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### **the light manifest as photoinhibition? Do oxidative stress conditions impairing photosynthesis in**

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Éva Hideg, Kálai Tamás, Kálmán Hideg and Imre Vass

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## **DO OXIDATE SURFE ROTAL**<br> **DO OXIDATIVE STRESS CONDITIONS IMPAIRING**<br> **DO OXIDATIVE STRESS CONDITIONS IMPAIRING poditions**<br>**photosynthesis in the light manifest**<br>**photosynthesis in the light manifest** re stress conditions im<br>**thesis in the light mai**<br>as photoinhibition?

# **E**va Hideg<sup>1\*</sup>, Tamás Kálai<sup>2</sup>, Kálmán Hideg<sup>2</sup> and Imre Vass<sup>1</sup>

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<sup>2</sup>Department of Organic and Medicinal Chemistry, University of Pécs, H-7643 Pécs, PO Box 99, Hungary<br>We compared the effect of photoinhibition by excess photosynthetically active radiation (PAR), UV-B<br>irradiation combine We compared the effect of photoinhibition by excess photosynthetically active radiation (PAR), UV-B<br>irradiation combined with PAR, low temperature stress and paraquat treatment on photosystem (PS) II.<br>Although the experime We compared the effect of photoinhibition by excess photosynthetically active radiation (PAR), UV-B<br>irradiation combined with PAR, low temperature stress and paraquat treatment on photosystem (PS) II.<br>Although the experime irradiation combined with PAR, low temperature stress and paraquat treatment on photosystem (PS) II.<br>Although the experimental conditions ensured that the four studied stress conditions resulted in<br>approximately the same e Although the experimental conditions ensured that the four studied stress conditions resulted in approximately the same extent of PS II inactivation, they clearly followed different molecular mechanisms. Our results show t approximately the same extent of PS II inactivation, they clearly followed different molecular mechanisms. Our results show that singlet oxygen production in inactivated PS II reaction centres is a unique characteristic of mechanisms. Our results show that singlet oxygen production in inactivated PS II reaction centres is a unique characteristic of photoinhibition by excess PAR. Neither the accumulation of inactive PS II (as in paraquat reac unique characteristic of photoinhibition by excess PAR. Neither the accumulation of inactive PS II<br>reaction centres (as in UV-B or chilling stress), nor photo-oxidative damage of PS II (as in paraquat<br>stress) is able to pr photoinhibition.

**Keywords:** photosynthesis; oxidative stress; singlet oxygen; DanePy fluorescence

#### **1. INTRODUCTION**

Increased production of reactive oxygen species (ROS) **HERODOCTION**<br>Increased production of reactive oxygen species (ROS)<br>has been associated with a number of stress conditions in<br>plants (for reviews see Hendry 1994: Inze & Van Increased production of reactive oxygen species (ROS)<br>has been associated with a number of stress conditions in<br>plants (for reviews, see Hendry 1994; Inze & Van<br>Montagu 1995; Smirnoff 1995; Hideg 1997) ROS bave has been associated with a number of stress conditions in<br>plants (for reviews, see Hendry 1994; Inze & Van<br>Montagu 1995; Smirnoff 1995; Hideg 1997). ROS have<br>been recognized in at least one of the following roles: plants (for reviews, see Hendry 1994; Inze & Van<br>Montagu 1995; Smirnoff 1995; Hideg 1997). ROS have<br>been recognized in at least one of the following roles: Montagu 1995; Smirnoff 1995; Hideg 1997). ROS have<br>been recognized in at least one of the following roles:<br>elicitors or propagators of oxidative damage or signal<br>molecules for repair processes. Their production site and been recognized in at least one of the following roles:<br>elicitors or propagators of oxidative damage or signal<br>molecules for repair processes. Their production site and<br>function may be different under different stress cond elicitors or propagators of oxidative damage or signal<br>molecules for repair processes. Their production site and<br>function may be different under different stress condi-<br>tions and also depend on the antioxidant capacity of molecules for repair processes. Their production site and<br>function may be different under different stress condi-<br>tions, and also depend on the antioxidant capacity of the<br>plant. The toxicity of ROS is caused by their reac function may be different under different stress condi-<br>tions, and also depend on the antioxidant capacity of the<br>plant. The toxicity of ROS is caused by their reactivity,<br>which also makes them difficult to detect and iden tions, and also depend on the antioxidant capacity of the plant. The toxicity of ROS is caused by their reactivity, which also makes them difficult to detect and identify, especially *in vivo*. plant. The toxicity of ROS is caused by their reactivity,<br>which also makes them difficult to detect and identify,<br>especially *in vivo*.<br>Unusually, light also appears to be a stress factor and<br>the primary target of light st

especially *in vivo*.<br>
Unusually, light also appears to be a stress factor and<br>
the primary target of light stress is the site of light energy<br>
use: the photosynthetic apparatus (Powles, 1984) Unusually, light also appears to be a stress factor and<br>the primary target of light stress is the site of light energy<br>use: the photosynthetic apparatus (Powles 1984).<br>Although in the field all stress conditions occur in l the primary target of light stress is the site of light energy<br>use: the photosynthetic apparatus (Powles 1984).<br>Although in the field all stress conditions occur in light,<br>the interpretation is probably not simple. Photoin use: the photosynthetic apparatus (Powles 1984).<br>Although in the field all stress conditions occur in light,<br>the interpretation is probably not simple. Photoinhibition<br>by excess photosynthetically active radiation (PAR) Although in the field all stress conditions occur in light,<br>the interpretation is probably not simple. Photoinhibition<br>by excess photosynthetically active radiation (PAR)<br>damages the reaction centre of the photosystem (PS) the interpretation is probably not simple. Photoinhibition<br>by excess photosynthetically active radiation (PAR)<br>damages the reaction centre of the photosystem (PS) II<br>complex of the photosynthetic apparatus which is located by excess photosynthetically active radiation (PAR)<br>damages the reaction centre of the photosystem (PS) II<br>complex of the photosynthetic apparatus, which is located<br>in the thylakoid membrane of higher plant chloroplasts damages the reaction centre of the photosystem (PS) II<br>complex of the photosynthetic apparatus, which is located<br>in the thylakoid membrane of higher plant chloroplasts.<br>Photoiphibition has been extensively studied in vitro complex of the photosynthetic apparatus, which is located<br>in the thylakoid membrane of higher plant chloroplasts.<br>Photoinhibition has been extensively studied *in vitro*, in in the thylakoid membrane of higher plant chloroplasts.<br>Photoinhibition has been extensively studied *in vitro*, in isolated membrane preparations and a definitive sequence<br>of events has emerged. In this model, excess PAR Photoinhibition has been extensively studied *in vitro*, in isolated membrane preparations and a definitive sequence of events has emerged. In this model, excess PAR leads to impairment of PS II electron transport, which i isolated membrane preparations and a definitive sequence<br>of events has emerged. In this model, excess PAR leads to<br>impairment of PS II electron transport, which is followed<br>by selective degradation of the DLPS II reaction of events has emerged. In this model, excess PAR leads to impairment of PS II electron transport, which is followed by selective degradation of the D1 PS II reaction centre impairment of PS II electron transport, which is followed<br>by selective degradation of the DI PS II reaction centre<br>protein and by more general membrane protein and lipid<br>damage (for reviews see Barber & Andersson 1992: Pra by selective degradation of the DI PS II reaction centre<br>protein and by more general membrane protein and lipid<br>damage (for reviews, see Barber & Andersson 1992; Prasil<br> $_{et}$  al 1999: Aro et al 1993) protein and by more genera<br>damage (for reviews, see Ba<br>*et al.* 1992; Aro *et al.* 1993).

Direct observation of ROS by spin trapping electron Direct observation of ROS by spin trapping electron<br>paramagnetic resonance (EPR) spectroscopy demon-<br>strated that photoiphibition by excess PAR is an Direct observation of ROS by spin trapping electron<br>paramagnetic resonance (EPR) spectroscopy demon-<br>strated that photoinhibition by excess PAR is an<br>ovidative stress (Hider et al. 1994a: Hirayama et al. paramagnetic resonance (EPR) spectroscopy demonstrated that photoinhibition by excess PAR is an oxidative stress (Hideg *et al.* 1994*a*; Hirayama *et al.* 1996: Vruela *et al.* 1996). Among the particular path strated that photoinhibition by excess PAR is an oxidative stress (Hideg *et al.* 1994*a*; Hirayama *et al.* 1996; Yruela *et al.* 1996). Among the particular pathoxidative stress (Hideg *et al.* 1994*a*; Hirayama *et al.* 1996; Yruela *et al.* 1996). Among the particular pathways of damage an important one is recognized as acceptor side induced photoinhibition, which occurs 1996; Yruela *et al.* 1996). Among the particular pathways of damage an important one is recognized as acceptor-side-induced photoinhibition, which occurs when photosynthetically active over a system acceptor-side-induced photoinhibition, which occurs<br>when photosynthetically active, oxygen-evolving acceptor-side-induced photoinhibition, which occurs<br>when photosynthetically active, oxygen-evolving<br>preparations are illuminated with excess PAR in the<br>presence of oxygen In this process double reduction when photosynthetically active, oxygen-evolving<br>preparations are illuminated with excess PAR in the<br>presence of oxygen. In this process, double reduction<br>of the first PS II quinone acceptor O results in presence of oxygen. In this process, double reduction<br>of the first PS II quinone acceptor  $Q_A$  results in<br>increased reaction centre chlorophyll triplet formation of the first PS II quinone acceptor  $Q_A$  results in (Vass *et al.* 1992; Vass & Styring 1992) and, consequently, in singlet oxygen production. There are (Vass *et al.* 1992; Vass & Styring 1992) and, consequently, in singlet oxygen production. There are strong indications that the above ROS are involved in the specific clasure of the  $\overline{D}l$  protein: singlet quently, in singlet oxygen production. There are<br>strong indications that the above ROS are involved in<br>the specific cleavage of the D1 protein: singlet<br>oxygen generating substances cause D1 protein from strong indications that the above ROS are involved in<br>the specific cleavage of the D1 protein: singlet<br>oxygen-generating substances cause D1 protein frag-<br>mentation to the same specific fragments as does the specific cleavage of the D1 protein: singlet<br>oxygen-generating substances cause D1 protein fragmentation to the same specific fragments as does<br>acceptor side induced photoiphibition by excess PAR oxygen-generating substances cause D1 protein fragmentation to the same specific fragments as does<br>acceptor-side-induced photoinhibition by excess PAR<br>(Mishra et al. 1994: Okada et al. 1996) Since the prinmentation to the same specific fragments as does<br>acceptor-side-induced photoinhibition by excess PAR<br>(Mishra *et al.* 1994; Okada *et al.* 1996). Since the prin-<br>cipal cause of photoinhibition in this model is the acceptor-side-induced photoinhibition by excess PAR (Mishra *et al.* 1994; Okada *et al.* 1996). Since the principal cause of photoinhibition in this model is the imbalance between energy intake and processing, it is plausible, that any stress condition, that suppresses cipal cause of photoinhibition in this model is the<br>imbalance between energy intake and processing, it is<br>plausible that any stress condition that suppresses<br>photosynthetic electron transport or exhausts excess imbalance between energy intake and processing, it is<br>plausible that any stress condition that suppresses<br>photosynthetic electron transport or exhausts excess<br>energy relaxation mechanisms would manifest as plausible that any stress condition that suppresses<br>photosynthetic electron transport or exhausts excess<br>energy relaxation mechanisms would manifest as<br>photoinhibition. The aim of the present work was to photosynthetic electron transport or exhausts excess<br>energy relaxation mechanisms would manifest as<br>photoinhibition. The aim of the present work was to<br>analyze this question on the basis of POS detection in energy relaxation mechanisms would manifest as<br>photoinhibition. The aim of the present work was to<br>analyse this question on the basis of ROS detection *in*<br>ring analyse this question on the basis of ROS detection *in vivo*.<br>Singlet oxygen production in acceptor-side-induced

photoinhibition has been confirmed *in vitro*, in isolated<br>photoinhibition has been confirmed *in vitro*, in isolated<br>photosynthetically active membrane preparations Singlet Singlet oxygen production in acceptor-side-induced photosinhibition has been confirmed *in vitro*, in isolated photosynthetically active membrane preparations. Singlet oxygen has been detected with various techniques photoinhibition has been confirmed *in vitro*, in isolated<br>photosynthetically active membrane preparations. Singlet<br>oxygen has been detected with various techniques

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Figure 1. Chemical structure of DanePy and its nitroxide radical form DanePyO.

including spin trapping EPR spectroscopy (Hideg *et al.* including spin trapping EPR spectroscopy (Hideg *et al.* 1994*b*), infrared chemiluminescence (MacPherson *et al.* 1993) and chemical trapping (Telfer *et al.* 1994) Extension including spin trapping EPR spectroscopy (Hideg *et al.* 1994*b*), infrared chemiluminescence (MacPherson *et al.* 1993) and chemical trapping (Telfer *et al.* 1994). Extension of our EPR results to *in ring* (leaf studies 1994*b*), infrared chemiluminescence (MacPherson *et al.* 1993) and chemical trapping (Telfer *et al.* 1994). Extension of our EPR results to *in vivo* (leaf studies) was hampered by technical difficulties caused by the bi 1993) and chemical trapping (Telfer *et al.* 1994). Extension of our EPR results to *in vivo* (leaf studies) was hampered by technical difficulties caused by the high water content of the leaves and the short lifetime of

of our EPR results to *in vivo* (leaf studies) was hampered<br>by technical difficulties caused by the high water content<br>of the leaves and the short lifetime of the probe within<br>the leaf (Hidea *et al* 2000) A new technique by technical difficulties caused by the high water content<br>of the leaves and the short lifetime of the probe within<br>the leaf (Hideg *et al.* 2000). A new technique was needed.<br>DangPy is a double sensor consisting of a fluo of the leaves and the short lifetime of the probe within<br>the leaf (Hideg *et al.* 2000). A new technique was needed.<br>DanePy is a double sensor consisting of a fluorophore,<br>dansyl and a sterically hindered amine attached t the leaf (Hideg *et al.* 2000). A new technique was needed.<br>DanePy is a double sensor consisting of a fluorophore,<br>dansyl, and a sterically hindered amine attached to a<br>nyrroline ring (figure 1) (Kálai *et al.* 1998). Thi DanePy is a double sensor consisting of a fluorophore,<br>dansyl, and a sterically hindered amine attached to a<br>pyrroline ring (figure 1) (Kálai *et al.* 1998). This<br>compound is fluorescent and diamagnetic Reaction with dansyl, and a sterically hindered amine attached to a an antibody raised against a synthetic peptide corresponding to pyrroline ring (figure 1) (Kálai et al. 1998). This the C-terminus of pea D1 protein, a kind gift from singlet oxygen converts the amine into a nitroxide radical. compound is fluorescent and diamagnetic. Reaction with<br>singlet oxygen converts the amine into a nitroxide radical.<br>The resulting compound (DanePyO) is paramagnetic<br>(data not shown) and has lower fluorescence than singlet oxygen converts the amine into a nitroxide radical.<br>The resulting compound (DanePyO) is paramagnetic<br>(data not shown) and has lower fluorescence than<br>DanePy (figure  $2a$ ) Using this sensor we were able to The resulting compound (DanePyO) is paramagnetic<br>(data not shown) and has lower fluorescence than<br>DanePy (figure 2*a*). Using this sensor, we were able to<br>demonstrate singlet over production in leaves exposed (data not shown) and has lower fluorescence than<br>DanePy (figure 2*a*). Using this sensor, we were able to<br>demonstrate singlet oxygen production in leaves exposed<br>to photoiphibition by excess  $\frac{p_{AB}}{q}$  (Hideo *et al.* 1 DanePy (figure 2*a*). Using this sensor, we were able<br>demonstrate singlet oxygen production in leaves expos<br>to photoinhibition by excess PAR (Hideg *et al.* 1998*a*).<br>Our earlier *in vitro* studies showed that among varia monstrate singlet oxygen production in leaves exposed<br>photoinhibition by excess PAR (Hideg *et al.* 1998*a*).<br>Our earlier *in vitro* studies showed that among various<br>ht stress conditions singlet oxygen production seemed t

to photoinhibition by excess PAR (Hideg *et al.* 1998*a*).<br>Our earlier *in vitro* studies showed that among various light stress conditions singlet oxygen production seemed to Our earlier *in vitro* studies showed that among various<br>light stress conditions singlet oxygen production seemed to<br>be a unique characteristic of PS II under acceptor-side-<br>induced photoinhibition. Neither donor-side-indu light stress conditions singlet oxygen production seemed to<br>be a unique characteristic of PS II under acceptor-side-<br>induced photoinhibition. Neither donor-side-induced<br>photoinhibition (Hideg et al. 1994a) por ultraviolet be a unique characteristic of PS II under acceptor-side-<br>induced photoinhibition. Neither donor-side-induced<br>photoinhibition (Hideg *et al.* 1994*a*) nor ultraviolet B<br>(UVR) irradiation (Hideg & Vass 1996) appeared as sing induced photoinhibition. Neither donor-side-induced<br>photoinhibition (Hideg *et al.* 1994*a*) nor ultraviolet B<br>(UVB) irradiation (Hideg & Vass 1996) appeared as singlet<br>oxygen-mediated stress: both were associated with ot photoinhibition (Hideg *et al.* 1994*a*) nor ultraviolet B<br>(UVB) irradiation (Hideg & Vass 1996) appeared as singlet<br>oxygen-mediated stress; both were associated with other<br>types of ROS mainly hydroxyl radicals. In the pre (UVB) irradiation (Hideg & Vass 1996) appeared as singlet<br>oxygen-mediated stress; both were associated with other<br>types of ROS, mainly hydroxyl radicals. In the present<br>study we carried out a comparative study of PS II fu oxygen-mediated stress; both were associated with other earlier (Hideg *et al.* 1998*a*). Singlet oxygen types of ROS, mainly hydroxyl radicals. In the present ized as relative fluorescence quenching, -<br>study, we carried types of ROS, mainly hydroxyl radicals. In the present<br>study, we carried out a comparative study of PS II function<br>and D1 protein degradation, *in vivo*, in photoinhibited leaves<br>and in leaves exposed to other types of oxi study, we carried out a comparative study of PS II function<br>and D1 protein degradation, *in vivo*, in photoinhibited leaves<br>and in leaves exposed to other types of oxidative stress (low<br>temperature, herbicide or UVB irradi and D1 protein degradation, *in vivo*, in photoin<br>and in leaves exposed to other types of oxidat<br>temperature, herbicide or UVB irradiation).

#### **2. MATERIAL AND METHODS**

Tobacco (*Nicotiana tabacum* L.) plants were grown in a Tobacco (*Nicotiana tabacum* L.) plants were grown in a<br>greenhouse, under 80–100 $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PAR, 20–25°C. Leaf Tobacco (*Nicotiana tabacum* L.) plants were grown in a<br>greenhouse, under 80–100 $\mu$ molm<sup>-2</sup>s<sup>-1</sup> PAR, 20–25°C. Leaf<br>disks were cut from the tip region of six-week-old leaves<br>symplic the midnih section. These semples were disks were cut from the tip region of six-week-old leaves Q avoiding the midrib section. These samples were kept on wet tissue paper on a thin layer of water with their adaxial sides up and exposed to one of the following stress conditions: (i) photo- $\bigcirc$  tissue paper on a thin layer of water with their adaxial sides up tissue paper on a thin layer of water with their adaxial sides up<br>and exposed to one of the following stress conditions: (i) photo-<br>inhibition by 1500 µmol m<sup>-2</sup>s<sup>-1</sup> PAR at room temperature,<br>(ii) impediation with 25 µmol and exposed to one of the following stress conditions: (i) photo-<br>inhibition by 1500  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PAR at room temperature,<br>(ii) irradiation with 25  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> UVB (280-320nm) and<br>100 umol m<sup>-2</sup>s<sup>-1</sup> PAR at r  $100 \,\mathrm{\mu mol\,m}^{-2}\,\mathrm{s}^{-1}$  PAR at room temperature, (iii) chilling at 1500  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PAR at room temperature,<br>
i with 25  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> UVB (280-320nm) and<br>
s<sup>-1</sup> PAR at room temperature, (iii) chilling at (ii) irradiation with 25  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> UVB (280-320nm) and<br>100  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PAR, at room temperature, (iii) chilling at<br>5 °C under 400  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PAR, or (iv) 7.5 mM paraquat at 100 µmol m<sup>-2</sup>s<sup>-1</sup> PAR at room temperature, (ii)<br>5 °C under 400 µmol m<sup>-2</sup>s<sup>-1</sup> PAR, or (iv) 7.5 mN<br>room temperature under 100 µmol m<sup>-2</sup>s<sup>-1</sup> PAR.<br>- PAR was provided through an antical fibre guid C under 400  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PAR, or (iv) 7.5 mM paraquat at<br>om temperature under 100  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PAR.<br>PAR was provided through an optical fibre guide from a KL-<br>00 (DMR Suitzerland) lamp. This illumination sexue

1500 (DMP, Switzerland) lamp. This illumination caused no PAR was provided through an optical fibre guide from a KL<br>1500 (DMP, Switzerland) lamp. This illumination caused no<br>local warming of the samples, even when  $1500 \,\mu\text{mol m}^{-2} \text{s}^{-1}$ <br>PAP, were explied. In parameterizations 1500 (DMP, Switzerland) lamp. This illumination caused no<br>local warming of the samples, even when  $1500 \mu$ mol m<sup>-2</sup>s<sup>-1</sup><br>PAR were applied. In paraquat stress, leaf disks were preincubated with paraquat in the dark for 1h before illumination<br>and papement was contained in the floating medium incubated with paraquat in the dark for 1h before ill<br>and paraquat was contained in the floating medium.<br>En PS H estivity and DI pretain measurements For Ps II activity and D1 protein measurements, thylakoid<br>For PS II activity and D1 protein measurements, thylakoid<br>mehranes were propered from the leaf disks immediately

and paraquat was contained in the floating medium.<br>
For PS II activity and D1 protein measurements, thylakoid<br>
membranes were prepared from the leaf disks immediately<br>
of the stress. Photographics guygen membranes were prepared from the leaf disks immediately<br>after the cessation of the stress. Photosynthetic oxygen membranes were prepared from the leaf disks immediately<br>after the cessation of the stress. Photosynthetic oxygen<br>evolution was measured with oxygen polarography (Hansatech,<br> $V_K$ ) wing dimethyl hangoguinane as electron acce after the cessation of the stress. Photosynthetic oxygen<br>evolution was measured with oxygen-polarography (Hansatech,<br>UK) using dimethyl-benzoquinone as electron acceptor. Net<br>DJ protein centent was determined wing Western-UK) using dimethyl-benzoquinone as electron acceptor. Net D1 protein content was determined using Western blotting with an antibody raised against a synthetic peptide corresponding to Nixon (Imperial College of Science, Technology and Medicine, London, UK) after separation of thylakoid membrane proteins by SDS polyacrylamide gel electrophoresis. mdon, UK) after separation of thylakoid membrane proteins<br>SDS polyacrylamide gel electrophoresis.<br>Singlet oxygen detection was based on the reaction of singlet<br>was usited DanaPa (2 (M distribulationing that) M danaph

by SDS polyacrylamide gel electrophoresis.<br>
Singlet oxygen detection was based on the reaction of singlet<br>
oxygen with DanePy (3-(*N*-diethylaminoethyl)-*N*-dansyl)-<br>
ominomethyl 9.5 dihydro 9.9.55 tatamathyl 1*H* numals) oxygen with DanePy (3-(N-diethylaminoethyl)-N-dansyl)-<br>aminomethyl-2,5-dihydro-2,2,5,5-tetramethyl-lH-pyrrole), yielding a nitroxide radical (DanePyO, 3-(*N*-diethyl-aminoethyl)-*N*-<br>dansyl) aminomethyl-2,5-dihydro-2,2,5,5-tetramethyl-*H*-pyrrol-<br>1-yloxyl), which is EPR active and has lower fluorescence than dansyl) aminomethyl-2,5-dihydro-2,2,5,5-tetramethyl- $H$ -pyrroldansyl) aminomethyl-2,5-dihydro-2,2,5,5-tetramethyl-*H*-pyrrol-<br>1-yloxyl) which is EPR active and has lower fluorescence than<br>DanePy (Kálai *et al.* 1998). Leaf disks were infiltrated with 1-yloxyl), which is EPR active and has lower fluorescence than<br>DanePy (Kálai *et al.* 1998). Leaf disks were infiltrated with<br>50 mM DanePy and fluorescence emission spectra were recorded<br>with a Quanta Mastar OM 1 (Photon 50 mM DanePy and fluorescence emission spectra were recorded<br>with a Quanta Master QM-1 (Photon Technology Int., Inc., South Brunswick, NJ, USA) using 345 nm excitation as described with a Quanta Master QM-1 (Photon Technology Int., Inc.,<br>South Brunswick, NJ, USA) using 345 nm excitation as described<br>earlier (Hideg *et al.* 1998*a*). Singlet oxygen trapping is character-South Brunswick, NJ, USA) using 345 nm excitation as described<br>earlier (Hideg *et al.* 1998*a*). Singlet oxygen trapping is character-<br>ized as relative fluorescence quenching,  $-\Delta F/F$ , at the 532 nm<br>emission maximum of Dan ized as relative fluorescence quenching,  $-\Delta F/F$ , at the 532 nm

### **3. RESULTS**

Figure 2 illustrates the measurement of singlet oxygen 5. **RESOLTS**<br>Figure 2 illustrates the measurement of singlet oxygen<br>production in leaves using DanePy. As shown in figure 2*a*,<br>there is a marked difference between the fluorescence emis-Figure 2 illustrates the measurement of singlet oxygen<br>production in leaves using DanePy. As shown in figure 2a,<br>there is a marked difference between the fluorescence emis-<br>sion of DanePy and its nitrovide radical form. D production in leaves using DanePy. As shown in figure 2*a*, there is a marked difference between the fluorescence emission of DanePy and its nitroxide radical form, DanePyO. In the experiment shown in figure 2*a*, leaves w there is a marked difference between the fluorescence emission of DanePy and its nitroxide radical form, DanePyO.<br>In the experiment shown in figure 2*a*, leaves were infil-<br>trated by one of these two forms of the double se sion of DanePy and its nitroxide radical form, DanePyO.<br>In the experiment shown in figure 2a, leaves were infiltrated by one of these two forms of the double sensor. On<br>the basis of this difference in fluorescence intensit In the experiment shown in figure  $2a$ , leaves were infiltrated by one of these two forms of the double sensor. On the basis of this difference in fluorescence intensity, singlet trated by one of these two forms of the double sensor. On<br>the basis of this difference in fluorescence intensity, singlet<br>oxygen production can be characterized as fluorescence<br>quenching of DanePy (Kálai et al. 1998; Hide the basis of this difference in fluorescence intensity, singlet<br>oxygen production can be characterized as fluorescence<br>quenching of DanePy (Kálai *et al.* 1998; Hideg *et al.* 1998*a*).<br>Figure 2h shows that this quenching oxygen production can be characterized as fluorescence<br>quenching of DanePy (Kálai *et al.* 1998; Hideg *et al.* 1998*a*).<br>Figure 2*b* shows that this quenching occurred when a<br>DanePy-infiltrated leaf was exposed to photoin quenching of DanePy (Kálai *et al.* 1998; Hideg *et al.* 1998*a*).<br>Figure 2*b* shows that this quenching occurred when a<br>DanePy-infiltrated leaf was exposed to photoinhibition.<br>In order to characterize the stress-induced Figure 2b shows that this quenching occurred when a DanePy-infiltrated leaf was exposed to photoinhibition.<br>In order to characterize the stress-induced damage to PS II we measured the loss of electron transport activity DanePy-infiltrated leaf was exposed to photoinhibition.

and the relative amount of D1 protein. Experimental PS II we measured the loss of electron transport activity<br>and the relative amount of D1 protein. Experimental<br>parameters were set in order to ensure that the loss of<br>PS II activity followed approximately the same timeand the relative amount of DI protein. Experimental<br>parameters were set in order to ensure that the loss of<br>PS II activity followed approximately the same time-<br>course during the four different types of stress conditions parameters were set in order to ensure that the loss of<br>PS II activity followed approximately the same time-<br>course during the four different types of stress conditions<br>applied (data not shown). Inactive reaction centres w PS II activity followed approximately the same time-<br>course during the four different types of stress conditions<br>applied (data not shown). Inactive reaction centres were<br>defined as ones containing DI protein but unable to course during the four different types of stress conditions

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Figure 2. (a) Fluorescence emission spectra of DanePy and its<br>nitroxide radical form DanePyO infiltrated into tobacco leaf<br>disks (b) Fluorescence emission spectrum of DanePy in Figure 2. *(a)* Fluorescence emission spectra of DanePy and<br>nitroxide radical form DanePyO infiltrated into tobacco le:<br>disks. *(b)* Fluorescence emission spectrum of DanePy in<br>tobacco leaves before and after 60 min photoi nitroxide radical form DanePyO infiltrated into tobacco leaf<br>disks. (b) Fluorescence emission spectrum of DanePy in<br>tobacco leaves before and after 60 min photoinhibition (PI)<br>by 1500 umol m<sup>-2</sup>s<sup>-1</sup> PAR at room temperatu disks. (*b*) Fluorescence emission spectrum of DanePy in<br>tobacco leaves before and after 60 min photoinhibition (PI)<br>by 1500 µmol m<sup>-2</sup>s<sup>-1</sup> PAR at room temperature. All spectra tobacco leaves before and after 60 min photoinhibition (PI)<br>by 1500 µmol m<sup>-2</sup>s<sup>-1</sup> PAR at room temperature. All spectra<br>were measured using  $\lambda_{\text{exc}} = 345$  nm and are shown normalized<br>to the fluorescence emission maximum by 1500  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PAR at room temperature. All spectivere measured using  $\lambda_{\text{exc}} = 345 \text{ nm}$  and are shown normaliz<br>to the fluorescence emission maximum of DanePy in the<br>untreated leaf. Parameters used in the de were measured using  $\lambda_{\text{exc}} = 345 \text{ nm}$  and are shown normalized<br>to the fluorescence emission maximum of DanePy in the<br>untreated leaf. Parameters used in the definition of relative<br>fluorescence quenching.  $\Delta E/E$  are also to the fluorescence emission maximum of DanePy in the untreated leaf. Parameters used in the definition of relative fluorescence quenching,  $\Delta F/F$  are also shown in (*b*).

more<br>secolve oxygen upon illumination. When leaves were<br>exposed to photoiphibition at room temperature, there evolve oxygen upon illumination. When leaves were<br>exposed to photoinhibition at room temperature, there<br>was no marked decrease in the amount of Dl protein thus evolve oxygen upon illumination. When leaves were<br>exposed to photoinhibition at room temperature, there<br>was no marked decrease in the amount of D1 protein, thus<br>the accumulation of inactive centres corresponded to the exposed to photoinhibition at room temperature, there<br>was no marked decrease in the amount of DI protein, thus<br>the accumulation of inactive centres corresponded to the<br>loss of PS II activity was no marked decrea<br>the accumulation of in<br>loss of PS II activity.<br>Figure 3, shows the Execumulation of inactive centres corresponded to the<br>
Second PS II activity.<br>
Figure 3 shows the comparison of the amount of<br>
Rective PS II centres and singlet over production in

loss of PS II activity.<br>
Figure 3 shows the comparison of the amount of<br>
inactive PS II centres and singlet oxygen production in<br>
the case of four different oxidative stress conditions: Figure 3 shows the comparison of the amount of inactive PS II centres and singlet oxygen production in the case of four different oxidative stress conditions:<br>mhotoinhibition IIVR irradiation low temperature and inactive PS II centres and singlet oxygen production in<br>the case of four different oxidative stress conditions:<br>photoinhibition, UVB irradiation, low temperature and<br>treatment with paraguat. Figure  $3a$  shows that singlet the case of four different oxidative stress conditions:<br>photoinhibition, UVB irradiation, low temperature and<br>treatment with paraquat. Figure 3*a* shows that singlet<br>oxygen production during photoinhibition *in nine* is photoinhibition, UVB irradiation, low temperature and<br>treatment with paraquat. Figure 3a shows that singlet<br>oxygen production during photoinhibition *in vivo* is<br>proportional to the amount of inactive PS II centres treatment with paraquat. Figure  $3a$  shows that singlet<br>oxygen production during photoinhibition *in vivo* is<br>proportional to the amount of inactive PS II centres<br>(Hideg et al. 1998a) This result supports the model postuoxygen production during photoinhibition *in vivo* is<br>proportional to the amount of inactive PS II centres<br>(Hideg *et al.* 1998*a*). This result supports the model postu-(Hideg *et al.* 1998*a*). This result supports the model postu-<br>*Phil. Trans. R. Soc. Lond.* B (2000)

oxygen. Similarly to photoinhibition by PAR, the other lating functionally inactive centres as sources of singlet<br>oxygen. Similarly to photoinhibition by PAR, the other<br>type of light stress, irradiation with UVB, also resulted in<br>the accumulation of inactive PS II centres, alt oxygen. Similarly to photoinhibition by PAR, the other<br>type of light stress, irradiation with UVB, also resulted in<br>the accumulation of inactive PS II centres, although to a<br>smaller extent. Contrary to photoinhibition, the type of light stress, irradiation with UVB, also resulted in<br>the accumulation of inactive PS II centres, although to a<br>smaller extent. Contrary to photoinhibition, there was no<br>marked singlet overen production until the la the accumulation of inactive PS II centres, although to a smaller extent. Contrary to photoinhibition, there was no marked singlet oxygen production until the late phase of smaller extent. Contrary to photoinhibition, there was no<br>marked singlet oxygen production until the late phase of<br>UVB treatment (figure 3*b*). Chilling at 5 °C resulted in<br>hoth the accumulation of inactive PS II and singl marked singlet oxygen production until the late phase of UVB treatment (figure  $3b$ ). Chilling at  $5^{\circ}$ C resulted in both the accumulation of inactive PS II and singlet oxygen production, although to a smaller extent th UVB treatment (figure  $3b$ ). Chilling at  $5^{\circ}$ C resulted in both the accumulation of inactive PS II and singlet oxygen production, although to a smaller extent than photoinhibition (figure  $3c$ ) while neither inactive P both the accumulation of inactive PS II and singlet oxygen production, although to a smaller extent than photoinhibition (figure  $3c$ ), while neither inactive PS II accumulation, nor singlet oxygen production was characoxygen production, although to a smaller extent than<br>photoinhibition (figure  $3c$ ), while neither inactive PS II<br>accumulation, nor singlet oxygen production was charac-<br>teristic of treatment by paraguat (figure  $3d$ ) photoinhibition (figure 3*c*), while neither ina<br>accumulation, nor singlet oxygen production v<br>teristic of treatment by paraquat (figure 3*d*). teristic of treatment by paraquat (figure 3*d*).<br>**4. DISCUSSION** 

Photoinhibition by excess PAR may deactivate photo-**4. DISCUSSION**<br>Photoinhibition by excess PAR may deactivate photo-<br>synthetic electron transport and cause oxidative damage<br>in plants (Prasil et al. 1992: A ro et al. 1993: K rause 1994) Photoinhibition by excess PAR may deactivate photosynthetic electron transport and cause oxidative damage<br>in plants (Prasil *et al.* 1992; Aro *et al.* 1993; Krause 1994).<br>The amount of PAR that is canable of initiating ph synthetic electron transport and cause oxidative damage<br>in plants (Prasil *et al.* 1992; Aro *et al.* 1993; Krause 1994).<br>The amount of PAR that is capable of initiating photo-<br>inhibition depends on the efficiency of the e in plants (Prasil *et al.* 1992; Aro *et al.* 1993; Krause 1994). The amount of PAR that is capable of initiating photo-inhibition depends on the efficiency of the energy dissipa-The amount of PAR that is capable of initiating photo-<br>inhibition depends on the efficiency of the energy dissipa-<br>tion mechanisms and the capacity of the antioxidant<br>defence system (for reviews see Demmin-Adams  $\&$ inhibition depends on the efficiency of the energy dissipa-<br>tion mechanisms and the capacity of the antioxidant<br>defence system (for reviews, see Demmig-Adams &<br>Adams 1992: Asada 1994) The primary site of damage is tion mechanisms and the capacity of the antioxidant<br>defence system (for reviews, see Demmig-Adams &<br>Adams 1992; Asada 1994). The primary site of damage is<br>in PS II where excess PAR generates stable reduced defence system (for reviews, see Demmig-Adams &<br>Adams 1992; Asada 1994). The primary site of damage is<br>in PS II, where excess PAR generates stable reduced<br>abnormal quinone states that lead to the production of Adams 1992; Asada 1994). The primary site of damage is<br>in PS II, where excess PAR generates stable reduced<br>abnormal quinone states that lead to the production of<br>singlet oxygen via chlorophyll triplet states (Vass & in PS II, where excess PAR generates stable reduced<br>abnormal quinone states that lead to the production of<br>singlet oxygen via chlorophyll triplet states (Vass &<br>Styring 1992–1993). In agreement with the established abnormal quinone states that lead to the production of singlet oxygen via chlorophyll triplet states (Vass & Styring 1992, 1993). In agreement with the established singlet oxygen via chlorophyll triplet states (Vass & Styring 1992, 1993). In agreement with the established model of acceptor-side-induced photoinhibition, we have recently shown that singlet oxygen is produced under Styring 1992, 1993). In agreement with the established model of acceptor-side-induced photoinhibition, we have recently shown that singlet oxygen is produced under high light intensities in vine in inactive  $PS$  II reactio model of acceptor-side-induced photoinhibition, we have<br>recently shown that singlet oxygen is produced under<br>high light intensities *in vivo*, in inactive PS II reaction<br>centres (Hidea *et al.* 1998*a*). Because stress con recently shown that singlet oxygen is produced under high light intensities *in vivo*, in inactive PS II reaction centres (Hideg *et al.* 1998*a*). Because stress conditions in high light intensities *in vivo*, in inactive PS II reaction<br>centres (Hideg *et al.* 1998*a*). Because stress conditions in<br>the field usually combine, it is feasible to ask whether the<br>impairment of PS II by another stres centres (Hideg *et al.* 1998*a*). Because stress conditions in<br>the field usually combine, it is feasible to ask whether the<br>impairment of PS II by another stress could decrease the<br>threshold intensity above which PAR is i the field usually combine, it is feasible to ask whether the<br>impairment of PS II by another stress could decrease the<br>threshold intensity above which PAR is in excess for<br>photosynthesis and thus generate singlet oxygen-med impairment of PS II by another stress could decrease the threshold intensity above which PAR is in excess for photosynthesis and thus generate singlet oxygen-mediated photoinhibition. In the experiments demonstrated above, photosynthesis and thus generate singlet oxygen-mediated photosynthesis and thus generate singlet oxygen-mediated<br>photoinhibition. In the experiments demonstrated above,<br>three different types of oxidative stress were combined<br>with PAR and their ability to promote singlet oxygen photoinhibition. In the experiments demonstrated above,<br>three different types of oxidative stress were combined<br>with PAR and their ability to promote singlet oxygen<br>production was examined. In order to perform comparathree different types of oxidative stress were combined<br>with PAR and their ability to promote singlet oxygen<br>production was examined. In order to perform compara-<br>tive experiments conditions were set in a way that with PAR and their ability to promote singlet oxygen<br>production was examined. In order to perform compara-<br>tive experiments, conditions were set in a way that<br>ensured that PS II electron transport activity, was production was examined. In order to perform comparative experiments, conditions were set in a way that ensured that PS II electron transport activity was tive experiments, conditions were set in a way that<br>ensured that PS II electron transport activity was<br>damaged to the same extent during the same periods of<br>time by the various stress conditions ensured that PS II electron trans<br>damaged to the same extent during the<br>time by the various stress conditions.<br>IIVR irradiation affects plants at ma maged to the same extent during the same periods of<br>the by the various stress conditions.<br>UVB irradiation affects plants at many levels causing a<br>mplex oxidative stress (for review see Vass 1997). In the

time by the various stress conditions.<br>UVB irradiation affects plants at many levels causing a<br>complex oxidative stress (for review, see Vass 1997). In the<br>thulakoid membrane, the primary target of UVB is at the UVB irradiation affects plants at many levels causing a<br>complex oxidative stress (for review, see Vass 1997). In the<br>thylakoid membrane, the primary target of UVB is at the<br>Mn cluster of the oxygen-evolving system (Renger complex oxidative stress (for review, see Vass 1997). In the thylakoid membrane, the primary target of UVB is at the Mn cluster of the oxygen-evolving system (Renger *et al.*) 1989: Vass *et al.* 1999), although the quinon thylakoid membrane, the primary target of UVB is at the Mn cluster of the oxygen-evolving system (Renger *et al.* 1989; Vass *et al.* 1999), although the quinone acceptors of PS II are also affected (Greenberg et al. 1989; Vass et al. 1989; Vass *et al.* 1999), although the quinone acceptors of PS II are also affected (Greenberg *et al.* 1989; Vass *et al.* 1996). As we showed earlier, in isolated thylakoid membranes this PS II impairment by  $IUXB$  does PS II are also affected (Greenberg *et al.* 1989; Vass *et al.* 1996). As we showed earlier, in isolated thylakoid membranes this PS II impairment by UVB does not result in marked singlet oxigen production (Hideg  $\&$  Vas 1996). As we showed earlier, in isolated thylakoid<br>membranes this PS II impairment by UVB does not result<br>in marked singlet oxygen production (Hideg & Vass 1996).<br>Our present data confirm this observation in leaves Moremembranes this PS II impairment by UVB does not result<br>in marked singlet oxygen production (Hideg & Vass 1996).<br>Our present data confirm this observation in leaves. Morein marked singlet oxygen production (Hideg & Vass 1996).<br>Our present data confirm this observation in leaves. Moreover, we found that a combination of UVB irradiation and<br>PAR-did not promote singlet oxygen production. Sing Our present data confirm this observation in leaves. More-<br>over, we found that a combination of UVB irradiation and<br>PAR did not promote singlet oxygen production. Singlet<br>oxygen appeared only in severely damaged leaves, af over, we found that a combination of UVB irradiation and<br>PAR did not promote singlet oxygen production. Singlet<br>oxygen appeared only in severely damaged leaves, after<br>long irradiation, probably accompanying membrane linid PAR did not promote singlet oxygen production. Singlet oxygen appeared only in severely damaged leaves, after long irradiation, probably accompanying membrane lipid peroxidation. There is no marked singlet oxygen producoxygen appeared only in severely damaged leaves, after<br>long irradiation, probably accompanying membrane lipid<br>peroxidation. There is no marked singlet oxygen produc-<br>tion at the earlier phase of damage, although inactive r long irradiation, probably accompanying membrane lipid peroxidation. There is no marked singlet oxygen production at the earlier phase of damage, although inactive reaction centres accumulated (figure 3*b*). The absence of singlet oxygen production supports the recent model tion at the earlier phase of damage, although inactive reaction centres accumulated (figure 3*b*). The absence of singlet oxygen production supports the recent model based on studies of cyanobacteria in which the initial tion centres accumulated (figure  $3b$ ). The absence of singlet oxygen production supports the recent model based on studies of cyanobacteria in which the initial site of PS II inactivation by  $IWR$  light in vira is not at singlet oxygen production supports the recent model<br>based on studies of cyanobacteria in which the initial<br>site of PS II inactivation by UVB light *in vivo* is not at

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Figure 3. Comparison of singlet oxygen production and the amount of inactive PS II reaction centres in leaves under various<br>stress conditions. The latter are defined as centres with intact protein structure but functional Figure 3. Comparison of singlet oxygen production and the amount of inactive PS II reaction centres in leaves under<br>stress conditions. The latter are defined as centres with intact protein structure but functionally impai stress conditions. The latter are defined as centres with intact protein structure but functionally impaired. Singlet oxygen<br>production was measured as quenching of DanePy fluorescence (see § 2 for details). Leaves were e production was measured as quenching of DanePy fluorescence (see § 2 for details). Leaves were exposed to (*a*) photo-<br>inhibition by 1500 µmol m<sup>-2</sup> s<sup>-1</sup> PAR at room temperature, (*b*) irradiation with 25 µmol m<sup>-2</sup> s<sup>-1</sup> inhibition by 1500 µmol m<sup>-2</sup> s<sup>-1</sup> PAR at roon<br>100 µmol m<sup>-2</sup> s<sup>-1</sup> PAR at room temperature,<br>temperature under 100 µmol m<sup>-2</sup> s<sup>-1</sup> PAR.

temperature under  $100 \mu \text{mol m}^{-2} \text{s}^{-1}$  PAR.<br>the quinone acceptors (Vass *et al.* 1999). Damage by the quinone acceptors (Vass *et al.* 1999). Damage by UVB may be facilitated by PAR in centres with inacti-<br>vated oxygen-evolving complexes according to the the quinone acceptors (Vass *et al.* 1999). Damage by UVB may be facilitated by PAR in centres with inactivated oxygen-evolving complexes according to the mechanism of donor-side-induced photoinhibition but UVB may be facilitated by PAR in centres with inactivated oxygen-evolving complexes according to the mechanism of donor-side-induced photoinhibition, but this hypothesis needs further testing vated oxygen-evolving complexes a<br>mechanism of donor-side-induced ph<br>this hypothesis needs further testing.<br>Chilling results in an oxygen-an mechanism of donor-side-induced photoinhibition, but<br>
Uthis hypothesis needs further testing.<br>
Chilling results in an oxygen- and light-dependent

this hypothesis needs further testing.<br>Chilling results in an oxygen- and light-dependent<br>inactivation of photosynthesis. It has been reported that<br>the extent of PS II inhibition by low temperature depends Chilling results in an oxygen- and light-dependent<br>inactivation of photosynthesis. It has been reported that<br>the extent of PS II inhibition by low temperature depends<br>on the photon flux density during chilling and no inhib inactivation of photosynthesis. It has been reported that<br>the extent of PS II inhibition by low temperature depends<br>on the photon flux density during chilling and no inhibi-<br>tion was observed in the dark (Bowles *et al.* the extent of PS II inhibition by low temperature depends<br>on the photon flux density during chilling and no inhibi-<br>tion was observed in the dark (Bowles *et al.* 1983). In this<br>way, chilling may be regarded as low-tempera on the photon flux density during chilling and no inhibition was observed in the dark (Bowles *et al.* 1983). In this way, chilling may be regarded as low-temperature-<br>enhanced photoinhibition (Hetherington *et al.* 1989) tion was observed in the dark (Bowles *et al.* 1983). In this way, chilling may be regarded as low-temperature-<br>enhanced photoinhibition (Hetherington *et al.* 1989). Our<br>results show that although inactive PS II reaction way, chilling may be regarded as low-temperature-<br>enhanced photoinhibition (Hetherington *et al.* 1989). Our<br>results show that although inactive PS II reaction centres<br>accumulated during illumination at low temperature and enhanced photoinhibition (Hetherington *et al.* 1989). Our results show that although inactive PS II reaction centres accumulated during illumination at low temperature and singlet oxygen was produced both occurred to a sm results show that although inactive PS II reaction centres<br>accumulated during illumination at low temperature and<br>singlet oxygen was produced, both occurred to a smaller<br>extent than during PAR at room temperature (figure 3 accumulated during illumination at low temperature and singlet oxygen was produced, both occurred to a smaller extent than during PAR at room temperature (figure  $3c$ ). singlet oxygen was produced, both occurred to a smaller<br>extent than during PAR at room temperature (figure  $3c$ ).<br>This suggests that although chilling can be partially<br>regarded as causing enhancement of photoinhibition by extent than during PAR at room temperature (figure  $3c$ ).<br>This suggests that although chilling can be partially<br>regarded as causing enhancement of photoinhibition by *Phil. Trans. R. Soc. Lond.* B (2000) *Phil. Trans. R. Soc. Lond.* B (2000)

low temperature, this is not the only mechanism of PS II damage by low temperature. Observation of hydroxyl (How temperature, this is not the only mechanism of PS II<br>damage by low temperature. Observation of hydroxyl<br>(Hideg & Björn 1996) and superoxide (Hodgson &<br>Raison 1991) radical production in chilling exposed damage by low temperature. Observation of hydroxyl<br>(Hideg & Björn 1996) and superoxide (Hodgson &<br>Raison 1991) radical production in chilling exposed<br>leaves as well as the retarding effect of various added (Hideg & Björn 1996) and superoxide (Hodgson & Raison 1991) radical production in chilling exposed leaves, as well as the retarding effect of various added antioxidants (Wise & Navlor 1995) supports the role of Raison 1991) radical production in chilling exposed<br>leaves, as well as the retarding effect of various added<br>antioxidants (Wise & Naylor 1995), supports the role of<br>alternative ROS-mediated damage leaves, as well as the retarding effect of various added<br>antioxidants (Wise & Naylor 1995), supports the role of<br>alternative ROS-mediated damage.<br>Contrary to the above three stress conditions, oxidative tioxidants (Wise & Naylor 1995), supports the role of<br>ternative ROS-mediated damage.<br>Contrary to the above three stress conditions, oxidative<br>mage by paraquat originates outside PS II. Paraquat is

damage by paraquat originates outside PS II. Paraquat is Contrary to the above three stress conditions, oxidative<br>damage by paraquat originates outside PS II. Paraquat is<br>univalently reduced by PS I to its cation radical, which<br>rapidly donates electrons to overen producing super damage by paraquat originates outside PS II. Paraquat is<br>univalently reduced by PS I to its cation radical, which<br>rapidly donates electrons to oxygen, producing superoxide<br>radicals (Babbs et al. 1989). Such superoxide prod univalently reduced by PS I to its cation radical, which<br>rapidly donates electrons to oxygen, producing superoxide<br>radicals (Babbs *et al.* 1989). Such superoxide production at<br>PS I exceeds the antioxidant ability of the s rapidly donates electrons to oxygen, producing superoxide radicals (Babbs  $et al.$  1989). Such superoxide production at PS I exceeds the antioxidant ability of the superoxide radicals (Babbs *et al.* 1989). Such superoxide production at PS I exceeds the antioxidant ability of the superoxide dismutase-ascorbate-peroxidase system (for a review, see  $\Delta$ sada 1994) and excess superoxide and other PS I exceeds the antioxidant ability of the superoxide<br>dismutase–ascorbate–peroxidase system (for a review, see<br>Asada 1994) and excess superoxide and other ROS propa-<br>gate oxidative damage to other membrane components dismutase–ascorbate–peroxidase system (for a review, see<br>Asada 1994) and excess superoxide and other ROS propagate oxidative damage to other membrane components.<br>In this way, PS II is also affected and Dl is degraded Asada 1994) and excess superoxide and other ROS propagate oxidative damage to other membrane components.<br>In this way, PS II is also affected and D1 is degraded (Hiden et al. 1998b) but there is practically no inactive gate oxidative damage to other membrane components.<br>In this way, PS II is also affected and Dl is degraded<br>(Hideg *et al.* 1998*b*), but there is practically no inactive

PS II accumulation (figure 3d). Accordingly, singlet oxygen production was also low (figure 3d). Although PS II accumulation (figure  $3d$ ). Accordingly, singlet oxygen production was also low (figure  $3d$ ). Although PAR is necessary for the action of paraquat, damage caused by this internal radical source (the paraquat oxygen production was also low (figure 3d). Although<br>PAR is necessary for the action of paraquat, damage<br>caused by this internal radical source (the paraquat<br>radical) results in almost simultaneous electron transport PAR is necessary for the action of paraquat, damage<br>caused by this internal radical source (the paraquat<br>radical) results in almost simultaneous electron transport<br>inactivation and DI decradation. In this way, functional caused by this internal radical source (the paraquat radical) results in almost simultaneous electron transport inactivation and D1 degradation. In this way, functional PS II impairment is probably the result of general (D inactivation and D1 degradation. In this way, functional inactivation and D1 degradation. In this way, functional<br>PS II impairment is probably the result of general (D1<br>and other) protein and membrane degradation, and<br>acceptor-side-induced photoiphibition does not occur PS II impairment is probably the result of general (1 and other) protein and membrane degradation, and acceptor-side-induced photoinhibition does not occur.<br>It is important to note that inactive PS II reaction d other) protein and membrane degradation, and<br>ceptor-side-induced photoinhibition does not occur.<br>It is important to note that inactive PS II reaction<br>patres are not the only potential sources of singlet over a

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acceptor-side-induced photoinhibition does not occur.<br>It is important to note that inactive PS II reaction<br>centres are not the only potential sources of singlet oxygen.<br>Stress-induced membrane disinteration may lead to fre It is important to note that inactive PS II reaction<br>centres are not the only potential sources of singlet oxygen.<br>Stress-induced membrane disintegration may lead to free<br>chlorophyll formation, which readily reacts with ox centres are not the only potential sources of singlet oxygen.<br>Stress-induced membrane disintegration may lead to free<br>chlorophyll formation, which readily reacts with oxygen to<br>form singlet oxygen (Halliwell 1982). Prolong Stress-induced membrane disintegration may lead to free<br>chlorophyll formation, which readily reacts with oxygen to<br>form singlet oxygen (Halliwell 1982). Prolonged exposure<br>of isolated chloroplasts to photo-oxidative stress chlorophyll formation, which readily reacts with oxygen to<br>form singlet oxygen (Halliwell 1982). Prolonged exposure<br>of isolated chloroplasts to photo-oxidative stress caused<br>linid peroxidation, which was accompanied by sin form singlet oxygen (Halliwell 1982). Prolonged exposure<br>of isolated chloroplasts to photo-oxidative stress caused<br>lipid peroxidation, which was accompanied by singlet<br>oxygen production (Takahama & Nishimura 1975). The of isolated chloroplasts to photo-oxidative stress caused<br>lipid peroxidation, which was accompanied by singlet<br>oxygen production (Takahama & Nishimura 1975). The<br>singlet oxygen production that we observed after prolonged lipid peroxidation, which was accompanied by singlet<br>oxygen production (Takahama & Nishimura 1975). The<br>singlet oxygen production that we observed after prolonged<br>exposure to UVB (for an array of the stress (for the 3d) oxygen production (Takahama & Nishimura 1975). The<br>singlet oxygen production that we observed after prolonged<br>exposure to UVB (figure 3*b*) or paraquat stress (figure 3*d*),<br>and found unrelated to the accumulation of inact singlet oxygen production that we observed after prolonged<br>exposure to UVB (figure  $3b$ ) or paraquat stress (figure  $3d$ ),<br>and found unrelated to the accumulation of inactive PS II centres, may originate from such reactions.

#### **5. CONCLUSIONS**

Besides being the driving force of photoinhibition, PAR **is. CONCLUSIONS**<br>Is essential for damage by low temperature and by para-<br>quat and it may modify the effect of UVB light. Although Besides being the driving force of photoinhibition, PAR<br>is essential for damage by low temperature and by para-<br>quat and it may modify the effect of UVB light. Although<br>experimental conditions for the four studied stresses is essential for damage by low temperature and by para-<br>quat and it may modify the effect of UVB light. Although<br>experimental conditions for the four studied stresses were<br>set to result in the same time-course of PS II ina quat and it may modify the effect of UVB light. Although<br>experimental conditions for the four studied stresses were<br>set to result in the same time-course of PS II inactivation,<br>damage clearly followed different pathways. I experimental conditions for the four studied stresses were<br>set to result in the same time-course of PS II inactivation,<br>damage clearly followed different pathways. Inactive PS II<br>reaction, centres, accumulated, during, pho react to result in the same time-course of PS II inactivation,<br>damage clearly followed different pathways. Inactive PS II<br>reaction centres accumulated during photoinhibition,<br>IIVR irradiation and chilling, while there was damage clearly followed different pathways. Inactive PS II<br>reaction centres accumulated during photoinhibition,<br>UVB irradiation and chilling, while there was little accu-<br>mulation in paraquat stress. Singlet oxygen product reaction centres accumulated during photoinhibition,<br>UVB irradiation and chilling, while there was little accumulation in paraquat stress. Singlet oxygen production<br>correlated well with the growth in the amount of inactive UVB irradiation and chilling, while there was little accumulation in paraquat stress. Singlet oxygen production<br>correlated well with the growth in the amount of inactive<br>PS II reaction centres during photoinhibition and al mulation in paraquat stress. Singlet oxygen production<br>correlated well with the growth in the amount of inactive<br>PS II reaction centres during photoinhibition and also to<br>some extent in chilling. There was no such correlat correlated well with the growth in the amount of inactive PS II reaction centres during photoinhibition and also to some extent in chilling. There was no such correlation in PS II reaction centres during photoinhibition and also to some extent in chilling. There was no such correlation in leaves exposed to UVB irradiation and PAR. Our results show that singlet oxygen production in inactivated some extent in chilling. There was no such correlation in<br>leaves exposed to UVB irradiation and PAR. Our results<br>show that singlet oxygen production in inactivated PS II<br>reaction centres is a unique characteristic of photo leaves exposed to UVB irradiation and PAR. Our results<br>show that singlet oxygen production in inactivated PS II<br>reaction centres is a unique characteristic of photo-<br>inhibition by excess PAR. We found that although PAR show that singlet oxygen production in inactivated PS II<br>reaction centres is a unique characteristic of photo-<br>inhibition by excess PAR. We found that, although PAR<br>may make chilling stress more severe by promoting reaction centres is a unique characteristic of photo-<br>inhibition by excess PAR. We found that, although PAR<br>may make chilling stress more severe by promoting<br>photoinhibition, this was not the exclusive pathway of inhibition by excess PAR. We found that, although PAR<br>may make chilling stress more severe by promoting<br>photoinhibition, this was not the exclusive pathway of<br>oxidative damage at low temperature. Moreover, pether may make chilling stress more severe by promoting<br>photoinhibition, this was not the exclusive pathway of<br>oxidative damage at low temperature. Moreover, neither<br>the accumulation of inactive PS II reaction centres by photoinhibition, this was not the exclusive pathway of oxidative damage at low temperature. Moreover, neither the accumulation of inactive PS II reaction centres by IIVR por photo-oxidative damage of PS II by paraquation oxidative damage at low temperature. Moreover, neither<br>the accumulation of inactive PS II reaction centres by<br>UVB, nor photo-oxidative damage of PS II by paraquat<br>was able to produce the special oxidizing conditions the accumulation of inactive PS II reaction centres by<br>UVB, nor photo-oxidative damage of PS II by paraquat<br>was able to produce the special oxidizing conditions<br>characteristic of accentor-side-induced photoinbibition UVB, nor photo-oxidative damage of PS II by paraqua<br>was able to produce the special oxidizing condition<br>characteristic of acceptor-side-induced photoinhibition.

characteristic of acceptor-side-induced photoinhibition.<br>This work was supported by the Hungarian National Research<br>Foundation OTK A (T-030232) This work was supported by the<br>Foundation OTKA (T-030232).

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