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Variation in Response of *Arabidopsis thaliana* Lines to Atmospheric SO_2 Exposure

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S u m m a r y

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Thirteen lines of *Arabidopsis thaliana* L. of world-wide origin were exposed to 0.65 µl l^{-1} SO_2 for 11 days. Shoot growth of most lines was hardly affected. Growth of one line, originating from Tadjikistan, was negatively affected upon SO_2 exposure and this line developed acute injury symptoms as leaf necrosis. There was some variation in the sulfate and water-soluble non-protein thiol content between the lines. SO_2 exposure resulted in a 2- to 3-fold increase in shoot sulfate content, whereas there was only a slight increase in the thiol content. *A. thaliana* appeared to be a rather SO_2 tolerant species with little intraspecific variation.

I n t r o d u c t i o n

Sulfur dioxide is a common and widespread air pollutant and considered as a phytotoxic gas for most plants (MANSFIELD & FREER-SMITH 1981, DE KOK 1990). Chronic levels of SO_2 may negatively affect plant growth, whereas acute high levels may result in visible injury (POSTHUMUS 1998). There is large inter- and intraspecific variation in SO_2 tolerance, however, the underlying physiological basis is still largely unclear (DE KOK 1990, ERNST 1993). Variation in tolerance may be due to differences in the rate of deposition (BRESSAN & al. 1978) and sensitivity to bisulfite, acidification of the plant interior, (ANDERSON & DUGAN

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1977), oxidation of SO_2 and accumulation of sulfate (HUVE & al. 1995), or formation of active oxygen species (ALSCHER & al. 1987). On the other hand SO_2 may be beneficial and it may contribute to the sulfur nutrition of plants under sulfur deficient conditions (SCHNUG 1998) and stimulate the performance of high sulfur demanding plants, e.g. cruciferous species (ERNST 1998).

Arabidopsis thaliana appeared to be a rather SO_2 tolerant species, its growth and development were hardly affected upon prolonged exposure to levels ranging from 0.1 - 0.7 µl l^{-1} (VAN DER KOOIJ & al. 1997). The shoot rapidly absorbed SO_2 and the uptake rate was solely determined by the stomatal conductance and hardly affected by the ambient temperature (VAN DER KOOIJ & al. 1997, VAN DER KOOIJ & DE KOK 1998). The absorbed SO_2 was either oxidized to sulfate or metabolized and incorporated into organic sulfur compounds (VAN DER KOOIJ & al. 1997). SO_2 exposure resulted in a strongly enhanced total sulfur content in the shoots of *A. thaliana*. The major proportion of the absorbed SO_2 could be revealed in the sulfate fraction, and there was a 25 % increase in the organic sulfur fraction, irrespective of the SO_2 concentration. Only a minor proportion (2 % or less) was revealed as secondary sulfur compounds (glucosinolates) and thiols (VAN DER KOOIJ & al. 1997).

Plant populations may develop SO_2 tolerance by natural selection upon continuous exposure to SO_2, which has a quantitative genetic basis (TAYLOR 1978, AYAZLOO & BELL 1981, ERNST 1998). In the present study 13 lines of *A. thaliana* with a world-wide origin were compared in their response to exposure to a high SO_2 concentration of 0.65 µl l^{-1} for 11 days, in order to assess the intraspecific variation in tolerance. In addition to biomass production the impact of SO_2 on sulfate and thiol levels in shoots were evaluated.

Materials and Methods

Seeds of thirteen *Arabidopsis thaliana* (L.) Heynh. lines from world-wide origin were obtained from self-reproduced single seed propagated lines (F1-generation). Most lines were gifts from either the Nottingham *Arabidopsis* Stock Centre (University of Nottingham, Nottingham, UK) or the Arabidopsis Biological Resource Center at Ohio State (Ohio State University, Columbus, USA). The line Landsberg (erecta) was obtained from Prof. dr. J. KOORNNEEF (Agricultural University, Wageningen, The Netherlands). The line Wezep was collected in Wezep (The Netherlands) in a garden on a sandy soil. The line Glimmen was collected in Glimmen (The Netherlands) on a sandy soil along a railway track.

Seeds were sown in 0.25 l pots in commercial potting soil and vernalized for four days at 4°C and germinated in a climate controlled room at a day and night temperature of 20 °C for 14 days. The photoperiod was 14 hours at a photon fluence rate of 230 µmol m^{-2} s^{-1} (within the 400-700 nm range). The number of plants was reduced to four plants per pot eight days after germination.

Fumigations were carried out in cylindrical stainless steel cabinets (65 cm diameter, 185 l volume) with polycarbonate tops. The temperature of the cabinets was controlled by pumping cooling fluid (1,2 ethanediol), itself cooled by a beer cooler (Fridina, Groningen, The Netherlands), through the double wall and bottom of the cabinet. The day and night temperature were 19.5 and 14°C, respectively. The photoperiod was 14 hours at a photon fluence rate of 300 ± 20 µmol m^{-2} s^{-1} (within the 400-700 nm range) at plant height, provided by a 400 W lamp (Philips, HPI BUS). The relative humidity in the cabinets was 50-60 % and plants were daily watered. The airflow through

the cabinets was 45 l min^{-1}. The air inside the cabinets was mixed by two fans placed on the bottom (59 m^3 h^{-1} each). Pressurized SO_2 (1000 µl l^{-1} in N_2, Hoekloos, The Netherlands) was injected into the incoming air stream by mass flow controllers (AFC-260, ASM, Bilthoven, The Netherlands).

The experimental setup consisted of four cabinets, which contained two pots with eight individual plants of all thirteen lines in each. Plants were acclimated for 3 days and thereafter, plants were exposure to 0 and 0.65 µl l^{-1} SO_2 for 11 days. SO_2 concentrations were daily checked by using a SO_2 analyzer (ML 9850, Lear Siegler Measurement Controls Corporation, Englewood, USA).

Shoots were harvested at the start and at the end of the exposure period. Dry matter content was determined and the relative growth rate (RGR) was calculated using the ln transformed shoot weights. There were no significant differences ($p<0.05$) in biomass production of individual lines between and within the cabinets upon exposure. Differences between means were statistically analyzed by a Student-t test and linear fist order fits by Slide 5.0 software.

Sulfate was extracted and determined refractrometrically after HPLC separation as described by TAUSZ & al. 1996. Water-soluble non-protein thiol compounds were extracted according to STUIVER & al. 1992. The content of DTNB (5,5-dithiobis-2-nitrobenzoic acid) reactive compounds was determined as described by DE KOK & GRAHAM 1989.

Results and Discussion

There was only little variation in growth of the tested lines of *Arabidopsis thaliana* and the RGR's of the shoots ranged from 25 to 29 % day^{-1} (Table 1). Growth of the majority of the lines was hardly affected upon exposure to 0.65 µl l^{-1} SO_2, for some lines shoot fresh weight production even slightly increased (Table 1). Similar to previous observations for *A. thaliana* cv. Landsberg (VAN DER KOOIJ & al. 1997) there was a slight but significant decrease in dry matter content in most lines upon SO_2 exposure (Table 1). Growth of solely one of the tested line, N922 originating from Tadjikistan, was negatively affected. In addition, this line the old leaves had become necrotic as an acute toxic response. Interestingly, leaves that had developed during the SO_2 exposure exhibited no visible damage.

Table 1. Effect of 0.65 µl l^{-1} SO_2 on shoot biomass production of various *Arabidopsis thaliana* lines. Plants were exposed for 11 days. Shoot fresh (FW) and dry weight (DW) represent the mean of 16 and 8 measurements, respectively (±SD). RGR was calculated over the 11 days exposure interval and is expressed as % day^{-1}. Dry matter content (DMC) is expressed as % of the fresh weight. Significant differences ($p<0.05$) between treatments within a line are indicated by an asterisk*.

Line	Origin	Initial FW (mg)	0 µl l^{-1} SO_2 FW (mg)	(RGR) (% day^{-1})	0.65 µl l^{-1} SO_2 FW (mg)	(RGR) (% day^{-1})	0 µl l^{-1} SO_2 DM (mg)	(DMC) (%)	0.65 µl l^{-1} SO_2 DM (mg)	(DMC) (%)
Landsberg	*Laboratory*	9 ± 2	216 ± 46	*(29)*	274 ± 38*	*(31)*	26 ± 4	*(11.2)*	28 ± 5*	*(9.7)**
N900	*Germany*	14 ± 2	236 ± 40	*(26)*	278 ± 36*	*(27)*	28 ± 4	*(11.0)*	28 ± 4	*(9.9)**
N911	*Estonia*	15 ± 3	226 ± 38	*(25)*	255 ± 58	(26)	25 ± 3	*(10.8)*	27 ± 5	*(9.4)*
N921	*Tadjikistan*	12 ± 3	200 ± 37	*(26)*	177 ± 39	*(25)*	21 ± 4	*(11.2)*	18 ± 6	*(10.4)**
N922	*Tadjikistan*	16 ± 4	328 ± 44	*(28)*	212 ± 114*	*(24)*	34 ± 4	*(10.0)*	25 ± 12	*(9.1)**
N929	*Tadjikistan*	16 ± 2	330 ± 66	*(28)*	376 ± 81	*(29)*	40 ± 6	*(10.0)*	33 ± 5	*(8.0)**
N3109	*Denmark*	12 ± 2	278 ± 58	*(29)*	292 ± 60	*(29)*	35 ± 5	*(11.5)*	31 ± 5*	*(10.5)**
N3180	*Portugal*	12 ± 2	278 ± 68	*(28)*	309 ± 46	*(29)*	30 ± 9	*(10.1)*	32 ± 6	*(9.8)*
Cs6075	*England*	15 ± 3	363 ± 80	*(29)*	369 ± 127	*(29)*	37 ± 10	*(10.6)*	43 ± 10	*(9.5)**
Cs6094	*France*	6 ± 1	145 ± 32	*(29)*	166 ± 32	*(30)*	16 ± 4	*(11.0)*	15 ± 3	*(8.3)**
Cs6188	*USA*	18 ± 4	370 ± 47	*(28)*	395 ± 68	*(28)*	36 ± 5	*(9.9)*	41 ± 5	*(9.7)*
Glimmen	*Netherlands*	15 ± 3	347 ± 62	*(29)*	344 ± 46	*(29)*	34 ± 5	*(9.1)*	29 ± 2	*(8.8)*
Wezep	*Netherlands*	10 ± 4	293 ± 37	*(25)*	326 ± 47*	*(26)*	37 ± 2	*(11.8)*	39 ± 6	*(10.8)**

In most herbaceous species, including *A. thaliana*, there is in general a nearly linear relation between SO_2 deposition to the shoot and the atmospheric concentration (DE KOK 1990, VAN DER KOOIJ & DE KOK 1998, VAN DER KOOIJ & al. 1998). For *A. thaliana* cv. Landsberg it has been estimated that the foliar sulfur deposition at an atmospheric level of approx. 0.1 $\mu l\ l^{-1}$ SO_2 should be sufficient to cover the organic sulfur need for growth at a RGR of 24 % day^{-1} (VAN DER KOOIJ & al. 1997). The major proportion of the excessive absorbed SO_2 is presumably accumulated in the vacuolar sulfate pool (DE KOK 1990, VAN DER KOOIJ & al. 1997, 1998, VAN DER KOOIJ & DE KOK 1998). In *A. thaliana* cv. Landsberg the accumulation of sulfate roughly accounted for 75 % of the excess of sulfur taken up from the atmosphere (VAN DER KOOIJ & al. 1997). All *A. thaliana* lines showed a substantial increase in sulfate content upon exposure to 0.65 $\mu l\ l^{-1}$ SO_2 (Table 2). Despite the variation in the sulfate content, in most lines there was a 2- to 3-fold increase in sulfate content after the 11 days exposure period. Sulfate accumulation was the lowest in the SO_2-sensitive line N922 (1.5-fold), that might in part be explained by a possible lower rate of SO_2 deposition in the necrotic tissue rather than by differences in SO_2 metabolism.

Exposure of plants to SO_2 may result in an enhanced water-soluble non-protein thiol content, mainly due to an accumulation of glutathione (GRILL & al. 1979, DE KOK 1990, HUVE & al. 1995). It has been proposed that high thiol (glutathione) levels might protect plants against oxidative stress induced by SO_2 (ALSCHER & al. 1987, ALSCHER & AMTHOR 1988). On the other hand enhanced thiol levels might reflect an overload of sulfur assimilation upon exposure to atmospheric sulfur (DE KOK & STULEN 1993, VAN DER KOOIJ & al. 1997). There was some variation in thiol levels in shoots of the *A. thaliana* lines and it ranged from 0.7 – 1.3 $\mu mol\ g^{-1}$ fresh weight (Table 2). SO_2 exposure resulted in slightly, but in most lines not significantly, higher thiol levels (Table 2). Solely in the sensitive line, N922, there was a substantial increase in thiols upon SO_2 exposure. Evidently, SO_2 has little effect on thiol metabolism in the tolerant species *A. thaliana*, as has been reported previously for cv. Landsberg (VAN DER KOOIJ & al. 1997). Nevertheless, it illustrates again that thiols do not play a direct role in the SO_2 tolerance of *A. thaliana* as discussed previously (VAN DER KOOIJ & al. 1997).

The extreme vacuolar sulfate accumulation might cause a decrease of the dry matter content via increased vacuolar osmotic potential as also discussed by HUVE & al. 1995 for coniferous species. However, there was no direct correlation ($r = 0.240$) between the difference in dry matter content of plants at control conditions and exposed to SO_2, and the corresponding difference in sulfate content. Furthermore, there was also no correlation between both sulfate content or sulfate accumulation and plant size ($r = 0.020$ and 0.345, respectively). In addition, there was no correlation ($r = 0.501$) between the accumulated amount of sulfate and the difference in thiol content between control and SO_2 exposed plants.

Intraspecific variation in SO_2 tolerance has been reported for several plant species (TAYLOR 1978, AYAZLOO & BELL 1981, ERNST 1998). All these species were SO_2 sensitive and developed SO_2 tolerance by natural or artificial selection.

Table 2. Effect of 0.65 µl l^{-1} SO_2 on sulfate and thiol content in shoots of various *Arabidopsis thaliana* lines. Plants were exposed for 11 days. Sulfate and thiol content represent the mean of 4 measurements (±SD). Significant differences ($p<0.05$) between treatments within a line are indicated by an asterisk*.

Line	0 µl l^{-1} SO_2		0.65 µl l^{-1} SO_2		SO_2-induced Sulfate Accumulation	
	Sulfate (µmol g^{-1} FW)	Thiols (µmol g^{-1} FW)	Sulfate (µmol g^{-1} FW)	Thiols (µmol g^{-1} FW)	(µmol g^{-1} FW)	(%)
Landsberg	10.2 ± 1.1	0.94 ± 0.08	26.7 ± 1.8*	0.98 ± 0.08	16.5	(262)
N900	10.8 ± 1.4	1.17 ± 0.09	28.2 ± 4.3*	1.06 ± 0.07	17.4	(261)
N911	12.7 ± 1.7	1.12 ± 0.06	27.1 ± 0.8*	1.33 ± 0.03*	14.4	(213)
N921	11.7 ± 0.3	1.33 ± 0.06	23.9 ± 1.4*	1.54 ± 0.08*	12.2	(204)
N922	9.7 ± 0.8	0.82 ± 0.07	14.6 ± 1.2*	1.33 ± 0.45	4.9	(151)
N929	9.8 ± 1.5	0.77 ± 0.02	17.8 ± 0.6*	0.61 ± 0.09*	8.0	(182)
N3109	10.4 ± 2.2	1.08 ± 0.09	25.0 ± 1.3*	1.21 ± 0.15	14.6	(240)
N3180	14.3 ± 2.4	0.76 ± 0.05	34.5 ± 4.1*	0.88 ± 0.01*	20.2	(241)
Cs6075	11.0 ± 2.2	0.87 ± 0.10	24.2 ± 2.9*	1.08 ± 0.16	13.2	(220)
Cs6094	11.0 ± 1.0	1.21 ± 0.19	23.7 ± 5.7*	1.09 ± 0.07	12.7	(215)
Cs6188	10.7 ± 0.9	0.67 ± 0.02	26.7 ± 1.8*	0.81 ± 0.07*	16.0	(250)
Glimmen	7.6 ± 0.5	0.83 ± 0.07	27.0 ± 2.2*	0.91 ± 0.07	19.4	(355)
Wezep	10.3 ± 0.5	1.07 ± 0.17	27.2 ± 4.0*	1.22 ± 0.11	16.9	(264)

From the present study it is obvious that in general *A. thaliana* is a SO_2 tolerant species, with rather little intraspecific variation. High atmospheric SO_2 concentrations appear not to be a threat for the survival of *A. thaliana* as a species. The widespread occurrence of SO_2 pollution might in part even be responsible for the increased distribution of members of *Brassicaceae* (including *A. thaliana*) over the last century (ERNST 1993). This might not solely be due to SO_2 tolerance but also to the ability of these high-sulfur demanding species, to utilize the deposited atmospheric sulfur as source for growth and survival. Amongst the tested lines one *A. thaliana* line (N922) appeared to be rather SO_2 sensitive. Further comparative studies might open new ways of studying the mechanism underlying phytotoxicity of SO_2, especially with regard to the huge amount of molecular information on *A. thaliana*, which is available nowadays.

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