Changes in CO_2 exchange rate, stomatal conductance, activities of photosystems 1 and 2, and chloroplast polypeptide profile induced by simulated acidic rain

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Abstract

In seedlings of *Vigna radiata* (L.) R.Wilczek cultivars ADT-1 and CO-5 exposed to acidic showers (H_2SO_4 : HNO_3 : HCl, 4 : 2 : 1, v/v) of different pH (7.0, 5.5, 4.0, and 2.5) for 10 d, net CO_2 uptake and stomatal conductance were reduced. The chlorophyll (Chl) *a* and *b* contents were reduced but the carotenoid (Car) content increased. *In vivo* Chl *a* fluorescence patterns of both the cultivars were altered. No significant change in photosystem (PS) 1 activity was observed except at pH 2.5 where an inhibition was evident. By contrast, PS2 activities declined rapidly with increasing acidity. The room temperature absorption spectra of isolated chloroplasts showed very little changes. SDS-PAGE analysis revealed depletion of 23, 33, and 55 kDa polypeptides. Cultivar CO-5 was more sensitive to acidic rain than cv. ADT-1

Additional key words: absorption spectra; chlorophyll fluorescence; photosynthesis; *Vigna radiata*.

Introduction

The problem of acid rain, which has been confined to Europe and North American countries till recently, now threatens to engulf the entire world. Already, it has devastated aquatic and forest ecosystems. In crop plants, it damages the leaves by eroding the cuticular waxes and leaching cell metabolites. Localised acidity affects the underlying mesophyll causing chlorosis followed by necrosis, forming discrete lesions (Evans *et al.* 1977). It reduces the Chl contents (Ferenbaugh 1976, Takemoto *et al.* 1987, Muthuchelian *et al.* 1994), stomatal conductance (Saxe 1991,

Received 21 July 1997, *accepted* 27 October 1997.
Acknowledgements: The authors thank Professor G. Kulandaivelu, Head of the Department of Plant Sciences, Madurai Kamaraj University for his keen interest in the work and constructive suggestions. Technical assistance from Dr. K. Muthuchelian, Dr. A. Premkumar, and Dr. K. Eswaran is also gratefully acknowledged.

Muthuchelian *et al.* 1994), and net photosynthetic rate (Takemoto *et al.* 1987, Martens *et al.* 1989, Muthuchelian *et al.* 1994). Activities of nitrate reductase and ribulose-1,5-bisphosphate carboxylase (RuBPC) (Muthuchelian *et al.* 1993), and glutathione reductase, ascorbic acid reductase, and peroxidase (Chen *et al.* 1991) have also been affected.

Most of the information on the biological and metabolic effects have been obtained employing sulphuric acid either alone or in combination with nitric acid. Studies employing mixtures of sulphuric, nitric, and hydrochloric acids for simulating acid rain are not only rare but have concentrated on growth and yield characteristics only (Kohno and Kobayashi 1989). Hence, an investigation was undertaken on the influence of simulated acid rain (SAR) on the photosynthetic process in two grain legume cultivars, *Vigna radiata* cv. ADT-1 and CO-5.

Materials and methods

Plants: Seeds of green gram [Vigna *radiata* (L.) R. Wilczek cv. ADT-1 and CO-5] were sown in earthenware pots (25×25 cm) filled with a mixture of sand, red soil, and farmyard manure (2 : 1 : 1, v/v). Plants (5 per pot) were irrigated with borewell water daily just enough to keep the soil moist. They were maintained under natural greenhouse conditions [day-temperature 38 ± 2 °C, night-temperature 18 ± 2 °C, relative humidity 60 ± 5 %, maximum irradiance (PAR) 1400 μmol m^{-2} s^{-1}, photoperiod 12-14 h].

Simulation of acid rain: Fifteen-day-old plants at 2-leaf stage [expanded primary leaf (PL) and emerging first trifoliate leaf (TF1)] were exposed to simulated acid rain (SAR) of 6 min duration for 10 d. The rain drop size ranged from 0.35 to 1.35 mm at a flow rate of 7.8 mm h^{-1}. Double distilled water (DH_2O) adjusted to pH 5.5, 4.0, or 2.5 using a diluted mixture of H_2SO_4, HNO_3, and HCl (4 : 2 : 1, v/v) in the molar ratios of 6 : 3 : 1 was used for simulating rain events. DH_2O adjusted to pH 7.0 by adding one or two drops of 0.1 M NaOH served as control.

Pigments: After the termination of showers, TF1 from treated plants were taken and the pigments were extracted into dimethyl sulphoxide (DMSO) at 60 °C for 1 h. Chl content was estimated following the method of Shoaf and Lium (1976). Car content was quantified following Ikan (1969).

In vivo Chl *a* fluorescence was induced in dark-adapted (28 °C, 10 min) intact leaves (TF1) after excitation with the broad-band blue radiation (100 W m^{-2}, 400-460 nm) attached to a photomultiplier recorder as described by Kulandaivelu and Daniell (1980) at a photon flux density of 700 μmol m^{-2} s^{-1}.

Room temperature spectra of chloroplasts were recorded directly using a double beam spectrophotometer (*Hitachi* model *557*) with normalisation at 540 nm.

Measurement of gas exchange: Net photosynthetic CO_2 uptake (P_N) and stomatal conductance (g_s) were monitored on fully expanded terminal leaflet of TF1 using a *LI-6200* portable infra-red gas analyser (*LI-COR*, USA). A steady state system was used to measure the CO_2 concentrations in a 1000 cm^3 leaf chamber under natural

irradiation between 10:30 and 11:30 h. Ten readings were taken for each sample at 5 s intervals. The g_s was measured with a steady state porometer (*LI-COR-1600*, *LI-COR*, USA).

Measurement of electron transport activities: Type 2 broken chloroplasts were isolated from control and SAR treated plants according to the method of Reeves and Hall (1973). Whole electron chain transport ($H_2O \rightarrow$methyl viologen, MV) was measured as described by Armond *et al.* (1978) using a *Hansatech* oxygen electrode. PS1 and PS2 mediated electron transport was measured as described by Noorudeen and Kulandaivelu (1982).

SDS-PAGE analysis of chloroplast polypeptides: The chloroplasts were precipitated with 10 % trichloroacetic acid (TCA) and kept in ice for 20 min before centrifugation in order to collect the pellets. Traces of TCA in the pellet were removed by washing it three times with ice-cold acetone. The dried pellet was then solubilized in a small volume of 10 % SDS (Nedunchezhian and Kulandaivelu 1991). SDS-PAGE was performed following the method of Laemmli (1970) using a polyacrylamide gradient of 7.5-15 % gel.

Statistical analysis was done using the Tukey's multiple range test (TMRT) at 5 % level of significance (Zar 1984).

Results and discussion

Our results confirm the harmful effects of SAR on photosynthesis. The Chl contents in the two green gram cultivars treated with SAR were reduced at all acidic levels, though not significantly. Reduction in Chl contents in plant exposed to SAR has also been reported in other crop species (Ferenbaugh 1976, Hindawi *et al.* 1980, Muthuchelian *et al.* 1993, 1995). Conversely, Car contents increased over the control in cv. ADT-1 but only marginally in cv. CO-5 (Table 1).

P_N of both cultivars dropped significantly below the control values (Table 1). This conforms to the general inhibition of P_N in crop species exposed to H_2SO_4 mist described by Muthuchelian *et al.* (1993, 1995). These authors also noticed a positive correlation between reduced P_N and decreased g_s. Here, g_s of both cultivars decreased (Table 1).

Chl *a* fluorescence induction provides an intrinsic probe into the changes in photosynthetic reactions. Typical fast (0.2 s) fluorescence induction curves of dark adapted leaves show a rapid initial rise to F_0 followed by a sigmoidal increase to a temporary maximum level (F_m) which corresponds to the status of photochemical reactions. Under prolonged irradiation (60 s) certain enzymes of the electron transport chain and Calvin cycle are activated which is reflected in the slow fluorescence transients (Govindjee and Papageorgiou 1971, Krause and Weis 1984). The SAR-treated plants exhibited significant alterations in the slow and fast fluorescence patterns suggesting an impairment of photosynthetic systems (Fig. 1). Though both cultivars responded similarly, the cv. CO-5 was more sensitive.

Room temperature absorption spectra of isolated chloroplasts showed peaks at 434 and 674 nm, but there was no shift in the spectral peaks in treated samples of both cultivars which indicated that SAR did not affect the thylakoid organization. However, the small differences in spectral heights indicated minor quantitative changes in pigment contents.

Table 1. Effect of simulated acidic rain on chlorophyll (Chl) *a*+*b* and carotenoid (Car) contents, net photosynthetic rate (P_N), stomatal conductance (g_s), and photochemical activities [mol(O_2) kg^{-1}(Chl) s^{-1}] of two cultivars of green gram plants. Values in a row followed by different letters are significantly different according to Tukey's HSD multiple range test at 5 % level (n = 10).

Parameter	Cultivar	pH 7.0	5.5	4.0	2.5
Chl (*a*+*b*) [g kg^{-1}(f.m.)]	ADT-1	2.22^a	1.82^a	2.10^a	2.02^a
	CO-5	2.39^a	1.70^a	1.80^a	1.91^a
Car [g kg^{-1}(f.m.)]	ADT-1	0.26^a	0.40^b	0.37^{bc}	0.32^c
	CO-5	0.33^a	0.41^b	0.40^b	0.39^b
P_N [mg(CO_2) m^{-2} s^{-1}]	ADT-1	0.34^a	0.33^a	0.28^b	0.26^b
	CO-5	0.39^a	0.28^b	0.20^c	0.18^c
g_s [cm s^{-1}]	ADT-1	0.017^a	0.016^a	0.013^b	0.008^c
	CO-5	0.013^a	0.012^a	0.009^b	0.008^b
whole chain (H_2O→MV)	ADT-1	37.9^a	31.4^a	27.4^b	25.8^b
	CO-5	39.3^a	36.2^a	31.4^b	17.6^c
PS2 (H_2O→BQ)	ADT-1	63.7^a	60.8^a	51.4^b	41.9^c
	CO-5	81.2^a	72.6^a	64.6^b	32.9^c
PS1 (DCPIPH_2→MV)	ADT-1	78.3^a	72.9^a	72.2^a	56.1^b
	CO-5	99.8^a	96.3^a	88.3^a	65.8^b

In *Vigna sinensis* and *Phaseolus mungo*, H_2SO_4 alone and in combination with HNO_3 affects the whole chain electron transport, especially PS2 mediated by benzoquinone (BQ); the effect on PS1 was milder (Muthuchelian *et al.* 1993, 1995); the trends were impressive at pH 2.0. In the two green gram cultivars employed in this study, the overall electron transport (H_2O→MV) was affected significantly at all acidities, especially at pH 2.5. At pH 4.0 and 2.5, the cv. ADT-1 showed 17.7 and 31.9 % decline compared to *ca.* 20 and 55 % reductions in cv. CO-5, respectively.

The polypeptide profiles of chloroplasts from SAR-treated plants revealed depletion of 23, 33, and 55 kDa fractions in cv. ADT-1 at pH 4.0 and 2.5. In cv. CO-5 a general depletion has occurred apart from a substantial decline in 43 and 55 kDa fractions (Fig. 2). Since the 55 kDa polypeptide is the large subunit of the RuBPCO, the decline in photosynthetic capacity noted here might be linked to the changes in chloroplast polypeptides. Similar structural impacts have been reported by Muthuchelian *et al.* (1993) on *Vigna mungo* and *V. sinensis* with sulphuric acid mist. Employing an acid mixture (H_2SO_4 : HNO_3), they observed a more severe effect on those plants wherein 5 polypeptides have been lost and 3 more depleted

(Muthuchelian *et al.* 1995). The adverse impact on polypeptide profile was confirmed in the present study too, where the acid mixture included also HCl. Nevertheless, the degree and magnitude of damage was rather mild. How far the incorporation of HCl in the acid mixture could have influenced the events and mitigated the erosions is not clear.

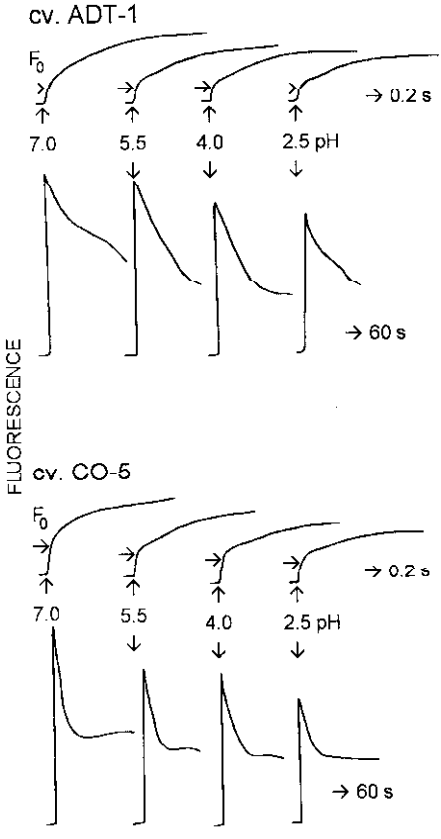

Fig. 1. Typical fluorescence transients (fast and slow) of intact leaves of *Vigna radiata* cv. ADT-1 and CO-5 exposed to simulated acidic rain daily for 10 d (all leaf samples were incubated in dark for 10 min prior to fluorescence measurements, $n = 6$).

Our results suggest that the reduction of P_N of green gram seedlings consequent to acidic deposition might be due to declining photosynthetic pigment contents or decreased g_s or reduced capacity of PS2 (Martens *et al.* 1989, Van Elsacker and Impens 1989, Muthuchelian *et al.* 1990, 1993, 1995).

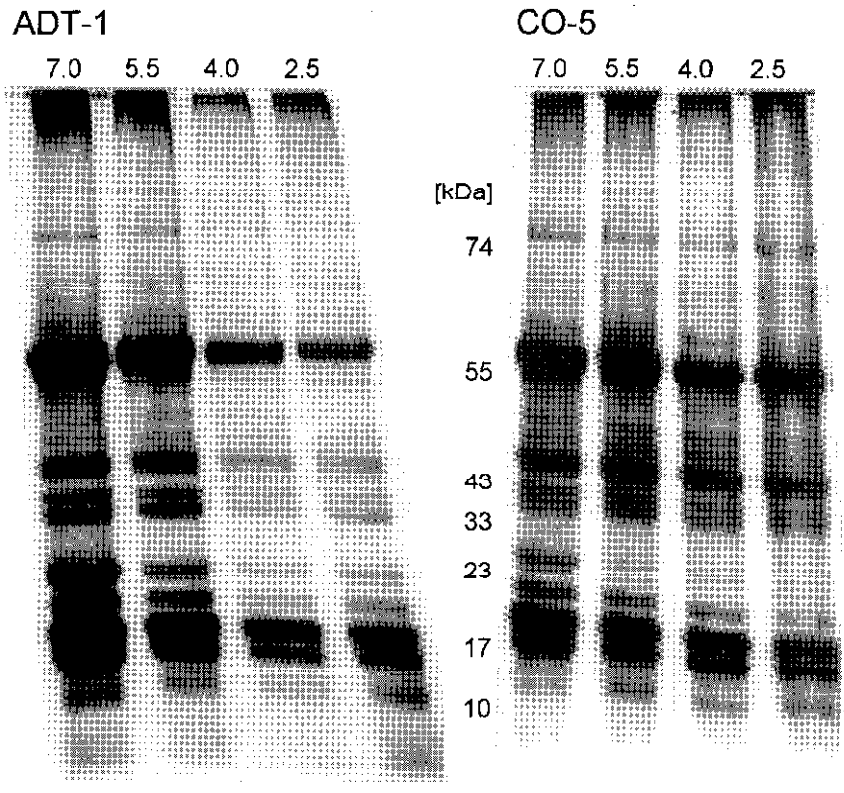

Fig. 2. SDS-PAGE profiles of chloroplast proteins in *Vigna radiata* cv. ADT-1 and CO-5 exposed to pH 7.0 as control and simulated acid rain of pH 5.5, 4.0, and 2.5.

References

Armond, P.A., Schreiber, U., Björkman, O.: Photosynthetic acclimation to temperature in the desert shrub, *Larrea divaricata*. II. Light-harvesting efficiency and electron transport. - Plant Physiol. **61**: 411-415, 1978.

Chen, Y.M., Lucas, P.W., Wellburn, A.R.: Relationship between foliar injury and changes in antioxidant levels in red and Norway spruce exposed to acidic mists. - Environ. Pollut. **69**: 1-15, 1991.

Evans, L.S., Gmur, N.F., Da Costa, F.: Leaf surface and histological perturbation of leaves of *Phaseolus vulgaris* and *Helianthus annuus* after exposure to simulated acid rain. - Amer. J. Bot. **64**: 903-913, 1977.

Ferenbaugh, R.W.: Effects of simulated acid rain on *Phaseolus vulgaris* L. (*Fabaceae*). - Amer. J. Bot. **63**: 283-288, 1976.

Govindjee, Papageorgiou, G.: Chlorophyll fluorescence and photosynthesis: Fluorescence transients. - In: Giese, A.C. (ed.): Photophysiology. Vol. VI. Pp. 1-46. Academic Press, New York - London 1971.

Hindawi, I.J., Rea, J.A., Griffis, W.L.: Responses of bush bean exposed to acid mist. - Amer. J. Bot. **67**: 168-172, 1980.

Ikan, R.: Natural Products. A Laboratory Guide. - Academic Press, New York 1969.

Kohno, Y., Kobayashi, T.: Effect of simulated acid rain on the growth of soybean. - Water Air Soil Pollut. **43**: 11-19, 1989.

Krause, G.H., Weis, E.: Chlorophyll fluorescence as a tool in plant physiology II Interpretation of fluorescence signals. - Photosynth. Res. **5**: 139-157, 1984.

Kulandaivelu, G., Daniell, H.: Dichlorophenyl dimethylurea (DCMU) induced increase in chlorophyll *a* fluorescence intensity. An index of photosynthetic oxygen evolution in leaves, chloroplasts and algae. - Physiol. Plant. **48**: 385-388, 1980.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. - Nature **227**: 680-685, 1970.

Mariens, K.C., Landuydt, K., Impens, I.: Direct effects of acidic wet deposition on photosynthesis and stomatal conductance of two *Populus clones* (*P.* cv. Beaupre and *P.* cv. Robusta). - Ann. Sci. forest. **46** (Suppl.): 586s-589s, 1989.

Muthuchelian, K., Murugan, C., Harigovindan, R., Nedunchezhian, N., Kulandaivelu, G.: Growth, $^{14}CO_2$ fixation, activities of photosystems, ribulose 1,5-bisphosphate carboxylase and nitrate reductase in trees as affected by simulated acid rain. - Biol. Plant. **37**: 355-362, 1995.

Muthuchelian, K., Nedunchezhian, N., Kulandaivelu, G.: Effect of simulated acid rain on $^{14}CO_2$ fixation, ribulose-1,5-bisphosphate carboxylase and nitrate and nitrite reductases in *Vigna sinensis* and *Phaseolus mungo*. - Photosynthetica **28**: 361-367, 1993.

Muthuchelian, K., Nedunchezhian, N., Kulandaivelu, G.: Acid rain: acidic mist-induced response in growth and photosynthetic activities on crop plants. - Arch. Environ. Contam. Toxicol. **26**: 521-526, 1994.

Muthuchelian, K., Paliwal, K., Gnanam, A., Mitchel, R.K.: The effect of three long-chain aliphatic alcohols on photosynthesis and growth of *Pennisetum polystachyeon* Schult. - Photosynthetica **24**: 257-260, 1990.

Nedunchezhian, N., Kulandaivelu, G.: Evidence for the ultraviolet-B (280-320 nm) radiation induced structural reorganization and damage of photosystem II polypeptides in isolated chloroplasts. - Physiol. Plant. **81**: 558-562, 1991.

Noorudeen, A.M., Kulandaivelu, G.: On the possible site of inhibition of photosynthetic electron transport by ultraviolet-B (UV-B) radiation. - Physiol. Plant. **55**: 161-166, 1982.

Reeves, S.G., Hall, D.O.: The stoichiometry (ATP/2e$^-$ ratio) of non-cyclic photophosphorylation in isolated spinach chloroplasts. - Biochim. biophys. Acta **314**: 66-78, 1973.

Saxe, H.: Photosynthesis and stomatal responses to polluted air, and the use of physiological and biochemical responses for early detection and diagnostic tools. - Adv. bot. Res. **18**: 1-128, 1991.

Shoaf, W.T., Lium, B.W.: Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. - Limnol. Oceanogr. **21**: 926-928, 1976.

Takemoto, B.K., Shriner, D.S., Johnston, I.W.: Physiological responses of soybean to simulated acid rain and ambient ozone in the field. - Water Air Soil Pollut. **33**: 373-384, 1987.

Van Elsacker, P., Impens, I.: Response of shoot growth and gas exchange of *Picea abies* clones to rain acidity and the addition of ions. - Ann. Sci. forest. **46**(Suppl.): 595s-598s, 1989.

Zar, J.H.: Bio-Statistical Analysis. - Prentice-Hall, Englewood Cliffs 1984.