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AN ELECTRON SPIN RESONANCE INVESTIGATION OF INTERNAL RUST SPOT, A PHYSIOLOGICAL DISORDER OF THE POTATO TUBER

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ESR spectroscopy has been used to investigate the formation of paramagnetic species during the development of internal rust spot (IRS) in the potato tuber. Production of free radicals and oxidation of metal ions such as Fe(II) and Mn(II) occur when necrotic tissue is formed. However, since IRS develops in periods of calcium stress (low calcium supply), it is suggested that the principal cause of the disorder is a loss of cell membrane integrity which is brought about by a lack of calcium. Cell senescence and the formation of necrotic tissue may then result either from increased oxygen radical production within the cell or from oxidation of metal complexes in the extracellular regions of the tissue.

KEY WORDS: Potato, internal rust spot, ESR, radicals, Fe, Mn.

INTRODUCTION

Internal rust spot (IRS) is a physiological disorder of the potato plant in which externally healthy-looking tubers exhibit discrete rust-coloured lesions in the medullary tissue of the flesh. Development of IRS has been observed during the bulking phase of tuber growth, although there is also evidence that lesions may appear for the first time or worsen after harvesting.[1] The primary events leading to cell death are not known, but there is circumstantial evidence from nutrient culture experiments which implicates inadequate calcium supply to the growing tubers.[2] Conditions of low calcium supply may occur in the field in hot, dry weather, when transpirational demand diverts the water transporting this nutrient away from growing tubers into the leaves. Calcium is a macronutrient that has been shown to exhibit several important physiological functions, including a role in membrane integrity.[3] Disruption of cellular organisation by a loss of membrane integrity, brought about by a lack of calcium, would allow uncontrolled chemical reactions to occur. These would include deleterious oxidative reactions, arising from cellular metabolic processes. Since it is likely that such reactions, culminating in cell death, would proceed via free radical processes, investigations have been commenced using electron spin resonance (ESR) spectroscopy.

In this initial investigation the rust-coloured necrotic lesions, which represent the visible symptom of the disorder known as IRS, were examined. The potato genotype

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chosen for the work was the SCRI clone 10337 de 40, which has a marked susceptibility to IRS, both in the field and under controlled nutrient conditions.[4]

EXPERIMENTAL

The SCRI clone 10337 de 40 was grown in an unheated greenhouse in pots containing vermiculite. Plants were watered twice weekly with one litre of the following nutrient solution: KNO_3 (4.0 mol m^{-3}), $NaNO_3$ (1.5 mol m^{-3}), $NaH_2PO_4.2H_2O$ (1.3 mol m^{-3}), $MgSO_4.7H_2O$ (0.75 mol m^{-3}), $CaCl_2$ (20 mol m^{-3}), Fe-EDTA (0.02 mol m^{-3}), and other essential trace elements (after Collier *et al.*[2]). A low calcium regime, full nutrient solution but with only 1.0 mol m^{-3} $CaCl_2$, was implemented when the plants were ten weeks old and the developing tubers were entering the main bulking phase. Tubers were harvested at the end of the season and stored for three months, prior to analysis.

ESR spectra were recorded as first derivatives on a Varian E 104 A X-band spectrometer. Samples of medullary tissue[5] at ambient temperature were mounted as thin sections within a quartz tissue cell and those at 77 K were prepared as cubes of tissue with dimensions of approximately 0.25 cm^3 and immersed in a flask of liquid nitrogen. Spectral conditions were as shown in the captions to Figures 2 and 3.

RESULTS AND DISCUSSION

Figure 1 shows a typical manifestation of IRS with plentiful necrotic lesions across the potato tuber. ESR spectra of samples of healthy and necrotic tissue from six

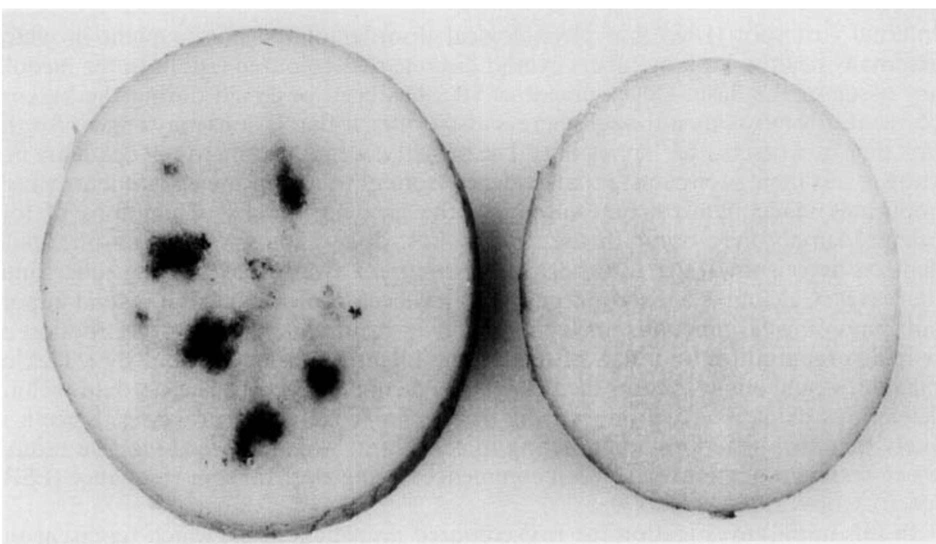

FIGURE 1 Transverse section of a potato tuber from clone 10337 de 40 grown under a low calcium nutrient regime, showing internal necrotic lesions which constitute the physiological disorder Internal Rust Spot.

different potato tubers were recorded at room temperature. Similar spectra were obtained for each of the samples in either group and representative spectra are shown in Figure 2. For the healthy tissue, the spectrum (Figure 2a) consists of six peaks with a hyperfine splitting of 9.3 ± 0.1 mT and a g-value of 1.997 ± 0.002. This almost certainly corresponds to the solvated Mn(II) ion, which is often seen in plant tissue (B.A. Goodman and D.B. McPhail, unpublished observations). With the necrotic tissue the spectrum (Figure 2b) also contains this Mn(II) signal, but in addition there is a single peak feature with a g-value of 2.0043 ± 0.0005. This g-value is consistent with the presence of an O-centred organic free radical species[6] and is similar to that seen in biological polymers such as melanin[7] and humic acid.[8]

Representative spectra from samples at 77 K are shown in Figure 3. Here the spectrum of the healthy tissue (Figure 3a) consists of three distinct features. In addition to peaks from an immobilised Mn(II) ion, there is a broad feature centred

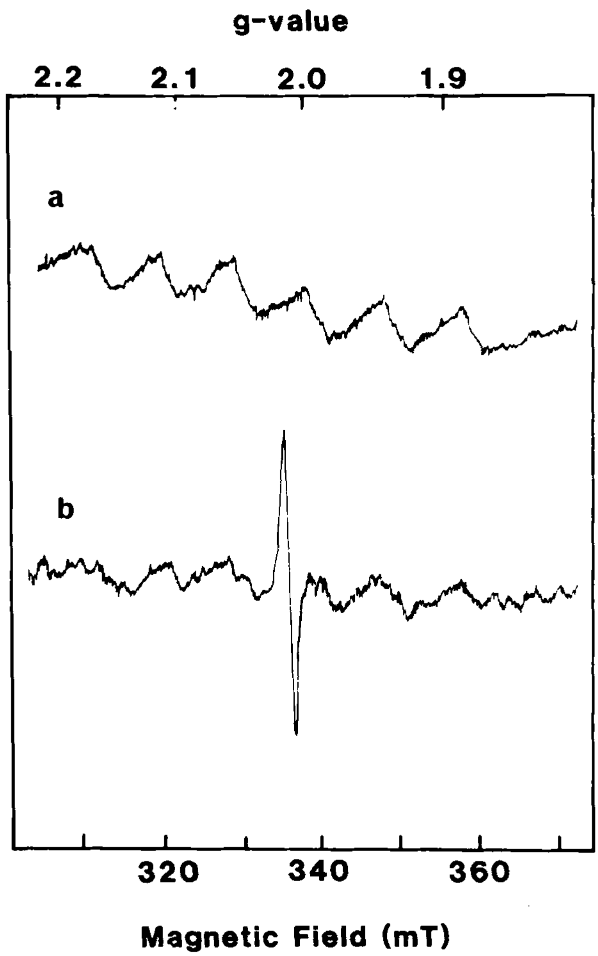

FIGURE 2 ESR spectra at ambient temperature of (a) healthy and (b) necrotic tissue from the potato clone 10337 de 40. Both spectra were recorded at approximately 9.5 GHz with 10 mW microwave power, 100 KHz modulation frequency and 1 mT modulation amplitude.

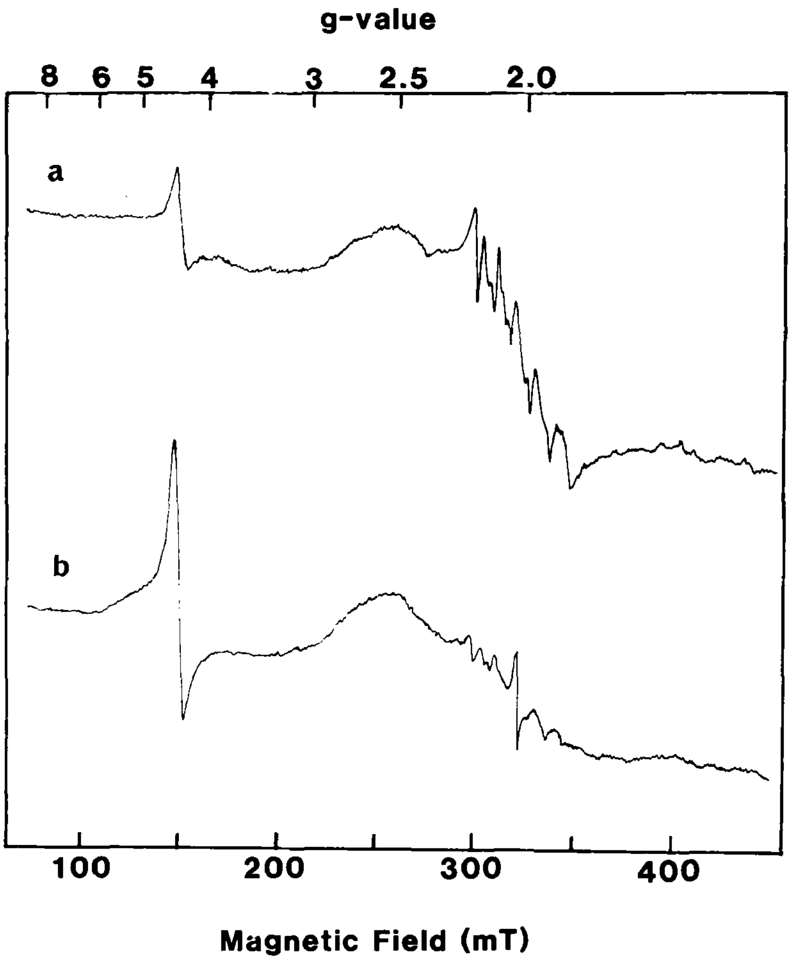

FIGURE 3 ESR spectra at 77 K of (a) healthy and (b) necrotic tissue from the potato clone 10337 de 40. Both spectra were recorded at approximately 9.19 GHz with 2 mW microwave power, 100 Hz modulation frequency and 1 mT modulation amplitude.

on g = 2.0 and a weak feature with g = 4.3. Both of these components probably originate from Fe(III), the former being typical of species in which extensive magnetic exchange interactions occur[9,10] and the latter being characteristic of many mononuclear high spin Fe(III) chelates.[10-12] The spectrum of the necrotic tissue (Figure 3b) shows several differences from that of the healthy tissue. The intensity of the Mn(II) signal is decreased relative to that of the broad Fe(III) signal with g = 2.0 and there is an appearance of a single peak free radical signal with g = 2.004 ± 0.001 consistent with the observations with the room temperature spectra. However, by far the most dramatic change is seen in the intensity of the Fe(III) feature with g = 4.3, where a major increase in intensity is seen with the necrotic tissue.

Thus, the ESR results show that the formation of necrotic tissue is accompanied by (i) the production of a free radical signal, (ii) an increase in intensity of Fe(III) features and (iii) a probable decrease in intensity of Mn(II). The changes in metal signals can be explained by the occurrence of oxidation reactions, with Fe(II) in healthy tissue being oxidized to Fe(III) and Mn(II) to either Mn(III) or Mn(IV). Fe(II) and Mn(III) would not be expected to be seen in ESR spectra under the conditions of the present experiment, and in fact are rarely seen at temperatures greater than that of liquid helium[13] whereas any Mn(IV) would be expected to disproportionate or precipitate (see Baes and Mesmer,[14]) and possibly contribute to the intensity of the broad $g = 2$ features. Although it is difficult to obtain quantitative information from ESR spectra, it is quite clear that the number of free radical centres formed in the necrotic tissue is very much smaller than the number of Fe(III) ions produced, especially when one considers that much of the Fe(III) signal intensity in rigid limit spectra is lost because of the high degree of anisotropy of the major resonance transitions.[11] Therefore the formation of internal rust spot appears to be primarily the result of metal ion oxidation (largely Fe(II) to Fe(III)), and is accompanied by some free radical production. However, the possibility of a more significant role for organic free radicals cannot be discounted because of the likely mutual annihilation of free radicals during polymerization reactions.

The major question raised by these results is whether the changes observed in the ESR spectra are able to provide new information on the cause of the internal rust spot disorder, or are simply the consequence of cell senescence produced by the disorder. Formation of necrotic tissue has been reported to occur during periods of rapid cell growth when the supply of calcium to tuber tissue may become limiting.[1] It has also been shown by spin label ESR studies that calcium has a significant effect on membrane fluidity.[15,16] It is possible, therefore, that the onset of IRS has its primary cause associated with the integrity of the plasma membrane and the endomembrane system. Loss of integrity would predispose the cell to metabolic disturbance, upsetting the balance of oxygen free radical production and quenching by endogenous antioxidants. This could lead to the oxidation of metal and organic components with the cell, which would result in rapid cell death. Such a sequence of events could explain why individual cells are often affected, while neighbouring cells remain in a healthy state.

The occurrence of uncontrolled oxidative reactions is exacerbated by the presence of iron, of which there are significant amounts in the potato tuber.[17] Low molecular weight iron complexes with the ability to catalyse the formation of $OH\cdot$ from O_2^- and H_2O_2 are thought to exist within the cell.[18] Normally this reaction is limited by the presence of endogenous antioxidants, such as ascorbate or α-tocopherol and the enzymes superoxide dismutase, catalase and peroxidases. These compounds are localised within various cellular compartments and control the intracellular levels of O_2^- and H_2O_2. If membrane integrity is lost, the increased radical production occurring as a result of reactions with low molecular weight iron complexes could prove lethal. Production of $OH\cdot$ from O_2^- and H_2O_2 results in redox cycling between the Fe(II) and Fe(III) oxidation states and consequently may explain the large increase in Fe(III) in the necrotic tissue. Differences in susceptibility of different potato genotypes to IRS[19] could be explained by differences in membrane composition which produces different effects on fluidity in calcium stress situations. In future work it is planned to investigate whether such differences exist between susceptible and resistant cultivars by using the spin-label ESR method.

CONCLUSIONS

ESR spectra have shown that the internal rust spot metabolic disorder of the potato tuber is accompanied by the formation of free radicals and major changes in the oxidation states of metal ions. However, since the disorder is associated with calcium stress situations which may have an adverse effect on membrane fluidity, it is suggested that the primary cause of the disorder is a loss of membrane integrity.

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