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Gamma-ray-induced ESR signals in 'dry' and 'wet' seeds

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Data are presented herein on ESR spectral characteristics of 'dry' (~3.2 per cent moisture content) and 'wet' (~12.4 per cent moisture content) irradiated barley seeds. The probable nature of the radiation-induced, oxygen-sensitive species is briefly discussed in the light of the reports in the literature that the 'dry', but *not* the 'wet' seeds develop post-irradiation oxic damage.

IN the physicochemical characterization of radiobiological oxygen effect(s), dormant barley seeds have been most successfully employed. The demonstration that the magnitude of post-irradiation oxygen effect is maximal in seeds of very low moisture content, and that it decreases with increasing seed moisture content to finally disappear in seeds of >11 per cent moisture content[1,2] led to investigations on the stability of radiation-induced, oxygen-sensitive sites (free radicals) in seeds of varying moisture contents[3]. Subsequent studies in our laboratory[4] have elucidated the interrelationship between seed moisture content and post-hydration temperature on the kinetics of reactivity towards oxygen or decay of oxygen-sensitive sites. It is now evident that the radiation-induced O_2-sensitive sites decay rapidly with increasing initial seed moisture content[5].

We have now initiated studies in order to gain an insight into the physicochemical nature of the radiation-induced free radicals in seeds of low and high moisture contents, as it might help in characterizing the radiation-induced O_2-sensitive species. Distinct differences in the ^{60}Co-gamma-ray-induced ESR signals between the 'dry' (moisture content ~3.2%) and 'wet' (moisture content ~12.4%) seeds have been consistently observed, and preliminary data are reported.

The pureline seeds (karyopsis) of *Hordeum vulgare* of hulless strain (1B 65) were used in these experiments. The moisture contents of two sets of seeds were equilibrated to ~3.2% and ~12.4% respectively following an earlier method[6]. For each treatment, four seeds (wt. = 117 ± 2 mg) were put into vials of 5 ml capacity and these were placed in an ice-bath before exposure to 1400 Gy of gamma-rays using ^{60}Co, 204TBq (5500 Ci) gamma-chamber obtained from Bhabha Atomic Research Centre, Bombay, India. The dose-rate as determined by Fe^{2+}/Fe^{3+} dosimetry was 0.78 Gy s^{-1}. One set of the irradiated seeds was immediately subjected to ESR studies and the other after storing in air or vacuum for 48 h. The ESR measurements were carried out using JEOL FE2XG-X band spectrometer operated at 9.445 GHz. Minimum detection limit of the instrument is 1.5×10^{10} spins mm^{-2}. DPPH (1,1 diphenyl-2-picrylhydrazyl) used as reference for measuring g-values ($g = 2.0028$) was obtained from Sigma Chemicals, USA. The ESR spectra were recorded at 20°C. Response time, sweep time and modulation width were appropriately adjusted for optimum signal-to-noise ratio. Baseline corrections and standardization were carried out using unirradiated barley seeds.

The ESR spectra of 'dry' and 'wet' seeds recorded within 10 min of irradiation as also of those stored for 48 h at room temperature (~20°C) in air or in vacuum desiccator are shown in Figure 1 *a*, *b*. It is noted that

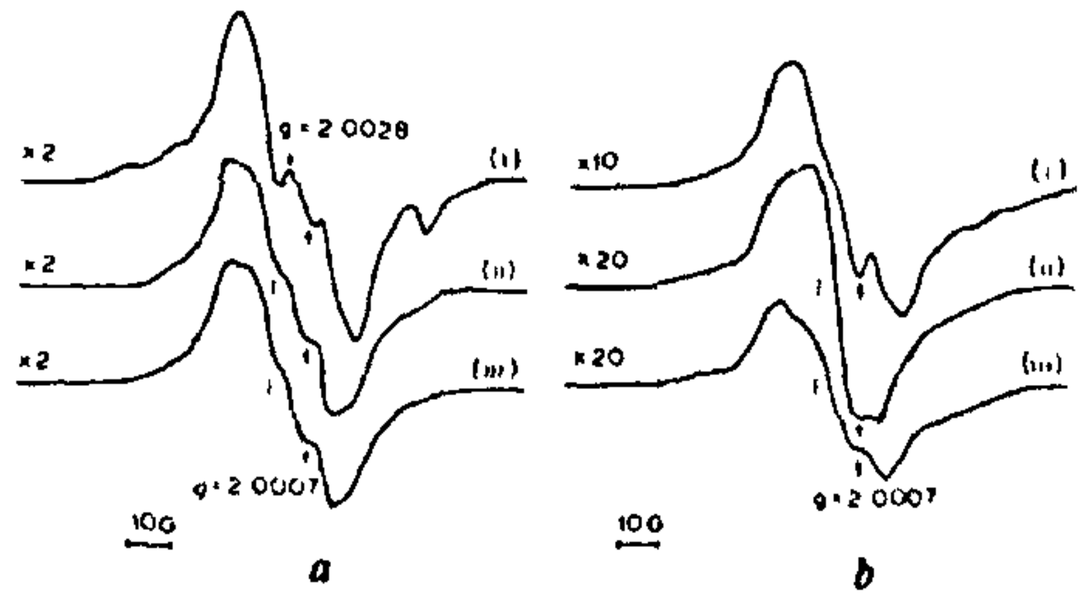

Figure 1. *a*, ESR spectra of 'dry' irradiated seeds. A sharp line I in spectra indicates central magnetic field (3368G). (i) within 10 min in air; (ii) after storage in vacuum for 48 h; (iii) after storage in air for 48 h. *b*, ESR spectra of 'wet' irradiated seeds. A sharp line I in spectra indicates central magnetic field (3368G). (i) within 10 min in air; (ii) after storage in vacuum for 48 h; (iii) after storage in air for 48 h.

ESR spectrum of dry seeds recorded within 10 min of irradiation consists of a doublet with g-value of 2.0028 which rapidly assumes the shape of a shoulder. Two tail components with g-values of 2.0216 and 1.9868 are also the distinct features of this spectrum. On the other hand, the ESR spectra of the 'wet' seeds recorded under the same experimental conditions do not show the g-values corresponding to the doublet and the tail components as observed for 'dry' seeds.

Storage at ~20°C of the 'dry' irradiated seeds either in vacuum or in air for 48 h results in the disappearance of the doublet ($g=2.0028$) and tail components ($g=2.0216$; $g=1.9868$). A discernible shift in the g-values of principal resonance from 2.0087 to 2.0099, also occurs. In the case of 'wet' irradiated seeds, the presence or absence of air during storage greatly influences the spectral features. The details of the g-values corresponding to various treatment schedules are given in Table 1.

Figure 2 (inset) shows that within 5–10 min of irradiation, the 'wet' seeds contain only ~20 per cent of the magnitude of ESR signals found in 'dry' seeds. Further, the rate of decay of signals in the irradiated 'dry' seeds is *not* appreciably influenced by the presence or absence of air during storage, whereas in 'wet' seeds, the decay of the signal is ~6-fold greater in air than in vacuum. The mechanistic aspects for the occurrence of *large magnitude of post-irradiation oxygen effect in 'dry' seeds*, and the lack of the same in 'wet' seeds[2–4,7,8] seem now explicable in terms of yield, stability and g-value components of the radiation-induced ESR signals (Figures 1, 2 and Table 1).

It seems reasonable to view that the g-value components observed in the 'dry' but *not* in the 'wet' irradiated seeds are possibly the oxygen-reactive free radicals. The g-value component of 2.0028 (refs. 9–11) corresponds to carbon-centred radicals and is observed only in 'dry' seeds subjected to ESR analysis within 10 min of irradiation. It is noteworthy that it is not found after 48 h of storage. The g-value components of 2.0099–2.0096, 2.0007, 1.9977–1.9971 possibly correspond to sulphur-centred radicals. The microwave power saturation characteristics of these radicals are in accordance with these assignments[12]. Complexity of the system does not permit an exact determination of hyperfine splitting constants for the multiplet spectra of irradiated barley seeds.

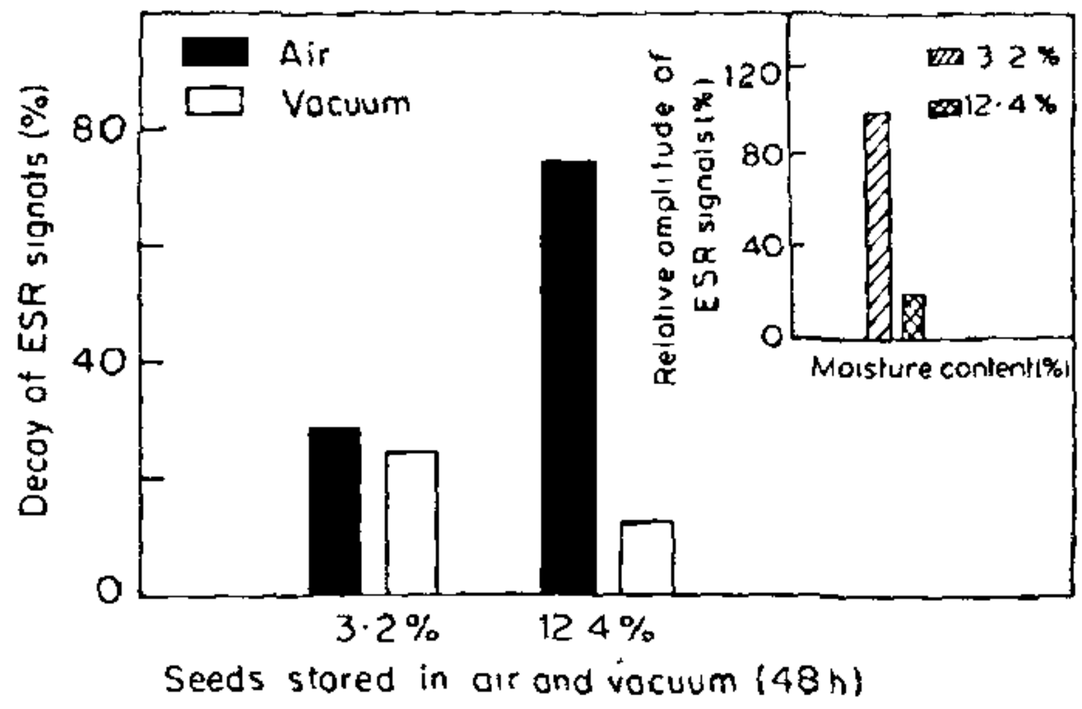

Figure 2. Percentage decay of ESR signals in 'dry' (3.2%) and 'wet' (12.4%) seeds following storage in air or in vacuum for 48 h after irradiation. Inset, Relative amplitude of signals immediately (5 min) after irradiation in 'dry' and 'wet' seeds.

In considering the mechanism of post-irradiation oxygen-dependent damage in 'dry' seeds, the present study reveals a role for carbon- and sulphur-centred radicals. And earlier studies have shown that carbon-centred radicals react with oxygen much faster than the sulphur-centred radicals by one to two orders of magnitude[13]. Considering the presence of hordeins in endosperm, albumins and glutelins in embryos and sulphur-containing enzymes in the aleurone layers[14], predominance of the radiation-induced sulphur- and carbon-centred radicals is readily understandable.

The influence of storage on ESR signals is most striking in 'wet' seeds (Table 1). With appropriate experiments, it should be possible to correlate the changes in the g-value components of the irradiated seeds with enhancement or diminution of oxygen-dependent and oxygen-independent components of radiobiological damage.

Table 1. 'g'*-value components of ESR spectra of 'dry' (3.2% moisture) and 'wet' (12.4% moisture) barley seeds irradiated at 1400 Gy

Duration/ type of storage	g-value components in seeds with moisture content of	
	3.2%	12.4%
5–10 min (Air)	2.0216, 2.0153, 2.0087, 2.0028, 2.0007, 1.9957, 1.9868	2.0129, 2.0096, 2.0063, 2.0007, 1.9974, 1.9957, 1.9945
48 h (Vacuum)	2.0129, 2.0099, 2.0081, 2.0007, 1.9971, 1.9957, 1.9909	2.0162, 2.0096, 2.0051, 2.0007, 1.9977, 1.9909
48 h (Air)	2.0129, 2.0099, 2.0081, 2.0007, 1.9971, 1.9957, 1.9909	2.0096, 2.0051, 2.0007, 1.9962

*g-value is taken as a measure of various peak positions in corresponding ESR spectrum.

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Effect of crop residue management on microbial biomass accumulation in the soil

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The present study was undertaken to evaluate the influence of applications of straw and chemical fertilizer on the soil microbial biomass under reduced tillage, dryland conditions. Straw incorporation in combination with chemical fertilizer resulted in the greatest microbial biomass level, which is the source for nutrients in the soil.

For developing countries, residue management is especially important because the amount of nutrients in crop residue is several times higher than the quantities of these nutrients applied as high cost fertilizers. In the present study, four treatments with three replicates each were established in randomized block design using a plot size of 5 m × 4.2 m in the dryland experimental farm of the Institute of Agricultural Sciences, Banaras Hindu University (25° 18′ N lat. and 80° 1′ E long., 76 m above msl) using a rice-lentil crop rotation. The treatments were: (a) control; (b) chemical fertilizer–NPK (80 kg N ha^{-1}, 40 kg P ha^{-1}, 30 kg K ha^{-1}; for N urea, for P single super phosphate, and for K muriate of potash); (c) wheat straw (C = 37.8%, N = 0.48%, P = 0.09%) – 2 kg m^{-2} (the amount of N was equivalent to that under treatment b); (d) wheat straw (1 kg m^{-2}) + fertilizer (50% of b). Applications of inputs to plots were made on 24 June 1990 and again on 30 June 1991. Straw was lightly incorporated into soil while chemical fertilizer was applied on the soil surface. Rice (*Oryza sativa* var. Akashi) was sown on 17 July 1991 and harvested on 26 October. Lentil (*Lens esculenta* var. Pant 209) was sown on 20 November 1991 and harvested on 17 March 1992.

Two soil samples were randomly collected to a depth of 10 cm from each plot, sieved through a 2 mm mesh screen, and mixed together into composite samples. Microbial biomass in field-moist samples was determined by chloroform fumigation extraction method[1]. All the results are expressed on oven dry soil (105° C, 24 h) basis. Statistical analysis was done by using SPSS/PC statistical software on an IBM compatible microcomputer[2]. Significant differences between means were examined by using ANOVA and LSD range test.

Microbial biomass C was maximum in the straw + fertilizer treatment (408–420 $\mu g\ g^{-1}$) followed by straw (360–392 $\mu g\ g^{-1}$) and fertilizer treatments (272–357 $\mu g\ g^{-1}$). The values for control were minimum (238-246 $\mu g\ g^{-1}$). With time straw+fertilizer treatment accumulated more microbial biomass C in the soil (77% over control), followed by straw treatment (51% over control) (Figure 1). The effect of treatments was significant at $P < 0.01$. However the effect of fertilizer was transient in nature with the microbial C value at the end of the experiment not being statistically different from the control (Figure 1).

Microbial biomass was maximum in straw + fertilizer treatment because limitations due to nutrients as well as carbon were overcome by combined exogenous application of chemical fertilizer and plant residue.

A very large and rapid increase is reported in the size of the microbial biomass following straw incorporation[3]. The

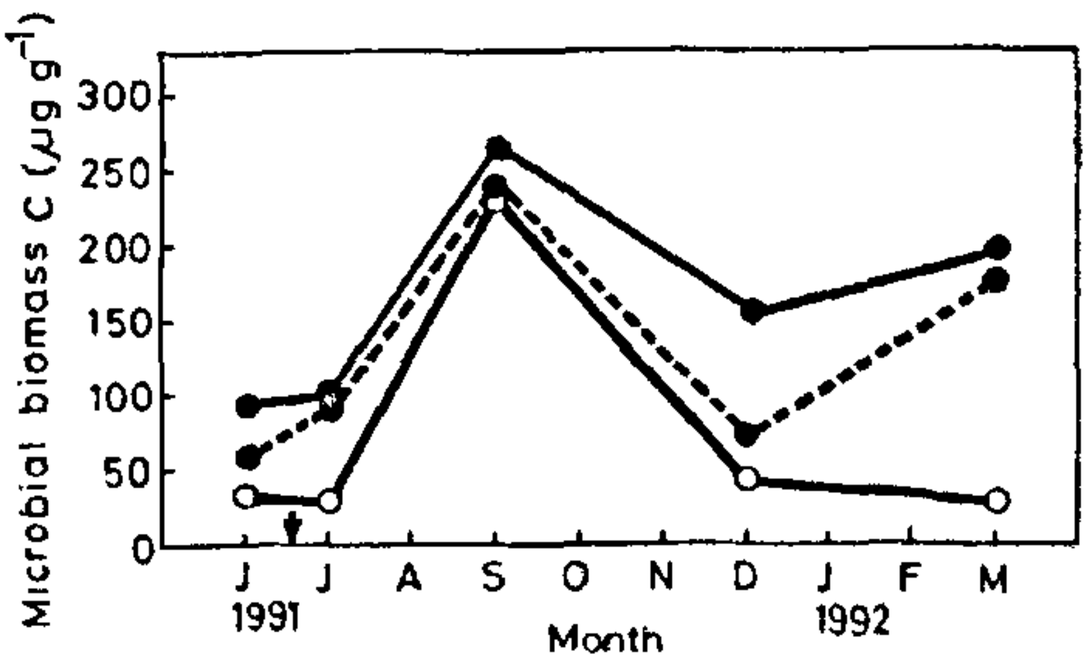

Figure 1. Effect of applications of straw and fertilizer, singly and in combinations (arrow indicates time of second application), on microbial biomass C ($\mu g\ g^{-1}$). The values for control have been subtracted from the treatment values. Solid circles connected by solid line are for straw + fertilizer; solid circles connected by broken line are for straw and open circles connected by solid line are for fertilizer.