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Influence of SO_2 on changes in the content of proline and hydroxyproline in the leaves of rooted *Weigela* cuttings

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Abstract

The effect of sulphur dioxide on the level of free proline, protein-bound proline and hydroxyproline in the leaves of rooted cuttings from the genus *Weigela* differing in sensitivity to this gas was investigated. It was found that SO_2 causes an increase in the level of free proline and a decline in the level of bound proline and hydroxyproline. These changes are greater in the more susceptible plants than in the more tolerant ones.

Key words: amino acids, susceptibility, air pollution.

INTRODUCTION

In the leaves of several plants the action of sulphur dioxide causes an increase in the content of free amino acids (Godzik and Linskens 1974, Malhotra and Sarkar 1979). This increase following SO_2 treatment could be the result of protein hydrolysis (Malhotra and Sarkar 1979) or a reduction in protein synthesis (Mudd 1979).

The action of SO_2 on plants causes a release of large quantities of toxic ammonia from them (Godzik and Linskens 1974, Jäger and Klein 1977). As in the case with plants that have a water deficit, the NH_3 formed may be neutralized by its incorporation into synthesized amino acids. The increase in the level of free amino acids, particularily of proline in plants under a water stress has been observed among others by Naylor (1972) and Britikov (1975). These authors believe that this amino acid may perform some protective role in plants endangered by environmental stresses.

Ethylene is released in plants under the influence of SO_2 (Guttenberger et al. 1978, Mudd 1979). Studies conducted by Ridge and

Osborne (1971) indicate that ethylene, a natural hormonal inhibitor of cell growth, causes a reduction in the elasticity of cell walls. These phenomena are explained by these authors as the influence of ethylene on increased hydroxylation of proline bound into peptides. The resultant hydroxyproline, which is associated with the proteins of cell walls conditions the changes in the maturation of tissues and in the termination of cell growth. Cell walls particularily rich in hydroxyproline are found in mature and ageing tissues (Chrispeels et al. 1974).

It was the aim of this study to determine the influence of SO_2 on the changes in free and protein-bound proline and hydroxyproline in leaves during exposition to this gas and during regeneration.

MATERIALS AND METHODS

PLANT MATERIAL

The plant material consisted of 2-year-old rooted green cuttings from the genus *Weigela* (Thunb.), namely: *Weigela florida* A. DC and *Weigela* × Bailey cv. 'Van Houtte'. In order to prevent SO_2 absorption and reaction with soil components the pots with the plants were tightly covered with polythene. Experiments conducted earlier have shown that *Weigela florida* is more sensitive to SO_2 than *W.* × Bailey cv. 'Van Houtte' (Karolewski 1984).

EXPOSITION TO SO_2

The experiments were conducted under controlled laboratory conditions. The plants were exposed to SO_2 using a complex of equipment composed of chambers within a climatized greenhouse coupled with a dosing-measuring system for SO_2 (Białobok et al. 1978).

The plants were placed simultaneously in two chambers one with SO_2 and the other without it (control). The concentration of SO_2 applied was 2 ppm, relative air humidity was 60-70%, illumination 70-80 $W \cdot m^{-2}$ (natural plus artificial light, 6 a.m.-9 p.m.), air temperature 19-23°C and the rate of air exchange in the chamber 15 times $\cdot h^{-1}$. The experiment was performed in June.

CHEMICAL ANALYSES

The plant material was collected for analysis either 1) directly before exposition, 2) after 3, 6, 9 and 12 hours of exposition to SO_2 or 3) after 6, 12, 18, 24, 48, 72, 96 and 120 hours following exposition. Simultaneously,

for the same period of time, leaves of control plants were collected for analysis. The analyses were performed on whole leaves of untreated plants and on those parts of leaves which were visually uninjured following treatment with SO_2. For each *Weigela* variety in one variant of the experiment, 5 g of leaves were collected from two plants.

Leaf samples were homogenized in 100% acetone at a temperature of —15°C. The homogenate was filtered through a Schott filter, the residue was washed with acetone until all the pigments were extracted. The dried residue was used for measuring the levels of bound proline and hydroxyproline. Free proline was determined in water solutions using ninhydrine according to the method described by Bergman and Loxley (1970) after prior evaporation of acetone under vacuum. The absorption of the solutions was determined at 512 nm using a Spekol spectrophotometer (DDR).

Bound proline, after prior hydrolysis of the samples with 12 N HCl for 18 h at a temperature of 107°C and evaporation of HCl under vacuum, was determined identicaly as free proline.

To determine the level of hydroxyproline, use was made of the method of Stegemann and Stalder (1967) based on the colorimetric measurement of absorption by the complex of the amino acid with p-dimethylaminobenzoic aldehyde.

Measurments of the individual amino acid contents were performed trice.

RESULTS

After 12 hours from the moment of termination of a 12 hour exposition of the plants to 2 ppm SO_2 leaves of the less sensitive variety *W.* 'Van Houtte' have had necroses on over 15% of the leaf surface while the more sensitive variety, *W. florida,* had necroses on over 60% of the surface.

Changes in the levels of amino acids in the leaves during exposition to SO_2 as well as after its termination have been presented as the difference (Δ_{SO_2-C}) between the amino acids in treated and control plants (Fig. 1).

In the leaves of plants of the less sensitive variety (*W.* 'Van Houtte') during a 12 hour exposition to this gas, a slight increase in the level of free proline relative to the level of this amino acid in the leaves of control plants resulted (Fig. 1A). The action of SO_2 on the more sensitive variety *(W. florida)* resulted in a rapid increase in the level of free proline until the termination of the exposition time. Ninety hours after transfer of the SO_2 treated plants to an atmosphere free of the gas the

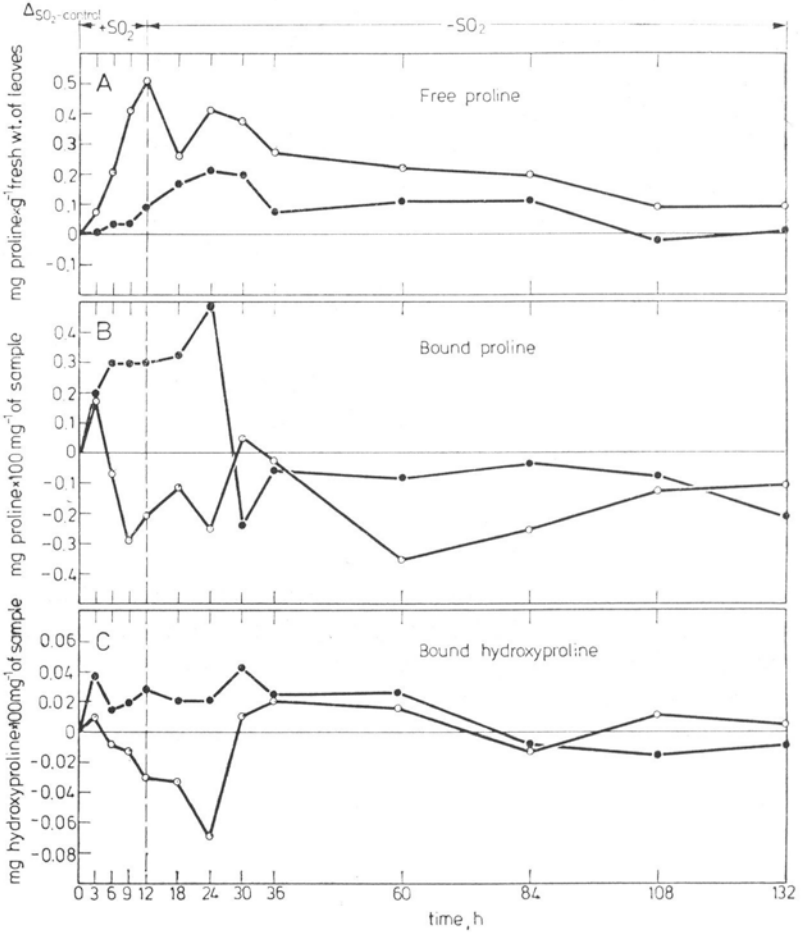

Fig. 1. Changes in the level of proline and hydroxyproline in leaves of *Weigela* 'Von Houtte' (—●—) and *W. florida* (—○—) exposed to SO_2, relative to the control during and after exposition to this gas

level of free proline variety in the leaves of the less sensitive returned to the level of the control. In the more sensitive variety, throughout the observation period the level of this amino acid was higher than in the control plants.

The action of SO_2 in the initial period caused an increase in the level of bound proline in the leaves of plants of both varieties (Fig. 1B). The level of this amino acid in the leaves of variety *W.* 'Van Houtte' continued to increase, while in the leaves of *W. florida* it declined rapidly until the termination of the exposition time. After transfer of the plants to an atmosphere free of SO_2 the content of bound proline in the leaves of plants of both the varieties remained generally at a lower level than in the control plants throughout the observation period.

The content of bound hydroxyproline in the leaves of both varieties during the first period of exposition to SO_2 was on the increase (Fig. 1C). In the case of the less sensitive variety during exposition and for a long period after its termination, it remained at a level higher than in the control. In the leaves of the more sensitive variety, the content of hydroxyproline already declined rapidly during the exposition and then, with some fluctuations, it remained at a level close to that of the control till the end of the observation period.

Compared with the control, the ratio of bound proline to hydroxyproline (PRO:HYPRO) in the leaves of the less sensitive variety was on the increase during exposition to SO_2 and for some time later (Fig. 2). In the case of the more sensitive variety, the ratio fluctuated, however it never exceeded the level for the value reported for the less sensitive variety. After this period a decline was observed in the PRO:HYPRO ratio in the leaves of both varieties to below the value typical for the controls. There was a tendency for the return to a ratio similar to that of the control to be more rapid in the case of the less sensitive variety.

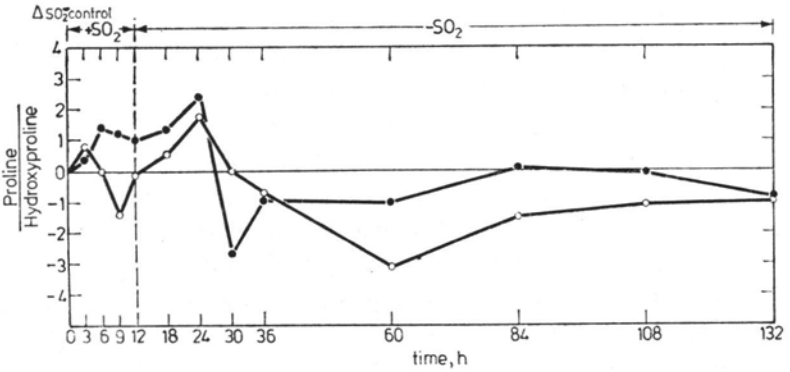

Fig. 2. Changes in the ratio of bound proline to hydroxyproline in the leaves of *Weigela* 'Van Houtte' (—●—) and *W. florida* (—○—) subjected to the action of SO_2, relative to the control during and after exposition to this gas

DISCUSSION

The results presented indicate that in the leaves of *Weigela* rooted cuttings following the action of SO_2, an accumulation of free proline occurs. This accumulation is greater in the case of the more sensitive *W. florida* than in the less sensitive *W.* 'Van Houtte' regardless of the fact that the exposition time to SO_2 was the same.

The phenomenon of accumulation of large quantities of free proline in the leaves of plants is not a specific reaction to the action of toxic gases but also occurs under the influence of other stresses. The accumu-

lation of free proline in the needles of *Picea abies* when water is deficient, following freezing or after action of SO_2 has been observed following single stresses as well as after combined action of several factors when the response is synergistic (Tesche 1979).

Palfi and Juhasz (1968) have shown that when leaves of many cultivated species wilt, free proline accumulates in quantities several times greater than is the normal level.

Studying the influence of SO_2 on seedlings of peas, Jäger and Pahlich (1972) have found an increase in the level of glutamic acid after action with 1 ppm SO_2 for 24 hours. The studies of Pahlich et al. (1972) indicate that as a result of action of SO_2 glutamine synthetase is activated in the direction of reductive amination and it is inactivated in the direction of oxidative desamination:

$$\alpha\text{-ketoglutarate} + NH_3 + H^+ + NADH^+ \underset{\text{inactivation}}{\overset{\text{activation}}{\rightleftharpoons}} \text{glutamate} + H_2O + NAD^+$$

This reaction should also bind the toxic ammonia released as a result of the action of SO_2 on plants (Godzik and Linskens 1974, Jäger and Klein 1977). However, using a higher concentration of SO_2 and a longer exposition period on seedlings of peas. Jäger and Pahlich (1972) have found a substantial increase in the level of glutamine, independent of the magnitude of the concentration and the duration of action of this gas. On the other hand Godzik and Linskens (1974) as well as Malhotra and Sarkar (1979) have observed a reduction in the level of glutamic acid with a parallel increase in the level of glutamine, ornithine, proline, citrulline and γ-aminobutyric acid.

An increase in the level of free proline after action with SO_2 on *Picea abies* has been observed by Lück and Pavlik (1966). The more sulphur has been absorbed by the needles the higher was the content of this amino acid.

Studying the possible pathways of forming free proline when plants wilt, Naylor (1972) has found that the synthesis of proline was associated with an accumulation of sugars and the formation of α-kateglutaric acid when they were oxidized. An increase in the level of mono- and polysaccharides was also observed following the action of SO_2. This was observed by Börtitz (1968) who has shown that under field conditions the action of SO_2 on larch and pine resulted in the complete hydrolysis of starch in the needles of these trees. Mudd (1979) too reports that the action of SO_2 at a concentration of 0.77 ppm on seedlings of beans has caused an increase in the incorporation of ^{14}C into soluble sugars while at the same time the level of labelled starch declined. The increase in the content of sugars may be the result of rapid hydrolysis of starch caused by SO_2 or inhibited synthesis of it.

The results presented in this paper indicate that SO_2 hastens the accumulation of free proline to a greater extent in more sensitive plants than in plants less sensitive to this gas. This leads to changes proportional to the level of accumulation, characteristic for ageing processes in plants, that is, to malfunctions in the water management leading to wilting and death of leaves.

The results obtained indicate that following SO_2 treatment, the reduction in the level of proline and hydroxyproline bound in proteins is related to the degree of plants to this gas. This is most probably caused by a hydrolysis of proteins following exposition to SO_2 (Malhotra and Sarkar 1979).

A rapid decline in the level of proline and hydroxyproline was observed in the leaves of the more sensitive variety *W. florida*. In the less sensitive variety *W.* 'Van Houtte' the level of both these bound amino acids increased, particularily that of proline. This possibly occurs in the cases where proteolysis has not taken place yet, due to free proline, accumulating in the leaves, adjoining to proteins.

In the less sensitive variety during the duration of exposition to SO_2, an increase occured in the level of bound hydroxyproline, while in the more sensitive variety this was true only in the initial period after which a decline in the level of this amino acid was observed. This would suggest that when the injury caused by SO_2 is not severe enough to cause proteolysis, the gas causes an increase in the hydroxylation process of peptide-bound proline, similarily to what is observed in ageing plant tissues (Chrispeels et al. 1974).

The action of SO_2 on plants causes exudation by them of ethylene (Guttenberger et al. 1978, Mudd 1979), a hormonal inhibitor, the release of which characterises ageing of plants (Aharont and Lieberman 1979). According to Ridge and Osborne (1971) ethylene is a factor increasing the cytoplasmic hydroxylation of proline, participating in the enrichment of cell wall proteins in hydroxyproline. This would explain why an increase in hydroxylation following SO_2 treatment was observed.

As can be seen from the results presented in this paper, both the amino acids studied, proline and hydroxyproline, most probably play a significant role in the mechanism of resistance of plants to SO_2. However presently it is difficult to define clearly the role of these amino acids in the response of plants. Sulphur dioxide is a compound, affecting several metabolic processes. Thus, changes in the levels of proline and hydroxyproline taking place under the influence of this gas may take place as a result of the direct action of SO_2 or due to effects on some earlier more sensitive links in the metabolic chain.

The results of this investigation appear to indicate that the differences in the degree of sensitivity of plants may be caused by differences

in the intensity of the course of the ageing processes in cells which have been induced by SO_2.

These results indicate that the level of proline and hydroxyproline in leaves may by one of the indicators useful in the evaluation of the influence of SO_2 on plants.

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Wpływ SO_2 na zmiany zawartości proliny i hydroksyproliny w liściach sadzonek z rodzaju Weigela

Streszczenie

Badano wpływ dwutlenku siarki na zmiany poziomu wolnej proliny i związanych z białkiem proliny i hydroksyproliny w liściach sadzonek z rodzaju *Weigela* różniących się pod względem wrażliwości na ten gaz. Ustalono, że SO_2 powoduje zwiększenie zawartości wolnej proliny i zmniejszenie zawartości obydwu związanych aminokwasów. Stwierdzone zmiany wywołane działaniem SO_2 są większe u roślin bardziej wrażliwych.