polluted water from Homan-zan, a closed copper mine. The paper was cut into strips of 4×20 cm. For easier manipulation they were fixed to plastic plates. Each set, consisting of 5 strips, was buried lengthwise, keeping 5 cm of the top above the land surface. They were placed at several points in the field. At the end of the incubation period, the strips were removed and soil particles were washed away by showering. After the top of each strip was cut off at the land surface, the residue was dried for 12 h at 70 °C, and the remaining cellulose of each paper was weighed,together with the polyethylene back. The weight of cellulose per unit size of the paper had been measured prior to the incubation.

Results and discussion. The burying of the strips in the soil, the measurement of the growth of the rice plants, and the collection of soil samples from the points measured were carried out on July 3, 1979. The copper content in soil collected from the various points ranged from a minimum value of 144.0 μg/g dry soil to a maximum value of 272.0 μg/g dry soil as shown in table 1. There was a close correlation between the copper content and the growth of rice plants, as shown in table 2. The strips of Benchkote were removed 16 and 23 days after the start of the incubation. As shown in table 1, cellulose decomposition was clearly inhibited in soil polluted excessively by copper. In particular, the amount of cellulose remaining on the strip removed after 23 days was closely related to the copper content of the soil, as shown in table 2. Judging from the observation that the remaining cellulose is easily removed without loss and the remaining amount is closely related to the soil pollution level, it seems that the polyethylene-backed paper is very suitable as a source of cellulose.

Table 2. Correlation coefficients between copper content in soil, growth of rice plants and amount of cellulose remaining on paper in the polluted paddy soil

	Length of rice plants	Number of tillers	Remaining amount of cellulose	
			16 days	23 days
Copper content in soil	−0.836*	−0.816*	0.615	0.919**
Length of rice plants	-	-	−0.665	−0.935**
Number of tillers	-	-	−0.431	−0.872*

* Significant at the 5% level; ** significant at the 1% level.

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Modifying effects of divalent ions on the sulfhydryl content of normal and tumorous beet root tissue under thermal and γ-irradiation stress

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Summary. Temperature and γ-irradiation stresses result in the loss of DTNB (5-5′ Dithionitrobenzoic acid) - reactive sulfhydryl groups in the TCA (trichloroacetic acid) - insoluble protein of normal and tumorous beet root tissue. Metal ions like Ca^{2+}, Zn^{2+} and Pb^{2+} have been found to partially prevent the change of -SH quantity under stress conditions. An attempt has been made to correlate the observed loss of DTNB - reactive sulfhydryl content with the loss of membrane permeability under thermal and irradiation conditions.

Zn^{2+} and Ca^{2+} are known to stabilize membranes under various physico-chemical stresses[2,3]. Divalent metal ions like Ca^{2+}, Zn^{2+}, Mg^{2+} and Pb^{2+} have already been shown to inhibit the heat-induced efflux of betacyanin in beet roots by stabilising membranes to thermal stress[4-6]. Ca^{2+} also causes an inhibition of the UV- and γ-irradiated efflux of betacyanin[6,7]. Thermal and radiation resistance in the presence of metal ions seems to be due to the interaction of these ions with protein or lipid components of both. Bresciani et al.[8] have demonstrated that some lipid-requiring enzymes like membrane ATPase, possessing -SH groups, are inactivated by radiation and hence the observed change in permeability. Rothstein[9] suggested earlier the existence of a relationship between cation transport and membrane-bound -SH groups in yeast cells. The relationship between γ-irradiation induced K^+ loss and decrease in -SH groups found by Rink[10] also lent support to such a possibility.

Alterations in the tissue -SH content due to neoplastic growth in animals are well documented[11] but little information is available on the -SH content in plant tumors and even less on the quantity of -SH under thermal and radiation stress. The present work was carried out to see the changes in the -SH content in both normal and tumorous beet root tissue under thermal and radiation stress, which was found to cause damage to the membrane permeability. The extent of damage was monitored in terms of the efflux of betacyanin[6], Na- and K-ions, and 260 and 280 nm absorbing materials (unpublished data of this laboratory already communicated). The present experiments were designed to make a comparative study on the role of divalent metal ions under similar circumstances.

Sulfhydryl and protein content in normal tissue, tumorous tissue and normal tissue adjacent to a tumor

	SH (nM mg^{-1} protein)	SH content nM g^{-1} fresh weight of tissue	Protein mg protein g^{-1} weight of tissue
Normal tissue	15.0	18.26	1.217
Tumor	4.17	8.67	2.0869
Normal tissue (adjacent to tumor)	6.57	6.268	0.954

The values are the mean determinations of 4 values.

Materials and methods. Sets of 6 g of beet root discs, prepared as already described[6], were incubated at 45 °C in 12 ml tricine-NaOH (10 mM, pH 6.8) buffer for 60 min in a shaking water bath. The buffer in different sets was supplemented with the desired concentrations (figure 2) of Ca^{2+}, Zn^{2+} or Pb^{2+}, based upon earlier observations[8]. γ-irradiation of discs was carried out at a dose rate of 125 rads/sec (estimated by Fricke Ferrous sulphate dosimetry[11]) in a 4000 (CO^{60}, 5500 Ci) Gamma Chamber (supplied by BARC, Bombay) for 65 min (total dose, 487.50 krads) in buffer, and in buffers supplemented with different cations. Post-irradiation incubation was carried out at 25±2 °C. In buffered controls, -SH was assayed at 2-h intervals for a period of 8 h after irradiation.

Tumors were induced by the inoculation of healthy beets with *Agrobacterium tumefaciens* LBA 201 (supplied by Prof. S.C. Maheswari, Botany Department, Delhi University) cultured on Nutrient broth (Nutrient broth (Difco) 8 g; NaCl 5 g; yeast extract (Difco) 5 g/l). Sulfhydryl quantity was assayed in samples from 2-month-old tumors and the adjoining normal tissues, with and without calcium (50 mM), subjected to thermal and radiation stress.

Sulfhydryl quantity was estimated using Ellman's reagent[13] in TCA-insoluble proteins pelleted at 4000 rpm for 15 min. TCA soluble protein could not be assayed for -SH because of an intense 'blank' colour due to the betacyanin content. The proteins were solubilized in 3% SDS (sodiumdodecyl sulfate) by incubation at 37 °C for 30 min. Sulfhydryl content was estimated by adding 0.1 ml of 5-5′ dithiobis(dithio nitrobenzoic acid, 3.96 mg/ml in 0.1 M phosphate buffer, pH 7.0) to 1 ml SDS extract and 1.0 ml of 0.1 M phosphate buffer (pH 8.0) in a total vol. of 3.0 ml. The change in A_{415}, 15 min after the addition of the reagent, was read against a reagent blank in an ECIL spectrophotometer. Protein was estimated by Vernon and Roberts' method[14] from the absorption at 230 and 260 nm. Sulfhydryl content mg^{-1} protein was estimated by using a molar absorptivity value of 13,600 M^{-1} cm^{-1} (Ellman[13]).

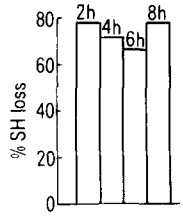

Fig. 1. Effect of the duration (h) on the post-irradiation (487.50 krads) incubation at 25±2 °C on the loss of -SH content. Each value represents the mean of 3 values.

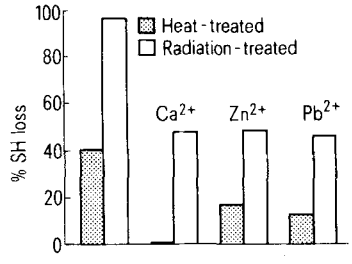

Fig. 2. Effect of $CaCl_2$ (50 mM), $ZnSO_4$ (10 mM) and $Pb(NO_3)_2$ (5 mM) on the loss of -SH content from temperature (45 °C/60 min) and γ-irradiation (487.50 krads) stress. Post-irradiation incubation was carried out at 25±2 °C for 2 h before -SH estimation. Values are the mean determination of 3 values.

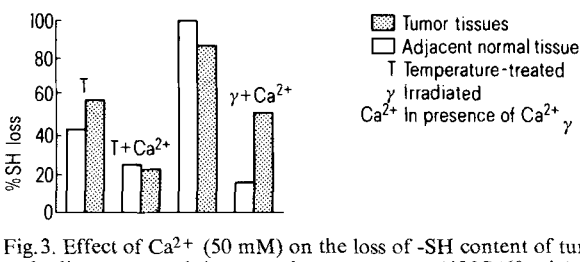

Fig. 3. Effect of Ca^{2+} (50 mM) on the loss of -SH content of tumor and adjacent normal tissues under temperature (45 °C/60 min) and γ-irradiation (487.50 krads) stresses. Post-irradiation incubation was carried out for 2 h at 25±2 °C. Values are the mean determination of 2 sets, 1 with 3 replicates.

Results and discussion. Temperature and γ-irradiation stress caused a decrease in the DTNB reactive -SH quantity (figures 1–3) thus confirming earlier reports[15–17]. Post-irradiation incubation at 25±2 °C did not make a significant difference (figure 1). However, there was a rise in the -SH content between 4 and 6 h which may be due to reduction of oxidized sulfur centres to -SH since formation of the latter through normal biosynthetic paths (eg. sulfate reduction etc.) seems less likely to occur over such a short interval of time.

Tumor tissue was found to contain more protein g^{-1} of tissue but the -SH content mg^{-1} protein was less than that of the adjacent normal tissues (table). Again, adjacent normal tissues showed less -SH groups mg^{-1} of protein compared to the normal uninoculated tissues (table).

It is reported that in erythrocytes the presence of external -SH reagents like cysteine and cysteamine protects against radiation-induced damage of membrane permeability[18]. The presence of metal ions during thermal and radiation stress partially prevented the oxidation of -SH groups (figures 2 and 3). They are in accordance with the earlier observations which show that Pb^{2+} and Zn^{2+} interact with -SH groups[19,20]. Thus, they may prevent partially the oxidation of tissue -SH groups under thermal and radiation stress.

Metal ions like Ca^{2+} might cap or protect the sulfhydryl groups by means of a physical interaction, thereby inhibiting the increased loss of betacyanin from tissues under heat and irradiation stress[5]. Recently it has been demonstrated that Ca^{2+} induces a modification in the -SH environment of sarcoplasmic reticulum ATPase, suggesting a new mechanism for possible conformational changes during the transport cycle of membranes[19].

Divalent metal ions are known to elevate the phase transition temperature of membrane lipids[21] and are also known to stabilize beet root membranes at higher temperatures[6]. Hence from the results obtained, it can be conjectured that the divalent metal ions may indirectly stabilize the activity of the lipid-requiring enzymes like Na^+, K^+ ATPase of membranes possessing free -SH groups, or directly interact with the -SH groups by means of physical interaction and thus partially prevent the oxidation of -SH to SS due to temperature and radiation stresses. It has also been established that Pb^{2+} interacts with phospholipids of membrane[19]. This may prevent the alteration of membrane fluidity due to stress conditions and thus stabilise the protein -SH groups.

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Role of visual and auditory cues in mating behavior of two desert species of *Drosophila*[1]

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Summary. The effects of visual and auditory cues on mating behavior was examined for 2 members of the *Drosophila nannoptera* species group. Results indicate that visual and auditory signals do not play a significant role in mating success in *Drosophila acanthoptera* and *D.* species W.

Visual, auditory, and olfactory cues are 3 major forms of stimuli affecting mating behavior in *Drosophila.* Bennet-Clark and Ewing[2] indicated that auditory cues are essential to stimulate the courtship of wingless males. Manning[3] reported that reduction in mating frequency was observed when the aristae and funiculi of the female (which function as receivers of auditory cues), were removed or immobilized. Petit and Nouaud[4] however, obtained results which indicated a nonessential role of auditory cues in stimulating courtship.

Averhoff and Richardson[5,6] found that airborne chemosignals are necessary for the initial stages of male courtship in *D. melanogaster.* Wingless males behaved as normal males and courted females when exposed to airborne chemosignals. The existence of sex pheromones in *D. pseudoobscura* have also been found[7,8].

Spieth[9] has found that other environmental cues such as visual signals and tactile sensations are also important in mating behavior. Some species of *Drosophila* are light-dependent with respect to their mating and oviposition behaviors.

Preliminary experiments were set up to examine the effects of visual and auditory cues on mating behavior of 2 members of *Drosophila nannoptera* species group. The *nannoptera* species group consists of 4 species. *D. pachea* is distributed throughout the Sonoran desert of southwestern Arizona, northwestern Mexico, and Baja California. The other 3 species (*D. nannoptera, D. acanthoptera* and species W) are sympatric in arid habitats of Guatemala and the Southern Mexican states of Oaxaca and Puebla. This study focuses on *D. acanthoptera* and species W.

Materials and methods. Both species W and *D. acanthoptera* were collected from rotting cactus tissue in Ixtepec, Oaxaca, Mexico and designated strains A585.1b and A585.2b, respectively. Inbred lines were established for each species by pair mating brothers and sisters for 7 generations. Upon eclosion, males and females were separated and aged to 2 days. To test for the role of visual cues, 60 pairs of a brother and sister were placed in total darkness while another 60 pairs were placed in an environment of 12 h of light and 12 h of darkness. To test for the role of auditory cues, 30 males from each of the above 2 environments were dewinged with ultramicro surgical scissors. For each species 120 brother-sister pair matings were examined. One male and female were placed in a 2.5 cm diameter × 9.5 cm high glass specimen tube containing 7 ml of our standard laboratory *Drosophila* media. All pair matings remained at 25 °C in their particular environments for 17 days after which the specimen tubes were examined for the presence of fertilized eggs. A successful mating was one that had larvae present within 17 days after the pair mating had been set up.

Results. Table 1 shows the effects of visual and auditory cues on successful matings in *D. acanthoptera.* Although a decline in the number of successful matings occurs when pairs are kept in the dark or when males are dewinged, a contingency chi-square test ($\chi^2 = 2.78$, d.f. = 3) indicates no significant differences between pairs of different treatments.

Table 2 shows test results for *D.* species W. A slight increase in the number of successful matings was found for pair matings kept in total darkness. However, a contingency chi-square test ($\chi^2 = 0.663$, d.f. = 3) indicates no significant differences between pairs of different treatments.

Discussion. The experiments show that *D. acanthoptera* and *D.* species W are not lightdependent with respect to mating

Table 1. Effects of visual and auditory cues on successful matings in *Drosophila acanthoptera*

Treatment	N*	Successful matings (%)
12-h light: normal pairs	30	70.0
Total darkness: normal pairs	30	63.3
12-h light: dewinged males	30	50.0
Total darkness: dewinged males	30	56.7

* N equals number of brother-sister pair matings set up.

Table 2. Effects of visual and auditory cues on successful matings in *Drosophila* species W.

Treatment	N*	Successful matings (%)
12-h light: normal pairs	30	60.0
Total darkness: normal pairs	30	63.3
12-h light: dewinged males	30	53.3
Total darkness: dewinged males	30	60.0

* N equals number of brother-sister pair matings set up.