- Decagonal $Al_{75}Ni_{15}Co_{10}$ – 25.0%

     $Al_{71}Ni_{24}Fe_5$ – 29.0%

     $Al_{70.5}Mn_{16.5}Pd_{13}$ – 29.5%

     $Al_{70}Ni_{15}Rh_{15}$ – 30.0%

     $Al_{62}Cu_{17.5}Cu_{17.5}Si_3$ – 38.0%

2. Crystalline phases

- $Al_{10}Fe_2Ce$, $Al_{20}Fe_5Ce$, $Al_{10}Cr_2Ca$ and $Al_{10}Cu_2La$ – 23.1%

- Al_9Mn_3Si, $Al_9Mn_2Si_2$, Al_9Mn_3Ca and Al_9Fe_3Sm – 30.8%

- Al_8Fe_4Ca, Al_8Cu_4Ca, Al_8Mn_4Ca and many dozen others with rare earth elements   – 38.5%

In conclusion, it is hoped that the above facts, figures and postulates, particularly the encouraging preliminary analysis of compositions, will be considered adequate for justification for further work by scientists in this field to explore systematically and understand clearly the special features of the proposed new species of Icosahedral Cluster Compounds, not only in aluminium alloys, but in other related alloy systems.

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Modification of genotoxicity of radiation by post-irradiation treatment with caffeine

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The effect of caffeine during post-irradiation growth period on survival, gene conversion, back mutation, aberrant colony formation (ACF) and mitotic crossing over (MCO) was investigated in the diploid yeast strain *Saccharomyces cerevisiae* D7. The presence of caffeine in growth media reduced the radiation-induced cell killing and the frequency of all the genetic end points except MCO. The increase in MCO frequency might be related to the enhanced cell survival brought about by caffeine. It is suggested that post-irradiation division delay introduced by caffeine may alter the post-irradiation repair processes and may help the process of recovery by error-free repair pathways.

CAFFEINE is an environmental chemical to which people are exposed through food items, beverages and medicines[1]. A number of workers have reported a mutagenic activity of caffeine in bacteria, yeast and cultured mammalian cells[2]. In addition, caffeine has been shown to modify the mutagenic activity of several chemical mutagens, UV and ionizing radiations in these systems[3–8]. Caffeine also modifies carcinogenic effects of chemicals[9]. In yeast, caffeine has been reported to modify the lethal, mutagenic and recombinagenic effects of UV and N-methyl-N'-nitro-N- nitrosoguanidine (MNNG)[10–12]. Caffeine enhances cytotoxicity of non-ionizing radiation in mammalian cells[13,14]. With ionizing radiation, caffeine has been shown to exert both protecting and sensitizing effect. When present during irradiation, caffeine offers protection in cell culture systems[15], plant seeds[16,17], and bacterial spores[18,19]. Caffeine also exibits radioprotection

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for bone marrow chromosomes in mice given whole body gamma radiation[20]. However, caffeine has also been shown to act as a sensitizer in some other studies with cultured mammalian cells[21,22].

Since radiation is one of the standard mutagens and people are exposed to radiation due to occupational or medical reasons, the modulation of radiation-induced genotoxicity by this chemical is a subject of great interest.

Among the various genetic events, mutation is a very well studied end point. It has been shown that there is a high degree of correlation between carcinogenicity and mutagenicity of chemicals and it is now generally accepted that most of the carcinogens are mutagens[23-26]. Further, it has been suggested that recombination may be involved in carcinogenesis and teratogenesis[27-30] and that expression of deleterious genes following recombination may also result in heritable changes. Formation of aberrant colonies in yeast can be due to mitotic gene conversion, point mutation, chromosome deletion or aneuploidy induced by a mutagen[31]. It has been suggested that aberrant mitotic segregation events like mitotic recombination, non-disjunction, and other chromosome events might be involved in tumour promotion[28]. These genetic events occurring in the eukaryotic yeast test system are important as similar events may occur in man. Homologous genes controlling recombinational pathways (e.g. HsRAD52) have been identified in human cells[32].

In this paper, we describe the effect of caffeine on gamma radiation-induced gene conversion (non-reciprocal recombination), back mutation, aberrant colony formation (ACF), mitotic crossing over (MCO) or reciprocal recombination and cell killing using the diploid yeast strain *Saccharomyces cerevisiae* D7.

Saccharomyces cerevisiae D7 (a/α ade2-40/ade2-119, trp5-12/trp5-27, ilv1-92/ilv1-92) was donated by F. K. Zimmermann. The strain is heteroallelic at the *trp5* locus and requires tryptophan in the growth medium. Induction of gene conversion is detected by the appearance of colonies on medium lacking tryptophan. D7 detects back mutation at the *ilv* locus. Appearance of prototrophic colonies on isoleucine-free medium indicates induction of back mutation. In addition, the strain carries two different alleles of the gene locus *ade2*. Defective mutants of *ade2* cause a requirement for adenine and accumulate a red pigment. Allele *ade2-40* causes an absolute growth requirement for adenine and forms deep red colonies on medium containing a small amount of adenine. The other allele *ade2-119* causes a leaky requirement for adenine so that the cells grow at a slower rate in the absence of adenine and form pink colonies on low-adenine supplemented medium. Since these two alleles are complementing, the diploid cells carrying these two alleles can grow in the absence of adenine and form

white colonies. Mitotic crossing over between the centromere and the *ade2* locus leads to the formation of two daughter cells which are homoallelic for *ade2-40* and *ade2-119* in 50% of the cases. This results in the formation of red and pink sectored colonies. In addition to pink and red sectored colonies, red-pink-white, white and pink, white and red sectored, entirely red or pink and hairline sectored colonies are also formed following treatment with mutagens. Out of these, red-pink and red-pink-white sectored colonies represent MCO. The other types of pigmented colonies can be due to gene conversion, point mutation, chromosomal deletion and aneuploidy induced by a mutagen. MCO and all the other pigmented colonies together represent total aberrant colonies. These events are scored on synthetic complete media containing a low concentration of adenine. Details of media and procedures used for scoring these events are described elsewhere[31,33,34].

Caffeine (Fluka AG, Switzerland) at the concentrations of 5, 7.5, or 10 mM was added to synthetic complete and omission media.

Stationary phase cells were washed and resuspended in sterile distilled water at a concentration of 10^7 cells/ml. Cell suspensions were distributed in 4 ml aliquots in glass vials and were maintained at 0–4°C. Irradiation was carried out in a ^{60}Co gamma chamber at a dose rate of 40 Gy/min. Control and irradiated cell suspensions were suitably diluted and plated on media with and without caffeine.

The effect of 5 and 10 mM caffeine on stationary phase cells of strain D7 is shown in Table 1. Caffeine at these concentrations did not induce significant levels of cell killing. There was also no significant change in the background frequency of gene conversion. However, there was a significant reduction in the frequency of back mutation and ACF. MCO events were not detected in the absence or presence of caffeine although around 10,000 colonies were scored in total on synthetic complete media in two independent experiments.

The effect of 5 and 10 mM caffeine on survival of irradiated cells is shown in Figure 1. Caffeine at both these concentrations enhanced the survival of irradiated cells almost to the same extent. In the presence of

Table 1. Effect of caffeine on cell survival and different genetic end-points in *S. cerevisiae* D7[a]

End-point	Caffeine concentration		
	0	5 mM	10 mM
% Survival	100	94 ± 2.5	98 ± 3.6
Gene convertants/10^6 s	35 ± 13	32 ± 10	27 ± 5
Back mutants/10^7 S	42 ± 14	16 ± 5	5 ± 1
Aberrant colonies/10^4 S	11 ± 2	9 ± 4	4 ± 2
Mitotic cross overs/10^4 S	ND	ND	ND

[a] Values indicate mean ± SDm; ND, Not detected.

caffeine, the survival of cells exposed to 400 Gy increased from 36 to 58%, whereas at 600 Gy, the increase was from 23 to 42%.

Figure 2 shows the effect of caffeine on radiation-induced gene conversion frequencies. Caffeine decreased the gene conversion frequencies significantly at 400 and 600 Gy. At 600 Gy, the decrease was about 30% and 50% for caffeine concentrations of 5 and 10 mM respectively.

Back mutation frequencies induced by radiation in the absence or presence of caffeine are shown in Figure 3. Caffeine decreased the back mutation frequencies induced by 400 and 600 Gy of radiation. The decrease was dependent on caffeine concentration. For 600 Gy, the decrease was 40% for 5 mM and 65% for 10 mM caffeine.

Figure 4 shows the effect of caffeine on frequencies of radiation-induced total aberrant colonies per 10^4 survivors. Caffeine at 5 mM concentration did not alter the frequencies of radiation-induced aberrant colonies significantly, the decrease at 600 Gy was only 13%. However, at 10 mM, caffeine reduced the frequency by about 50%. Total aberrant colonies include colonies having pink and red twin sectors which represent MCO events. The frequencies of MCO induced by 300–600 Gy of radiation are shown in Figure 5. Caffeine at 5 mM concentration enhanced the frequency of MCO by a
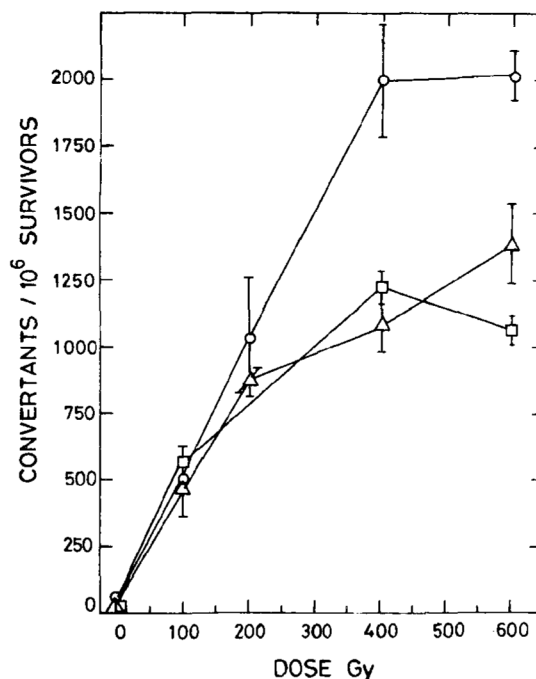

Figure 2. Induction of gene conversion in S. cerevisiae D7 irradiated with gamma radiation and plated on medium without caffeine (o), with 5 mM caffeine (Δ) and 10 mM caffeine (□). Values represent mean of 3 experiments ± SD.
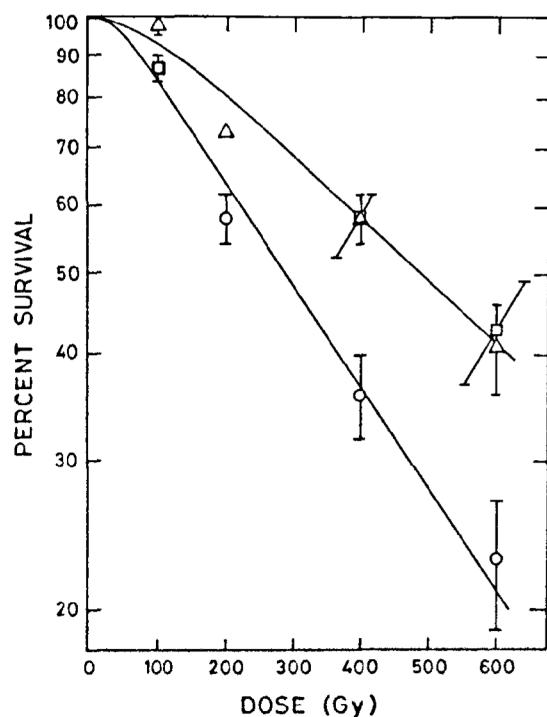

Figure 1. Survival response of Saccharomyces cerevisiae D7 irradiated with gamma radiation and plated on medium. Without caffeine (o), with 5 mM caffeine (Δ) and 10 mM (□) caffeine. Values represent mean of 3 experiments ± SD.
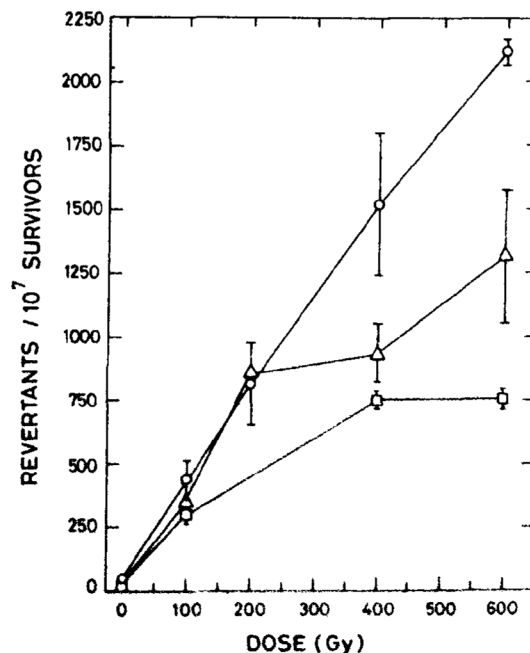

Figure 3. Induction of back mutation in S. cerevisiae D7 following gamma irradiation on medium without caffeine (o), with 5 mM caffeine (Δ) and 10 mM caffeine (□). Values represent mean of 3 experiments ± SD.

factor of 2–3 at radiation doses of 400 and 600 Gy. However, the frequencies observed with 10 mM caffeine were only slightly higher than those induced by radiation alone. At 7.5 mM, caffeine enhanced the radiation-induced MCO frequency to an intermediate level. MCO frequencies induced by 50 and 100 Gy were very low (2–3 colonies per 3000–4000 survivors). Hence it was not possible to detect any modifying effect of caffeine at these lower radiation doses.

It was observed that when 10 mM caffeine was present in the growth media, appearance of colonies was significantly delayed. In order to quantify the division delay, the growth kinetics of control and irradiated cells (600 Gy) in synthetic complete media containing 0, 5 and 10 mM of caffeine were investigated. The results are shown in Figures 6 and 7. It can be seen that the appearance of budding cells is delayed in media containing 5 and 10 mM caffeine in both control and irradiated cells. The delay was highly significant with 10 mM caffeine (Figure 6). Figure 7 shows the kinetics of cell division in the absence and presence of caffeine. The duration of lag phase, time required for 50% of the cells to undergo budding and first cell division following lag phase and also the doubling time of cells (in the log phase) under different conditions are presented in Table 2. In unirradiated cells, 5 and 10 mM caffeine induced significant division delay. Although duration of lag phase and doubling time of irradiated cells in the absence and presence of 5 mM caffeine is almost the same, caffeine introduced a delay of 2 h in the first post-irradiation budding process. On the other hand, 10 mM caffeine induces significant increase in the lag

phase and more than 8 h of delay in the budding process. This is consistent with the observation that colonies appeared almost simultaneously on the media plates containing no caffeine and those containing 5 mM caffeine. On the contrary, it took about five more days to observe countable colonies on media plates containing 10 mM caffeine. For 7.5 mM, the delay was less than that observed with 10 mM. For each dose, colony counts on plates with or without caffeine were done on the same day.

Caffeine has been reported to induce diverse genetic effects, viz. mutagenic[2], comutagenic[6] and anti-mutagenic[35]. Moreover, it has been shown to enhance, as well as inhibit, neoplastic transformation[36,37]. The basis for these effects is thought to be that caffeine interferes with DNA repair and other metabolic processes[6,38]. Depending upon the repair process affected, either enhancement or reduction in the effect of an agent is seen.

Caffeine potentiates the effects of ionizing radiation in mammalian cells[6,21,22], probably by directly inhibiting DNA repair[39] or by reducing either the G_2 block[40] or radiation-induced depression of DNA synthesis, and thereby decreasing the time available for repair before the damage becomes fixed by replication[41]. However, it has also been demonstrated that the above effects of caffeine and increased radiosensitivity may not be directly related[42]. It should be noted that potentiation of radiation effects and the reduction in cell cycle block observed in mammalian cells by caffeine are just the opposite of the results observed with yeast. Hence, our results cannot be directly compared with those obtained with mammalian cells.
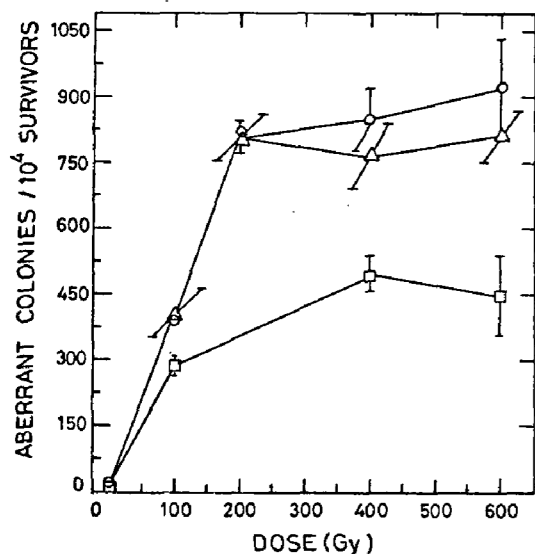

Figure 4. Induction of aberrant colonies in *S. cerevisiae* D7 following gamma irradiation on medium: without caffeine (o), with 5 mM caffeine (Δ) and 10 mM caffeine (□). Values represent mean of 2–5 experiments ± SD.
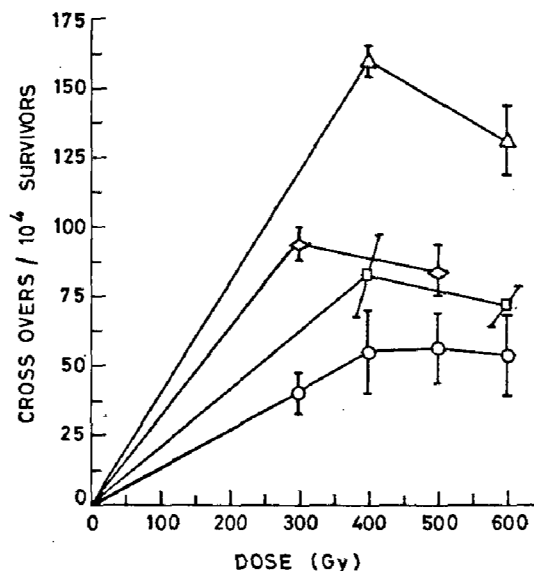

Figure 5. Induction of mitotic crossing over in *S. cerevisiae* D7 by gamma radiation on medium: without caffeine (o), with 5 mM caffeine (Δ), 7.5 mM caffeine (◇) and 10 mM caffeine (□). Values represent mean of 3 experiments ± SD.

Figures 1 to 4 indicate that caffeine increases the survival level of irradiated cells on the one hand, and on the other, it reduces the frequencies of induced gene conversion, back mutation and aberrant colonies. It appears that caffeine brings about some modification in the repair processes operating after irradiation. It can be suggested that caffeine facilitates the repair of radiation damage via an error-free repair pathway which reduces the cellular damage leading to these effects.

In contrast to the results seen for the above genetic end-points, the frequency of MCO increased 2–3 fold in the presence of 5 mM caffeine. It should be noted that MCO is detected as the appearance of red- and pink-sectored colony. At lethal doses of 300 to 600 Gy, death of one of the daughter cells would lead to the formation of either entirely pink or red colony which would be counted as an aberrant colony but not as a MCO event. Zimmermann has also indicated the possi-

bility of observing colonies which are entirely red or pink when MCO takes place with a lethal segregation of one of the two reciprocal products at lethal doses[33]. Since caffeine enhances the survival level of irradiated cells, the probability of both the daughter cells surviving increases, which may possibly lead to an enhancement of the appearance of red-pink sectored colonies. Hence, 5 mM of caffeine does not alter the frequency of ACF significantly but it increases the frequency of MCO by 2–3 fold. Caffeine at 10 mM introduces a significantly greater delay in the post-irradiation budding process and cell division as shown in Figures 6, 7 and Table 2. Any delay introduced prior to first post-irradiation cell division may provide an opportunity for the cells to recover from some part of the radiation-induced damage. In this system, caffeine appears to facilitate the repair of radiation damage via an error-free repair pathway. It is possible that medium containing 10 mM caffeine may
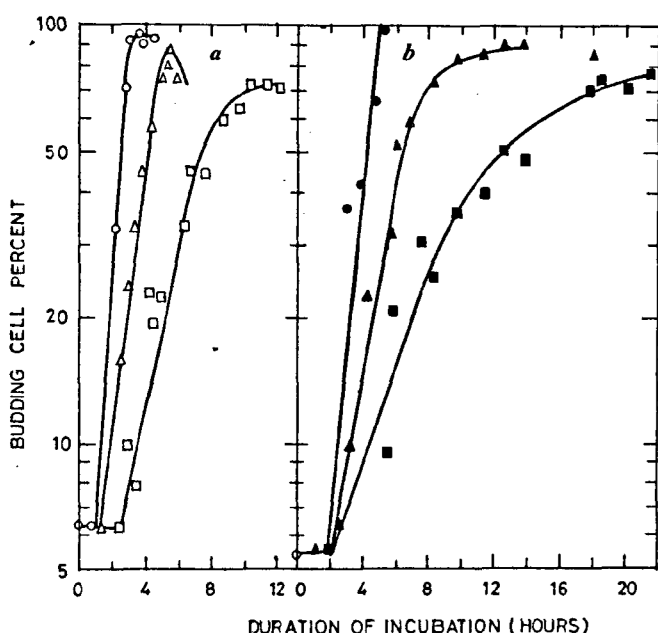

Figure 6. Budding cell kinetics of S. cerevisiae D7 in synthetic complete medium. a, unirradiated cells: without caffeine (o), 5 mM caffeine (Δ). 10 mM caffeine (□); b, Cells irradiated to 600 Gy: without caffeine (●), 5 mM caffeine (▲) and 10 mM caffeine (■).
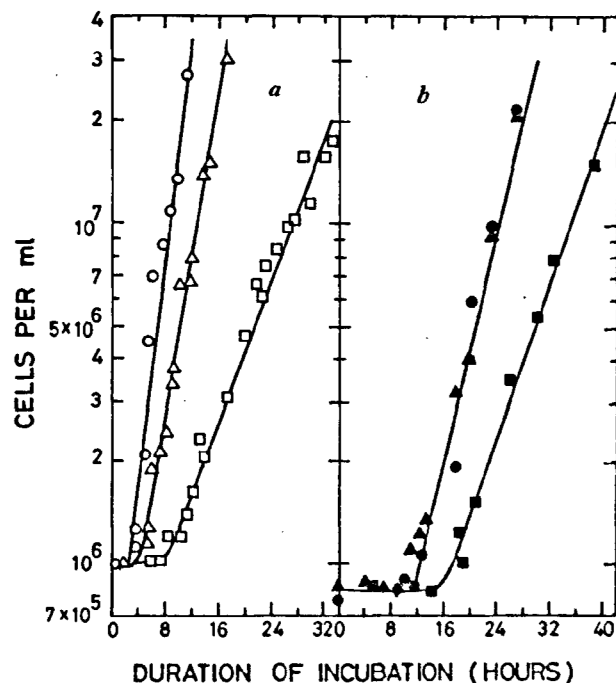
Figure 7. Cell division kinetics of S. cerevisiae D7 in SC medium. a, Unirradiated cells. b, Cells irradiated to 600 Gy. Symbols same as in Figure 6.

Table 2. Parameters of cell division kinetics of control and irradiated cells in the absence and presence of caffeine

	Control cells			Cells irradiated to 600 Gy		
	Caffeine concentration			Caffeine concentration		
Parameters	0	5 mM	10 mM	0	5 mM	10 mM
Lag phase	2 h 28 min	3 h 58 min	7 h 45 min	10 h 49 min	10 h 34 min	16 h 11 min
Time required for 50% budding	2 h 30 min	4 h	7 h 12 min	4 h 12 min	6 h 18 min	12 h 30 min
Time required for first division	4 h	6 h 48 min	11 h	14 h 24 min	15 h	21 h 36 min
Doubling time	1 h 48 min	2 h 29 min	5 h 42 min	3 h 30 min	3 h 42 min	5 h 13 min

further facilitate post-irradiation error-free repair processes by introducing a significant division delay. As a result of which probably the frequency of ACF and MCO is significantly reduced with 10 mM caffeine compared to that observed with 5 mM caffeine. When 7.5 or 10 mM caffeine is used, two opposing factors might be operating simultaneously for the induction of MCO: firstly the improved survival of both the daughter cells may enhance the expression of red and pink twin-sectored colonies (MCO) and secondly, an improved error-free repair during enhanced division delay, may reduce the lesions leading to MCO event. The net effect of these two opposing processes at the higher caffeine concentration, results in the MCO frequency being greater than radiation alone value but significantly less than that observed with 5 mM caffeine.

Since MCO is included in aberrant colonies, the relative percentage of these two events in the irradiated cell populations was analysed (Table 3), in order to study whether ACF or MCO was selectively inhibited by caffeine. It can be seen that frequencies of aberrant colonies induced by 600 Gy decrease to 87.9% and 48.5% in the presence of 5 and 10 mM of caffeine respectively (column 3). The percentage of MCO events in the total aberrant colonies can be examined in each case. Among the 923 radiation-induced aberrant colonies, MCO events constituted 53 (5.7%). Due to the possibility of improved survival of daughter cells, the MCO events with 5 mM caffeine increases to 131 out of 811 (16.2% of total aberrant colonies). Even with 10 mM caffeine following reduction in aberrant colony frequency to 48.5% of radiation alone value, the MCO events were 71 out of 448 (15.8% of total aberrant colonies), indicating that 10 mM caffeine reduces the frequency of aberrant colonies and MCO in the same proportion so that the relative percentage of MCO in the total aberrant colonies remains

Table 3. Effect of caffeine on the relative frequencies of radiation-induced aberrant colonies and mitotic crossing over events

Radiation dose	Caffeine concentration (mM)		Abrerrant colonies/10^4 survivors*	Mitotic crossing-over/10^4 survivors*
400 Gy	0	(a)	851 ± 168 (100%)	55 ± 20 (6.5% of a)
	5	(b)	762 ± 170 (89.5% of a)	159 ± 8 (20.9% of b)
	10	(c)	497 ± 66 (58.4% of a)	82 ± 21 (16.5% of c)
600 Gy	0	(a)	923 ± 217 (100%)	53 ± 21 (5.7% of a)
	5	(b)	811 ± 119 (87.9% of a)	131 ± 21 (16.2% of b)
	10	(c)	448 ± 157 (48.5% of a)	71 ± 10 (15.8% of c)

*Values represent mean ± SD.

the same, at about 16%. A similar pattern was seen in cells exposed to 400 Gy.

In another strain of yeast *Schizosaccharomyces pombe*, caffeine has been shown to increase UV-induced cell killing[10]. In addition, caffeine decreased UV and MNNG-induced forward and reverse mutation and MCO[11,12]. However, the mechanisms by which UV and MNNG act are different from those of ionizing radiation. Moreover, the repair pathways operating on UV-induced damage are different from those operating on ionizing radiation-induced damage even though some steps may be common. In addition, it has been indicated that different repair mechanisms of UV lesions are present in different eukaryotic test systems including *S. pombe* and *S. cerevisiae*[11]. In view of the above reasons, results obtained in other systems cannot be directly compared with our results.

It became evident some years ago that more than one repair pathways control the process of recovery in yeast cells[43,44]. The repair pathway mediated by the expression of RAD 51, 52, 54 genes gives rise to recombination, whereas the pathway mediated by the RAD6 and RAD18 gene does not yield recombination[45]. It has been shown that mammalian cells possess homologous recombinational pathways[32]. Evolutionary conservation of DNA repair genes has enabled the cloning of human genes homologous to ionising radiation-sensitive yeast *S. cerevisiae* mutants: RAD6, which is cross-sensitive to other DNA-damaging agents and the RAD52 epistasis group of yeast mutants defective in double strand break repair[46,47]. Homologous genes of RAD52 epistasis group have recently been identified in fission yeast[48], *D. melanogaster*[49], chicken[50] mouse and human[51,52] which indicates that the recombinational repair pathway is conserved throughout evolution. Fahrig had reported that the only suitable test system to detect reciprocal recombination was the spot test with mice[53]. Subsequently, it has become possible to detect mitotic reciprocal recombination at the dilute locus of mice *in vivo* by using methods of molecular biology[54]. In light of the above observations, it would be interesting to determine the manner in which caffeine may modify radiation-induced recombinational events in higher eukaryotic organisms including man.

It may be suggested on the basis of present results that caffeine may interfere with the repair processes by altering the proportion of the damage repaired by these pathways. In order to determine which of the pathways are specifically affected, further work is required involving construction and use of mutant strain of *S. cerevisiae* having the same marker end-points.

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Agrobacterium rhizogenes-mediated gene transfer in mungbean (*Vigna radiata* L. (Wilczek))

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Stable transformation and expression of transgenes was achieved in mungbean using *Agrobacterium rhizogenes*-mediated system. Seedling hypocotyls, on cocultivation with different *A. rhizogenes* strains containing Ri plasmid and a binary vector (pBin9GUSint) with neomycin phosphotransferase II and GUS (with a intron in coding region) as marker genes, produced roots which showed GUS expression and rapid growth on hormone free-medium containing 50 mg l^{-1} kanamycin and 500 mg l^{-1} cefotaxime. Transformation frequency varied with the age of the explants, duration of pre-induction of explants, cocultivation time and medium, concentration and strain of *Agrobacterium* but did not vary too much with different genotypes of mungbean. The transgenic roots produced calli on callusing medium containing 50 mg l^{-1} kanamycin and 500 mg l^{-1} cefotaxime. The calli showed expression of *nptII* and *Gus* genes. Transfer of GUS DNA into mungbean genome was confirmed by Southern blot analysis. However, neither transgenic roots nor their calli could regenerate shoots on either the B_5 or B_5-containing different cytokinins or auxins alone or in combination.

GENETIC transformation of grain legumes has, so far, been a difficult and challenging problem probably due

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