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Anatomical and functional responses of *Calendula officinalis* L. to SO_2 stress as observed at different stages of plant development

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Abstract

Growth responses of field-grown marigold (*Calendula officinalis* L.) plants to SO_2 (0.5, 1.0 and 2.0 ppm) stress were studied at pre-flowering, flowering and post-flowering stages. Low concentrations of SO_2 did not cause any noticeable difference in shoot length, flowers and fruits per plant, and in seeds per fruit, but enhanced leaf number, leaf area and root length. With higher concentrations, however, all these parameters declined. Compared with controls, dry mass of leaves and roots was higher with 0.5 ppm SO_2 but significantly lower with higher doses at each stage. With a high SO_2 dose stomatal pore size increased on adaxial epidermis but decreased on abaxial epidermis. The net photosynthetic rate significantly increased, whereas stomatal conductance and intercellular CO_2 concentration decreased with 0.5 ppm SO_2 treatment, the reverse being the case with higher concentrations. The photosynthetic pigments declined significantly under high SO_2 stress at each stage of plant development, although 0.5 ppm concentration had a stimulatory effect. SO_2 stress delayed the development of interfascicular cambium and altered the proportion of the component tissues in the plant axis. The areas of cortex and vasculature in stems and roots decreased, while pith area increased in the treated plants. The length of fibres and vessel elements increased in the stem and decreased in the root, compared with the control. Vessel density and diameter declined in large vascular bundles and increased in small vascular bundles of the stem. In roots, both the parameters gained under heavy SO_2 stress.

Keywords: *Calendula officinalis*; Chlorophyll; Photosynthesis; Stomatal characterstics; Sulphur dioxide; Vascular tissues

Introduction

In addition to the normal sulphur uptake in the form of sulphate from the soil, plants may receive additional sulphur through leaves from the atmosphere. SO_2, normally 0.05–0.5 ppm in the urban areas and up to 2.0 ppm or more around point sources of air pollution, is the major source of atmospheric sulphur (Khan and Khan, 2000; Khan et al., 2006). SO_2 absorption by shoots may lead to a decrease in sulphate uptake by roots and sulphate transport from roots to shoots (Herschbach, 1992). Leaves experience the maximum brunt of exposure and accordingly undergo structural and functional alterations with changing habitat environment. On reaching the mesophyll through stomata, SO_2 combines with water to form sulphurous acid, which, on dissociation, produces phytotoxic sulphite and bisulphite ions (Rennenberg and Herschbach, 1996; Rennenberg and Polle, 1994). SO_2 can cause foliar

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injury, disturb stomatal behaviour and transpiration, inhibit photosynthesis and reduce the final yield (Dhir et al., 2001; Wali et al., 2004; Yunus and Iqbal, 1996).

Alteration in plant growth patterns often involves changes in anatomical and physiological characteristics, which in turn have a bearing on the quality of plant products. Information is deficient on all these aspects, particularly with reference to medicinal plants. This study on marigold (*Calendula officinalis* L.), a plant of considerable medicinal utility, evaluates plant growth performance, foliar characteristics and the anatomy of root and stem at different stages of plant development under the influence of SO_2 stress.

Materials and methods

Plant description

Marigold (*C. officinalis* L., Asteraceae), is an annual herb with corymbosely branched stem; acute, alternate and hispid leaves; yellow to orange flowers (female ray-flowers and hermaphrodite disc flowers); and long curved achenes (Bisset, 1994; Keville, 1991; Kirtikar and Basu, 1993). Various preparations from this plant are used as antiseptic, diaphoretic, stimulant, antispasmodic and antipyretic agents (Kirtikar and Basu, 1993; Weiner, 1990).

Experimental design

New Delhi, situated at 28.38′N latitude and 77.11′E longitude, at an altitude of 228 m asl, has a semi-arid and subtropical climate with extremes of hot weather in summer and cold weather in winter. The soil at Jamia Hamdard campus, the study site, is a coarse-textured sandy loam with pH around 8.0, EC 0.198 mmhos cm^{-1}, and the nitrogen and sulphur contents are around 10.4 and 2.8 ppm, respectively. Experiments were laid out in plots of 4 m × 3 m, each having three rows of plants maintaining a row-to-row distance of 30 cm and a plant-to-plant distance of 15 cm. Seeds were sown in October, with monthly means of the minimum and maximum temperatures lying around 19 °C and 32 °C, while those of relative humidity around 49% and 80%, respectively. Thirty-day-old seedlings of *C. officinalis* were fumigated for a week, for 1 h daily in the morning, in a specially fabricated 4 m × 3 m × 1 m fumigation chamber with 0.5, 1.0 and 2.0 ppm SO_2 concentrations released from a gas cylinder controlled by a regulator. The untreated plants were maintained as the control. Sampling (10 plants of each treatment) was done at 30 DAF (days after fumigation) (pre-flowering stage), 60 DAF (flowering stage) and 90 DAF (post-flowering stage).

Plant growth and yield

For growth and biomass estimations, plants with intact roots were dug at random from the control as well as the treated plots and washed thoroughly to remove the soil. Leaf area was measured by a Li-3000A Leaf Area Meter (LICOR, Lincoln, USA). For analysing the dry mass, the component plant parts were separated and oven-dried completely at 65 °C. In the last harvesting (post-flowering), treatment effects on yield components such as flower and fruits per plant and seeds per fruit were also assessed.

Stomatal and photosynthetic performance

Epidermal peels, obtained by the method of Ghouse and Yunus (1972) using hot nitric acid, were processed in the customary ethanol series for dehydration and stained with safranin and then mounted in Canada balsam for microscopic study. Stomatal index (SI) was calculated as

$$\mathrm{SI} = \frac{S}{S+E} \times 100,$$

where, S and E represent the number of stomata and epidermal cells per mm^2, respectively (Salisbury, 1927). The chlorophyll and carotenoid contents of fresh leaves were estimated by the method of Hiscox and Israelstam (1979) using dimethylsulphoxide and calculated using the formulae of MacLachlan and Zalik (1963), and Duxbury and Yentsch (1956).

Stomatal conductance, intercellular CO_2 concentration and net photosynthetic rate were measured by clamping the leaf in situ in the leaf chamber (6000-13, quarter litre) of a portable Li-6200 Photosynthesis System (LICOR, Lincoln, USA), which measured the transient exchange rate of water vapour and CO_2 in a closed system. Leaf gas exchange measurements were made on cloud-free days at 8.00–9.00 am.

Microtomy and maceration

The collected samples (roots and stems) were fixed in FAA (formaldehyde: acetic acid: alcohol: 5: 5: 90) for a week, and then preserved either in alcoglycerol mixture (1:1 mixture of 70% ethyl alcohol and glycerol) or in 70% ethyl alcohol only, depending on the hardness of the material. To study the cell size variation in vascular tissues, small tangential slices of the roots and stems were macerated in hot nitric acid (Ghouse and Yunus, 1972). The macerated xylem fibre and vessel elements were measured with an ocular micrometer scale fitted in a compound light microscope. Also, the fixed samples of the root and the third internode of stem, washed with distilled water, were sectioned in transverse plane at a thickness of 10 μm on a Reicherts Sliding Wood Microtome. The sections were dehydrated in a graded ethanol series,

stained with Heidenhain's haematoxylin and safranin/Bismarck brown and mounted in Canada balsam on glass slides. The statistical significance of variations observed was determined by applying the Student's *t* test.

Following the formulae of Patel (1956), the vulnerability ratio was determined by dividing the mean vessel diameter by the mean vessel frequency (number of vessels per mm^2 of xylem), whereas the mesomorphic ratio was obtained by multiplying the vulnerability ratio with the mean length of corresponding vessel elements. Mean length of fibres was divided by mean length of vessel elements to obtain the (F/V) length ratio.

Results

Growth measurements

Compared with the control, the length of roots increased (15–45%) under the influence of 0.5 ppm of

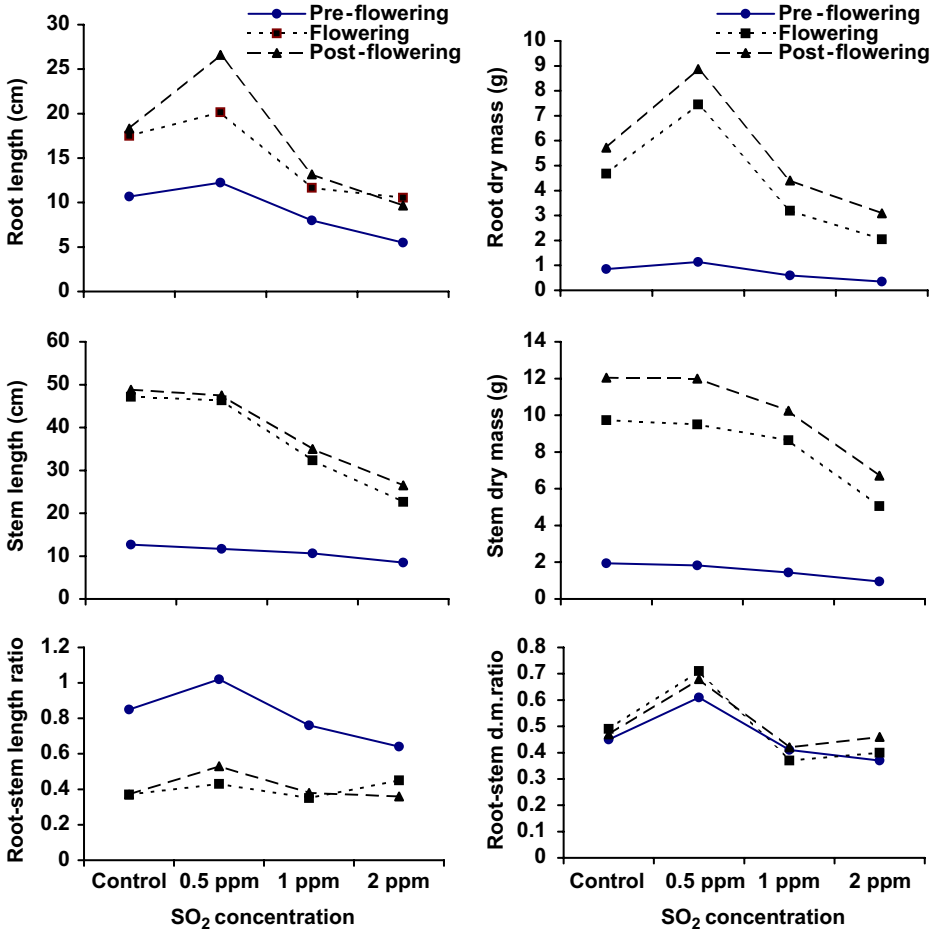

Fig. 1. Changes in length and dry mass of root and stem of *Calendula officinalis* in response to SO_2 treatments, as observed at pre-flowering, flowering and post-flowering stages of plant development. Values are the means of 10 independent readings.

Table 1. Data on flower, fruit and seed production in the control and SO_2-treated plants of *Calendula officinalis*

Parameters	Control	0.5 ppm SO_2	1 ppm SO_2	2 ppm SO_2
Flowers per plant	59.66 ± 6.651	57.00 ± 5.11 (4.45^{NS})	44.33 ± 7.76 (25.69**)	23.60 ± 3.00 (61.45**)
Fruits per plant	35.00 ± 8.88	32.40 ± 4.25 (7.14^{NS})	25.33 ± 5.03 (27.62**)	15.66 ± 2.08 (55.25**)
Seeds per fruit	39.20 ± 4.76	39.00 ± 4.35 (0.005^{NS})	35.80 ± 3.70 (8.67*)	32.80 ± 4.08 (16.32**)

The means ± SD are based on readings from ten plants. Parentheses include per cent variation.
** = Significant at 1% level, * = significant at 5% level, NS = non-significant.

SO_2, but decreased (25–48%) significantly with higher concentrations. For instance, 0.5 ppm treatment showed its maximum effect (45% gain) in the post-flowering stage, 1 ppm did so (33% decline) in the flowering stage, whereas 2 ppm did so (48% decline) in the pre-flowering stage. Root dry mass was significantly increased (34–59%) with low SO_2 doses, but decreased significantly with higher doses. The lower doses (0.5 and 1 ppm) caused the maximum variation (up to 59%) in the flowering stage, while the highest dose did so in pre-flowering stages (Fig. 1). On the other hand, stem length did not vary significantly with the 0.5 ppm dose and was reduced by 16–52% with higher doses. The maximum reduction (31% with 1 ppm and 52% with 2 ppm) occurred during the flowering stage. Stem dry mass in treated plants was always low with respect to the control, but significantly so with higher doses only (Fig. 1). For this parameter, pre-flowering stage was invariably more sensitive than others.

The root-length to stem-length ratio was significantly higher with the 0.5 ppm SO_2, but showed inconsistent and often non-significant variation from the control with the higher SO_2 concentrations. Variation was maximal in the post-flowering (43% increase with 0.5 ppm) and pre-flowering (11% and 25% decline with 1 and 2 ppm) stages. The root–stem dry mass ratio showed little variation with plant age. It was significantly higher (up to 45%) with 0.5 ppm SO_2 and markedly low (up to 25%) with other doses, as compared with the control (Fig. 1).

The number of flowers and fruits per plant and the number of seeds per fruit remained almost unaffected with the lowest SO_2 dose, but declined significantly with the higher ones (Table 1).

The number of leaves per plant increased with plant age. Compared with the control, leaf density was affected by SO_2 treatments, being relatively higher with the 0.5 ppm dose whereas being much smaller with other doses (Fig. 2). Maximum leaf loss (48% with 1 ppm; 61% with 2 ppm) was noticed in the pre-flowering stage. Variation in leaf size at different stages of plant development was not consistent under the influence of 0.5 ppm SO_2 concentration, but the higher doses stimulated leaf expansion to a varied extent. The trend of variation in total leaf area and in leaf dry mass was similar to that in leaf density (Fig. 2). For these parameters, the pre-flowering stage of plant life was most sensitive to high SO_2 levels.

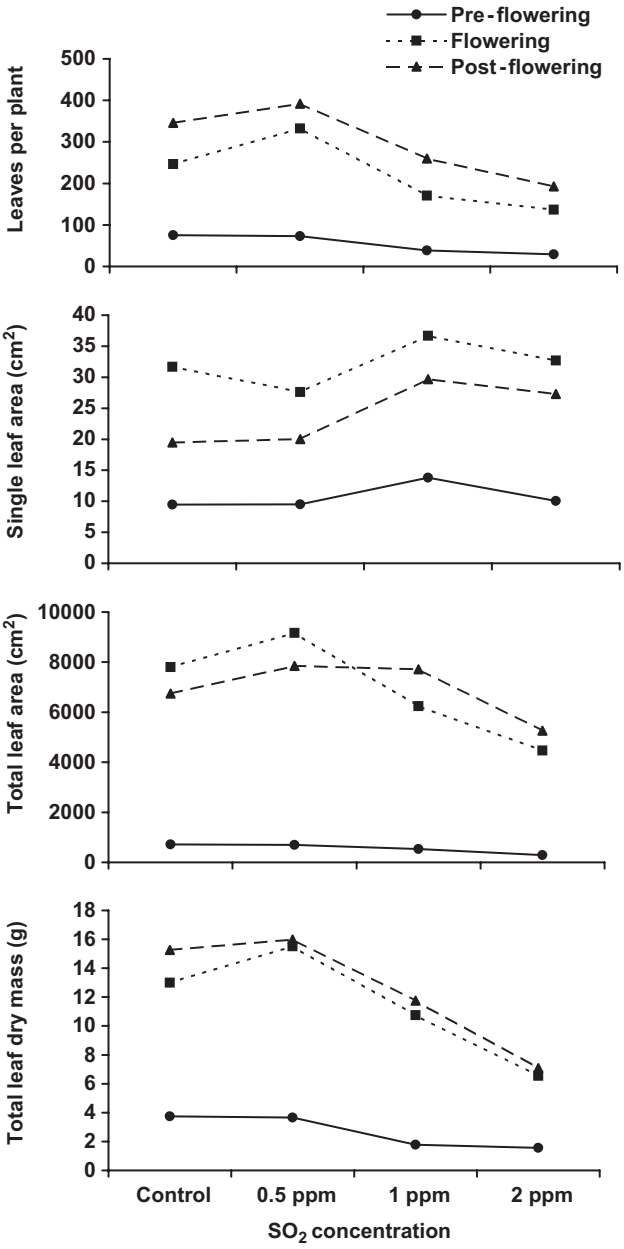

Fig. 2. Changes in size and dry mass of leaves of *Calendula officinalis* in response to SO_2 treatments, as observed at pre-flowering, flowering and post-flowering stages of plant development. Mean values are based on observations from 10 plants of each treatment.

Stomatal features and photosynthetic efficiency

The size of stomatal aperture increased with plant age on both leaf surfaces (Fig. 3), and remained almost unaffected by low SO_2 treatment. However, high concentrations of SO_2, especially 2 ppm, caused apertures to be larger on the adaxial surface and smaller on the abaxial surface of leaf. Stomatal density (SD) on the leaf increased with age of the plant. Exposure to 0.5 ppm SO_2 caused a negligible variation on both epidermises. Under higher stress, SD decreased (up to 29%) on the adaxial epidermis and increased (up to 15%) on the abaxial epidermis, with maximum variation in the post-flowering stage. However, SI on either of the leaf surfaces was little affected by SO_2 treatments (Fig. 3).

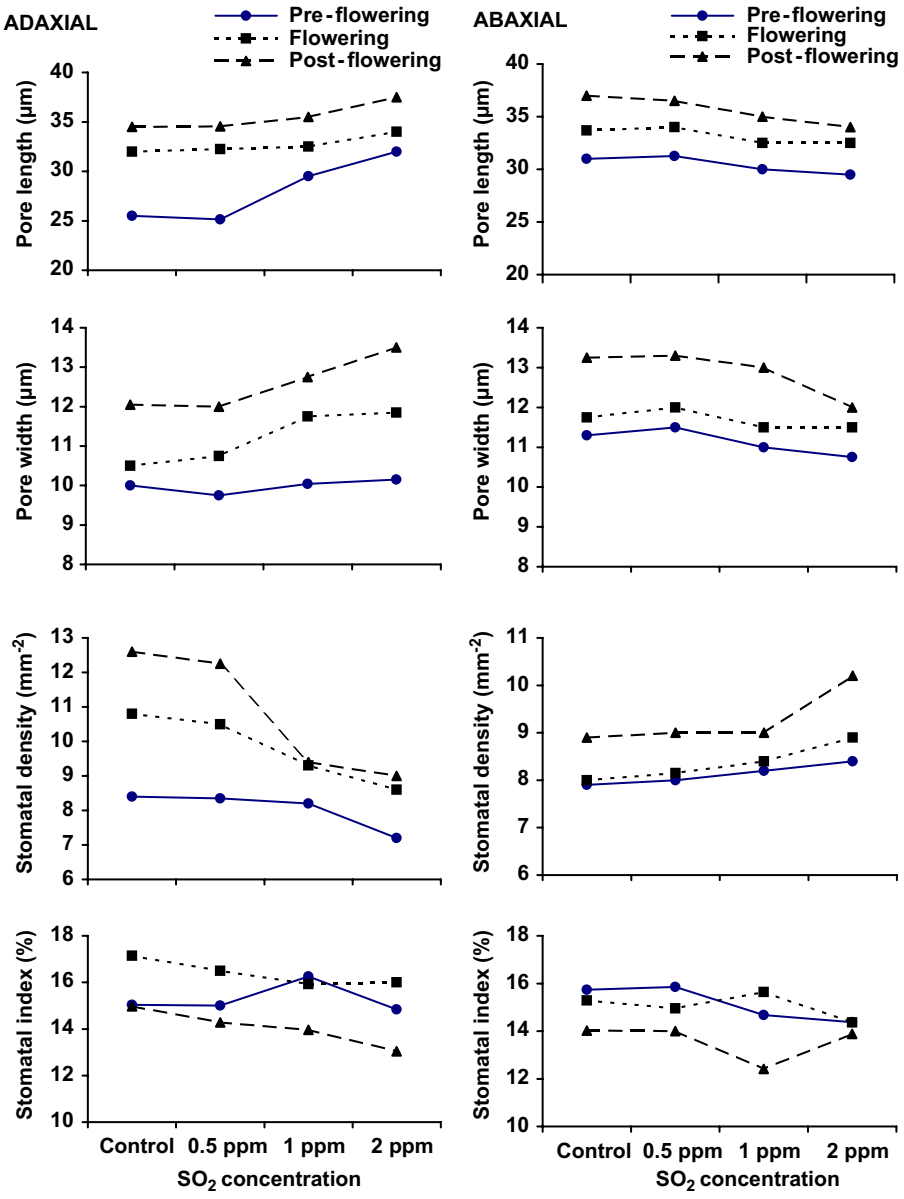

Fig. 3. Changes in dimensions and density of stomata on the adaxial and abaxial surfaces of leaves of *Calendula officinalis* in response to SO_2 treatments, as observed at pre-flowering, flowering and post-flowering stages of plant development. Values are means of 25 readings for each parameter.

Chlorophyll concentration in the leaves of the control as well as the treated plants was maximum at the time of flowering. Low SO_2 concentration (0.5 ppm) had a stimulatory effect, which was most prominent in the pre-flowering stage for chlorophyll *a* and in the post-flowering stage for chlorophyll *b*, but higher SO_2 concentrations were clearly depressive (Fig. 4). The decline in concentration of chlorophyll *a* and chlorophyll *b* under high stress levels was up to 46% and 48% (both with 2 ppm), respectively. Variation in the total chlorophyll concentration reached the maximum (77% increase with 0.5 ppm; 38% decline with 2 ppm) in the post-flowering stage. Carotenoid content also showed a similar variation trend, showing a significant increase with the 0.5 ppm dose and a significant decline with the higher doses, compared with the control (Fig. 4).

As indicated in Fig. 5, stomatal conductance increased with the age of the plant. It was almost unaffected with low SO_2 stress but rose considerably over the control with higher levels of stress (up to 30% increase with 1 ppm and 57% increase with 2 ppm). The maximum variation figured in the post-flowering stage. Intercellular CO_2 concentration also increased with age of the growing plant. The low SO_2 dose had a depressive effect; the maximum (41%) decline was in the flowering stage, whereas high doses elevated the internal CO_2 level

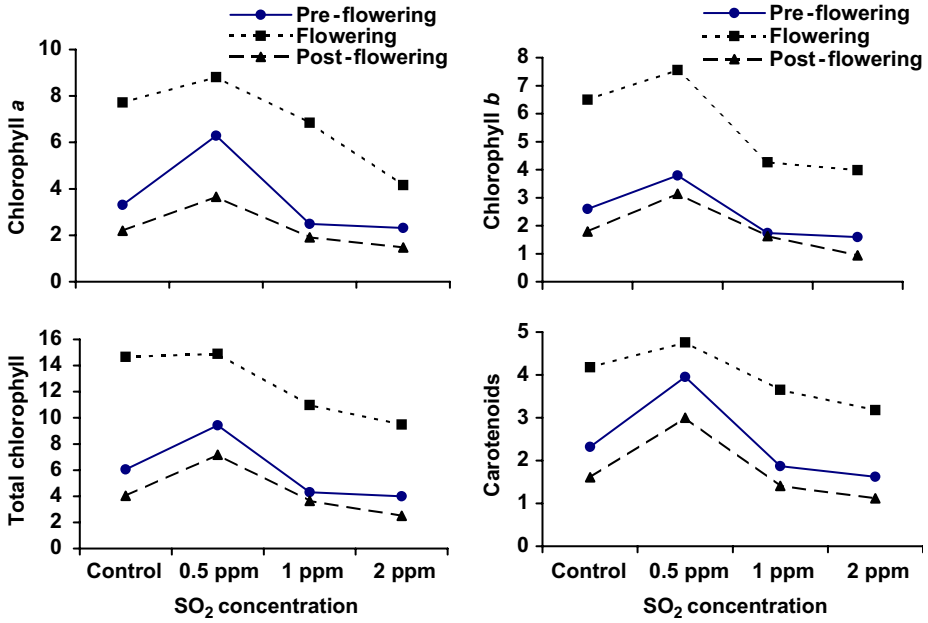

Fig. 4. Changes in the chlorophyll and carotenoid contents (mg g^{-1} dry mass) of the leaves of *Calendula officinalis* in response to SO_2 treatments, as observed at pre-flowering, flowering and post-flowering stages of plant development. Values are means of three replications.

in the leaf at each stage, with higher values at the flowering stage (with 1 ppm) and post-flowering stage (with 2 ppm). Photosynthetic rate was invariably high in the flowering stage. Compared with the control, it was higher under the influence of low SO_2 stress, but significantly lower under high SO_2 stress at each stage of plant growth. The 0.5 ppm dose caused maximum variation (42%) in the flowering stage, whereas higher doses did so (66% with 1 ppm and 82% with 2 ppm) in the post-flowering stage.

Histological variations

Relative proportions of cortex and vasculature in roots increased with plant age, the rate of increase being relatively low under SO_2 stress. The pith area, visible in pre-flowering stage only, was significantly reduced in the treated material (data not presented). Xylem fibres in the roots became longer with increasing plant age, the extent of elongation being low in the SO_2-treated plants. Difference from the control was maximum (21% with 1 ppm and 29% with 2 ppm) in the post-flowering stage. The length of vessel elements, almost constant at all developmental stages, was consistently low under SO_2 stress; the maximum and significant differences figured with 2 ppm treatment (Table 2). Vessel diameter and vessel density increased with plant age as well as with SO_2 stress. Increase in vessel diameter due to plant age or a low SO_2 stress was only marginal. It became conspicuous with 2 ppm concentration and reached the maximum in the post-flowering stage. Similarly, increase in vessel abundance was non-significant with 0.5 and 1 ppm and significant with 2 ppm SO_2 concentration (Table 2).

Development of vascular cambium in the stem was delayed under heavy SO_2 stress. Fig. 6 shows that at a given point of time when interfascicular strips of the cambium had been formed in the control and were developing in plants exposed to 1 ppm SO_2 concentration, their formation was yet to start under stress caused by 2 ppm concentration. The relative proportions of cortex and vasculature in the stem increased, whereas pith area decreased consistently with growing age of the plant. The rate of the respective increase/decrease was relatively low in the treated plants. The effect of SO_2 treatments was, on the whole, inhibitive on cortical and vascular tissues and promotive on the pith (data not presented).

The vascular bundles in the stem, that could be well categorized as large vascular bundles and small vascular bundles, increased in number with the growing plant age. Under high SO_2 stress, the large bundles were less abundant in late stages of plant development. However, the number of small bundles increased markedly under stressful condition, as compared with the control (data not presented). As regards the cell size, fibre length in the stem increased with plant age as well as with SO_2 stress (except with 0.5 ppm where variation from the control was inconspicuous and non-significant). The

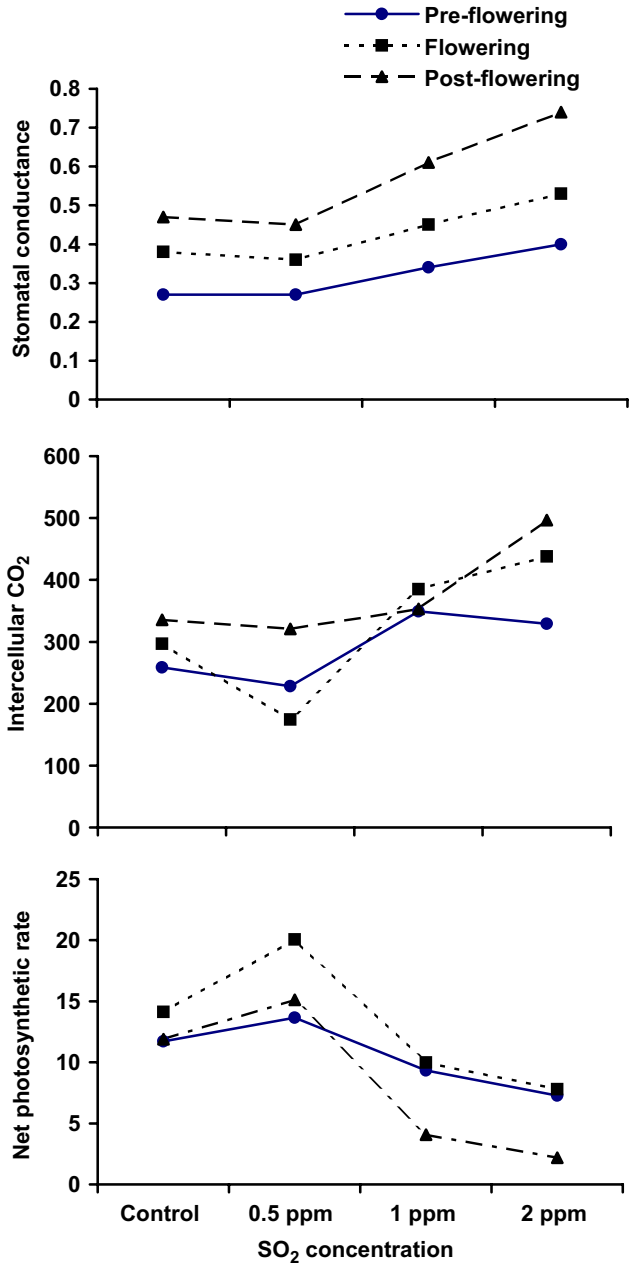

Fig. 5. Changes in stomatal conductance ($mol\,m^{-2}\,s^{-1}$), intercellular CO_2 concentration (ppm) and net photosynthesis rate ($\mu mol\,CO_2\,m^{-2}\,s^{-1}$) of mature leaves of *Calendula officinalis* in response to SO_2 treatments, as observed at pre-flowering, flowering and post-flowering stages of plant development. Values are the means of 10 independent measurements.

same was true for the length of vessel elements; the difference being maximum with 2 ppm, particularly during the post-flowering stage (Table 3). Likewise, the diameter as well as the number of vessel elements increased as the plant grew older. The vessels were generally narrower in the stressed plants than in the control, the variation being maximum (16% with 1 ppm and 22% with 2 ppm) in the post-flowering stage. Variations due to the 0.5 ppm treatment were mostly non-significant. Under SO_2 stress, their abundance was less than in the control till the flowering stage and more than in the control during the subsequent phase of growth, the difference becoming larger with increasing age of the plant (Table 3).

The vulnerability and mesomorphic ratios of vessels were calculated to determine the effect of SO_2 stress on the level of efficiency and ecological correlation of stem wood. These ratios did not exhibit any consistent rise or fall with respect to plant age but did show a slight decline with increasing stress level. Vulnerability ratio in the control was lowest during flowering. It was relatively low in treated plants compared with the control, showing large differences under heavy SO_2 stress. A more or less similar variation was registered by the mesomorphic ratio of the wood (Fig. 6). However, a reverse trend was shown by the ratio of fibre length to vessel-element length (F/V) in relation to plant age and SO_2 stress. It increased under high stress and was the largest at the flowering stage (Fig. 7).

Discussion

The leaves of *C. officinalis* developed chlorosis, necrosis and tip burn when exposed to high SO_2 doses. The injury was more severe just after completion of fumigation; newly formed leaves developed patches of chlorosis that later turned to greyish necrotic spots. SO_2, dissolved in water in the leaf tissues, possibly causes local injury by generating toxic ions (Manninen et al., 1996).

Plant growth efficiency

The results have shown that high SO_2 concentrations inhibited root growth of *C. officinalis*, though a low concentration was stimulatory, as has been reported earlier for *Althea officinalis* (Wali et al., 2004). An improved root system should improve absorption of water and the nutrients dissolved, thus facilitating the overall plant performance. High SO_2 concentrations also reduced stem length significantly, whereas low concentrations did not. SO_2 stress possibly retards the rate of cell division and cell expansion (Chang and Thompson, 1966). The root–stem length ratio in treated plants of the present study was markedly high, thus suggesting a shift in assimilation allocation. Dry mass of both roots and stems increased with 0.5 ppm SO_2, but declined under a heavy stress. This reduction in phytomass could be a consequence of the suppressed photosynthetic activity.

Table 2. Data on xylem fibres and vessel elements in the roots of *Calendula officinalis*, as recorded at different developmental stages of the control and SO_2-treated plants

Parameters	Control	0.5 ppm SO_2	1 ppm SO_2	2 ppm SO_2
Fibre length (μm)				
Pre-flowering	401.60 ± 52.26	397.88 ± 37.78 (0.93^{NS})	392.00 ± 43.03 (2.39^{NS})	352.00 ± 35.31 (12.35**)
Flowering	431.20 ± 62.05	435.00 ± 31.34 (0.88^{NS})	418.40 ± 60.88 (2.96^{NS})	407.93 ± 52.05 (5.39^{NS})
Post-flowering	476.00 ± 61.26	465.18 ± 47.17 (1.87^{NS})	452.00 ± 50.87 (21.52**)	408.66 ± 48.59 (29.05**)
Vessel element length (μm)				
Pre-flowering	120.83 ± 30.29	120.60 ± 36.66 (0.19^{NS})	101.60 ± 25.50 (15.91*)	100.93 ± 22.71 (16.46**)
Flowering	120.93 ± 31.59	121.04 ± 29.54 (0.09^{NS})	114.40 ± 24.58 (5.39^{NS})	102.40 ± 21.28 (15.32*)
Post-flowering	121.60 ± 31.44	121.05 ± 32.80 (0.45^{NS})	116.80 ± 23.95 (3.94^{NS})	108.00 ± 20.12 (11.18^{NS})
Vessel density (number mm^{-2})				
Pre-flowering	8.68 ± 2.83	8.70 ± 1.59 (0.23^{NS})	9.16 ± 1.91 (5.52^{NS})	11.04 ± 2.37 (27.18**)
Flowering	10.04 ± 2.22	10.11 ± 1.79 (0.69^{NS})	10.60 ± 2.29 (5.57^{NS})	12.00 ± 2.10 (19.52**)
Post-flowering	10.48 ± 2.14	10.53 ± 1.34 (0.47^{NS})	11.20 ± 2.52 (6.87^{NS})	12.88 ± 1.76 (20.90**)
Vessel diameter (μm)				
Pre-flowering	41.32 ± 9.69	41.22 ± 7.30 (0.24^{NS})	47.37 ± 11.19 (14.64^{NS})	49.82 ± 12.74 (20.57^{NS})
Flowering	44.35 ± 11.83	43.97 ± 10.66 (0.85^{NS})	53.56 ± 14.26 (20.76^{NS})	55.29 ± 13.76 (24.66**)
Post-flowering	50.68 ± 17.78	50.81 ± 15.64 (0.25^{NS})	61.91 ± 18.63 (22.15^{NS})	69.12 ± 17.66 (36.12^{NS})

Values represent a mean of 100 readings. Parentheses include per cent variation.
** = Significant at 1% level, * = significant at 5% level; NS = non-significant.

On entering the plant tissues, either from the soil via roots or from the air via leaves, SO_2 affects plant growth by altering the production and distribution of photosynthates (Khan and Khan, 1993). Excessive sulphur levels in plants can hamper photosynthesis, which may even impair the reproductive phase by reducing the number of flowers per plant (Bergmann, 1992), as was the case in the present study. An observed decline in fruit/seed yield under heavy stress might result from failure of pollination or fertilization. Reduction in the number of flowers and fruits per plant by SO_2 exposure substantiates some earlier works like those of Deepak and Agrawal (1999) and Wali et al. (2004).

Photosynthetic efficiency

In the present study, the lowest SO_2 dose has shown stimulatory effects with respect to number of leaves per plant, as observed at the flowering and post-flowering stages. Consequently, the total leaf area and the total leaf dry mass also increased at these stages. The higher doses, however, proved to be inhibitive at all three stages of plant development. In earlier studies, increased leaf area has at times been interpreted as a strategy of the plant to compensate for other negative physiological changes (Capron et al., 2000). The size of stomatal aperture increased on the adaxial surface and decreased on the abaxial surface of the *C. officinalis* leaf under SO_2 stress. Shrinking or closure of stomatal pores could be due to accumulation of CO_2 in sub-stomatal cavities following inhibition of photosynthesis (Wali et al., 2004). Compared with the control, SD in treated plants was significantly low on the adaxial epidermis but high on the abaxial one, contrary to the findings with *Catharanthus roseus* (Khan et al., 1990).

The amounts of total chlorophyll and carotenoids in fumigated *C. officinalis* plants were significantly low under high SO_2 stress at each stage of plant

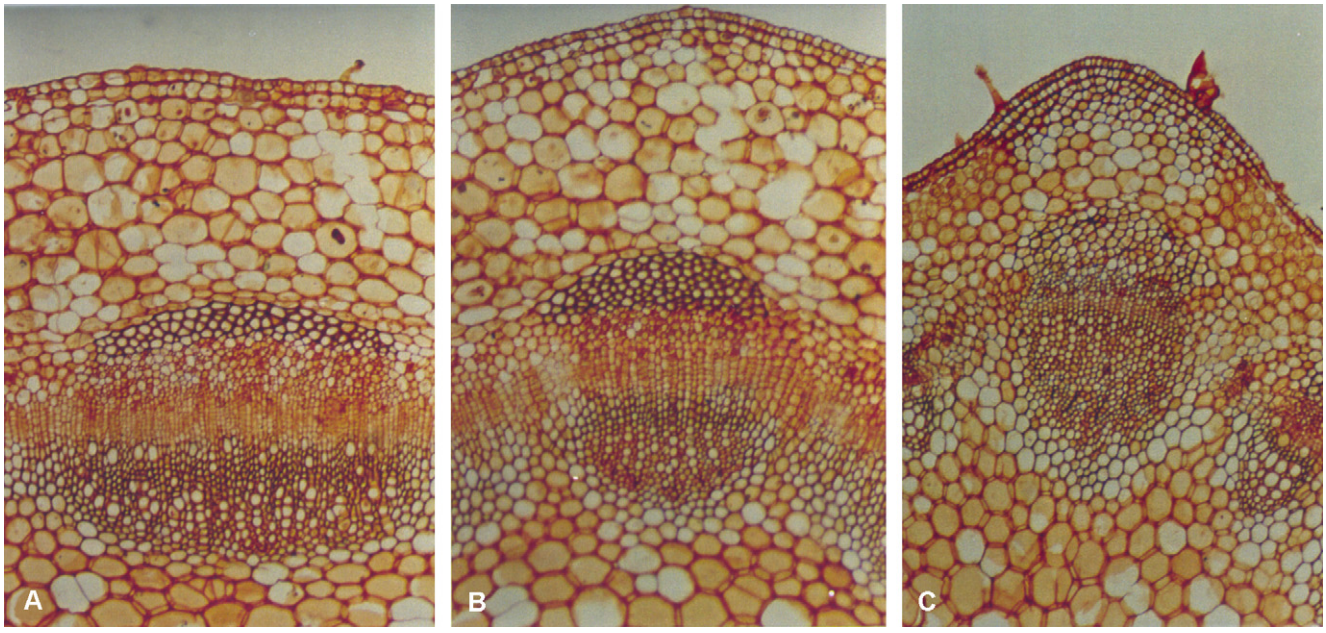

Fig. 6. Transverse sections of the third internode of *Calendula officinalis* stems, showing the effect of SO_2 treatments on the formation of interfascicular cambium and secondary vascular tissues during the post-flowering phase (magnification: × 100). (A) The cambium ring is well developed in the untreated (control) plants. (B) Formation of interfascicular cambium has just started in plants treated with 1 ppm SO_2. (C) Formation of interfascicular cambium is yet to begin in plants treated with 2 ppm SO_2.

development, though the lowest SO_2 dose proved stimulatory. Sulphites are known to react with chlorophyll to produce superoxide radicals (Shimazaki et al., 1980; Williams and Banerjee, 1995). Chlorophyll *a* is degraded to phaeophytin through replacement of Mg^{2+} ions in chlorophyll molecules, while chlorophyll *b* forms chlorphyllide *b* through removal of the phytol group of the molecule (Rao and Le Blanc, 1966).

Increase in stomatal conductance and internal CO_2 concentration under high SO_2 stress may be related to size and condition of stomatal apertures. A gain with 0.5 ppm and a significant decline in the subsequent stages conform to several previous studies. The retarded photosynthetic efficiency may be correlated with a decrease in leaf nitrogen content (Nakano et al., 1997), phosphoenol pyruvate activity, chlorophyll content and/or leaf area (Joshi et al., 1993).

Presence of gaseous pollutants hampers CO_2 uptake and reduces photosynthetic activity, which, in turn, may inhibit cambial activity and, consequently, wood production (Iqbal et al., 2000a, b, 2005). Since the activity of cambium depends on availability of water, starch, soluble sugars, minerals and growth hormones (Berlyn and Battey, 1985; Iqbal, 1995), the reduced photosynthesis, resulting in a limited accumulation of carbohydrates, eventually affects cambial activity and the development of derivative tissues. Air pollutants may reduce xylem accumulation even in the absence of visible symptoms (Phillips et al., 1977).

Histological changes

Reductions in size and biomass of shoots and roots are correlative to the retarded growth of the component cells. An increase in the amount of xylem with increasing age of the plant ensures an efficient ascent of sap in the constantly growing plant body. In the present study, proportions of cortex and vasculature in the roots and stems of the SO_2-exposed plants decreased, whereas pith proportion increased. Physiological events leading to a decreased production of vascular tissues involve inhibition of photosynthesis and hormone synthesis, resulting in a limited transport of carbohydrates and hormones to the sites of tissue differentiation (Khudsar et al., 2000; Kozlowski and Constantinidou, 1986). Despite a decrease in the relative proportion of vasculature, the plants under study were able to withstand the stress, possibly because narrow vessels resist embolism and thus smoothen the conduction of water. Development of xylem cells is also affected by SO_2 concentration in the atmosphere (Khudsar et al., 2000; Pozgaj et al., 1996). A shortening of tracheids in pollution-affected *Pinus sylvestris* was ascribed by Tulik (2001) to the retarded rate of anticlinal division of fusiform initials in the cambium. Gupta and Iqbal (2005) have shown inhibition of dimensional growth but increase in frequency of vessel elements in the wood of *Mangifera indica* growing in a polluted air. Because of a high frequency and reduced size of vessel elements, values of vulnerability and mesomorphic ratios of the stressed mango wood were low and

Table 3. Data on xylem fibres and vessel elements in the stem of *Calendula officinalis*, as recorded at different developmental stages of the control and SO_2-treated plants

Parameters	Control	0.5 ppm SO_2	1 ppm SO_2	2 ppm SO_2
Fibre length (μm)				
Pre-flowering	399.84 ± 43.43	408.24 ± 40.00 (2.10^{NS})	478.56 ± 77.78 (19.68**)	510.24 ± 78.32 (27.61**)
Flowering	449.76 ± 52.87	439.82 ± 50.28 (2.21^{NS})	497.28 ± 61.43 (10.56**)	626.88 ± 85.68 (39.38**)
Post-flowering	468.96 ± 43.94	474.04 ± 42.84 (1.08^{NS})	583.12 ± 49.57 (9.41**)	744.96 ± 91.49 (58.85**)
Vessel element length (μm)				
Pre-flowering	112.66 ± 24.27	111.92 ± 20.12 (0.66^{NS})	116.88 ± 11.74 (3.74^{NS})	124.84 ± 25.42 (10.67*)
Flowering	113.76 ± 20.56	113.08 ± 14.10 (0.60^{NS})	114.24 ± 15.74 (0.42^{NS})	118.94 ± 29.01 (4.55*)
Post-flowering	130.56 ± 22.25	132.44 ± 21.96 (1.44^{NS})	139.84 ± 20.51 (7.10^{NS})	147.36 ± 24.06 (12.86*)
Vessel density (number mm^{-2})				
Pre-flowering	30.64 ± 7.12	30.82 ± 6.46 (0.59^{NS})	29.70 ± 6.75 (3.05^{NS})	28.82 ± 7.22 (5.94**)
Flowering	34.88 ± 7.59	34.73 ± 7.21 (0.43^{NS})	32.92 ± 7.95 (5.62^{NS})	31.20 ± 6.69 (10.55**)
Post-flowering	37.28 ± 9.68	37.05 ± 7.96 0.62^{NS})	46.95 ± 11.16 (25.94*)	49.26 ± 11.84 (32.13**)
Vessel diameter (μm)				
Pre-flowering	23.16 ± 6.52	23.05 ± 6.39 (0.47^{NS})	21.03 ± 7.71 (9.20^{NS})	18.78 ± 6.87 (18.91**)
Flowering	25.33 ± 6.15	25.32 ± 6.20 (0.04^{NS})	23.02 ± 5.05 (9.12^{NS})	20.18 ± 5.29 (20.33**)
Post-flowering	28.83 ± 6.01	28.69 ± 6.19 (0.48^{NS})	24.22 ± 6.32 (15.99*)	22.51 ± 5.31 (21.92**)

Values represent a mean of 100 readings. Parentheses include per cent variation.
** = Significant at 1% level, * = significant at 5% level, NS = non-significant.

declined further with increasing age of the tree (Gupta and Iqbal, 2005).

Since most of the mechanical strength of wood is based on fibres, long fibres in the stem of *C. officinalis* may be an adaptive response to the stress. Wood fibres in several dicotyledonous species occupy a larger transectional area of wood in the polluted samples than in the control (Mahmooduzzafar and Iqbal, 1993, 2000). Development of short vessel elements under stressful conditions, as observed in this and many other studies, may be of help to ensure a smooth and facilitated ascent of sap in the plant (Gupta and Iqbal, 2005; Husen et al., 1999).

Vessel width increases and vessel density decreases in several woody species facing air pollution, although the proportion of vessels per unit area of wood may not always evidence a drastic difference between healthy and polluted individuals (Pozgaj et al., 1996). In the present study, both vessel diameter and vessel density declined in the SO_2-exposed stem in general, whereas both the parameters experienced a considerable increase in the roots.

Ecological correlations

The stress-caused dimensional changes of vessel elements (shorter and broader in roots whereas longer and narrower in stems) are indicative of a behavioural change of xylem tissue in the treated *C. officinalis* plants; this could be indicative of an ontogenetic adaptive strategy of the plant to cope with environmental stress. The short and broad vessel elements in the root are likely to facilitate water conduction, whereas long and narrow elements in the stem confer resistance to embolism. Vulnerability ratio of the vessels was minimum during the flowering phase, which is a positive indication. The ratio declined with growing stress in the environment, thus showing an elaborate management of ensured water supply to the tissues under harsh

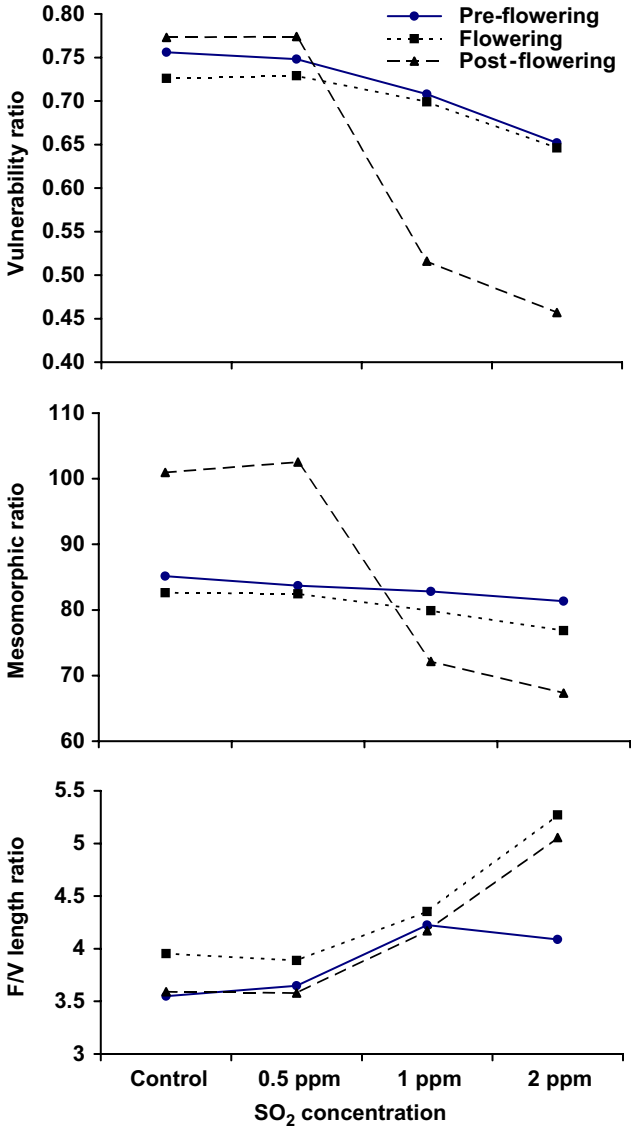

Fig. 7. The magnitude of vulnerability value, mesomorphic ratio and the *F*/*V* length ratio in the stem wood of *Calendula officinalis*, as observed in the pre-flowering, flowering and post-flowering stages in control and SO_2-treated plants.

environmental conditions. Carlquist (1977) proposed that low values of vulnerability ratio could be interpreted as a sign of high redundancy of vessels, showing an increased capability of the given plant species to withstand water stress. Likewise, low values of mesomorphic ratio could be considered as a tendency of the species concerned to move from mesomorphy to xeromorphy. Thus, a decrease in the vulnerability and mesomorphic ratios under SO_2 stress, as noted in this study, suggests that the test plant has experienced an increased water stress possibly due to enhanced aerial water loss via enlarged stomatal apertures. In consequence, a greater vessel redundancy as well as a shift to xeromorphy resulted as prime adaptations to environmental stress. The gain in *F*/*V* length ratio in SO_2-treated plants seems to suggest that the test plants were able to maintain and improve their mechanical strength while facing a hostile environment.

On the whole, *C. officinalis* slightly gains in growth when exposed to 0.5 ppm SO_2 concentration, whereas higher concentrations proved to be injurious. The positive effect of mild SO_2 treatment could be because the soil of the experimental site, with a 2.8 ppm sulphur content, was sulphur deficient; therefore SO_2 application must have compensated initially for sulphur deficiency. Toxicity of high doses of SO_2 compelled the plant to adopt protective measures and to develop adaptive traits. The delay involved in development of the cambial ring and consequently in the accumulation of secondary vascular tissues is a curious phenomenon. Perhaps the gas treatment was inhibitive for expression of certain relevant genes.

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