

Influence of Caffeine and Cysteine on Post-Irradiation Oxygen-Dependent and -Independent Components of Damage in *Hordeum vulgare*

In germinating seeds and other actively metabolizing systems, the application of caffeine (1,3,7-trimethylxanthine) during or immediately after irradiation potentiates the damage¹⁻⁶; under similar conditions, cysteine decreases the damage^{2,7,8}. But in dry (slowly metabolizing) seeds, recent studies have shown that caffeine eliminates part of the post-irradiation oxygen-dependent damage⁹ (POWERS¹⁰ class III damage) but enhances the oxygen-independent (class I¹⁰) damage¹¹. Furthermore, cysteine was found to have no influence whatsoever on this component of damage¹¹. Hence, it was felt necessary to investigate the influence of cysteine on the post-irradiation oxygen-dependent damage in the presence and absence of caffeine. Preliminary data reported herein suggest that cysteine exercises some protective action against oxic but not anoxic damage, and that when both caffeine and cysteine are applied together, the mode and magnitude of modification are determined by the former.

Materials and methods. Pure-line seeds (caryopses) of a hull-less barley strain (IB 65) were stored in a desiccator over calcium chloride until equilibrated to a moisture content of 7%. These seeds in vacuo (25 seeds in each of the 3 sealed ampoules per treatment) were exposed to 45 kR of γ -rays using a γ -cell (3680 Ci ⁶⁰Co) from Atomic Energy of Canada Ltd, at a dose-rate of 38.1 R/sec. For details of estimation of the moisture content of seeds, evacuation of the ampoules, sealing etc., see our earlier papers^{9,12}.

Post-irradiation hydration media consisted of oxygenated or oxygen-free water¹² which was also used for preparing 3.8×10^{-3} M solutions of caffeine (Sigma, USA) or L(+)-cysteine (E. Merck, Germany) or both in equimolar concentrations. Soon after irradiation the glass ampoules were broken and the seeds were placed in the respective hydration media at 0–2°C for 12 h. Appropriate controls were included in the experimental design. After the hydration period, the seeds of each replication were planted separately on moist filter paper in Petri dishes and grown in a growth chamber for 8 days at a temperature of $24 \pm 1^\circ\text{C}$ and under constant illumination. Statistically analyzed data on the 8-day seedling growth are presented in the Figure.

Results and discussion. The data show that caffeine and cysteine treatments as such have no effect on unirradiated seeds. Cysteine exercises approximately 60% more radio-

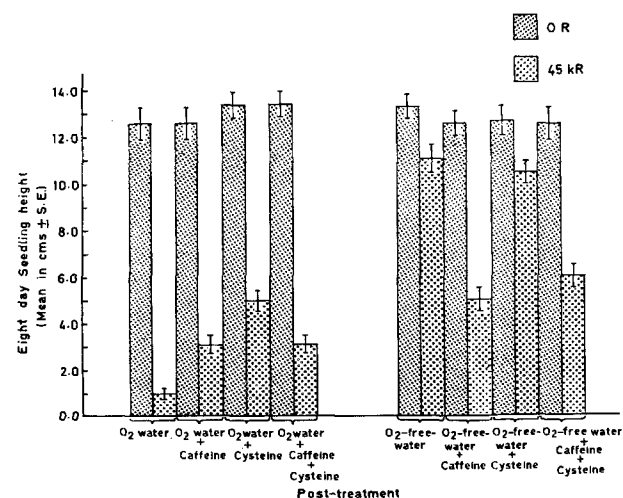
protection than caffeine against the class III damage; however, this additional protection is lost when caffeine is applied together with cysteine during oxygenated hydration. There is already a suggestion^{9,12,13} that the precursors of the post-irradiation oxic damage are not a homogeneous population but consist of different categories of which some are eliminated by heat shock and caffeine while others are not. It may well be that cysteine removes qualitatively more categories of the precursors than caffeine. Experiments are in progress to elucidate the mechanism by which caffeine removes the additional level of protection afforded by cysteine.

Caffeine potentiates the class I damage but cysteine has no influence on it. These observations are in accordance with our earlier findings^{9,11}. It is not known whether the radiation-induced free radicals responsible for the class I effect are so short-lived that they complete their reaction before cysteine post-treatment could exercise any protective action. Current interpretations of the molecular basis of radiosensitization by caffeine^{3-5,14-16} are largely due to experiments with actively metabolizing systems, and hence they may not be valid for the potentiation of an anoxic component in a slowly metabolizing system. There is no initiation of DNA synthesis when the seeds are subjected to various hydration media at 0–2°C for 12 h. Further experiments are necessary to elucidate the mechanisms of action of caffeine and cysteine on the oxic and anoxic components of radiation damage in slowly metabolizing systems.

Résumé. La cystéine est un radio-protecteur plus efficace que la caféine contre le dommage post-irradiatoire dû à l'oxygène. Mais la cystéine n'exerce aucune influence sur la partie du dommage qui ne dépend pas de l'oxygène et la caféine accentue ce dommage. Dans le traitement simultané de caféine et de cystéine, c'est seulement la caféine qui détermine l'amplitude et le genre de modification.

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Post-irradiation modification of oxygen-dependent and -independent components of seedling injury by caffeine and cysteine.

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