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# Effect of water deficit and sulphur dioxide on total soluble proteins, nitrate reductase activity and free proline content in sunflower leaves

K. TANKHA and R.K. GUPTA

*Department of Environmental Sciences, G.B. Pant University of Agriculture and Technology, Pantanagar - 263145, India*

## Abstract

Sunflower (*Helianthus annus* L. cv. PSH-7) plants were subjected to different osmotic potentials, using polyethylene glycol-6000 (PEG-6000), after, prior to and during $SO_2$ fumigation. Total soluble proteins and nitrate reductase activity (NRA) decreased, and free proline content increased with the increasing water stress. These biochemical parameters were more adversely affected in fumigated plants than in non-fumigated ones, when mild water stress was provided prior to and during fumigation. When severe water stress was given prior to and during fumigation, total soluble proteins, NRA and free proline content were nearly the same in fumigated and non-fumigated water-stressed plants; it is because the stomatal closure was observed in water-stressed plants. The leaf water potential decreased with the increasing water stress; however, it was not significantly affected due to $SO_2$ fumigation.

## Introduction

The atmospheric $SO_2$ adversely affects various morphological and physiological characteristics of plants. Though a lot of work has been published on the increase in free proline content in response to various environmental stresses in plants (*e.g.* Levitt 1972, Stewart 1981), no such work seems to be reported in relation to $SO_2$ pollution stress. It is quite likely that $SO_2$ pollution and water deficit interact with each other in plant life. High soil moisture and high relative humidity aggravated $SO_2$ injury in plants (McLaughlin and Taylor 1986).

The present investigation was therefore carried out to ascertain total soluble proteins, NRA and free proline content in sunflower plant leaves which was exposed to various osmotic potentials after, prior to and during $SO_2$ fumigation.

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* to whom correspondence should be addressed

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## Materials and methods

Seeds of sunflower (*Helianthus annus* L. cv. PHS-7), obtained from Plant Breeding Department of this University, were grown in the field. Uniform, three to four weeks old plants were collected from the field. Their roots were washed and immersed in water.

$SO_2$ fumigation was accomplished (Banerjee *et al.* 1980) by transfering plants to fumigation chamber (90 x 70 x 70 cm which was placed in the growth room (temperature 27 ± 2 °C, relative humidity 65 ± 3 %, irradiance 14 W $m^{-2}$). Control plants were kept in chamber that was identical in all respects, except no $SO_2$ was added to the air stream. After 2 h, control and fumigated plants were removed from their respective chambers, their roots were immersed in PEG-6000 solutions of -0.3, -0.5 and -0.75 MPa osmotic potentials (Zur 1966, Kumar and Gupta 1986a, 1986b) or distilled water. After 2 h of exposure to different osmotic potentials the plants (one fumigated and one unfumigated) were employed to measure the leaf water potential using pressure chamber (Boyer 1967, Kumar and Gupta 1986a, 1986b) and stomatal aperture using microscopic technique, and other plants (fumigated and unfumigated) were employed for the determination of total soluble proteins, NRA and free proline content. In another experiment plants were exposed to different osmotic potentials for 2 and 4 h prior to and during fumigation.

Plant leaves were homogenised in phosphate buffer (pH 7.5) and cell debris was removed by centrifugation at 3600 *g*. Protein precipitate was obtained and freed from non-protein impurities (Siminovitch 1986). The precipitate was dissolved in a definite volume of 0.5 M NaOH. The final solution was employed for total soluble protein estimation using the procedure of Lowry *et al.* 1951.

NRA was measured according to the procedure of Hageman and Hucklesby (1971). Plant leaves (1 g) were chopped into small pieces and put into 25 $cm^3$ cold infiltration medium (0.1 M $KNO_3$, 0.15 M potassium phosphate buffer pH: 7.5, 0.45 % butanol). The flask was evacuated twice for 3 min. Further the flask was incubated at 30 °C in dark with gentle shaking for 70 min. 0.2 $cm^3$ aliquot of the medium was removed and then added in test tubes containing 1.8 $cm^3$ distilled water. This was followed by addition of 2 $cm^3$ of 1:1 (v/v) mixture of 0.02 % N-(1-naphthyl) ethylene diamine dihydrochloride and 1 % sulfanilamide in 1.5 N HCl. Absorbance was read at 540 nm and NRA [μmole $g^{-1}$ (fresh mass) $h^{-1}$] was calculated.

Free proline content in plant leaves was determined according to Bates *et al.* (1973).

## Results

**Influence of different $SO_2$ concentrations:** Total soluble proteins and NRA decreased significantly with the increasing $SO_2$ concentration (Fig. 1). At 0.5, 1.0 and 1.5 $cm^3 m^{-3}$ $SO_2$ total soluble proteins was 83.5, 56.8 and 46.2 % and NRA was 85.2, 57.5 and 41.9 % of the control, respectively; free proline content increased markedly with the increasing $SO_2$ concentration; it was 156, 172 and 240 % of the control, respectively.

**Water stress after fumigation**: After 2 h of exposure at, 0, -0.3, -0.5 and -0.75 MPa osmotic potential, the leaf water potential was -0.1, -0.2, -0.3 and -0.5 MPa in both pre-fumigated and unfumigated plants, respectively (Table 1).

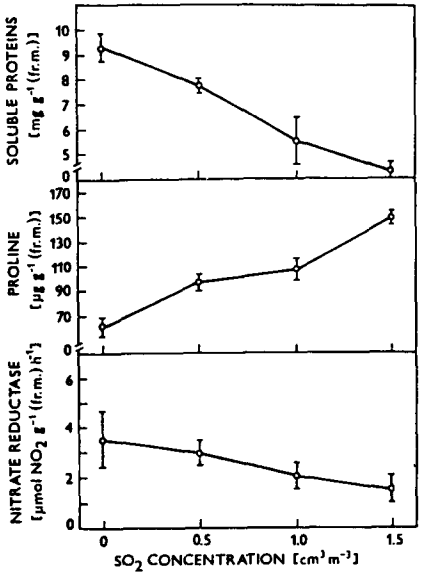


Fig. 1. Influence of different concentration of $SO_2$ on total soluble proteins, NRA and total free proline content in sunflower leaves. Each plotted value represents the mean of two replications. Bars represent the standard error.

Table 1. Leaf water potential [MPa] in sunflower leaves exposed to different osmotic potentials after, prior to and during $SO_2$ fumigation. Each value is the mean of two measurements.

| Treatments | Osmotic potential [MPa] | | | | |
|---|---|---|---|---|---|
| | 0 | -0.30 | -0.50 | -0.75 | -1.00 |
| 2 h water stress, unfumigated plant | -0.10 | -0.20 | -0.30 | -0.50 | - |
| 2 h water stress after fumigation | -0.10 | -0.20 | -0.30 | -0.45 | - |
| 4 h water stress, unfumigated plant | -0.10 | -0.40 | -0.65 | -0.75 | -1.05 |
| 4 h water stress (2 h before and 2 h during fumigation) | -0.10 | -0.35 | -0.60 | -0.75 | - |
| 6 h water stress, unfumigated plant | -0.10 | - | - | -0.95 | -1.20 |
| 6 h water stress (4 h before and 2 h during fumigation) | -0.10 | - | - | -0.90 | -1.25 |

Stomata are opened at all the water stress treatments in both pre-fumigated and unfumigated plants.

Total soluble proteins and NRA decreased, and free proline content increased with decreasing osmotic potential in pre-fumigated and unfumigated plants (Table 2). At all osmotic potentials, plants pre-fumigated with $SO_2$ revealed significantly lower value of soluble proteins and NRA, and significantly higher value of free proline content than the unfumigated plants.

Table 2. Effect of different osmotic potentials (treatment 2 h) on total soluble proteins [μg $cm^{-2}$ (leaf area)], NRA [μmol $NO_2$ $g^{-1}$(fresh mass) $h^{-1}$] and total free proline [μg $cm^{-2}$ (leaf area)] content in pre-fumigated and unfumigated sunflower leaves. Each value is the mean of two replications ± S.E.

| | | Osmotic potential [MPa] | | | |
|---|---|---|---|---|---|
| | | 0 | -0.30 | -0.50 | -0.75 |
| unfumigated plants | soluble proteins | 272.00±10.8 | 205.18±38.1 | 174.20±17.9 | 159.85±12.6 |
| | NRA | 4.69± 0.52 | 3.07± 0.005 | 2.54± 0.52 | 2.10± 0.25 |
| | proline content | 1.35± 0.15 | 1.37± 0.10 | 2.43± 0.03 | 2.43± 0.12 |
| pre-fumigated plants | soluble proteins | 141.43±30.9 | 133.36±22.9 | 132.9 ± 3.6 | 51.18± 2.7 |
| | NRA | 3.64± 0.53 | 2.13± 0.52 | 2.08± 0.43 | 2.02± 0.51 |
| | proline content | 1.54± 0.13 | 2.24± 0.05 | 2.69± 0.29 | 3.21± 0.23 |

**Water stress prior to and during fumigation**: The leaf water potential was generally same at all the osmotic potential treatments in fumigated and unfumigated plants (Table 1). Stomata are almost open even after 4 h of exposure upto -0.5 MPa osmotic potential (partially open at -0.75 MPa) in both fumigated and unfumigated plants; however, it was completely closed after 6 h of exposure at osmotic potential of -0.75 and -1.0 MPa.

Total soluble proteins and NRA decreased, and free proline content increased with the increasing water stress in fumigated and unfumigated plants (Table 3). At all the osmotic potential treatments when 4 h water stress (2 h prior to and 2 h during fumigation) was given to plants, total soluble proteins and NRA markedly decreased, and free proline content increased as compared to unfumigated plants with the same water stress; when water stress was increased to 6 h (4 h prior to and 2 h during fumigation) at various osmotic potential treatments, total soluble proteins, NRA and free proline content were nearly the same in fumigated and unfumigated plants.

## Discussion

The results of the present study show that total soluble proteins and NRA were adversely affected even after 2 h of fumigation at 0.5 $cm^3$ $m^{-3}$ $SO_2$ in sunflower plant (Fig. 1). Similar results have been reported in other plants (Mudd 1975, Chaphakar 1982). The decrease in RNA may reflect inhibition of protein synthesis due to $SO_2$

fumigation. It is interesting to note that free proline content accumulated in response to $SO_2$ fumigation.

Table 3. Total soluble proteins [μg $cm^{-2}$ (leaf area)], NRA [μmol $NO_2$ g $^1$(fresh mass) $h^{-1}$] and total free proline [μg $cm^{-2}$ (leaf area)] content in sunflower leaves exposed to different osmotic potentials prior to and during $SO_2$ fumigation. Each value is the mean of two replications ± S.E.

| | | Osmotic potential [MPa] | | | | |
|---|---|---|---|---|---|---|
| | | 0 | -0.30 | -0.50 | -0.75 | -1.00 |
| 4 h water stress | soluble proteins | 227.7 ±7.18 | 197.5 ±3.5 | 174.2 ±1.8 | 163.4 ±1.8 | - |
| unfumigated | NRA | 4.14±1.05 | 3.64±0.53 | 3.08±1.04 | 2.03±0.52 | - |
| plants | proline content | 0.84±0.05 | 1.83±0.05 | 2.19±0.20 | 2.19±0.10 | - |
| 4 h water stress | soluble proteins | 191.3 ±2.6 | 184.9 ±2.6 | 126.6 ±2.7 | 100.5 ±1.8 | - |
| 2 h prior to and | NRA | 3.07±0.01 | 2.04±0.01 | 1.56±0.48 | 0.54±0.50 | - |
| 2 h during fumig. | proline content | 1.49±0.29 | 2.04±0.05 | 2.48±0.06 | 2.66±0.10 | - |
| 6 h water stress | soluble proteins | 233.3 ±8.2 | - | - | 116.2 ±3.2 | 90.9 ±5.1 |
| unfumigated | NRA | 4.06±0.51 | - | - | 1.29±0.34 | 0.37±0.04 |
| plants | proline content | 0.80±0.03 | - | - | 2.76±0.05 | 3.25±0.10 |
| 6 h water stress | soluble proteins | 133.44±6.2 | - | - | 117.3 ±4.3 | 91.2 ±4.9 |
| 4 h prior to and | NRA | 2.46±0.46 | - | - | 1.27±0.06 | 0.39±0.03 |
| 2 h during fumig. | proline content | 1.06±0.01 | - | - | 2.79±0.10 | 3.08±0.10 |

Water stress in pre-fumigated plants on mild water stress during and after fumigation markedly decreased soluble proteins and NRA, and increased free proline content as compared to unfumigated plants. These results indicate that it has been cleary inconceivable to designate a harmless threshold toxic $SO_2$ concentration for leaves of a particular species since other environmental factors during or following fumigation profoundly affect the degree of damage.

When severe water stress was given prior to and during $SO_2$ fumigation then plant response in terms of soluble proteins, NRA and free proline content was not significantly affected compared to unfumigated water-stressed plants. Stomata are almost closed in water-stressed plants. Association of $SO_2$ damage with stomatal opening has been reported in number of plants (Fitter and Hay 1983). Soil moisture stress prior to $SO_2$ fumigation greatly reduced $SO_2$ visible injury (Krizek and Mirecki 1986). High soil moisture and high relative humidity accelerated $SO_2$ damage in plants (McLaughlin and Taylor 1981, Kender and Forsline 1983).

In nature, specially during summer, plants are frequently exposed in both water deficit and atmospheric $SO_2$ throughout the day time. In such circumstances plants are damaged by $SO_2$ especially in the morning, followed by water stress in the afternoon. In winter and rainy season, plants might be adversely affected by $SO_2$ throughout the whole day time, but not or rarely by water stress.

Applications of antitranspirants might protect the crop plants to reduce uptake of atmospheric $SO_2$.

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