Figure 2. *In vitro* regenerated shoots showing **a**, phylloid flowers from phyllody affected plant parts and **b**, normal flowers from unaffected plant parts.

Table 1. Effect of antibiotic tetracycline on remission of symptoms in phylloid cultures

Treatment	Percentage shoots showing Complete reversion	Partial reversion	No reversion
Control	—	—	100
100 ppm	20	40	40
250 ppm	87.5	—	12.5
500 ppm	83.33	—	16.67

field screening for phyllody using vectors and also in obtaining a sizable number of MLO's containing scions under natural conditions for mass scale graft transmission, the above technique should be a great boon to plant breeders.

1. Pal, B. P. and Nath, P., *Indian J. Agric. Sci.*, 1935, 5, 517.
2. Sternis, C. G. and Van, G. J., Flora Malesiana P. Noordhoff Ltd. Djakartha, Ser 1 4 XXVI.
3. Kolte, S. J., *Diseases of Annual Edible Oilseed Crops*, 1985, vol. III, p. 137.
4. Sastry, K. S., *Plant Virus and Mycoplasmal Diseases in India, A Bibliography*, Bharathi Publications, Delhi, 1980, p. 292.
5. Purohit, S. D. and Arya, H. C., *Agric. Digest*, 1980, 4, 5.

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Radioprotection by nitrous oxide: role of cellular water content

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Contrary to earlier reports that nitrous oxide (N_2O) potentiates radiobiological damage, we have observed radioprotection in very dry seeds. It is known that N_2O converts hydrated electrons (e^-_{aq}) into hydroxyl radicals (OH) probably accounting for the commonly observed radiosensitization. Considering the available data, the role of water in altering the N_2O-mediated modification of radiobiological damage is considered.

THAT nitrous oxide (N_2O) which converts electrons into hydroxyl radicals[1], also enhances radiosensitivity has been reported for the aqueous suspension of *Bacillus megaterium* spores[2-4], vegetative cells of *E. coli*[5-7] and mammalian cells[8]. However, a lack of radiosensitizing effect by N_2O has also been reported for lymphocytes[9], Chinese hamster V 79 cells[10] and vegetative cells of bacteria[11-13]. It has been shown that cell concentration, dose-rate and irradiation temperature affect the radiosensitization of *Pseudomonas radiora* 0.1 by N_2O[14]. There are also reports[3,15] that only a few strains of vegetative cells of bacteria and only two mutants of *E. coli* K-12, respectively were radiosensitized by N_2O. An entirely novel finding, however, was recently reported by Singh and Kesavan[16] that N_2O-saturated post-hydration affords radioprotection to a greater extent than N_2 to dry barley seeds exposed to ^{60}Co-gamma-rays *in vacuo*. This unexpected observation holds considerable implications to basic radiobiology.

We present in this paper data on the effect of varying seed moisture content in relation to the radioprotective action of N_2O.

Pure-line barley seeds (*Hordeum vulgare*) of a hull-less strain (IB 65) were used in these experiments. Seeds were equilibrated to the desired moisture contents following the method of Kesavan *et al.*[17] For each treatment, about 100 seeds were put into 10 ml glass ampoules which had been first evacuated at 10^{-2} torr for 4 h and then sealed off with a glass-working torch.

The glass ampoules containing the seeds *in vacuo* were exposed to 250, 350 and 450 Gy of gamma radiation [source ^{60}Co, 204 TBq (5500 Ci) obtained from the Bhabha Atomic Research Centre, Bombay, India]. The dose-rate was determined by Fricke Fe^{+2}/Fe^{+3} dosimeter.

Double-distilled water was degassed by boiling for 20 min and cooled down to 4°C. Then it was saturated with O_2, N_2 or N_2O gas (Indian Oxygen Limited, India) as described by Kesavan *et al.*[18] The oxygen contamination in the N_2 and N_2O gas was about 20–30 and 90–100 ppm respectively.

Immediately after irradiation, the glass ampoules were broken open and the seeds were quickly immersed in O_2-, N_2- and N_2O-saturated water at 4 ± 1°C. Seeds were left in the respective hydration media for 8 h and then washed thoroughly in running tap water. These were grown at 25 ± 1°C as reported by Singh and Kesavan[16]. At the end of 8 days, the seedling height was measured and the seedling injury was computed following Conger *et al.*[19] In Figure 1, the post-irradiation O_2-enhancement factor is obtained by dividing seedling injury in O_2-saturated water by seedling injury in N_2-saturated water and the protection index of N_2O by dividing seedling injury in N_2-saturated water by seedling injury in N_2O-saturated water. The peroxidase (EC.1.11.1.7) activity was assayed in the 8-day old seedlings as reported by Singh and Kesavan[16].

The nature of gas dissolved in water for post-hydration does not exert any effect whatever on the seedling growth and peroxidase activity of the 8-day-old seedlings raised from the unirradiated seeds (Table 1).

In the present experiments performed with dry barley seeds with varying moisture content, N_2O affords maximal radioprotection to the seeds with least moisture content (~3.3%) and considerably less to those with ~8.0% moisture content; it affords no radioprotection to the seeds with ~11.5% moisture. These inferences result from comparison of the seedling injury values observed in N_2O and N_2. The dose of irradiation is also an important factor. These are illustrated as 'protection indices' (defined as the ratio of seedling injury in N_2 post-irradiation hydration to that of N_2O post-irradiation hydration) in Figure 1. The magnitude of seedling injury ascribable to the N_2-mediated pathway of radiation action is not influenced by the seed moisture content.

Saturation of the water with O_2 for post-hydration results in the manifestation of the classical 'oxygen effect' only in the dry seeds. Data (Table 1) show that drier the seed, greater is the O_2-effect, and that seeds of ~11.5% moisture register no such oxic response at all.

The direct action of radiation on vital target molecules (RH_2) in seeds results in the following:

$$RH_2 \xrightarrow{\gamma} RH^{\cdot}, H^{\cdot}, e^- \text{ (trapped)}. \quad (1)$$

In 'wet' (metabolizing) systems, radiolysis of water also results in hydroxyl radicals ($OH^{\cdot}$) and hydrated electrons (e^-_{aq}). N_2O, if present, acts as follows[1]:

$$N_2O + e^-_{aq} \longrightarrow N_2 + OH^{\cdot} + OH^-. \quad (2)$$

$$k = 5.6 \times 10^9 \ M^{-1} \ s^{-1}$$

The enhanced generation of $OH^{\cdot}$ from the above reaction is often held responsible for the increased radiosensitivity observed under certain conditions in bacterial spores and mammalian cells irradiated in N_2O than in N_2 (ref. 2–7). The present data reveal that N_2O acts as even a radioprotector in cell systems with extremely low water content. Such a radioprotection by

Table 1. Influence of initial seed moisture content and radiation-dose on O_2-, N_2-, and N_2O-mediated damage in barley seeds.

		Gaseous state					
		Oxygen		Nitrogen		Nitrous oxide	
Dose (Gy)	Seed moisture content (%)	% seedling injury	Peroxidase activity (units mg^{-1} protein)	% seedling injury	Peroxidase activity (units mg^{-1} protein)	% seedling injury	Peroxidase activity (units mg^{-1} protein)
0	3.3	0.0	13.6	0.0	12.9	0.0	14.0
0	8.0	0.0	12.8	0.0	13.0	0.0	13.2
0	11.5	0.0	13.6	0.0	14.1	0.0	13.7
250	3.3	45.1	55.1	8.7	22.7	1.7	18.6
250	8.0	35.7	48.9	8.6	18.7	3.9	19.1
250	11.5	8.4	17.9	7.1	18.1	8.1	17.2
350	3.3	71.8	88.5	14.0	25.9	4.0	18.0
350	8.0	63.9	65.1	14.4	25.1	8.3	17.5
350	11.5	13.2	21.1	13.4	20.7	13.2	20.3
450	3.3	88.6	132.3	29.0	29.1	19.0	23.9
450	8.0	78.3	98.5	27.9	29.7	21.0	18.3
450	11.5	33.1	34.2	30.3	30.3	30.9	30.1

Dose-rate 1.25 $Gy s^{-1}$

Post-irradiation hydration at 4°C for 8 h

The growth response of unirradiated seeds with different moisture contents do not differ from each other

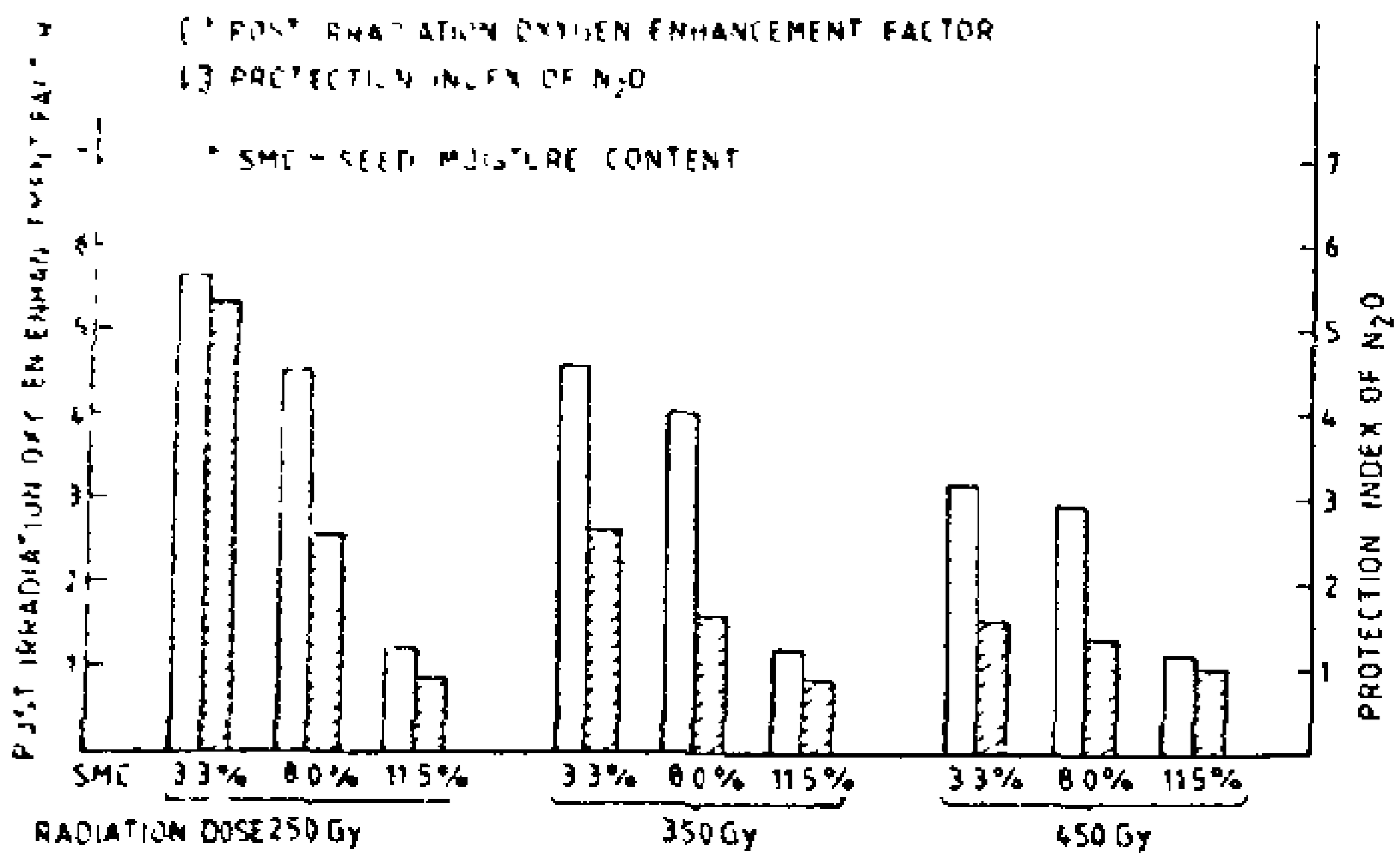

Figure 1. Influence of initial seed moisture content on post-irradiation oxygen enhancement factor and protection index of N_2O.

N_2O diminishes as the seed moisture content increases. It is indeed interesting to note that in barley seed N_2O acts as a radioprotector, or exerts no influence at all just by the circumstances regulated by the seed water content. In the irradiated situations, water profoundly alters the radiation chemical events[20-22] by way of promoting radical–radical recombinations.

So far as the radioprotection of the extremely dry ($\leq 8.0\%$) seeds by N_2O is concerned, Singh and Kesavan[16] *attributed it to the production of some OH˙* by the reaction of N_2O with H [23]. This would restore a favourable equilibrium between H˙ and OH˙ for harmless recombinations ($H^{\cdot} + OH^{\cdot} \longrightarrow H_2O$) to occur.

Several ways by which oxidizing and reducing species might mutually annihilate each other have been suggested[24]. One novel suggestion emanating from these studies is that anoxic radiation damage in dry seeds could possibly be due to excessive reducing events, and N_2O, which can generate a small amount of OH, could effectively balance these out. In support of this contention, it is pointed out (Table 1 and Figure 1) that N_2O exerts no effect whatsoever on seeds of high moisture content ($\sim 11.5\%$) and also metabolizing seeds (data not shown).

It is noted that peroxidase activity (Table 1) exhibits a close parallelism with seedling injury. That reactions involving radiation-induced O_2-sensitive sites (RH˙, H˙, e^-_{aq}) with O_2 would form oxygen reduction products ($RHO_2^{\cdot}$, $HO_2^{\cdot}$, $O_2^{\cdot -}$) is well known. However, an interest in the study of the degradative enzyme peroxidase has arisen from the recent demonstration by Singh and Kesavan[25] that these reduction products of oxygen possibly trigger the gene(s) coding for peroxidase. If so, we have now gained a new insight into the mechanism of induction of degradative enzymes.

1. Keene, J. P., *Radiat. Res.*, 1964, **22**, 1.
2. Powers, E. L. and Cross, M., *Int. J. Radiat. Biol*, 1970, **17**, 501.
3. Ewing, D., Fielden, E. M. and Roberts, P. B., *Radiat. Res.*, 1974, **58**, 481.
4. Kesavan, P. C. and Powers, E. L., Proc. Eighth Int. Congr. of Radiation Research, Edinburgh, Radiat. Res., (ed. Fielden *et al*), 1987, Abstract No. C30–4P, p. 1.
5. Brustad, T. and Wold, E., *Radiat. Res*, 1976a, **66**, 215.
6. Samuni, A. and Czapski, G., *Radiat. Res.*, 1978, **76**, 624.
7. Watanabe, H., Iizuka, H. and Takehisa, M., *Radiat. Res.*, 1981, **88**, 577.
8. Roots, R. *et al.*, *Radiat. Res.*, 1982, **92**, 245.
9. Antoku, S., *Radiat. Res.*, 1977, **71**, 678.
10. Ewing, D., Koval, T. M. and Walton, H. L., *Radiat. Res.*, 1986, **106**, 356.
11. Mullenger, L. and Ormerod, M. D., *Int. J. Radiat. Biol.*, 1969, **15**, 259.
12. Simone, G. and Quintiliani, M., *Int. J. Radiat. Biol.*, 1977, **31**, 1
13. Tilby, M. J., Loverock, P. S. and Fielden, E. M., *Radiat Res.*, 1982, **89**, 488.
14. Watanabe, H, Iizuka, H. and Takehisa, M., *Radiat. Res.*, 1982, **89**, 325.
15. Brustad, T. and Wold, E., in *Modification of Radiosensitivity of Biological Systems*, IAEA, Vienna, 1976b, p. 119.
16. Singh, S. P. and Kesavan, P. C., *J. Radiat. Res.*, 1990, **31**, 162.
17. Kesavan, P. C., Trasi, S. and Ahmad, A., *Int. J. Radiat. Biol.*, 1973, **24**, 581.
18. Kesavan, P. C., Sharma, G. J. and Afzal, S. M. J., *Radiat. Res.*, 1978, **75**, 18.
19. Conger, B. V., Nilan, R A. and Konzak, C. F., *Radiat. Res.*, 1968, **8**, 31.
20. Powers, E. L. and Tallentire, A., *Actions Chimiques et Biologiques der Radiation*, (ed. Haissinsky, M), Masson et Compagnie, Paris, 1968, vol. 12, p 3.
21. Donaldson, E., Nilan, R. A. and Konzak, C. F., *Environ Exp. Bot.*, 1979, **19**, 153.
22. Afzal, S. M. J. and Kesavan, P. C., *Int. J. Radiat. Biol.*, 1979, **36**, 161.
23. Anbar, M., Farhataziz and Ross, A. B, Reports NSRDS–NBS., U.S. Department of Commerce, Washington, D.C., 1975, p 51.
24. Ewing, D, *Radiat. Res.*, 1980, **21**, 288.
25. Singh, S. P. and Kesavan, P. C., *Int. J. Radiat. Biol.*, 1991, **59**, 1227.

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