Phyton (Horn, Austria)	**Vol. 35**	Fasc. 1	55–61	28. 7. 1995

Non-uniform Sensitivity to SO_2 within one Variegated Leaf of *Chlorophytum comosum*

By

Ewa NIEWIADOMSKA, Zbigniew MISZALSKI, Jan MORAŃDA*)

With 3 figures and 1 table

Received January 19, 1994

Accepted May 5, 1994

Key words: Free radicals, photosynthesis, SO_2, sulphite, variegated plants

Summary

NIEWIADOMSKA E., MISZALSKI Z. & MORANDA J. 1995. Non-uniform sensitivity to SO_2 within one variegated leaf of *Chlorophytum comosum*. – Phyton (Horn, Austria) 35 (1): 55-61, 3 figures. – English with German summary.

^{35}S-sulphite uptake by plastids isolated from white and green parts of variegated *Chlorophytum comosum* leaves does not differ greatly in spite of considerable higher differences in their sensitivity to SO_2 of the green parts of the leaves. The green parts produce more oxygen radicals and reveal lower SOD activity. Fumigation with SO_2 stimulates SOD activity in both green and white parts of the leaves. In this material sulphite oxidation ability is the highest in green parts in the light.

Zusammenfassung

NIEWIADOMSKA E., MISZALSKI Z. & MORANDA J. 1995. Unterschiedliche Empfindlichkeit gegen SO_2 innerhalb eines gestreiften Blattes von *Chlorophytum comosum*. – Phyton (Horn, Austria) 35 (1): 55–61, 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die ^{35}S-Aufnahme in Plastiden, welche von weißen und grünen Teilen gestreifter Blätter von *Chlorophytum comosum* isoliert wurden, unterscheidet sich nicht stark, obwohl die grünen Teile des Blattes wesentlich empfindlicher gegen SO_2 sind. Die grünen Teile produzieren mehr Sauerstoffradikale und zeigen geringere

*) E. NIEWIADOMSKA, Z. MISZALSKI, J. MORANDA, The F. Górski Department of Plant Physiology, Polish Academy of Sciences, Sławkowska 17, 31-016 Cracow, Poland

SOD-Aktivität. Eine Begasung mit SO_2 stimuliert die SOD-Aktivität sowohl in den grünen als auch in den weißen Teilen der Blätter. In diesem Probenmaterial können die grünen Teile im Licht am besten Sulfit oxidieren.

Introduction

As was shown by FISCHER 1967 in variegated (white and green parts) *Pisonia sp.* leaves there are great differences in sensitivity to SO_2. Also in experiments conducted on *Chlorophytum comosum* it has been established that the green parts of the leaf are more sensitive to oxidative stress caused by O_3 (MISZALSKI 1994).

It is also known that both SO_2 and O_3 stimulate the activity of superoxide dismutase. This enzyme can be used as a stress measure (WINGSLE & al. 1991, SCANDALIOS 1993). Resistance to SO_2 is connected with metabolic activity of the plants and consists, among other things, in the ability to oxidize sulphite (BAXTER & al. 1989).

The aim of the present work was to examine the rate of SO_2 uptake by isolated plastids and the subsequent changes in SOD activity in the white and green parts of the leaf. The second objective was to find out whether these parts differ much in their ability to oxidize sulphite.

Material and Methods

Leaves of 1 year old plants of variegated *Chlorophytum comosum* raised in growth chambers (25/18C: day/night; 12/12 h: 50 mol m^{-2} s^{-1} photosynthetically active radiation (PAR) range; 50% RH) were used for the experiments.

SO_2 fumigation

Fumigation was performed in a light intensity of 450 µmol m^{-2} s^{-1} PAR range, and at a temperature of 25°C and ambient concentration of CO_2 (350 ppm) as described previously (MISZALSKI 1991b). 45 minutes period of adaptation in fumigation chamber was given before fumigation. The plants were fumigated with 80 ppm SO_2 for 45 or 60 minutes. The concentration of SO_2 was determined according to the West-Gaeke rosaniline method (WEST & GAEKE 1956).

Superoxide dismutase(SOD) [E.C.1.15.1.1.] determination

Fresh green or white material (1 g) was homogenized with 0.2 M phosphate buffer (pH 7.8) containing 1.0 mM EDTA before centrifugation at 12000 g. The homogenate was dialyzed at about 5° C for 5.5 h against 0.2 M phosphate buffer pH 7.8. The amount of dismutated oxygen radicals was measured using buffer containing 0.01 mM xanthine, 0.1 U ml^{-1} xanthine oxidase and 0.5 mM hydroxylamine. The reaction was started with xanthine and after 30 min. in darkness it was stopped with 30 mM sulfanilamide in 5% HCl. N-1-naphtylethylenediamine was added to obtain the final concentration of 0.25 mM. The SOD activity was determined by measuring nitrite formation at 540 nm and comparing it with the standard curve (SOD, Sigma). Protein content was measured according to BRADFORD 1976.

Isolation of plastids

50 g of *Chlorophytum comosum* leaves were homogenized (3 × 2s) in 35 ml of 25 mM HEPES-KOH [N-(2-hydroxyethyl)1-piperazineethanesulfonic acid] pH 7.6 containing 0.45 M saccharose and 10 mM EDTA. The homogenate was filtered and washed according to MISZALSKI & NIEWIADOMSKA 1992.

Oxygen radicals determination ($O_2^{\cdot -}$)

The oxygen radicals level was measured according to ELSTNER & HEUPEL 1976 and MISZALSKI 1991a as a nitrite formation from hydroxylamine. The samples were illuminated for 30 min with 400 μmol m^{-2} s^{-1} (PAR range) on the surface of the vessels or were kept in darkness.

Sulphite oxidation

Sulphite oxidation was measured as described by HUMPHREY & al. 1970, using 5,5'-dithio*bis*-2nitrobenzene-2-oxa-1,3 diazole (DTNB). The samples were treated under light conditions as described above.

^{35}S-sulphite uptake

Sulphite uptake was determined in plastid suspension after 60 minute incubation with 0.1 mM ^{35}S-sulphite (74-296 kBq), in darkness. 23 μl of plastids suspension (containing about 10 μg of protein) was transferred into 450 μl Beckman tubes containing 50 μl 0.4 M sucrose and 200 μl silicon oil AR 200 Walker-Germany) density 1.040 g l^{-1} at 25°. The tubes were centrifuged for 15 s at 12000g, at 25°C. Tips with whole plastids were used to determine the amount of ^{35}S as described by MISZALSKI & ZIEGLER 1989.

Results and Discussion

As can be seen in fig. 1, after fumigating the variegated *Chlorophytum comosum* for 45 min. with 80 ppm SO_2, necroses are visible mostly on the green parts. Similar results were obtained by FISCHER 1967 working on *Pisonia sp.*. From these results it may be concluded that the green parts are more sensitive to SO_2 stress.

The effect of SO_2 should be proportional to the amount penetrating to the cells. As was shown by PFANZ & al. 1987, green plastids are the most important sink and accumulate sulphite very strongly. The ^{35}S-sulphite uptake measurements did not reveal any great differences between plastids isolated from the white and green parts (white and green plastids) of the leaf (fig. 2). However, slightly larger amounts were measured in experiments with plastids from white parts. It would seem that the more severe injury of the green parts of the leaf may not be attributed to a greater SO_2 uptake by the plastids as it might have been expected. The amounts of ^{35}S-sulphite penetrating into the white plastids, observed at pH 5.6 of the medium, were higher than those at pH 8.0. This can be attributed to the

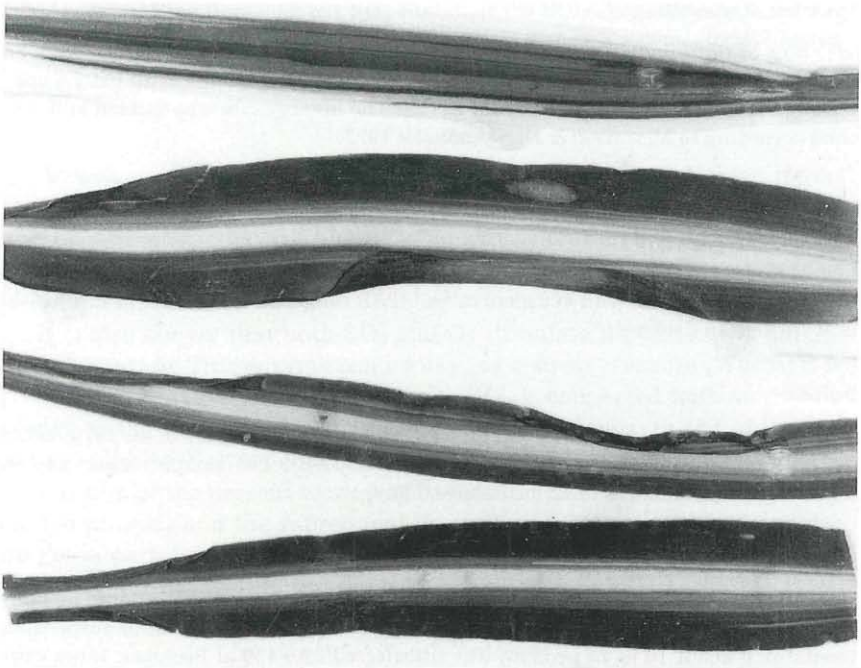

Fig. 1. *Chlorophytum comosum* leaves, 48 h after fumigation with 80 ppm SO_2 for 45 min.

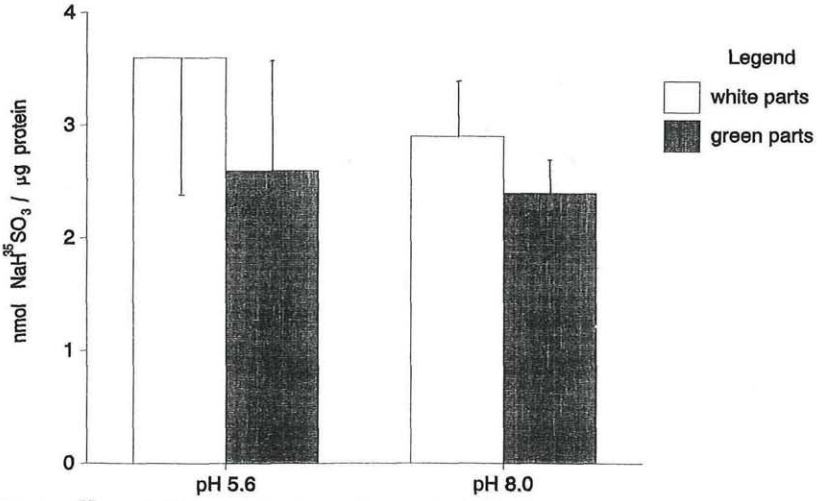

Fig. 2. ^{35}S-sulphite uptake by isolated plastids from white and green parts of *Chlorophytum comosum* leaves after 1h incubation with 0.1 mM sulphite at pH 5.6 or 8.0. Results are the averages of 4 independent experiments. Vertical bars represent standard deviation.

larger pH gradient between plastids and medium, as supposed by LAISK & al. 1988.

SO_2 can stimulate the activity of SOD in green cells (TANAKA & SUGAHARA 1980). This enzyme was also used in the present experiments to estimate the effect of SO_2 in *Chlorophytum comosum* leaves. SOD activity (expressed in SOD units per µg protein) is higher in the white parts of the leaf (fig 3., dialyzed samples). Its activity is stimulated by fumigation in

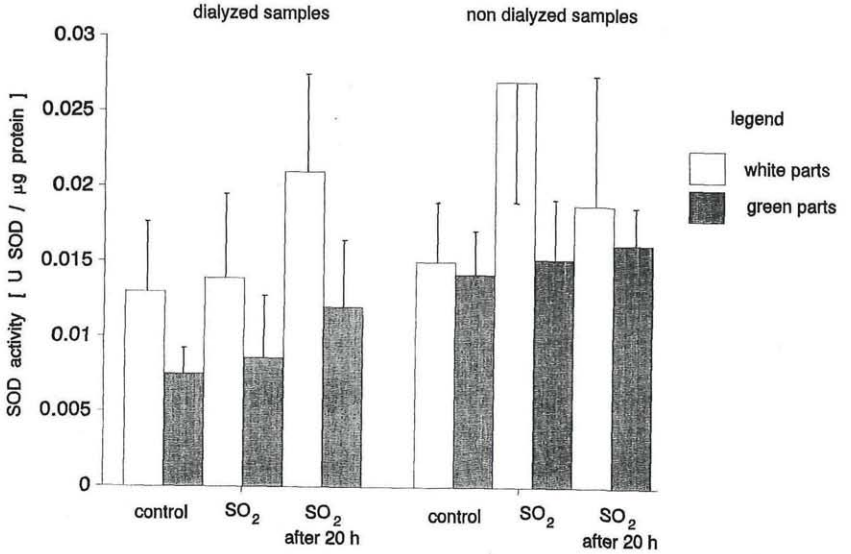

Fig. 3. The ability of scavenging $O_2^{\cdot-}$ (expressed in SOD activity) in dialyzed and nondialyzed homogenates of green and white tissues of *Chlorophytum comosum* measured directly and 20 h after fumigation with 80 ppm SO_2 for 60 min. Results are the averages of 5 independent experiments. Vertical bars represent standard deviation.

both green and white parts. The increase in SOD activity was observed immediately after fumigation and also 20h afterwards. This means that such a process develops during the short-term fumigation and is stable for at least 1 day. The increase in SOD activity occurs also in white parts and is not necessarily connected with the photosynthetic function. Similar changes were noticed in experiments in which non-dialyzed homogenates were used (fig 3.). Thus enhancement of SOD activity is accompanied by an increase in nonenzymatic free radicals scavenging substances, but only in the white parts and directly after fumigation, whereas in the green ones only an increase in SOD activity was observed. The higher SOD activity of the white parts may be due at least in part to the lower protein content in

Table 1

The production of $O_2^{\cdot-}$ in light (400 μmol m^{-2} s^{-1} PAR) and in darkness, during 30 min, in plastids isolated from white and green parts of *Chlorophytum comosum* leaves. Results are the averages of 7 independent experiments. Sulphite oxidation intensity (initial sulphite concentration 0.2 mM) in light (400 μmol m^{-2} s^{-1}) and in darkness, during 30 minutes, in plastids isolated from green and white parts of *Chlorophytum comosum* leaves. Results are the averages of 9 independent experiments. The signs – * – show the relative significances from the green control in light as revealed by the Mann-Whitney's test, where $p < 0.05$. The signs ** – show the relative significances from the green control in darkness.

	sulphite oxidation [μmol oxidized sulphite mg^{-1} protein]		$O_2^{\cdot-}$ production [μmol mg^{-1} protein]	
	light	darkness	light	darkness
green parts	0.831	0.226*	20.51	3.39*
white parts	0.251*	0.125	11.33*	10.21**

those samples, which amounts to 117.3 mg g^{-1} DW for green and 34.9 mg g^{-1} DW for white parts.

The highest oxygen radical ($O_2^{\cdot-}$) production occurs in green plastids in light (tab. 1), while in white parts this is much lower and does not change very much owing to illumination. One may suspect that oxygen radicals formation in green parts is connected with the electron transport chain in chloroplasts and in white parts it depends on mitochondrial activity. Fumigation with SO_2 can generate $SO_3^{\cdot-}$ (sulphite radical) formation in reaction with oxygen radicals. The production of oxygen radicals may contribute in developing stress. This can cause deleterious effects in the tissue.

The sulphite oxidation is much faster in light in the green plastids than in the white ones (tab. 1). One supposes that sulphite oxidation occurs through a reaction with oxygen radicals formed in light or with their dismutation products (MISZALSKI & ZIEGLER 1992). This reaction can be very intense, especially in green parts where SOD activity is low. Probably, the observed increase in SOD activity (in green parts) does not protect the cells against free radicals generated owing to sulphite. Our results does not correspond to TANAKA & SUGAHARA 1980, and BAXTER & al. 1989. In our experiments it has been shown in the present experiments that sulphite oxidation abilities do not relate to plant resistance against SO_2.

Acknowledgements

This work was supported by The Polish Research Grant Nr. KBN 6P205 016 04. We wish to thank Prof. H. ZIEGLER (Munich) for suggesting the experimental approach.

Literature

BAXTER R., EMES H. J. & LEE J. A. 1989. The relationship between extracellular metal accumulation and bisulfite tolerance in *Sphagnum cuspidatum* Hoffm.. – New Phytol. 111: 463–472.

BRADFORD A. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Anal. Biochem. 72: 248–255.

ELSTNER E. F. & HEUPEL A. 1976. Inhibition of nitrate formation from hydroxylammoniumchloride. A simple assay for superoxide dismutase. – Anal. Biochem. 70: 616–620.

FISCHER K. 1967. Cytologische und physiologische Wirkungen von Schwefeldioxyd auf höhere Pflanzen. – Dissertation. T.H. Darmstadt, Germany.

HUMPHREY R. E., MELBOURNE W. & HINZE W. 1970. Spectrophotometric determination of sulphite with 4,4-dithiopyridine and 5,5'-dithiobis(2nitrobenzoic acid). – Anal. Chem. 42: 698–702.

LAISK A., PFANZ H. & HEBER U. 1988. Sulfur-dioxide fluxes into different cellular compartments of leaves photosynthesizing in a polluted atmosphere. – Planta 173: 241–252.

MISZALSKI Z. 1991a. Sulphite oxidation in isolated chloroplasts lamellae. – Bull. Pol. Acad. Sci. 39: 221–229.

— 1991b. Influence of DDTC on SO_2 effect on tomato seedling (*Lycopersicon esculentum* Mill.) – Env. Exp. Bot. 31 (1): 125–130.

— 1994. Rapid evolution of stress ethylene only in the photosynthetically-competent tissues of variegated *Chlorophytum*. – Acta Physiol. Plantarum 16(1): 33–37.

— & ZIEGLER H. 1989. Uptake and efflux of ^{35}[S]sulfite by protoplasts and their chloroplasts of oat (*Avena sativa* L.). – Z. Naturforschung 44 c, 509–513.

— & — 1992. Superoxide dismutase and sulphite oxidation. Z. Naturforsch. 47c, 360–364.

— & NIEWIADOMSKA E. 1992. The effect of Fd-NADP$^+$ oxidoreductase on oxidation of SO_2. – Bull. Pol. Acad. Sci. 40 (4): 257–260.

PFANZ H., MARTINOIA E., LANGE O. L. & HEBER U. 1987. Mesophyll resistances to SO_2 fluxes into leaves. – Plant Physiol. 85: 922–977.

SCANDALIOS J. G. 1993. Oxygen stress and superoxide dismutases. – Plant Physiol. 101: 7–12.

TANAKA K. & SUGAHARA K. 1980. Role of superoxide dismutase in defence against SO_2 toxicity and an increase in superoxide dismutase activity with SO_2 fumigation. – Plant Cell Physiol. 21: 601–611.

WEST P. W. & GAEKE G. C. 1956. Fixation of sulphur dioxide as sulfitomercurate II and subsequent colorimetric estimation. – Anal. Chem. 28: 1816–1819.

WINGSLE G., GARDESTRÖM P., HÄLLGREN J-E. & KARPIŃSKI S. 1991. Isolation, purification, and subcellular localization of isozymes of superoxide dismutase from Scots pine (*Pinus sylvestris* L.) needles. – Plant Physiol. 95: 21–28.