Scavenging of Free Radicals and Radiation Protection by Nitric Oxide in Plant Seeds

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Under anoxic conditions nitric oxide (NO) is noteworthily untoxic to living tissue. In an exploratory study (unpublished) a treatment of resting barley seeds with NO at atmospheric pressure for several days was found neither to induce growth inhibition nor to raise the mutation rate significantly. Being a simple, fairly stable and easily diffusing free radical, *i. e.*, with possible ability to combine quantatively with free radicals in the tissue, NO is of potential value in radiobiological experiments. NO reacts in a 1:1 ratio with reactive free radicals [1]. This fact makes its modifying influence on radiation sensitivity more suited to interpretation on a quantitative basis than the corresponding [2-5] effects of the diradical oxygen, which might start chain reactions [1] and, in addition, affects the radiation sensitivity *via* its metabolic action [6].

In the present investigation the reactions of NO with free radicals was used to shed light on the mechanism by which water exerts an action on the radiation sensitivity of plant seeds. This effect consists of a decrease of the radiation sensitivity with increasing water content with a minimum at some value between 12 and 20 % water, dependent on the system studied (*cf.* Ehrenberg and Ehrenberg [7] and quoted literature).

In the latter publication measurements of electron spin resonance (esr) demonstrated that the same amount of free radicals is produced initially, irrespective of the water content, when seeds of *Agrostis stolonifera* are exposed to X-rays. In very dry seeds the radicals remain unchanged but, as the water content increases, an increasing proportion of the radicals decays in a second order reaction. The fact that the growth inhibition induced at the different water contents is found to be proportional to the (semiconstant) level of radical concentration after the decay, indicates

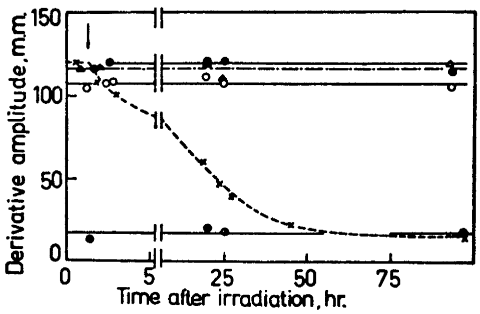

Fig. 1. Time dependence of derivative amplitude of esr absorption in dry seeds (4.5 % H_2O) of *Agrostis stolonifera*, X-irradiated and stored in different atmospheres. ◑ unirradiated seeds stored in N_2; ● irradiated in air, stored in air; O irradiated in N_2, stored in air; △ irradiated in N_2, stored in N_2; × irradiated in N_2, stored in NO. The arrow indicates the moment when the gas was changed over the three latter samples.

that the protective action of water is due to an increasing recombination of free radicals.

In the present studies we determined the rate of entrance into the seeds of NO and/or its rate of reaction with radiation produced paramagnetic centers. Dry seeds (4.5 % H_2O) were irradiated with 150 000 r of X-rays (185 kV, 2 mm Al filtration, 800 r min^{-1}) in N_2. After determination of the esr absorption the N_2 filling of the glass tube was replaced by NO, and the decay of the signal was followed for a few days. Total decay down to the control value [8] required about 48 h at 20°C (Fig. 1), at higher water contents the reaction was more rapid. The fact that the signal height was equal to the (stable) N_2 value when N_2 was replaced by air after irradiation or when irradiation was performed in the presence of air, indicates that free oxygen is not present in the tissue [7] or that the radicals are changed into an equal amount of peroxy radicals.

In a second experiment seeds were irradiated at different water contents, and the influence of the gas (N_2, NO, or air) present during irradiation was studied by determination of the dose causing 50 % reduction of the seedling growth (measurement at 15 mm control height). In order to secure penetration of NO into the tissue

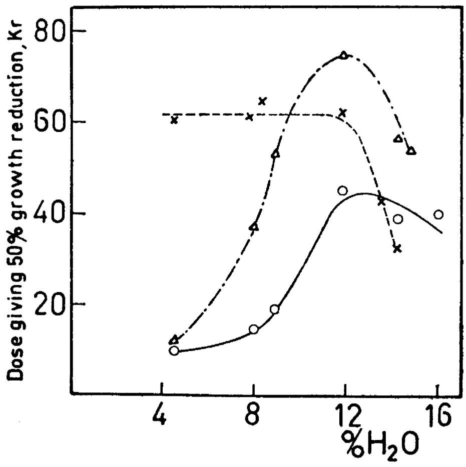

Fig. 2. The dose of X-rays required for 50 % growth inhibition of seeds of *Agrostis stolonifera* as a function of their water content. The seeds were irradiated and stored (see text) in air ○, in N_2 △, and in NO ×.

and a quantitative reaction with the radicals, the seeds were kept in the different gases for 48 h, and irradiation was performed in the middle of that period.

In Fig. 2 the radiation sensitivities are shown. In N_2 and air a clear minimum sensitivity (*i. e.*, maximum of 50 % dose) is registered at 12 % H_2O. In the presence of NO, however, about maximal protection was found at all water contents below 12 %, and under these conditions esr signals close to the control values were invariously found.

This influence of the radical scavenger, NO, connects directly the radiation produced free radicals with a large part of the biological effect. Within this range of water content the destruction of radicals leads to a protection, either this occurs by a recombination (which is facilitated by the presence of hydration water) or by reaction with a scavenger. The sensitivity increasing effect of oxygen does not necessarily depend on immediate reactions with radicals, since the air was not removed by evacuation before sowing. Thus the seeds irradiated in air had a higher oxygen content than the N_2 seeds, at the start of germination. Under similar conditions after-effects of O_2 have been observed [9].

At water contents above the minimum, where the radiosensitivity is again higher, NO increases the radiosensitivity in a manner similar to that of O_2, and in agreement with results of other investigators [2-5]. When the H_2O content exceeds 12 %, more loosely bound H_2O becomes present, permitting new types of reactions. Below 12 % H_2O, the reaction constant for radical recombination is independent of the water content [7], indicating that H_2O is exclusively present as a single, more or less complete layer of hydration water. Above 12 % H_2O, the reaction constant increases with increasing water content.

These preliminary investigations are being continued on a quantitative basis.

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