found in good condition even after six months. The details are as follows:

1. A peel (obtained from preserved materials) is placed on the lside and stained with 2–3 drops of aniline-blue or fast green in lactophenol (diluted to 50% with water if the peel bears glands, so as to avoid contraction of the latter) for 10–15 minutes or more depending on the material. Excess stain is drained off.

2. The peel is treated 2 or 3 times with 75% or 80% *t*-butanol. If the stain is too dense, the treatment could be for a longer period.

3. The peel is treated with 100% *t*-butanol two or three times for thorough dehydration.

4. About 3 drops of thin canada balsam in *t*-butanol, is placed on the peel and the cover glass is mounted.

Results

The nucleus, certain cytoplasmic granules and general cytoplasm all stain bright blue, but show decreasing intensity in that order, providing differentiation. The cell walls remain unstained or appear bluish, but are quite distinctive, from the other cell contents.

The method described has several advantages over the usual ethanol-xylol procedure[2] and cuts down much of the time normally needed. Since xylol is avoided, neihter brittleness, nor folding of peels is encountered. The ethanol-xylol treated peel mounts, do not show bright image of the cell characters, but the *t*-butanol treated ones are found to be quite translucent comparable to those with clove oil treatment[2]. The authors consider this to be due to *t*-butanol's nonshrinkage effects on cell components, and its superior clearing properties over xylol.

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MUTAGENIC, POTENTIATING AND ANTIMUTAGENIC ACTIVITY OF CERTAIN METALLIC IONS IN THE RICE GENETIC SYSTEM

THE grotesque poisoning of the biosphere caused by industrial effluents pose a grievous threat to the genetic hygiene of human population. The discovery that some of the metals, which are common constituents of industrial pollutants, mimic radiations[1,2] in their action has stimulated research on their role on the genetic damage in various test systems[3–12]. In the experiments reported here the genotoxicity of certain metallic ions was assessed in comparison and conjunction with gamma rays on rice seed (Cv. *Sona*).

Nine sets of rice seeds were treated with 10^{-4} M aqueous solutions (100 ml) of nine metallic salts for 24 hours at the room temperature (26° C). The salts used were chlorides of strontium (Sr), cadmium (Cd), mercury (Hg), barium (Ba), copper (Cu), lead (Pb), manganese (Mn), iron (Fe) and calsium (Ca). One set of seeds was immersed in distilled water for 24 hrs. which served as the control. In a second experiment, ten sets of seeds were irradiated with 20 kR gamma rays (^{60}Co source). Out of these one set was soaked in H_2O and the other nine were immersed in 100 ml each of 10^{-4} M solutions of the nine metallic salts, for 24 hrs. Selfed panicles were collected, treatment-wise, from surviving M_1 plants, and the M_2 generation was raised in panicle progenies. The chlorophyll mutation and mutant frequencies, as well as the amount of synergism (K values) in sequential treatments, were computed as reported earlier[13]. For testing the differences in mutation and mutant frequencies between gamma ray treatment *versus* sequential treatments, standard normal deviate (Z) test was applied.

Out of nine metals tested, seven, *viz.*, Sr, Cd, Hg, Ba, Cu, Pb and Fe, displayed mutagenic activity in the rice genetic system (Table 1). Salts of Ba and Cd produced chlorophyll mutation and mutant frequencies on par with those of 20 kR gamma rays. Chlorides of Cu and Hg also showed fairly potent mutagenic effects. Compounds of Sr, Fe and Pb, however, showed weak mutagenic effects. In contrast, two metals, *viz.*, Mn and Ca, failed to provoke chlorophyll mutations in rice seed.

Sequential treatments with gamma rays and five metals, *viz.*, Sr, Cd, Hg, Pb and Cu, produced synergistic yields of chlorophyll mutations in the M_2 generation. These results suggest that radiation- and metal-induced

TABLE I

Frequency of Chlorophyll Mutations induced by Metallic salts in Independent and Sequential treatments with Gamma Rays in Rice

Treatments	Total No. of M1 panicles	Mutation Frequency (%)	Total No. of M2 seedlings	Mutant Frequency (%)	Degree of synergism (K values)
H_2O (Control) 24 hrs	618	—	33,618	—	—
20 kR + H_2O 24 hrs	891	5·38	30,294	0·47	—
Sr 24 hrs	1248	1·68	33,696	0·09	—
20 kR + Sr 24 hrs	450	8·00[a]	10,350	1·94[a]	3·45
Cd 24 hrs	945	5·23	34,020	0·46	—
20 kR + Cd 24 hrs	428	14·49[a]	13,728	2·86[a]	3·07
Hg 24 hrs	480	3·12	17,280	0·26	—
20 kR + Hg 24 hrs	456	13·15[a]	15,504	1·52[a]	2·08
Ba 24 hrs	660	6·36	21,780	0·68	—
20 kR + Ba 24 hrs	878	6·08	27,528	0·91[b]	0·79
Cu 24 hrs	488	3·57	22,932	0·41	—
20 kR + Cu 24 hrs	444	6·75	15,540	1·12[a]	1·27
Pb 24 hrs	456	0·65	18,696	0·04	—
20 kR + Pb 24 hrs	408	10·29[b]	15,096	1·03[a]	2·02
Mn 24 hrs	455	—	21,312	—	—
20 kR + Mn 24 hrs	540	5·55	19,980	0·79[c]	1·68
Fe 24 hrs	648	0·92	27,864	0·07	—
20 kR + Fe 24 hrs	488	3·57	18,816	0·47	0·87
Ca 24 hrs	324	—	13,284	—	—
20 kR + Ca 24 hrs	612	2·94[c]	23,256	0·29[b]	0·61

a = significant at 0·1% p level; *b* = significant at 1% p level; *c* = significant at 5% p level.

genetic lessions interact synergistically in the incidence of chlorophyll mutations. On the other hand, two genetically active metals—Ba and Fe—showed less than additive effects ($K < 1$) when post-treated after gamma irradiation. Even though Mn *per se* failed to induce mutations, it potentiated the mutagenic activity of gamma rays as indicated by the K value of 1·68 in sequential treatment of gamma rays + Mn. The mutagenic potentiation, conceivably, involves direct amplification of the initial radiation damage and/or inactivation of the repair-enzymes. In sequential treatment with gamma rays + Ca, significant reduction in both mutation and mutant frequency was observed (Table I). Calcium, thus, seems to confer marked protection (of about 40%) against gamma-ray induced genetic lesions. It is difficult to visualize as to how Ca is able to counter the mutagenic activity of gamm rays. However, it is well known that Ca and/or Mg ions play a critical role in maintaining the sirucutral integrity of chromosome[14,15] by bridging the nucleic acid and protein molecules in chromosome fibrils[1]. It is also reported that, in plants grown under Ca or Mg deficiency, there is substantial increase in the number of spontaneous chromosome breaks[16–18]. In the absence of definite evidence it may be assumed that the presence of calcium ions promotes more rapid repair of gamma-induced genetic lesions.

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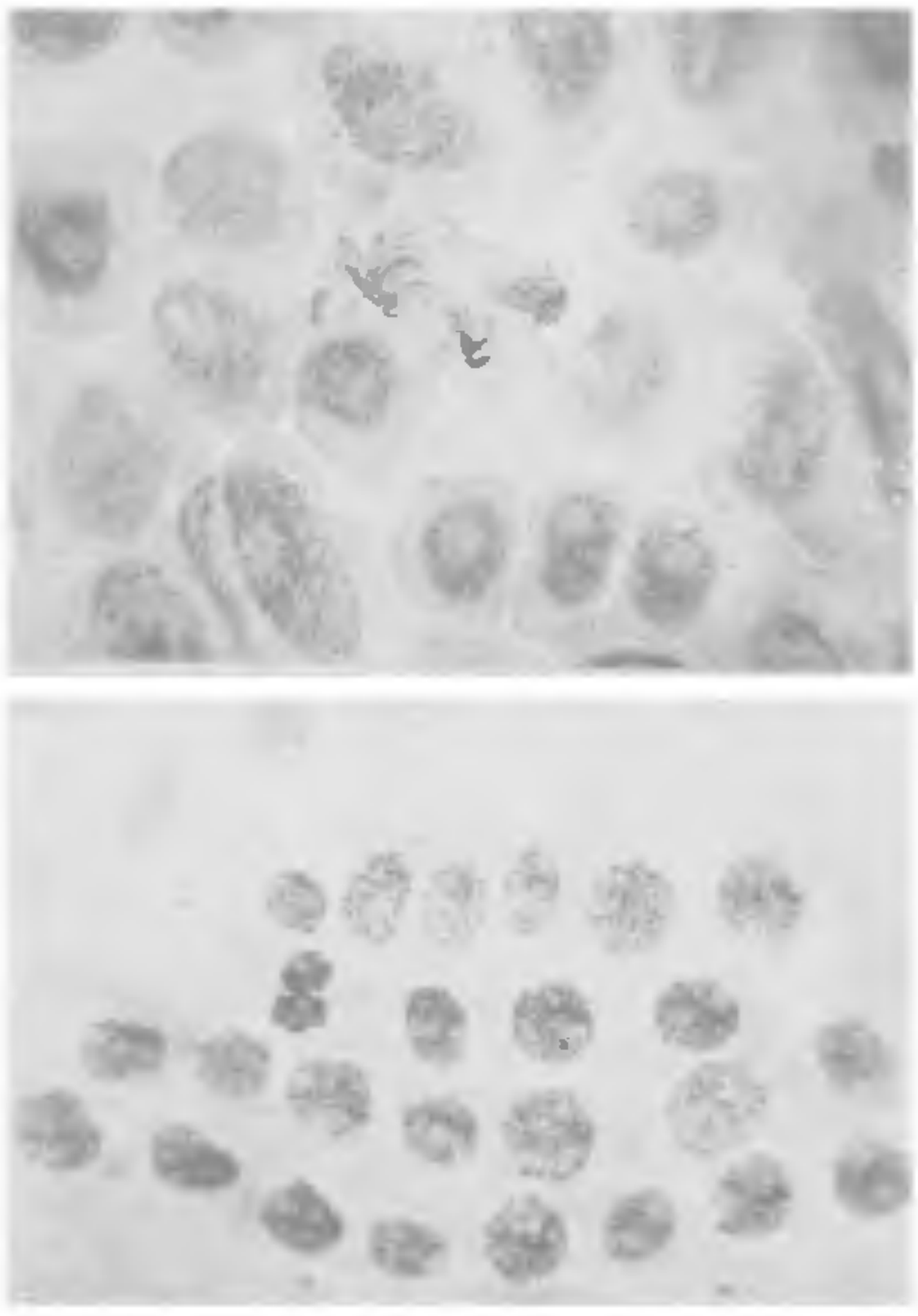
EFFECT OF ORAL CONTRACEPTIVE ON PLANT CHROMOSOMES

WHILE studying Twinning, it was noted that quite a good number of mothers who took oral pills as a contraceptive measure, gave birth to twins. It was deemed necessary to study the after effects of the drug on plant chromosomes.

The tablets* used as oral contraceptives were obtained from G.R. Medical College, Gwalior. Each tablet containing *Lynesternol* BP (1·0 mg and ethinyloestradiol 0·05 mg) was dissolved in warm distilled water. Trials to dissolve other combinations gave rise to suspended particles and hence discarded. The solution of the present drug was simultaneously administered on actively growing roots of *Allium cepa* (onion) and *Allium sativum* (garlic) for 2, 4 and 6 hours. Controls were maintained in distilled water.

Roots were fixed in equal parts of ethanol and acetic acid and stained by usual acetic-orcein method. Active mitotic rates [computed by adding meta and anaphases and dividing by the total number of cells observed *i.e.*, (M + A)/TC] have been compared in Table I.

The active mitotic rate goes down significantly especially in *Allium sativum*. The number of metaphases was considerably reduced in 6 hours while anaphase frequency was only halved. There were hardly any stages which could either exhibit C-metaphase or atleast the stickiness on the contours of chromosomes. Certain specific abervations like pseudo-translocations pseudo-Renner complex were also absent. A few cells possessing chromosomal breaks were observed in garlic ($f = 0{\cdot}005$) but not in onion in 4 *h* treatment. Multipolar spindle (Fig. 1) and a tendency for the nuclear cleavage (Fig. 2) have been observed, only in garlic roots ($f = 0{\cdot}011$; $0{\cdot}008$ respectively).



1

2

FIGS. 1–2. Fig. 1. *Allium sativum* root showing a tripolar spindle due to the treatment of the drug for 4 hours. Fig. 2. *Allium sativum* root exhibiting a tendency for nuclear cleavage.

Both *Allium cepa* and *A. sativum* have $2n = 16$ long chromosomes but *A. sativum* never sets any seed and its strain is regarded as a clone. Since *A, sativum* exclusively reproduces by vegetative propagation, it can be argued that it possesses higher inbreeding level than *Allium cepa* which reproduces by sexual and vegetative means. Observations of multipolar spindle and tendency of nuclear cleavage in *Allium sativum* and their absence in *Allium cepa* may be suggestive of the former's greater susceptibility to the contraceptive hormonal preparations.

That a drug can pass the placental barrier during the period of sensitive organogenesis in human being is well documented by *Thalidomide tragedy* in the German Federal Republic.[1] The drug was found to induce chromosome breakage in plant cells and it was realised that perhaps the Thalidomide disaster could have been avoided if its cytotoxic action on plant cells had been assessed earlier.