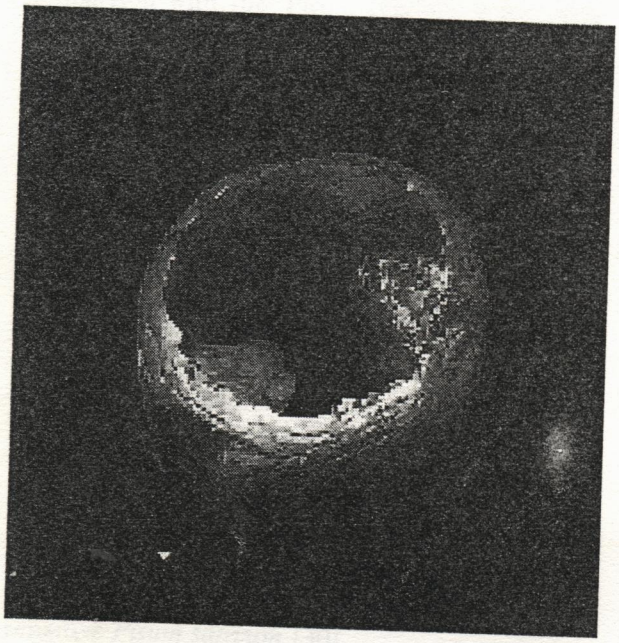
Figure 2

Reconstruction of the external surface of the healthy tissue in a tuber cv. Pentland Crown infected with *P. infestans* after 33 days' incubation

Removal of the healthy tissue ends creates an apparent hollow 'cylinder' with an ingress of diseased tissue.



large increase in the intensity of the Fe(III) signal along with the appearance of a single peak resonance with $g = 2.005$, which probably corresponds to a stable free radical produced as a result of tissue damage caused by the pathogen. The other signals all showed variations in intensities that could not be correlated with either the pathogen or cultivar, suggesting that there may be considerable local variations in tissue response to the infection processes.

NMR microimaging is able to give (near) microscopic information for intact potato tubers, image contrast differences between different tissue types being determined by a combination of water content and physical properties. Tissue infected by *P. infestans, P. foveata* or *F. sulphureum* could be discriminated by characteristic changes in NMR images, but the complexity of the processes responsible for determination of image contrast is too high for detailed interpretation at the present time, although useful information is obtained from EPR measurements. In the case of infection with *P. infestans* it was possible to follow the dynamics of disease development through the use of three-dimensional data sets combined with surface-rendered image reconstruction techniques.

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1 Brenchley, G. H. and Wilcox, H. J. (1979) Potato Diseases, HMSO, London
2 Goodman, B. A. (1994) Proc. Roy. Soc. Edin. **102B**, 479–493
3 Sutherland, M. W. (1991) Physiol. Mol. Plant Pathol. **39**, 79–93
4 Tzeng, D. D. and DeVat, J. E. (1993) Adv. Plant Pathol. **10**, 1–34
5 Wehrli, F. W., Shaw, D. and Kneeland, J. B. (eds.) (1988) Biomedical Magnetic Resonance Imaging: Principles, Methodology and Applications, VCH Publishers, New York
6 MacFall, J. S. and Johnson, G. A. (1994) Can. J. Botany **72**, 1561–1573
7 Goodman, B. A., Williamson, B. and Chudek, J. A. (1992) Protoplasma **166**, 107–109
8 Goodman, B. A., Williamson, B., Simpson, E. J., Chudek, J. A., Hunter, G. and Prior, D. A. M. (1996) Magn. Resonan. Imaging **14**, in the press
9 Griffith, J. S. (1964) Mol. Phys. **8**, 213–216
10 Monk, L. S., McPhail, D. B., Goodman, B. A. and Davies, H. V. (1989) Free Rad. Res. Commun. **5**, 345–350
11 Deighton, N., Johnston, D. J., Glidewell, S. M., Lyon, G. D. and Goodman, B. A. (1992) Protoplasma **171**, 167–169
12 Callaghan, P. T. (1991) Principles of Nuclear Magnetic Resonance Microscopy, Clarendon Press, Oxford
13 Labuza, T. P., Reineccius, G. A., Monnier, V., O'Brien, J. and Baynes, J. (eds.) (1994) Maillard Reactions in Chemistry, Food, and Health, Royal Society of Chemistry, Cambridge

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Superoxide generation in relation to dehydration and rehydration

F. Navari-Izzo*, M. F. Quartacci and C. M. L. Sgherri
Istituto di Chimica Agraria, Università di Pisa, I-56124 Pisa, Italy

General mechanism of superoxide anion production

Atmospheric oxygen ($^3O_2$) is relatively unreactive because it is a diradical with parallel spin state; thus its divalent reduction is kinetically limited by the relatively slow spin inversion process. The spin conservation rule states that spin must be conserved during the time for a chemical reaction to occur. This spin restriction means that when $^3O_2$ is involved in metabolic oxidation it has to be activated, allowing for spin inversion of one electron at a time, in order to have productive collision. This univalent pathway requires the generation of intermediates [1], among which superoxide ($O_2^{-\bullet}$) is the first reduced product.

The production of $O_2^{-\bullet}$ may be achieved by: (a) photodynamic reactions type I in which a non-reactive oxygen molecule in the triple ground state ($^3O_2$) is reduced by excited pigments that have undergone charge separation [2]; (b) univalent reduction of oxygen in the presence of a reductant with sufficiently negative electro-chemical potentials to donate electrons to oxygen (E_0 $O_2/O_2^{-\bullet} = -0.33$ V); or (c) univalent oxidation of hydrogen peroxide.

Generation of superoxide in plant cells

Oxygen activation may potentially occur in all compartments of plant cells, as recently reviewed by Elstner [3]. Two separate sites of $O_2^{-\bullet}$ generation have been identified in mitochondria. The first site is the flavoprotein region of the NADPH dehydrogenase segment of the respiratory chain and is insensitive to KCN and antimycin A. The second site is close to complexes I and III at the ubiquinone level [4,5], and when cyanide or antimycin A blocks electron transport beyond ubiquinone the rate of superoxide formation increases [6,7]. The electron transport system of microsomes can form superoxide at the expense of NADPH via cytochrome *P*-450 or cytochrome *P*-450 reductase [7]. Moreover, microsomal $O_2^{-\bullet}$ generation in soybean seedlings has been observed in the presence of either NADPH or NADH as cofactor [8]. It has been suggested that the superoxide found in microsomes of carnation senescent flowers is generated enzymatically, presumably by a membrane-associated oxidase [9]. NADH-dependent superoxide formation has also been shown in peroxisomal and glyoxysomal membranes [10,11]. In the chloroplasts, from a thermodynamic viewpoint, O_2 can accept electrons from both photosystems, forming superoxide [12]. This is discussed later.

Little is known about the enzymes responsible for the reduction of O_2 to form $O_2^{-\bullet}$. It has been suggested that cell wall peroxidases produce $O_2^{-\bullet}$ by means of apoplastic NADH, NAD radical and the NAD^+ pathway [3,13], and a plasma membrane peroxidase has been shown to be responsible for the formation of $O_2^{-\bullet}$ at the surface of plasmalemma [14,15]. More recently it has been demonstrated that the enzyme responsible for the synthesis of $O_2^{-\bullet}$ by elicitor-treated plant cells is similar to mammalian neutrophil NADPH oxidase [16], and that one function of the flavins and *b*-type cytochromes may be to produce $O_2^{-\bullet}$ in plant plasma membranes [17].

A common feature of different stresses, when the metabolism involving electron transfer is perturbed, is their potential to increase the production of superoxide species [12,18–23]. Evidence for increased generation of activated oxygen determining superoxide as the first product of radical-mediated reactions, is scanty. Such evidence comes mainly from measurements of the specific activities of enzymes, such as superoxide dismutase, peroxidases and Asada–Halliwell pathway recycling enzymes, as well as from changes in the concentrations of antioxidant molecules [18,20–23].

Measurements of $O_2^{-\bullet}$ in biological systems are technically difficult. Generally, the methods used to measure $O_2^{-\bullet}$ lack specificity and/or sensitivity, making it difficult to determine the rate of $O_2^{-\bullet}$ production accurately, as the radicals are very unstable and terminate quickly by disproportionation or other mechanisms [24]. However, 1,2-dihydroxybenzene-3,5-disulphonic acid (Tiron), a disulphonated catechol that is readily oxidized to the corresponding semiquinone (a more stable free radical) by $O_2^{-\bullet}$, can be used to detect the superoxide as soon as its production has been induced by illumination of isolated thylakoids or other biological systems [25–27]. The generation of the signal of the Tiron radical

*To whom correspondence should be addressed.

depends on oxygen and is obscured by scavengers of $O_2^{-\bullet}$, such as ascorbate, L-adrenaline or reduced glutathione, which shows that the Tiron radical is derived from $O_2^{-\bullet}$ [27]. Added superoxide dismutase is less effective in obscuring the signal, because the larger size of the enzyme, in comparison with the Tiron, renders it less able to permeate the site of $O_2^{-\bullet}$ production.

Attempts have been made to compare directly the formation of superoxide from stressed and unstressed plants, and these investigations provide some evidence for an increased rate of formation of $O_2^{-\bullet}$ in stressed plants. Isolated thylakoids from drought-stressed wheat show increased superoxide formation [19], and superoxide formation in thylakoids from wheat and sunflower seedlings subjected to increasing stress by water deficit is higher than in the controls [27,28]. Leprince and co-workers [4] found an increase in superoxide in wheat mitochondria after desiccation. A two- to five-fold increase in superoxide activity has been observed in microsomes isolated from lethally frozen crown tissue [29], and the NADH-dependent generation of $O_2^{-\bullet}$ is increased in the mitochondria of salt-sensitive *Pisum sativum* [30].

Generation of superoxide in chloroplasts

Chloroplasts contain photosensitizing pigments and are the most aerobic compartments, as they both produce and consume oxygen. As the chloroplast electron transport chain operates in a high O_2 environment, its propensity to leak electrons to oxygen is correspondently greater. Molecular O_2 possesses the physicochemical properties that permit this molecule to serve as an alternative Hill oxidant within chloroplasts *in vivo*. The solubility of O_2 in the aprotic interior of the membrane is higher than it is in water [31], and $O_2^{-\bullet}$ production is not limited by O_2 availability [32]. In the interior of chloroplasts the O_2 concentration has been estimated to range from 275 to 300 mM, and even at moderate light intensities (350 $\mu E \cdot m^{-2} \cdot s^{-1}$) the rate of superoxide formation may be as high as 15 mmol·(mg of chlorophyll)$^{-1} \cdot h^{-1}$ [33].

The production of $O_2^{-\bullet}$ by thylakoids has been recently reviewed [33,34]. Briefly, thermodynamically feasible sites for superoxide production in the chloroplasts can be either photosystem I (PSI) and/or photosystem II (PSII) [33,35,36]. Two mechanisms are associated with $O_2^{-\bullet}$ generation on the reducing side of the PSI. Most $O_2^{-\bullet}$ production proceeds via the univalent reduction of O_2 by reduced ferredoxin, and an alternative source is from the non-haem Fe-S centre [12]. At the PSII acceptor side, superoxide may be formed as the result of passing electrons from pheophytin to plastoquinone (Q_A) to O_2 [37].

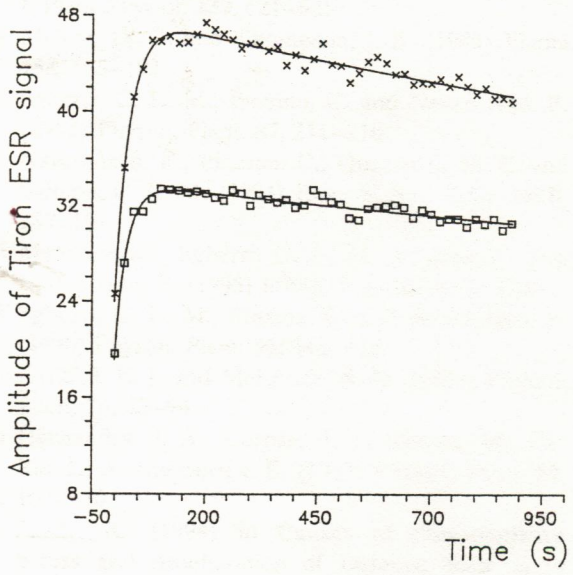
Generation of superoxide by thylakoids during dehydration and rehydration

When normal pathways and acceptors of photosynthetic electron transport are restricted under water stress conditions, photodynamic reaction type I may be intensified [2]. The reaction, initiated by light, is mediated by the photosynthetic electron transport chain [25,38–40]. The impairment by drought of photosynthesis without significantly decreasing the flow of electrons through the photosystems, and the transient disruption of the photosystems during dehydration, making them 'leaky', both result in the transfer of electrons to molecular oxygen, thus forming $O_2^{-\bullet}$ (Figure 1). Different factors seem to be involved in the electron leakage, such as electron transport rate, composition of membrane lipids and a greater exposure of chlorophyll molecules to

Figure 1

Kinetic measurements of $O_2^{-\bullet}$ production by illuminated thylakoids in plants of *Triticum durum* L. cv. Adamello subjected to water deficit

□, Control; x, stressed; continuous lines, least-squares-fit curves. Amplitude is given in arbitrary units.



oxygen [2,18,25], so that under drought conditions the production of superoxide is dependent on species, intensity of stress, repeated stress periods and the age of the plants [25–28].

One of the first consequences of dehydration is an alteration in the structure and function of cell membranes [41]. There is increasing evidence to support the view that during dehydration oxygen radicals play a role in mediating the degradative reactions that precede loss of membrane integrity [23,25,26,41,42]. The photoreduction of O_2 in thylakoids occurs not on the surface but within the membranes, so that the reduction rate of dioxygen is increased if the membrane structure is, even partially, destroyed [43]. Thus, whereas the pathology that follows drought is a consequence, at least in part, of the production of superoxide radicals in damaged chloroplasts, the increase in the $O_2^{-\bullet}$ production may reflect deteriorative molecular rearrangements in the thylakoid membranes [44,45]. A higher rate of $O_2^{-\bullet}$ production has been found in thylakoids, in which carotenoid and hydrophobic protein levels decreased and the lipid/protein ratio increased in comparison with control plants [28]. Changes in the chemical composition of membrane lipid bilayers can induce changes in lipid–protein interaction and protein conformation [45], as well as changes in the structural organization of the photosystem components in the lipid bilayer, so that they are more or less exposed to molecular oxygen. Table 1 shows that an altered chlorophyll *a/b* ratio and a high degree of lipid degradation caused greater conformational changes in thylakoid membrane proteins in a sensitive wheat cultivar than in a drought-tolerant cultivar. Moreover, the more rigid bilayer, partly because of an accumulation of free fatty acids and non-bilayer-forming lipids and partly as a result of changes in protein conformation, also increased superoxide production in the stressed drought-sensitive cultivar by about 25% in comparison with the control (Figure 1).

In contrast, the stressed plants of the tolerant wheat showed a dramatic decrease in superoxide production even when chlorophyll content was maintained and alteration in lipid composition was limited (Table 1).

When a lower $O_2^{-\bullet}$ production was monitored during dehydration [27,28,45], decreased thylakoid electron transport may have been involved. Moreover, a direct involvement of chlorophyll in the production of the Tiron radical signal, via superoxide, by type I photodynamic reaction may also have occurred [24]. As a consequence, the decline in superoxide production per unit of total chlorophyll might be due to a selective photobleaching of those chlorophyll molecules on the surface of the protein–chlorophyll complex, which are, therefore, more accessible to oxygen [24]. Decreased electron transport during anabiosis of resurrection plants [46] may be the cause of the reduced rate of $O_2^{-\bullet}$ production in slowly and rapidly dehydrated detached leaves of *Boea hygroscopica* in comparison with control and rehydrated leaves [26]. Furthermore, the

Table 1

Chemical and physical changes and $O_2^{-\bullet}$ formation rates in thylakoids of two wheat cultivars differently sensitive to drought subjected to water deficit conditions

For each cultivar, means followed by different letters (a/b) are significantly different from control by an analysis of variance test ($P \leq 0.01$). Ψ_w, leaf water potential (MPa); Chl, chlorophyll; MGDG/DGDG, monogalactosyldiacylglycerol/digalactosyldiacylglycerol (molar ratio); FFAs, free fatty acids (mol%); τ, spin label rotational correlation time (ns); k, $O_2^{-\bullet}$ formation rate (s^{-1}). *Adapted from [45].

	cv. Adamello (sensitive)		cv. Ofanto (tolerant)	
Parameter	Control	Stressed	Control	Stressed
Ψ_w*	-0.6^a	-1.7^b	-0.5^a	-1.8^b
Chl *a*/Chl *b*	3.1^b	1.5^a	3.6^a	3.3^a
MGDG/DGDG*	1.9^a	2.1^b	2.0^b	1.4^a
FFAs*	0.8^a	1.4^b	2.0^a	1.8^a
τ*	0.13^a	0.22^b	0.16^a	0.19^b
k (%)	100^a	125^b	100^b	69^a

cause of the higher $O_2^{-\cdot}$ production in rapidly dehydrated leaves, in comparison with those that are slowly dried, may lie in the changes observed in the lipid composition of thylakoid membranes [26].

The fact that different genotypes of sunflower seedlings experiencing the same level of water deficit stress produce different quantities of $O_2^{-\cdot}$ in comparison with their controls [25] may highlight the role played by structural factors in the release of $O_2^{-\cdot}$ from thylakoids. The maintenance of an adequate level of lipid unsaturation as well as a preferential rate of protein hydrolysis with a higher hydrophilic to hydrophobic protein ratio may be an adaptative feature to limit the production of $O_2^{-\cdot}$ [25]. Indeed, in embryo systems and in leaves of resurrection plants, desiccation tolerance leads to a rapid accumulation of both cytosolic and chloroplastic proteins, many of which are very hydrophilic [47].

Measurement of $O_2^{-\cdot}$ production by illuminated thylakoids gives an indication of the maximum capacity of thylakoids to transfer electrons to oxygen. By using thylakoid preparations without adding exogenous ferredoxin in any way the total extent of Mehler activity could be underestimated. However, in illuminated thylakoids of *Boea hygroscopica* plants to which ferredoxin has been added, the rate of $O_2^{-\cdot}$ production is not significantly different from that in thylakoids without added ferredoxin (F. Navari-Izzo, M. F. Quartacci and C. M. L. Sgherri, unpublished work). The rate determined in the presence of added ferredoxin and $NADP^+$ (about 30% of total $O_2^{-\cdot}$ production) most likely represents leakage of electrons from the normal pathway as a result of damage to thylakoids. In these conditions an increase in the real electron leakage to oxygen has been monitored in *Boea hygroscopica* during dehydration and rehydration of whole plant. This production again reaches the control level after 48 h of rehydration.

1 Taube, H. (1977) J. Gen. Physiol. **49**, 29–52
2 Elstner, E. F. and Osswald, W. (1994) Proc. R. Soc. Edin. **102B**, 131–154
3 Elstner, E. F. (1991) in Active Oxygen/Oxidative Stress and Plant Metabolism (Pell, E. J. and Steffen, K. L., eds.), pp. 13–25, American Society of Plant Physiologists, Rockville, MD
4 Leprince, O., Atherton, N. M., Deltour, R. and Hendry, G. A. F. (1994) Plant Physiol. **104**, 1333–1339
5 Rich, P. R. and Bonner, W. D., Jr. (1978) Arch. Biochem. Biophys. **188**, 206–213
6 Cadenas, E. (1989) Annu. Rev. Biochem. **58**, 79–110
7 Winston, G. W. (1990) in Stress Responses in Plants: Adaptation and Acclimation Mechanisms (Alscher, R. G. and Cumming, J. R., eds.), pp. 57–86, Wiley–Liss, New York
8 Simontacchi, M. and Puntarulo, S. (1992) Plant Physiol. **100**, 1263–1268
9 Mayak, S., Legge, R. L. and Thompson, J. E. (1983) Phytochemistry **22**, 1830–1833
10 Del Rio, L. A., Fernandez, V. M., Ruperez, F. L., Sandalio, L. M. and Palma, J. M. (1989) Plant Physiol. **89**, 728–731
11 Sandalio, L. M., Fernandez, V. M., Ruperez, F. L. and Del Rio, L. A. (1988) Plant Physiol. **87**, 1–4
12 Asada, K. and Takahashi, M. (1987) in Photoinhibition (Kyle, D., Osmond, C. and Arntzen, C., eds.), pp. 227–287, Elsevier, Amsterdam
13 Halliwell, B. (1987) Planta **130**, 175–180
14 Chalmers, J. D. C., Coleman, J. O. D. and Walton, N. J. (1986) Biochem. Soc. Trans. **14**, 108–109
15 Askerlund, P. C., Larsson, C., Widell, S. and Moller, I. M. (1987) Physiol. Plant. **71**, 9–19
16 Mehdy, M. C. (1994) Plant Physiol. **105**, 467–472
17 Anh, C. K. and Murphy, T. M. (1995) Plant Physiol. **107**, 1241–1247
18 Wise, R. and Naylor, A. (1987) Plant Physiol. **83**, 287–292
19 Price, A., Atherton, N. M. and Hendry, G. A. F. (1989) Free Radical Res. Commun. **18**, 61–66
20 Cakmak, I. and Marschner, H. (1988) J. Exp. Bot. **39**, 1449–1460
21 Doke, N. (1985) Physiol. Plant Pathol. **27**, 311–322
22 Heath, R. (1987) Adv. Phytochem. **21**, 29–54
23 Quartacci, M. F. and Navari-Izzo, F. (1992) J. Plant Physiol. **139**, 621–625
24 McRae, D. G. and Thompson, J. E. (1983) Planta **158**, 185–193
25 Sgherri, C. L. M., Pinzino, C. and Navari-Izzo, F. (1993) Physiol. Plant. **87**, 211–216
26 Navari-Izzo, F., Pinzino, C., Quartacci, M. F. and Sgherri, C. L. M. (1994) Proc. R. Soc. Edin. **102B**, 187–191
27 Menconi, M., Sgherri, C. L. M., Pinzino, C. and Navari-Izzo, F. (1995) J. Exp. Bot. **46**, 1123–1130
28 Sgherri, C. L. M., Pinzino, C. and Navari-Izzo, F. (1996) Physiol. Plant. **96**, 446–452
29 Kendall, E. J. and McKersie, B. D. (1989) Physiol. Plant. **76**, 86–94
30 Hernandez, J. A., Corpas, F. J., Gomez, M., Del Rio, L. A. and Sevilla, F. (1993) Physiol. Plant. **89**, 103–110
31 Asada, K. (1994) in Causes of Photooxidative Stress and Amelioration of Defence Systems in Plants (Foyer, C. H. and Mullineaux, P. M., eds.),

pp. 77–104, CRC Press, Boca Raton, FL
32 Egneus, H., Heber, U., Matthiesen, U. and Kirk, M. (1975) Biochim. Biophys. Acta **408**, 252–268
33 Robinson, J. M. (1988) Physiol. Plant. **72**, 666–680
34 Elstner, E. F. (1987) in The Biochemistry of Plants (Davies, D. D., ed.), vol. 11, pp. 253–317, Academic Press, San Diego
35 Ireland, C. R., Baker, M. R. and Long, S. P. (1987) Biochim. Biophys. Acta **893**, 434–443
36 Smirnoff, N. (1993) J. Exp. Bot. **125**, 27–58
37 Ananyev, G., Renger, G., Wacker, U. and Klimov, V. (1994) Photosynth. Res. **41**, 327–338
38 Alscher, R. G. and Amthor, J. J. (1988) in Air Pollution and Air Metabolism (Schulte-Hostede, S., ed.), pp. 91–126, Elsevier North Holland, Amsterdam
39 Grace, S. and Osmond, C. B. (1991) in Active Oxygen/Oxidative Stress and Plant Metabolism (Pell, E. J. and Steffen, K. L., eds.), pp. 13–25, American Society of Plant Physiologists, Rockville, MD
40 Walker, M. A., McKersie, B. D. and Pauls, K. P. (1991) Plant Physiol. **97**, 663–669
41 Navari-Izzo, F., Quartacci, M. F., Melfi, D. and Izzo, R. (1993) Physiol. Plant. **87**, 508–514
42 McKersie, B. D. (1991) in Active Oxygen/Oxidative Stress and Plant Metabolism (Pell, E. J. and Steffen, K. L., eds.), pp. 107–118, American Society of Plant Physiologists, Rockville, MD
43 Asada, K. (1992) Physiol. Plant. **85**, 235–241
44 Thompson, J. E., Legge, R. L. and Barber, R. F. (1987) New Phytol. **105**, 317–344
45 Quartacci, M. F., Pinzino, C., Sgherri, C. L. M. and Navari-Izzo, F. (1995) Plant Physiol. **108**, 191–197
46 Schwab, K. B., Schreiber, U. and Heber, U. (1989) Planta **177**, 217–227
47 Schneider, K., Wells, B., Schmelzer, E., Salamini, F. and Bartels, D. (1993) Planta **189**, 120–131

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Free radicals and metabolism associated with the acquisition and loss of desiccation tolerance in developing seeds

O. Leprince*‖, G. A. F. Hendryt, N. M. Atherton‡ and C. Walters-Vertucci§

*Department of Brassica and Oilseed Research, John Innes Centre, Colney, Norwich NR4 7UH, U.K., †NERC Unit of Comparative Plant Ecology, Department of Animal and Plant Sciences, ‡Department of Chemistry, The University, Sheffield S10 2TN, U.K., and §National Seed Storage Laboratory, USDA-ARS, Fort Collins, CO 80521, U.S.A.

Introduction

During the later stages of their development, seeds undergo extensive desiccation, during which more than 90% of the original water may be removed. These seeds are termed 'orthodox' and remain viable over long periods of time in dry, cold conditions [1,2]. During the early stages of germination, desiccation tolerance is generally maintained up to the point of radicle emergence. There are several challenges associated with surviving desiccation, as drying embryonic tissues must cope with structural, physical and biochemical stresses resulting from the progressive loss of water. It is apparent that desiccation tolerance is mediated by protective systems that prevent lethal damage during drying. Two mechanisms of protection have received extensive consideration [1–3]: (1) the accumulation of non-reducing carbohydrates and LEA (late embryogenesis abundant) proteins, which stabilize the cellular constituents during desiccation; and (2) the ability to evade damage from destructive reactions mediated by free radicals generated during desiccation. Several symptoms of free radical injury associated with the loss of viability after desiccation have been characterized and are summarized in Table 1. The origin of oxidative damage (Table 1) is likely to be the formation of transient but highly reactive, partially reduced or activated forms of O_2, as suggested by an extensive literature [2,3,7,12,13].

The physiological mechanisms that mediate the generation of activated O_2 during drying are poorly understood. Progress in this area has been slow for three main reasons. First, as recently argued [3], it is not always possible to distinguish experimentally between free radical processes arising from stress imposed by water loss and those occurring after death due to desiccation or those occurring in the dried state. The free radical reactions that take place during these three physiological conditions are likely to be different both quantitatively and qualitatively. They will not necessarily be reflected in the symptoms of injury that are assessed by biochemical assays. Thus, whether free radicals generated during

‖To whom correspondence should be addressed.