CPZ treatment separately (Figure 3). However, the repair of the damage produced by the combined treatment was much less than the repair of the damages produced by the agents individually.

A general model has been proposed to explain the action of intercalative and non-intercalative drugs, postulating their inhibitory effects on DNA nicking and closing enzymes[9]. It seems likely from our data that CPZ also inhibits some components of the cellular repair system. Further, this impairment of repair makes CPZ more attractive as a possible adjuvant to radiotherapy.

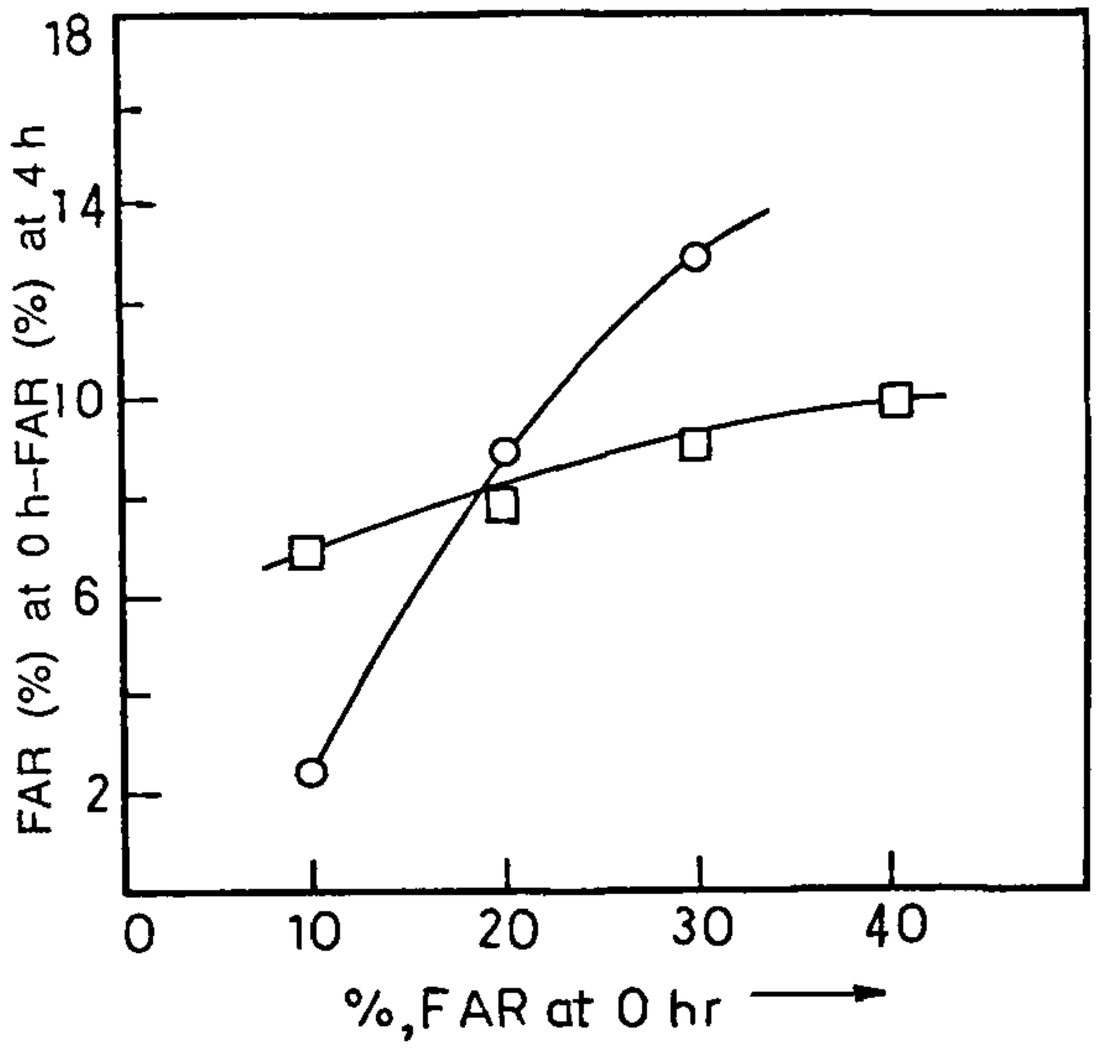

Figure 2. FAR (%) at 0 h–FAR (%) at 4 h against FAR (%) at 0 h Data derived from Figure 1. ○ X-rays, ❑ X-rays + CPZ for 1 h.

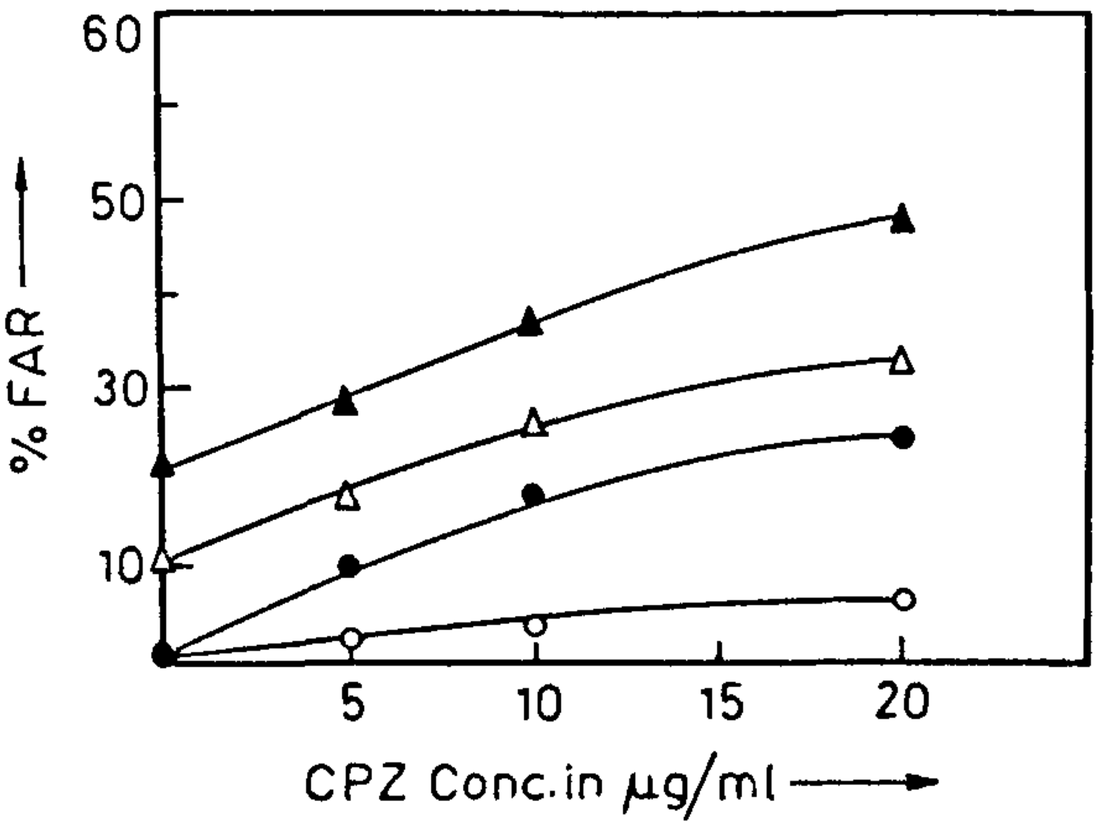

Figure 3. FAR (%) in cells incubated for 1 h with different doses of CPZ with or without X-irradiation (20 Gy). ● Blocks made immediately; ○ 4 h after incubation, ▲ cells X-irradiated during CPZ incubation and blocks made immediately; Δ 4 h after incubation.

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Gibberellic-acid-induced changes in α-amylase activity of barley seeds and their reversal by chlorflurenol

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α-amylase activity was measured in barley seeds soaked in water, gibberellic acid (GA, 10^{5} and 10^{-3} M) and chlorflurenol (CFL, 10^{-10} and 10^{-5} M) for 20 h. A high background activity was observed in controls. Seeds soaked in GA inhibited the enzyme activity, probably due to an endogenous superoptimal concentration. The higher concentration of CFL (10^{-5} M) reversed their inhibition. The action of CFL is attributed to interference in GA's action.

CHLORFLURENOL, a synthetic growth substance[1], inhibits seedling growth. In barley, this could be reversed by simultaneous application of GA, suggesting a role of GA in CFL-induced inhibition[2]. However, the lack of effect of CFL on GA-induced α-amylase activity in embryoless half seeds of barley, a specific bioassay for gibberellin action, tempted Krelle and Libbert[3] to question the mechanism of CFL action *vis-à-vis* GA mediation. Our findings (unpublished) also showed that CFL does not inhibit the induction of α-amylase activity by externally applied GA. Nevertheless, it was observed that

the background enzyme activity (in the absence of externally applied GA) was considerably reduced by CFL. These studies, however, were carried out on embryoless half seeds, while the studies on growth characteristics were conducted by presoaking treatment of whole seeds. The present study was, therefore, undertaken to examine the α-amylase activity in whole seeds as affected by CFL application.

Barley seeds (*Hordeum vulgare* c.v. 292) were soaked in cold (~ 9°C). After 20 h the seeds were cut transversely and the endosperm halves were used for α-amylase assay[4]. The half seeds were incubated for 72 h at 28°C over moistened sand in Petri dishes. Four half seeds per replicate were incubated in a medium consisting of $CaCl_2$, acetate buffer (pH 4.8) and chloramphenicol (to contain microbial growth) with or without GA (10^{-3} M) for 24 h, after which the enzyme activity was determined. The GA added at this stage is referred to as external GA. Enzyme activity is expressed as units by using standard α-amylase (Sigma). One unit hydrolyses 1 mg of maltose from starch in 3 min at pH 6.9 and at 20°C. The experiments, besides being replicated, were also repeated at least thrice.

The background α-amylase activity (without external GA) was 9.8 units in controls compared to 8.8 units in GA-treated sets (Table 1). As seen from the table, when the seeds were presoaked in GA, the enzyme activity was lowered by 25% in 10^{-5} M and by 50% in 10^{-3} M. At this stage external application of GA was ineffective.

Table 1. Effect of GA and CFL on α-amylase activity in barley seeds

Treatment (soaking)	External GA 0	External GA 10^{-3} M
DW	9.76 ± 0.323	8.78 ± 0.589
GA 10^{-5}	7.53** ± 0.336	7.56** ± 0.119
GA 10^{-3}	4.42** ± 0.535	5.25** ± 0.710
CFL 10^{-10}	8.79* ± 0.376	8 15 ± 0.187
CFL 10^{-5}	10.42 ± 0.190	9.36 ± 0.234
GA 10^{-5} + CFL 10^{-10}	2.61** ± 0.356	4.11** ± 0 110
GA 10^{-5} + CFL 10^{-5}	3 68** ± 0.527	3.79** ± 0.196
GA 10^{-3} + CFL 10^{-10}	2.65** ± 0.216	3.52** ± 0 000
GA 10^{-3} + CFL 10^{-5}	3 52** ± 0.511	2.73** ± 0.254

P values * = < 0.05; ** = < 0.01.

When seeds were soaked in CFL (10^{-10} M) there was 10% inhibition compared to control (Table 1). The inhibition was slightly more in the presence of applied GA. However, at 10^{-5} M a marginal stimulation (~ 7%) was observed. Here also the addition of GA caused a lowering of the enzyme activity. In the case of combination treatments, though there was a synergistic inhibition of the enzyme activity, the higher concentration of CFL (10^{-5} M) was less inhibitory (25–30% less) compared to the lower concentration (10^{-10} M) in the treatments where there was no application of external GA. This trend was reversed with the addition of GA externally.

Barley seeds, when soaked in GA (10^{-5} M and 10^{-3} M), inhibited α-amylase activity. This is probably due to a superoptimal level of endogenous GA. Chlorflurenol, though an inhibitor of growth, caused much less inhibition of the enzyme activity at 10^{-10} M and a slight enhancement at the higher concentration of 10^{-5} M. This effect may occur by lowering the endogenous GA level from a superoptimal to a normal level, which ultimately leads to an enhancement in α-amylase activity. Such an effect is supported by the observation that the higher CFL concentration is more effective in reversing the inhibition caused by a superoptimal level of GA.

When barley seeds were soaked in water, measurable amounts of α-amylase activity was observed (~ 10 units). It has been reported earlier[5] that sufficiently active gibberellins are present in the aleurone cells at the start of the incubation. In the present studies, since the seeds could not germinate due to the low temperature, the gibberellins produced became superoptimal. At this stage, any addition of GA from outside tends to inhibit the activity. Gibson and Paleg[6] observed in wheat that concentration of GA higher and lower than the optimal level caused inhibition of α-amylase activity. This point is vindicated in the present studies, where the enzyme activity was inhibited when seeds were soaked in GA. Further, the inhibition was more pronounced at higher concentration (10^{-3} M) of GA than at lower concentration (10^{-5} M).

Krelle and Libbert[3] observed no effect of CFL on GA-induced α-amylase activity in embryoless half seeds of barley. As evident from the work of Jackson[5] and Varner and Mense[7], this lack of response may be due to the presence of significant amounts of gibberellins or α-amylase as such in the aleurone cells, at the start of the incubation. Some of our findings (unpublished) had, however, shown that CFL reduces this background activity. An effect of CFL in similar direction is observed in the present studies also. The inhibition caused by a superoptimal concentration of GA is reversed by CFL.

It can be concluded from these studies as well as from our earlier observations[2] that the inhibition of seedling growth and enzyme activity by CFL and the reversal of inhibition of α-amylase activity caused by

a superoptimal concentration of GA are all due to a unidirectional effect, i.e. the inhibition of GA's action by CFL.

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Plant regeneration from protoplasts of scented indica rice using heterologous feeder system

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Protoplasts were isolated from 4–5-month-old embryogenic calli induced from mature seeds of the rice variety Pusa Basmati-1. The isolated protoplasts were cultured in the well formed by a ring of feeder cells of *Panicum maximum*, that were maintained as suspension cultures by periodic subculturing in R_2 medium. Six-month-old cell suspensions of *P. maximum* were found more suitable as a feeder for culturing of rice protoplasts than *Saccharum officinarum*. The microcalli obtained from protoplast culture were proliferated on modified N_6 medium and plantlets were obtained on MS regeneration medium supplemented with 1 mg l^{-1} IBA and 4% sucrose.

SEVERAL reports are available on regeneration of plants from protoplasts of rice[1–10]. These reports also suggested that feeder cells are a critical requirement for sustained division of protoplasts in the initial stage of culture. In most cases suspension cells of *japonica* rice varieties were used as feeder cells. When feeder cells and protoplasts of the same species are used together, often doubts are expressed with regard to the origin of calli and plantlets, whether, they are truly from protoplasts. In order to ensure that the plants regenerated are from protoplasts rather than from suspension cells, we used a heterologous system of feeder cell suspensions developed from a species distinctly different from cultured rice protoplasts.

Dehusked scented rice variety Pusa Basmati-1 (*Oryza sativa* ssp. indica) was surface-sterilized in 70% ethanol for 1 min and with 0.1% mercuric chloride for 5 min, followed by rinsing in sterile distilled water thrice. The sterilized seeds were cultured on modified MS medium[11] with 2 mg l^{-1} 2,4-D and variable concentration of either BAP or kinetin. After four weeks of incubation in the dark at 26 ± 1°C, the scutellar region of the seed embryos developed either nodular/globular whitish embryogenic calli (E-calli) or unorganized rough-surfaced light yellowish to creamy type of non-embryogenic calli (NE-calli). The embryogenic calli were separated under stereomicroscope and proliferated on the same callus induction medium. For isolation of protoplasts, the embryogenic calli were incubated in 15 ml of an enzyme mixture consisting of 2% cellulase 'Onozuka' RS (Yakult Honsha Co. Ltd, Tokyo, Japan) and 0.5% pectolyase Y-23 (Seishin Pharmaceutical Ltd. Co., Tokyo, Japan) in 5 mM MES buffer (2(*N*-morpholino)ethanesulfonic acid) and CPW salts[12] with 13% mannitol. The enzyme digestion was carried out in 90 mm Petri dishes for 4 h on a gyratory shaker at 50 rpm, followed by 1 h stationary period in the dark at 26 ± 1°C. The released protoplasts were sieved through a set of 64, 45 and 30 µm sterile nylon mesh and purified by sucrose density gradient[6]. The protoplasts were washed thrice in CPW salts[12] with 13% mannitol and pelleted by centrifugation at 1000 rpm. The viability and cell contamination of protoplasts were determined by staining with Trypan blue and Calcoflour white, respectively.

Calli were induced on MS medium[11] supplemented with 3.0 mg l^{-1} 2,4-D and 0.2 mg l^{-1} BAP from young inflorescence of *Panicum maximum*, leaf spindle of *Saccharum officinarum*, mature seed of *Triticum aestivum* and *Oryza barthii*. The 2-month-old calli of *P. maximum* and *S. officinarum* were used to develop a cell suspension for preparation of feeder cells. Suspension cultures were initiated in liquid R_2 medium[13] for *P. maximum* and AA medium[14] for *S. officinarum* and maintained through

Table 1. Effect of growth regulators on callus induction and embryogenic callus formation from seed embryos of Pusa Basmati-1

Basal MS medium with			Frequency of callus formation (%)	Growth of E-callus (%)
2,4-D (mg l^{-1})	BAP (mg l^{-1})	Kinetin (mg l^{-1})		
2	0 2	–	87.5	88 9
2	0 5	–	73.3	43 8
2	–	0 5	70 0	44 0
2	–	1	66 6	24 4
2	–	–	75 0	62 6

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