	SM	–	–	–	–	Cb	–	–	–	03
	60	–	–	–	–	–	–	–	–	cf	SM	–	cs	–	–	Cb	–	Lag	–	05
	90	–	–	–	–	–	–	–	–	–	–	cc	–	–	HS	Cb	Sf	Lag	–	05
	120	–	Db	Pn	Ml	–	cl	Mn	–	–	–	–	–	–	–	–	–	–	Bn	06

TABLE II.

Experimental taxon : *Nitella mirabilis* — Chromosome number : n = 6

Concentrations (ppm)	Duration of treatment (minutes	Induced Nuclear Aberrations																			Number of Aberrations
CONTROL	30 60 90 120	NO ABERRATIONS																			
2 ppm	30	–	–	–	–	–	–	–	–	–	SM	–	–	–	Ri	–	Cb	–	–	–	–	03
	60	–	–	–	–	–	–	–	–	–	SM	–	–	–	Ri	–	Cb	Lag	–	Poly	–	05
	90	–	–	–	–	–	–	–	–	–	SM	–	–	–	Ri	CB	Cb	Lag	Pa	Poly	PS	08
	120	–	–	–	–	MI	–	–	Mn	fcm	SM	cf	cc	cs	–	–	Cb	–	–	Poly	–	09
5 ppm	30	–	–	–	–	–	–	–	–	–	SM	–	–	–	–	–	Cb	–	–	–	–	02
	60	–	–	Pn	–	–	–	–	–	–	SM	–	–	–	–	–	Cb	–	Pa	–	–	04
	90	–	–	–	–	–	–	–	–	fcm	SM	cf	cc	cs	Ri	–	Cb	–	–	–	–	07
	120	ccm	–	–	SL	MI	–	Cl	–	–	–	–	–	–	Ri	CB	–	–	–	–	–	06
10 ppm	30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CB	–	–	Pa	–	–	02
	60	ccm	Db	–	–	–	–	Cl	Mn	–	–	–	cc	cs	–	–	–	–	–	Poly	–	07
	90	–	–	–	–	–	Er	Cl	–	–	–	–	–	–	–	–	–	–	–	–	–	02
	120	ccm	Db	Pn	–	MI	Er	Cl	–	–	–	–	–	–	–	–	–	–	–	–	–	06
15 ppm	30	ccm	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	01
	60	–	Db	Pn	–	–	Er	Cl	Mn	–	–	–	–	–	–	–	–	–	–	–	–	05
	90	–	Db	–	–	MI	–	Cl	–	–	–	–	–	–	–	–	–	–	–	–	–	03
	120	–	Db	–	–	–	Er	Cl	Mn	–	–	–	–	–	–	–	–	–	–	–	–	04

TABLE III.

Experimental taxon : *Tolypella prolifera* — Chromosome number : n = 11

Concentrations (ppm)	Duration of treatment (minutes)	Induced Nuclear Aberrations																		Number of Aberrations
CONTROL	30 60 90 120	NO ABERRATIONS																		
2 ppm	30	–	–	–	–	–	–	–	cf	–	–	–	–	CB	–	Sf	–	–	–	–	03
	60	ccm	–	–	–	–	–	–	cf	SM	–	–	–	CB	–	Sf	–	–	–	–	05
	90	–	–	–	Ml	–	–	Mn	cf	SM	cc	–	Ri	CB	–	–	Pa	Lag	HS	–	10
	120	–	–	–	–	–	–	–	cf	SM	cc	cs	Ri	–	–	–	–	Lag	–	–	06
5 ppm	30	–	–	–	–	–	–	–	cf	SM	–	–	–	–	Cb	–	Pa	–	–	PS	05
	60	–	–	–	–	–	–	–	cf	SM	cc	cs	–	CB	–	–	–	–	–	–	05
	90	ccm	–	–	Ml	–	Cl	–	cf	SM	–	cs	–	–	Cb	–	–	–	–	–	07
	120	–	–	–	–	–	Cl	Mn	cf	SM	–	–	Ri	–	Cb	–	–	Lag	–	–	07
10 ppm	30	–	–	–	–	–	Cl	–	–	SM	cc	–	–	–	Cb	–	–	–	–	–	04
	60	–	–	–	–	–	–	Mn	–	–	cc	cs	–	–	Cb	Sf	–	Lag	–	–	06
	90	–	–	–	–	–	Cl	–	cf	–	–	–	–	CB	Cb	–	–	–	–	–	04
	120	ccm	–	Pn	–	Er	Cl	–	cf	–	–	cs	Ri	–	Cb	–	–	–	–	–	08
15 ppm	30	ccm	–	–	–	–	Cl	–	cf	–	–	–	–	–	Cb	Sf	Pa	Lag	–	–	07
	60	–	–	–	–	Er	–	–	–	SM	–	–	–	–	Cb	–	Pa	–	–	–	04
	90	ccm	Db	Pn	–	–	Cl	–	–	–	–	–	–	–	–	–	–	–	–	–	04
	120	ccm	Db	–	Ml	–	Cl	–	–	–	–	–	–	–	–	–	–	–	–	–	04

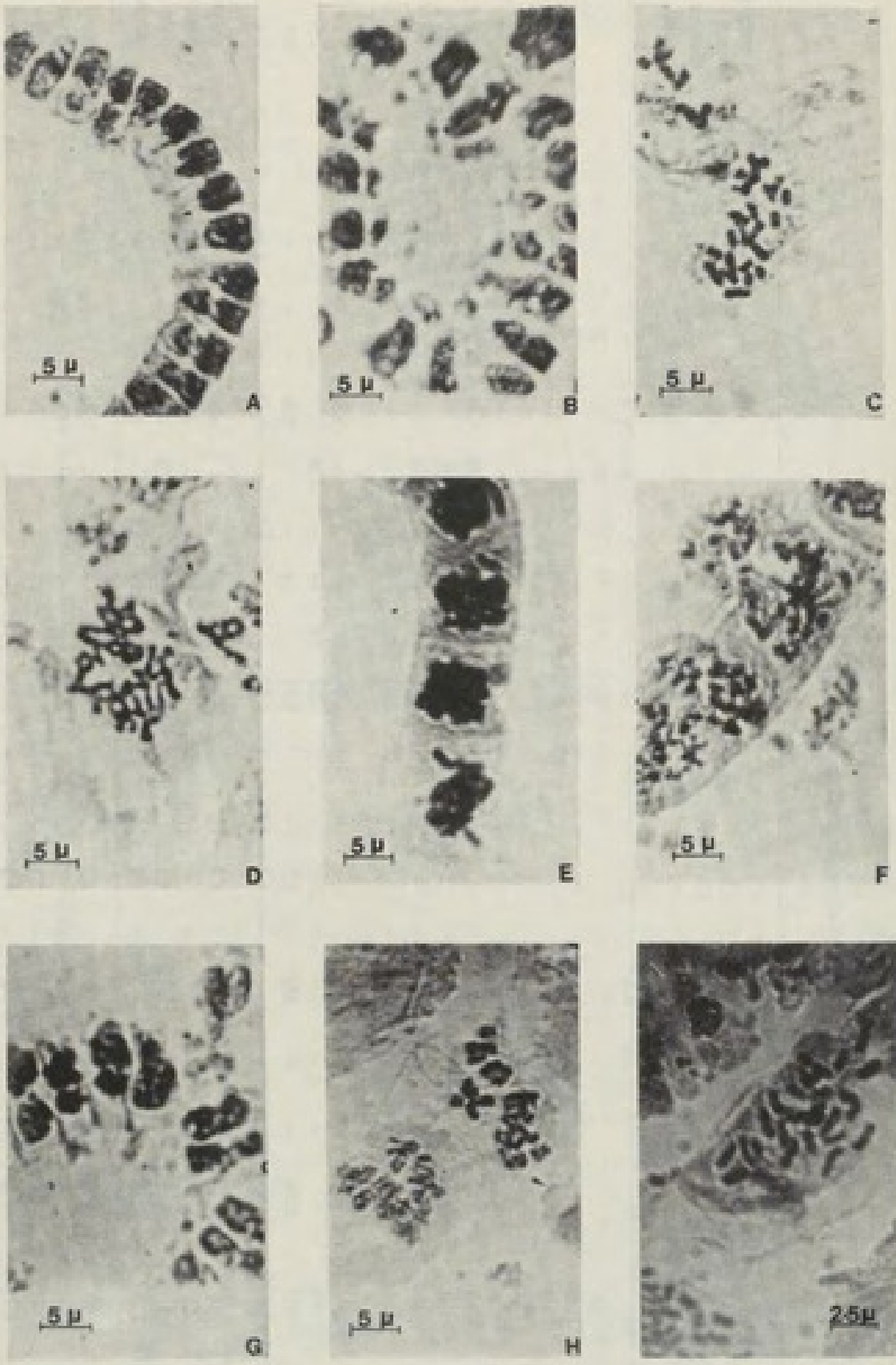

Plate 2. — Chlorflurenol : induced chromosomal aberrations. Taxon : *Chara vulgaris* f. *contraria*. — A. Nuclear erosion 2 ppm, 120 min.; B. Micronuclei 2 ppm, 120 min; C. Scattered metaphase 5 ppm, 60 min.; D. Chromosome rings 5 ppm, 120 min.; E. Chromosome forward & clumping 10 ppm, 120 min.; F. Chromatin fragmentation 10 ppm, 90 min.; G. Pycnotic nuclei 15 ppm, 120 min.; H. Chromatid separation 15 ppm, 60 min.; I. Control showing 28 chromosomes.

least aberrations during interphase except the clarity of chromatin material (2 ppm for 90 minutes, 5 ppm for 60 and 90 minutes and 10 ppm for 120 minutes). The frequency was quite low in 5 ppm for 90 minutes and 10 ppm for 120 minutes. Another significant change was the formation of dumb-bell shaped cells especially in 15 ppm (120 minutes) and 10 ppm (120 minutes). Onward concentrations were lethal causing death of the cells. Pyenotic nuclei (Pl. 2G) indicate lethality of chlorflurenol in 15 ppm (120 minutes). Reduction in mitotic activities or subsequent check of mitosis (mitotic inhibition) has been adjudged by the occurrence of interphase nuclei in all the cells rather than the synchronized mitosis in 15 ppm (120 minutes). The dilatation of nuclear material or its outward flow from the cell (nuclear erosion) has been noticed appreciably in 2 ppm (120 minutes, Pl. 2A), 5 ppm (120 minutes) and 10 ppm (120 minutes) concentrations.

During prophase, chromosome clumping was observed in all concentrations but its frequency was highest in 10 ppm (120 minutes, Pl. 2E) and lowest in 2 ppm (90 minutes). Micronuclei (Pl. 2B) were observed in 2, 10 and 15 ppm concentrations (Table I) while the fragmentation of chromatin material (Pl. 2F) was quite frequent in 2, 5 and 10 ppm concentrations.

Metaphase is much influenced by chlorflurenol thus producing maximum aberrations namely chromosome forward, scattered metaphase, chromosome condensation, chromatid separation, chromatin rings and heterogenous staining.

Chromosome forward (Pl. 2E) and scattered metaphase (Pl. 2C) were uniformly observed in all concentrations. Frequency of chromosome forward deteriorated with an increase in the concentration of chlorflurenol. The highest percentage of scattered metaphase was found in 2 ppm (120 minutes) and 5 ppm (60 minutes) while a few cells were seen in 15 ppm (60 minutes). Chromosome condensation was observed in 5, 10 and 15 ppm attaining highest frequency in 10 ppm (120 minutes). Chromatid separation (Pl. 2H) was another noticeable effect of chlorflurenol which may help in studying the chromosome morphology. This effect was pronounced in 10 ppm (90 minutes) though observed also in 5 and 15 ppm concentrations. Chromosome rings (Pl. 2D) were observed in 2 and 5 ppm while no rings could be seen in higher doses. Heterogenous staining in metaphase chromosomes was not a regular feature but a few cells were found to exhibit this effect in 2 ppm (120 minutes), 5 ppm (90 minutes) and 15 ppm (90 minutes).

During anaphase, chromatin bridge, spindle furcation, laggards and binucleate cells were the important aberrations. Chromatin bridges were frequent in 10 ppm (all durations) though found in all the concentrations in low percentage. In 2 and 5 ppm, spindle furcation was optimum which deteriorated with increasing concentrations.

Formation of acentric chromosome (laggard) is an after effect of breakage and was observed in 10 and 15 ppm concentrations. Frequency of such cells was maximum in 10 ppm (60 & 120 minutes) while no laggards could be seen in 2 ppm. The lethality of 15 ppm concentration (120 minutes) could be visualized in the form of binucleate cells.

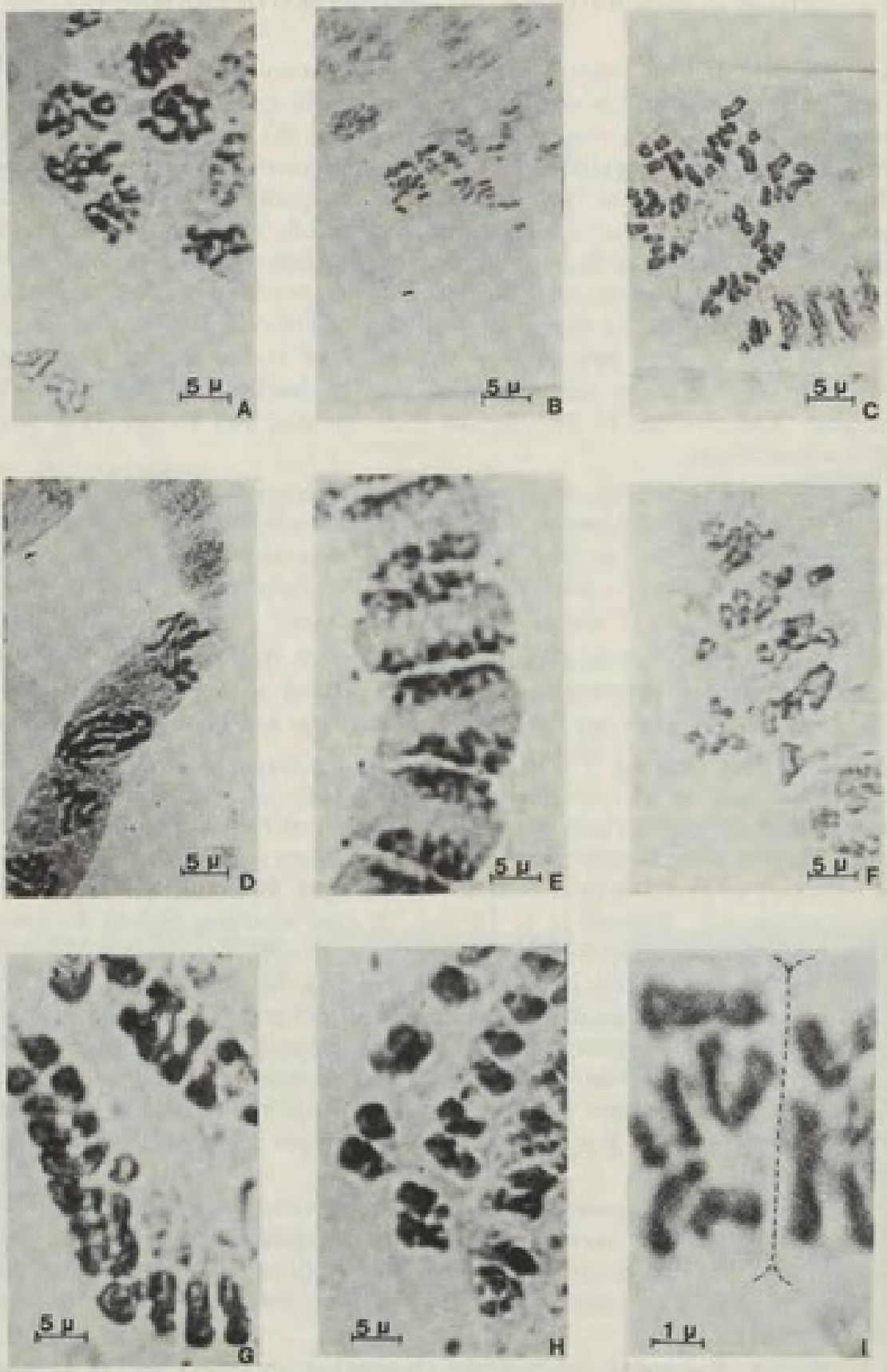

Plate 3. — Chlorflurenol : induced chromosomal aberrations. Taxon : *Nitella mirabilis*. A. Chromosome rings 2 ppm, 30 min.; B. Polyploid cells 2 ppm, 120 min.; C. Scattered metaphase 5 ppm, 60 min.; C. Chromatin bridge 5 ppm, 90 min.; E. Slits between cells 5 ppm, 120 min.; F. Chromatid separation 10 ppm, 60 min.; G. Pycnotic nuclei 15 ppm, 60 min.; H. Micronuclei 15 ppm, 90 min.; I. Control showing 6 chromosomes.

B. *Nitella mirabilis* (Nordst. ex Gr.) em. R.D.W.

N. mirabilis is characterised by anarthrodactylous (1-celled dactyls) habit. It possess n=6 but RAMJEE & BHATNAGAR (1978) reported n=9 and highlighted x = 3 as the basic number for genus *Nitella*. Present experimental plant shows a spherical nucleus 14.9-17.04 μm in diameter with 2(-3) darkly stained chromocentres. The prominent nucleolus measures 2.8 μm in diameter. Chromosomes are 3.66-10.22 μm long and 0.6-1.5 μm wide showing 5 submetacentric and 1 subtelocentric chromosomes (BHATNAGAR, 1981). Concentrations beyond 15 ppm proved to be lethal as usual. A detailed description of induced aberrations in this taxon is given below.

The cytological aberrations observed in *N. mirabilis* were clarity of chromatin material, erosion, dumb-bell shaped cells, mitotic inhibition, pycnotic nuclei, chromosome clumping, fragmented chromatin material, micronuclei, chromatid separation, scattered metaphase, chromosome forward, chromosome condensation, chromosome rings, chromatin bridge, laggards, polarity abolition, polyploidy and precocious separation.

Table II reveals insignificant changes owing to the non appearance of chromosomes. Visible changes were clarity of chromatin material in almost all the concentrations (except 2 ppm) and mitotic inhibition in 5, 10 and 15 ppm. Frequency of the later increased constantly with an increase in concentration and duration of treatment, terminating ultimately into the death of cells. Pycnotic nuclei and dumb-bell shaped cells were noticed in 5, 10 and 15 ppm concentrations having no sign of there appearance in 2 ppm. Highest frequency of pycnotic nuclei was observed in 10 ppm for 120 minutes and 15 ppm for 90 minutes. Dumb-bell shaped cells were negligible in 5 ppm and resulted into the death of cells at higher concentrations. Among noticeable aberrations, nuclear erosion was quite frequent in 10 and 15 ppm.

Despite of appreciably long chromosomes during prophase, chromosome clumping was seen in 5, 10 and 15 ppm concentrations showing its highest frequency in 10 ppm (90 minutes). Fragmented chromatin material (Pl. 3) and micronuclei were observed as the major influences of chlorflurenol. Fragmented chromatin was seen in 2 ppm (120 minutes) and 5 ppm (90 minutes) while micronuclei were frequent in 10 and 15 ppm. As many as 3 micronuclei were seen in some cells of *N. mirabilis*.

Metaphase, being represented by well defined chromosomes, is influenced largely by chlorflurenol. Most of the aberrations were observed in 2 and 5 ppm concentrations. Chromatid separation and chromosome condensation being the exception appear in 10 ppm (60 minutes). *N. mirabilis* plants do not survive well beyond 10 ppm.

Scattered metaphase was fairly good in 2 and 5 ppm (Pl. 3) at almost all durations while a few cells showing chromosome forward (Pl. 3) could be seen in 2 ppm (120 minutes) and 5 ppm (90 minutes). Chromosome condensation was noticed in 2, 5 and 10 ppm concentrations, increasing subsequently with an increase in duration of treatment. Chromosome rings, one of the most pronoun-

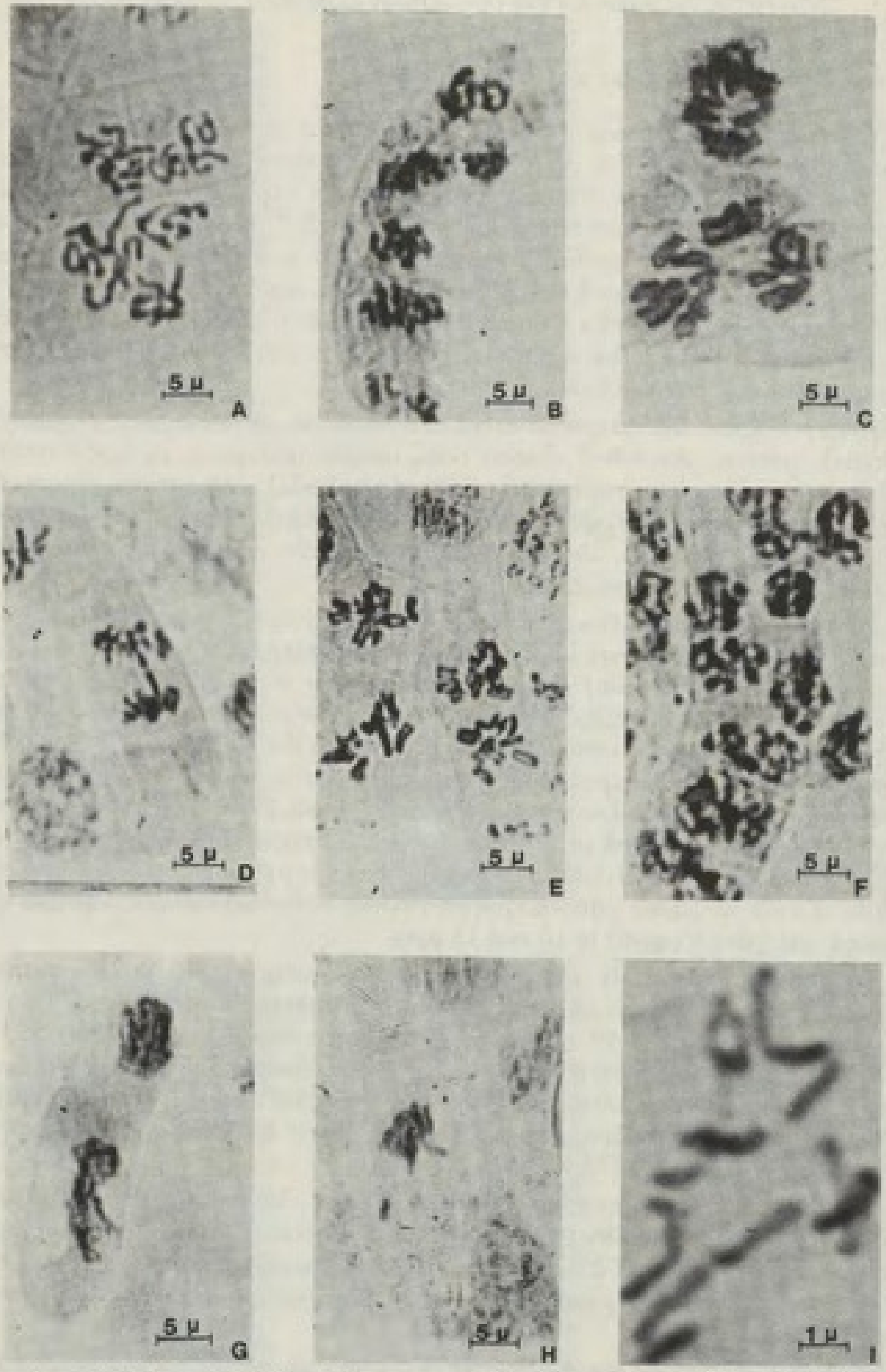

Plate 4. — Chlorflurenol : Induced chromosomal aberrations. Taxon : *Tolypella intricata* f. *prolifera*. — A. Clarity of chromatin material 2 ppm, 60 min.; B. Chromosome rings 2 ppm, 90 min.; C. Clumping of chromosomes 5 ppm, 90 min.; D. Chromatin bridge 9 ppm, 120 min.; E. Chromatid separation 10 ppm, 60 min.; F. Nuclear erosion 10 ppm, 90 min.; G. Pycnotic nuclei 15 ppm, 90 min.; H. Chromosome forward 15 ppm, 30 min.; I. Control showing 11 chromosomes.

ced effect, were frequently observed in 2 and 5 ppm while they were absent altogether in higher concentrations. Owing to the larger chromosomes, possibilities of chromosome breakage always exist and its maximum frequency was seen in 10 ppm (30 minutes) and 5 ppm (120 minutes) concentrations.

Chromatin bridges, the most significant aberrations during anaphase were uniformly observed at all durations in 2 and 5 ppm. At higher doses, they were altogether unnoticed (Pl. 3D). Lagging chromosomes (laggards) could be seen only in 2 ppm (60 and 90 minutes) while higher doses do not exhibit any laggard. Polarity abolition and precocious separation were randomly seen in 2, 5 and 10 ppm but their frequency was very low. In some cells, polyploidy or chromosome duplication was observed rather than the proper anaphasic movement. Disturbed metabolism of spindle formation results into the duplicated chromosome number in 2 ppm (60, 90 and 120 minutes) and rarely in 10 ppm (60 minutes). The exact nature of polyploidy is to be explored.

C. *Tolypella intricata* f. *prolifera* (Ziz. ex A. Br.) Leonh.

Tolypella is an interesting genus from cytotaxonomic and phylogenetic view point. Until recently, it was an unknown genus for republic of India, however ALLEN (1925) and GROVES (1924) reported a few species of *Tolypella* (cf. PAL et al., 1962) from Gangetic plains and Western Himalayas, long before India's independence. In the current years BHATNAGAR (1983), BHATNAGAR and JOHRI (1986) investigated in three species of *Tolypella* namely *T. nidifica* var. *glomerata*, *T. intricata* and *T. intricata* f. *prolifera* from Indian subcontinent. Chromosome number in all the three forms was n = 11.

Tolypella, phylogenetically stands in-between the tribes *Chareae* and *Nitelleae* but previously, it was included in the tribe *Nitelleae* by WOOD and IMAHORI (1965) and many of the authors. Its phylogeny and evolutionary status is debatable and has never been investigated in light of its chromosome susceptibility towards chemicals. Present investigations on *Tolypella prolifera* have been intended to explore their response towards mutagens which will place a milestone in deciding interrelationships to some extent. Experimental plant shows spherical nucleus, 12.7-13.8 μm in diameter with 2 chromocentres. Nucleoli are 1.2-1.7 μm in diameter. Chromosomes measure 1.67-2.8 μm long and 1.4 μm wide showing 3 metacentric, 6 submetacentric, no subtelocentric and 2 telocentric positions. Chlorflurenol beyond 15ppm was lethal like other taxa. Following are the details of chromosomal aberrations as induced by chlorflurenol.

Cytological aberrations observed in *Tolypella prolifera* were clarity and chromatin material, dumb-bell shaped cells, mitotic inhibition, nuclear erosion, pycnotic nuclei, chromosome clumping, micronuclei, scattered metaphase, chromosome forward, rings, chromatid separation, chromosome condensation, chromatin bridge, polarity abolition, spindle furcation, laggards, chromosome breakage, and precocious separation (Pl. 4E).

Clarity of chromatin material was regularly observed in all the concentrations (Pl. 4) while dumb-bell shaped cells could be seen in 15ppm (90 and 120 minutes). Mitotic inhibition could be adjudged in 2, 5 and 15 ppm, but it was not very pronounced. In 15 and 10 ppm concentrations nuclear erosion was seen which indicate «disintegrating influence» of chlorflurenol. Pycnotic nuclei (10 ppm for 120 minutes, 15 ppm for 90 minutes) indicate lethal indulgence of the chemical and have been observed in higher concentrations.

During prophase, chemical entered to cause chromosome clumping and formation of micronuclei. Clumping was a regular feature in all concentrations except 2 ppm but its highest degree was recorded in 15 ppm (90 minutes). Micronuclei formation, though not continuous in all durations, was found to exhibit nuclear fragmentation. The number of micronuclei was highly variable. Their frequency increased with an increase in concentration upto 10 ppm.

Scattered metaphase and chromosome forward were the most significant effects of chlorflurenol in all concentrations. Scattered metaphase was best seen in 2 ppm (90 minutes and 60 minutes) and 5 ppm (60 minutes) which goes on deteriorating with an increase in concentration. Frequency of chromosome forward was highest in 5 ppm (60 minutes) and 2 ppm (120 minutes). Another aberrations are chromosome rings which are quite frequent in 2, 5 & 10 ppm. Rings were constituted of 1 or 2 chromosomes usually. More rings were seen in 2 ppm (120 minutes).

Chromatid separation and chromosome condensation were found in all concentrations other than 15 ppm. Chromatid separation was quite significant in 2 and 5 ppm. Condensation of appreciably large chromosomes was noticed in 2 ppm (90 & 120 minutes), 5 ppm (60 minutes) and 10 ppm (30 & 60 minutes). Heterogenous staining was seen casually in 2 ppm (90 minutes).

Anaphase is the most affected stage of mitosis producing chromatin bridge and laggards uniformly in all concentrations. Chromatin bridges were however absent in 2 ppm while their highest frequency was observed in 10 ppm (30 & 60 minutes). Polarity abolition was predominantly found in all concentrations except in 10 ppm and spindle furcation could not be noticed in 5 ppm. Both these aberrations were characterised by the non-polar movement and more than 2 groups of chromosomes at anaphase. Lagging chromosomes, found in all concentrations are the result of breakage and formation of acentric chromosomes. Much laggards were seen in 10 ppm (60 minutes). Chromosome breakage at anaphase and rarely at metaphase was frequently observed in 2, 5 & 10 ppm but its highest degree was recorded in 2 ppm (60 minutes). However, no breakage could be seen in 15 ppm. Precocious separation was casually seen in 5 ppm (30 minutes) however, in other concentrations it could not be noticed.

DISCUSSIONS

Chlorflurenol was used as a synthetic bioregulant to study its influence on plant morphogenesis but the radiomimetic properties of this chemical could

be explored recently by BISARIA and BISARIA Jr. (1977) in the root apices of *Allium cepa*. They confirmed its karyological effects like mitotic inhibition and polarity abolition as already described by DENFFER et al. (1969), RINGE and DENFFER (1967), SCHNEIDER (1970, 1972), TREICHEL (1971), BOPP (1972), TOGNONI and ALPI (1969), KALDEWEY (1973) and ZIEGLER (1970).

The influences of chlorflurenol in nuclear and chromosomal metabolism of Cryptogams have never been studied. BHATNAGAR (1981), for the first time investigated on the mutagenic efficacies of this chemical on a few charophyte genera thus opening it for further investigation among Charophytes. Present investigations are in continuation with our previous attempts and have been carried out in 3 diverse taxa of division Charophyta.

During present studies, lethal effects of chlorflurenol at higher doses can be visualized by the occurrence of chromosome clumping, micronuclei, trinucleate cells, dumb-bell shaped cells, and pycnotic nuclei which finally terminate into death of the cells. Some aberrations like scattered metaphase, chromatid separation, spindle furcation, precocious separation indicate the impact of chlorflurenol on the formation and metabolism of spindles as referred earlier by BISARIA and BISARIA Jr. (1977). A viscosity balance between cytoplasm and microtubules which is responsible for spindle formation, suffers drastic changes and produces aberrations by chlorflurenol. This behaviour is similar to that of colchicine and results into the chromosome duplication as observed in *Nitella mirabilis* (Table II and Pl. 3B). It can be established, therefore, that chlorflurenol can induce polyploidy in plants at lower doses, like colchicine.

Condensation of chromosomes and chromatid separation can be best used in karyotypic analysis in ascertaining the position of centromeres, particularly in *Chara contraria*, which possess sticky and unseparable chromosomes. Low doses of chlorflurenol are more effective (Table I) and can be used successfully as pretreating agent in chromosome analysis.

Besides this, the mutagenic activities of chlorflurenol can be assessed by the formation of chromosome rings, breakage, laggards and chromatin bridges in all the three forms under investigation (Tables I, II and III). These mutations can be applicable equally well in higher plants for improving their varieties in various ways. But simultaneously in Charophytes, morphological diversities may be caused by these chromosomal changes and this diversity is the root cause of uneven speciation by WOOD and IMAHORI (1965).

Investigations made with *Chara contraria*, *Nitella mirabilis* and *Tolypella prolifera* as described earlier bring us to the following conclusions regarding their phylogeny and interrelationships. The corticated forms of *Chara contraria* are more resistant as compared to ecorticated forms of *Nitella* and *Tolypella* which can be witnessed by survival of the former in higher concentrations of chlorflurenol and production of more aberrations (Table I, Fig. 1). It indicates an advancement of corticated forms of *Chara* over ecorticated forms of *Nitella* and *Tolypella* on the basis of chromosome behaviour and their degree of susceptibility towards chemicals. Another important conclusion of this study is

the close affinity of *Tolypella* and *Nitella* as recognized by all charologists. The position of *Tolypella* in between the tribes *Chareae* and *Nitelleae* (BHATNAGAR, 1983) is strongly supported owing to the coinciding degrees of chromosomal susceptibilities. However, the establishment of a new tribe for *Tolypella* is untimely and a risk to complicate the very intricate taxonomy of Charophytes. These studies are of course scares and need much experimental verifications.

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