Original Article

Effect of Norflurazon on Responses of Superoxide Dismutase and Catalase in a Standard Maize Inbred Line and Superoxide Dismutase Mutant

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(Received December 19, 2002 ; Accepted March 13, 2003)

We compared the responses of superoxide dismutase (SOD) and catalase (CAT) to a herbicide norflurazon (NF)-induced oxidative stress in leaves and mesocotyls of a standard maize (*Zea mays*) inbred line (W64A) to those of a SOD mutant (A130-1) having a mutation in *Sod1*. The changes in activities of SOD and CAT with NF treatment did not differ between W64A and A130-1. The relative transcript levels of *Sod1* and *Cat1* increased greatly in response to NF in leaves and mesocotyls of the two maize lines. In leaf and mesocotyl tissues, the *Sod3* transcript increased only at a 33 μ M NF in W64A, whereas *Sod3* increased at 33 μ M and 100 μ M in A130-1. The *Sod4A* transcript increased only in mesocotyls of NF-treated A130-1, but the total *Sod4A* transcript level was much higher in W64A than in A130-1. *Cat2* increased in the leaves of NF-treated W64A but decreased significantly in the leaves of A130-1. In mesocotyls, the *Cat2* transcript increased only in A130-1 upon NF treatment. The maize variant with a mutation in *Sod1* demonstrated differences in the induction of *Sod* and *Cat* transcripts from the standard inbred line W64A in response to NF-induced oxidative stress.

Key words: catalase, maize (*Zea mays*), norflurazon, oxidative stress, superoxide dismutase.

INTRODUCTION

The combined action of superoxide dismutase (superoxide oxidoreductase, EC 1.15.1.1; SOD) and catalase (H_2O_2 oxidoreductase, EC 1.11.1.6; CAT) is critical in mitigating the effects of reactive oxygen species (ROS) whose production increases with various oxidative stresses. A crucial role of SOD in protection against oxidative processes has been shown in transgenic tobacco plants expressing either Mn-SOD or Fe-SOD.^{1,2)} In the Rpr 79/4 barley mutant which is not completely lacking catalase, the loss of its peroxisomal catalase (T-CAT) isozyme is evidently harmful, underscoring the protective role of the enzyme in plants.³⁾ The nonezymatic reductants consist of ascorbate, glutathione, α -tocopherol, carotenoids, and phenolic compounds.^{4,5)} Especially, carotenoid-dependent energy dissipation in the light harvesting antennae is thought to play an important protective role by mitigating oxidative damage.

Oxidative damage of plants is exacerbated by herbicides, either by direct involvement in radical production or by inhibition of biosynthetic pathways,^{6,7)} as well as atmospheric

pollutants and heavy metals.⁵⁾ In the present study a potent herbicide norflurazon (NF), which blocks carotenoid biosynthesis by non-competitively binding to phytoene desaturase,⁸⁾ was used. It eliminates important quenchers of the triplet chlorophyll (Chl) and singlet oxygen ($^1\text{O}_2$), thus initiating photooxidative processes. NF-induced oxidative damage in *Cucumis sativus* resulted in a reduction in Chls, carotenoids and CAT, with a concomitant increase in SOD activity.⁹⁾

The SOD comprises a class of metalloproteins that catalyze the disproportionation of the superoxide radical to hydrogen peroxide (H_2O_2) and molecular oxygen. Catalase degrades H_2O_2 to water and molecular oxygen. These enzymes have been found in all aerobic organisms and appear to serve a protective role against the detrimental effects of ROS by scavenging superoxide. The maize system provided an excellent model for determining the role of SOD and CAT in response to NF because of the multiple molecular forms of SOD in the plant.¹⁰⁾ SOD-1 is associated with plastids, SOD-3 with mitochondria, and SOD-2 and SOD-4 with the cytosol. Maize SOD consists of nine distinct isozymes encoded by *Sod1*, *Sod2*, *Sod3.1*, *Sod3.2*, *Sod3.3*, *Sod3.4*, *Sod4*, *Sod4A*, and *Sod5*.¹⁰⁾ Three CAT isozymes in maize are encoded by three genes *Cat1*, *Cat2* and *Cat3*. CAT-1 and CAT-2 are found in peroxisomes and glyoxysomes as well as cytosol.¹¹⁾ CAT-3 is associated with

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mitochondria. The expression of each maize catalase gene is tissue- and developmental stage-dependent.¹¹⁾ Upon imbibition and germination, CAT-1 is initially the only form expressed in the scutellum, but as the embryo grows, *Cat2* is rapidly induced, and by day 5 postimbibition, CAT-2 becomes the predominant isozyme.

To test the overall antioxidant responses of maize (*Zea mays* L.) to oxidative stress by NF, activities of SOD and CAT were investigated in leaves and mesocotyls of the standard maize inbred line W64A. We examined whether a deficiency in photosynthetic pigments by NF influences the isozymes of an H₂O₂-producing enzyme, SOD, and an H₂O₂-scavenging enzyme, CAT, in different subcellular compartments, and, if so, whether the NF-induced expression of these genes has a decisive role in NF-induced oxidative stress response. In addition, gene expression levels of *Sod* and *Cat* were compared between W64A and SOD mutant A130-1 to evaluate whether a mutation in *Sod1* affects responses of SOD and CAT during NF-induced oxidative conditions.

MATERIALS AND METHODS

1. Plant Material and Growth Conditions

Two maize lines (W64A, the standard inbred line; A130-1, SOD variant, which has a *Sod1B* mutation and slow electrophoretic form of SOD-1)¹²⁾ were used in this study. The fast (A) and slow (B) electrophoretic forms of SOD-1 are under the control of a single nuclear gene, designated *Sod1*.¹²⁾ Isozymes SOD-1A and SOD-1B are encoded in the alleles *Sod-1A* and *Sod-1B*, respectively.¹²⁾ Seeds were surface-sterilized in 20% Chlorox (1% sodium hypochlorite), rinsed and incubated in norflurazon [4-chloro-5-(methylamino)-2-(3-trifluoromethyl)phenyl-3(2H)-pyridazinone] (Novartis Inc., USA) solutions at room temperature and darkness for 24 hr and then planted in a soil mix (Bio Plug 2, Hungnong Seeds Inc., Korea). Plants were grown in a controlled environment in a growth chamber under a temperature of 25°C with a 16-hr photoperiod of 150 μ mol photons m⁻²s⁻¹. The leaves and mesocotyls of 12-day postimbibition (dpi) plants were used for experiments.

2. Superoxide Dismutase Assay

The tissues were homogenized in 50 mM HEPES buffer, pH 7.5. Extracts were centrifuged for 10 min at 14,000 $\times g$ and 4°C. The crude supernatant was used for enzyme assay. Total SOD activity in protein samples pooled from each NF treatment was determined using the xanthine oxidase-cytochrome *c* method at pH 10.0.¹³⁾ The assay was performed at 25°C in a 3-ml volume containing 50 mM sodium carbonate buffer (pH 10.0), 0.1 mM EDTA, 0.015 mM ferricytochrome *c*, and 0.05 mM xanthine. Enzyme activity (units/mg protein) is proportional to (V/v-1)/mg protein, where V and v equal the change in absorbance (550 nm) per minute (unit) in the absence and presence of SOD, respectively. Protein concentration was determined according to

Lowry *et al.*¹⁴⁾

For SOD analysis, equal amounts of protein were subjected to 10% non-denaturing polyacrylamide gels at 4°C for 1.5 hr with a constant current of 30 mA. After completion of electrophoresis the gels were stained for the enzymatic activities. Gels were stained for SOD isoforms by soaking in 50 mM potassium phosphate (pH 7.8) containing 2.5 mM nitroblue tetrazolium in darkness for 25 min, followed by soaking in 50 mM potassium phosphate (pH 7.8) containing 28 mM tetramethylethylenediamine and 28 μ M riboflavin in darkness for 30 min.¹⁵⁾ Gels were then exposed to light for approximately 15 min.

3. Catalase Assay

Total catalase activity was determined spectrophotometrically at 25°C in a 3-ml volume containing 50 mM potassium phosphate buffer (pH 7.0) and 20 mM H₂O₂ and by monitoring H₂O₂ destruction at 240 nm.¹⁶⁾ Total catalase activity is expressed as the change in absorbance per min (unit) per mg of protein.

Equal amounts of protein from plants exposed to different treatments were subjected to 10% non-denaturing polyacrylamide gels at 4°C for 1.5 hr with a constant current of 30 mA. After completion of electrophoresis the gels were stained for the catalase activities. Catalase activity was detected by incubating the gels in 3.27 mM H₂O₂ for 25 min, rinsing them in water, and staining them in a solution of 1% potassium ferricyanide and 1% ferric chloride for 4 min.¹⁷⁾

4. RNA Extraction and Analyses

All samples were frozen in liquid nitrogen and stored at -80°C for analysis. For studying the activation of specific *Cat* and *Sod* genes, total RNA was isolated from the control and NF-treated samples as described.¹⁸⁾ For Northern analysis, total RNA (20 μ g) from each sample was separated in denaturing 1.6% agarose gel and transferred onto nylon membranes (Schleicher and Schuell Nytran Plus). The blots were sequentially hybridized in modified Church buffer,¹⁹⁾ containing 7% SDS, 1 mM EDTA, 0.25 M NaH₂PO₄ and 1% BSA, with ³²P-labeled gene-specific probes for *Cat1*, *Cat2*, *Cat3*,^{20,21)} *Sod1*, *Sod2*, *Sod3*, *Sod4* and *Sod4A*.²²⁻²⁴⁾ After 2-hr prehybridization at 65°C, the prehybridization solution was substituted with hybridization solution. Hybridization proceeded at 65°C overnight, and washes were performed once for 20 min with 2 $\times$ SSC (sodium chloride/sodium citrate) and twice for 30 min with 0.1 $\times$ SSC at 65°C. Blots were finally probed with a DNA fragment from clone pHA2, containing an 18S ribosomal sequence.²⁵⁾

RESULTS

1. Effects of Norflurazon on Superoxide Dismutase in Leaves and Mesocotyls of W64A and SOD Mutant A130-1

Upon NF treatment, inhibition of carotenoid biosynthesis caused a characteristic bleaching of leaves and mesocotyls

of maize. In leaves of W64A, NF treatment significantly increased the staining of chloroplastic Cu/Zn SOD-1 while that of the other SOD isozymes including cytosolic Cu/Zn SOD-2 and SOD-4 and mitochondrial Mn SOD-3 remained constant (Fig. 1). By contrast, there were no change in the staining of any SOD isozymes in mesocotyls (Fig. 1).

The SOD activities were compared in the standard inbred line W64A and SOD variant A130-1 to determine whether A130-1, having a mutation in *Sod1B*, shows differential responses of SOD to NF-induced oxidative stress. In the untreated controls, total SOD activities in leaves and mesocotyls of A130-1 were similar to those of W64A (Table

1). NF significantly increased total SOD activities in leaves of W64A and A130-1, especially at 33 μM , but not in mesocotyls of the two maize lines (Table 1). Detection of the slow electrophoretic form of SOD-1 in A130-1 was not successful in the gel system used. In the untreated controls, the relative level of *Sod1* transcript in mesocotyls of W64A was higher than that in mesocotyls of A130-1 and in leaves of W64A and A130-1 (Fig. 2). The *Sod2*, *Sod3* and *Sod4* levels in leaves and mesocotyls were similar between W64A and A130-1 (Fig. 2). The *Sod4* level was much higher in untreated leaves than in mesocotyls, whereas the *Sod4A* level was higher in mesocotyls. In leaves of W64A and SOD-1 mutant A130-1, *Sod1* and in particular *Sod4* transcripts increased greatly with increasing concentrations of NF (Fig. 2). Relative transcript levels of *Sod2* and *Sod4A* were very low in leaves of W64A and A130-1 at the NF concentrations tested. *Sod3* transcript increased only at 33 μM NF in leaves of W64A, whereas it increased at 33 μM and 100 μM NF in leaves of A130-1. In mesocotyls, *Sod1* increased greatly in response to NF in W64A and A130-1 (Fig. 2). The *Sod4A* transcript increased only in mesocotyls of NF-treated A130-1, but the overall *Sod4A* transcript level was much higher in W64A. The *Sod3* transcript increased in mesocotyls of W64A and A130-1. Expression levels of *Sod2* and *Sod4* in mesocotyls were very low after all the treatments of the two maize lines (Fig. 2).

2. Effects of Norflurazon on Catalase in Leaves and Mesocotyls of W64A and SOD Mutant A130-1

In leaves of W64A, staining of CAT significantly decreased at NF concentrations above 100 μM , whereas in mesocotyls, it was relatively constant (Fig. 3). In the untreated controls, leaves and mesocotyls of W64A and SOD-1 mutant A130-1 did not differ in total CAT activity (Table 2). NF treatment significantly decreased total CAT activity in leaves of both W64A and A130-1 (Table 2). However,

Fig. 1. Effects of norflurazon on superoxide dismutase isozymes in leaves and mesocotyls of the standard maize inbred line W64A. Seeds were incubated with various concentrations of NF for 24 hr and grown in soil for 11 days. Non-denaturing activity gels were run 12 days after imbibition and NF treatment.

Table 1. Effects of norflurazon exposure on superoxide dismutase activity in leaves and mesocotyls of the standard maize inbred line W64A and SOD mutant A130-1

NF concentration (μM)	Superoxide dismutase (units mg^{-1} protein)			
	W64A		A130-1	
	Leaves	Mesocotyl	Leaves	Mesocotyl
0	23.5 $\pm$ 4.3b	40.3 $\pm$ 5.5a	28.0 $\pm$ 1.9b	40.2 $\pm$ 8.5a
33	55.2 $\pm$ 18.3a	36.6 $\pm$ 5.2a	44.3 $\pm$ 8.7a	44.5 $\pm$ 5.2a
100	42.8 $\pm$ 10.7a	37.5 $\pm$ 5.6a	38.8 $\pm$ 7.0a	45.8 $\pm$ 5.0a

Seeds were incubated with various concentrations of NF for 24 hr and grown in soil for 11 days. Each measurement was performed 12 days after imbibition and NF treatment. Data represent the mean $\pm$ SD of two independent experiments with repetitions. Within each column, means with the same letter are not significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

Fig. 2. Response to norflurazon exposure of the *Sod* genes in leaves and mesocotyls of the standard maize inbred line W64A and SOD mutant A130-1. The plants were subjected to the same treatments as in Fig. 1. Total RNA was extracted, separated on 1.6% agarose gels, at 20 μ g per lane, and transferred to membranes. Probing was done with [α - 32 P]-labeled gene-specific DNA fragments. The 18S rRNA is a loading control.

Fig. 3. Effects of norflurazon on catalase in leaves and mesocotyls of the standard maize inbred line W64A. The plants were subjected to the same treatments as in Fig. 1. Non-denaturing activity gels were run 12 days after imbibition and NF treatment.

in mesocotyls, CAT activity dropped slightly in response to NF (Table 2). Both CAT-1 and CAT-2 are localized in cytosol and glyoxysome, and CAT-3 is mitochondrially associated. In the untreated controls of W64A and A130-1, the *Cat1* transcript level was lower in leaf tissues than in mesocotyls (Fig. 4). The *Cat2* transcript level in the untreated leaves of W64A was lower than that of A130-1. Upon NF treatment, the relative level of *Cat1* transcript increased in a concentration-dependent manner in leaves and

mesocotyls of W64A and SOD-1 mutant A130-1 (Fig. 4). The *Cat1* transcript level was much higher in mesocotyls than leaves after the NF treatments of the two maize lines. *Cat2* increased in leaves of NF-treated W64A, but a significant decline was detected in leaves of NF-treated A130-1 (Fig. 4). In mesocotyls, the *Cat2* transcript increased only in mesocotyls of A130-1 treated with 33 μ M NF. *Cat3* decreased greatly in response to NF in mesocotyls of W64A and A130-1 (Fig. 4).

DISCUSSION

The treatment with norflurazon drastically reduces antioxidant carotenoids, which can effectively quench the excited triplet state of Chl and $^1\text{O}_2$,²⁶⁾ causes photooxidative stress in the bleached plants, and thus may trigger other components of the dissipative process. Upon NF treatment, inhibition of carotenoid biosynthesis caused a characteristic bleaching of leaves and mesocotyls of maize. The changes in activities of SOD and CAT induced by NF were not different between W64A and A130-1 (Tables 1 and 2), whereas the two maize lines exhibited different transcript levels of *Sod* and *Cat* (Figs. 2 and 4).

Chloroplasts are exposed to significant fluxes of ROS, and the ROS must be scavenged by effective antioxidant defenses, including both enzymatic and nonenzymatic antioxidant systems. A significant increase in SOD activity has been observed not only in NF-treated maize (Fig. 1), but also in response to treatment with the herbicides paraquat,

Table 2. Effects of norflurazon exposure on catalase activity of leaves and mesocotyls in the standard maize inbred line W64A and SOD mutant A130-1

NF concentration (μM)	Catalase (units mg^{-1} protein)			
	W64A		A130-1	
	Leaves	Mesocotyl	Leaves	Mesocotyl
0	$0.244 \pm 0.051\text{a}$	$0.218 \pm 0.052\text{a}$	$0.264 \pm 0.048\text{a}$	$0.196 \pm 0.034\text{a}$
33	$0.182 \pm 0.015\text{b}$	$0.165 \pm 0.029\text{b}$	$0.159 \pm 0.038\text{b}$	$0.200 \pm 0.027\text{a}$
100	$0.128 \pm 0.044\text{c}$	$0.167 \pm 0.035\text{b}$	$0.145 \pm 0.071\text{b}$	$0.178 \pm 0.032\text{a}$

Seeds were incubated with various concentrations of NF for 24 hr and grown in soil for 11 days. Each measurement was performed 12 days after imbibition and NF treatment. Data represent the mean $\pm$ SD of two independent experiments with repetitions. Within each column, means with the same letter are not significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

Fig. 4. Response to norflurazon exposure of the *Cat* genes in leaves and mesocotyls of the standard maize inbred line W64A and SOD mutant A130-1. The plants were subjected to the same treatments as in Fig. 1. Total RNA was extracted, separated on 1.6% agarose gels, at 20 μg per lane, and transferred to membranes. Probing was done with [α - ^{32}P]-labeled gene-specific DNA fragments. The 18S rRNA is a loading control.

diquat, diuron, and atrazine.^{27,28)} Unlike leaf tissues, mesocotyls did not show any change in staining activity of SOD in response to NF, suggesting that there is less photooxidative stress in the mesocotyl tissues (Fig. 1). Particularly, the leaves of NF-treated W64A were capable of increasing chloroplastic Cu/Zn SOD-1 (Fig. 1), suggesting that enzymatic removal of ROS by SOD-1 plays a dominant role in NF-induced enzymatic scavenging compared to the other SOD isozymes localized in the cytosol and mitochondria. The induction of individual SOD isozymes by NF was interpreted as a response to augmented ROS generation. Detection of the slow electrophoretic form of SOD-1 was not successful and total SOD activity was similar between W64A and A130-1 in response to NF (Table 1). However, differential responses of *Sod* genes between the two maize lines were observed upon treatment with NF, with increases

of *Sod3* and *Sod4A* only in A130-1 (Fig. 2). *Sod1* increased greatly with increasing concentrations of NF in leaves and mesocotyls of both W64A and A130-1 (Fig. 2), indicating the SOD-1 mutation does not affect the response of *Sod1* to NF.

Catalase takes part in an efficient protective action against oxidative stress.²⁹⁾ A significant decline in staining of CAT was observed in the leaves of W64A, not in mesocotyls, in response to NF (Fig. 3), consistent with reports of CAT inactivation in leaves exposed to high light or NF.^{9,30,31)} Total CAT activities in W64A were similar to those of A130-1 in leaves and mesocotyls of 12 dpi maize plants in the NF treatments (Table 2). The expression of the *Cat1* gene is low in almost all tissues and light-independent throughout development, and cytosolic CAT-1 may predominantly function as the base-level scavenger of H_2O_2 in

plant cells.³²⁾ The relative level of *Cat1* increased significantly in a NF concentration-dependent manner in leaves and mesocotyls of W64A and A130-1 (Fig. 4). The increase of *Cat1* appears to be dependent on excessive light energy caused by a lack of photoprotectant carotenoids. *Cat2*, which was rapidly induced with greening of the leaf tissue in the light,^{32,33)} increased in leaves of NF-treated W64A, whereas it decreased in leaves of NF-treated A130-1 (Fig. 4). *Cat3* decreased prominently in leaves and mesocotyls of the two maize lines at 33 and 100 μ M NF, except an increase of *Cat3* in leaves of A130-1 treated with 33 μ M NF (Fig. 4). This decline in *Cat3* transcript levels might arise either from reduced mitochondrial respiration, dependent on the metabolite decrease due to photooxidative stress, or from an inactivation of photosynthetic genes. The *Lhcb1* transcript was completely absent in the cells of NF-treated barley leaves.³⁴⁾

In maize, NF-induced oxidative stress is partially detoxified via enzymatic antioxidants. The marked accumulation of SOD-1 isozyme and *Sod1* transcript in leaves of NF-treated maize indicates that chloroplasts are greatly influenced via a decline in photosynthetic pigments, compared to other subcellular compartments. The requirement for effective CAT and SOD systems seems to be alleviated in mesocotyls of NF-treated maize, as indicated by less of an alteration in SOD and CAT activities than leaves. The *Cat1* responded dramatically to NF-induced photooxidative stress in the leaves and mesocotyls of both W64A and A130-1 lines. However, the maize mutant A130-1 with a slow electrophoretic form of SOD-1 exhibited a differential induction at other *Sod* and *Cat* transcript levels compared to the standard inbred line W64A in response to NF-induced oxidative stress and those responses appeared to be tissue-specific.

ACKNOWLEDGMENTS

This research was supported by the Korean Science and Engineering Foundation (KOSEF).

REFERENCES

- 1) L. Slooten, K. Capiou, W. Van Camp, M. Van Montagu, C. Sybesma and D. Inzé: *Plant Physiol.* **107**, 737-750 (1995).
- 2) W. Van Camp, K. Capiou, M. Van Montagu, D. Inzé and L. Slooten: *Plant Physiol.* **112**, 1703-1714 (1996).
- 3) A. Acevedo, R. W. Skadsen and J. G. Scandalios: *Physiol. Plant.* **96**, 369-374 (1996).
- 4) R. G. Alscher and J. L. Hess: "Antioxidant in Higher Plants," CRC Press, Boca Raton, 1993.
- 5) C. H. Foyer, M. Lelandais and K. J. Kunert: *Physiol. Plant.* **92**, 696-717 (1994).
- 6) E. F. Elstner, G. A. Wagner and W. Schutz: *Curr. Top. Plant Biochem. Physiol.* **7**, 159-187 (1988).
- 7) T. A. Ezhova, O. P. Soldatova, U. N. Ondar, L. B. Mamanova, N. L. Radyukina, A. V. Sof'in, V. I. Romanov and S. V. Shestakov: *Russ. J. Plant Physiol.* **44**, 575-579 (1997).
- 8) P. M. Bramley: "Carotenoids in Photosynthesis," ed. by A. Young and G. Britton, Chapman and Hall, London, pp. 127-159, 1993.
- 9) S. Jung, J. S. Kim, K. Y. Cho, G. S. Tae and B. G. Kang: *Plant Sci.* **153**, 145-154 (2000).
- 10) J. G. Scandalios: "Oxidative Stress and the Molecular Biology of Antioxidant Defenses," ed. by J. G. Scandalios, Cold Spring Harbor Laboratory Press, Plainview, pp. 527-568, 1997.
- 11) J. G. Scandalios, L. Guan and A. N. Polidoros: "Oxidative Stress and the Molecular Biology of Antioxidant Defenses," ed. by J. G. Scandalios, Cold Spring Harbor Laboratory Press, Plainview, pp. 343-406, 1997.
- 12) J. A. Baum and J. G. Scandalios: *J. Heredity* **73**, 95-100 (1982).
- 13) J. A. Baum and J. G. Scandalios: *Arch. Biochem. Biophys.* **206**, 249-264 (1981).
- 14) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall: *J. Biol. Chem.* **193**, 265-275 (1951).
- 15) M. V. Rao, G. Paliyath and D. P. Ormrod: *Plant Physiol.* **110**, 125-136 (1996).
- 16) P. F. Beers and I. W. Sizer: *J. Biol. Chem.* **195**, 133-138 (1952).
- 17) W. Woodbury, A. K. Spencer and M. A. Stahman: *Anal. Biochem.* **44**, 301-305 (1971).
- 18) W. F. Thompson, M. Everett, N. O. Polans, R. A. Jorgensen and J. D. Palmer: *Planta* **158**, 487-500 (1983).
- 19) G. M. Church and W. Gilbert: *Proc. Natl. Acad. Sci. USA* **81**, 1991-1995 (1984).
- 20) L. A. Bethards, R. W. Skadsen and J. G. Scandalios: *Proc. Natl. Acad. Sci. USA* **84**, 6830-6834 (1987).
- 21) M. G. Redinbaugh, G. J. Wadsworth and J. G. Scandalios: *Biochim. Biophys. Acta* **951**, 104-116 (1988).
- 22) R. E. Cannon, J. A. White and J. G. Scandalios: *Proc. Natl. Acad. Sci. USA* **84**, 179-183 (1987).
- 23) J. A. White and J. G. Scandalios: *Biochim. Biophys. Acta* **951**, 61-70 (1988).
- 24) R. E. Cannon and J. G. Scandalios: *Mol. Gen. Genet.* **219**, 1-8 (1989).
- 25) R. A. Jorgensen, R. E. Cuellar, W. F. Thompson and T. A. Kavanagh: *Plant Mol. Biol.* **8**, 3-12 (1987).
- 26) J. P. Knox and A. D. Dodge: *Phytochemistry* **24**, 889-896 (1985).
- 27) D. B. Harper and B. M. R. Harvey: *Plant Cell Environ.* **1**, 211-215 (1978).
- 28) B. Ye and J. Gressel: *Planta* **211**, 50-61 (2000).
- 29) A. Chaoui, S. Mazhoudi, M. H. Ghorbal and E. E. Ferjani: *Plant Sci.* **127**, 139-147 (1997).
- 30) J. Volk and J. Feierabend: *Plant Cell Environ.* **12**, 701-712 (1989).
- 31) N. P. Mishra, R. K. Mishra and G. S. Singhal: *Plant Physiol.* **102**, 867-872 (1993).
- 32) J. G. Scandalios: "Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants," ed. by C. H. Foyer and P. M. Mullineaux, CRC Press, Boca Raton, pp. 275-314, 1994.
- 33) R. W. Skadsen and J. G. Scandalios: *Proc. Natl. Acad. Sci. USA* **84**, 2785-2789 (1987).
- 34) V. La Rocca, F. Dalla Vecchia, R. Barbato, A. Bonora, E. Bergantino and N. Rascio: *Physiol. Plant.* **109**, 51-57 (2000).