Impact of simulated acidic rain on growth, photosynthetic pigments, cell metabolites, and leaf characteristics of green gram

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Abstract

Seedlings of green gram (*Vigna radiata* cv. ADT-1 and CO-5) were exposed to daily showers of simulated acidic rain ($H_2SO_4 : HNO_3 : HCl$, 4 : 2 : 1, v/v) for 10 d. The effects were analysed after 5 and 10 showers, respectively. Rain of pH 2.5 inhibited seedling growth and biomass accumulation, though in other acidic levels the effects were mostly inconsistent. Both cultivars had high degree of surface wettability indicated by high leaf surface contact angles and water-holding capacity. Treated leaves were thinner with smaller mesophyll cells. Stomatal index and trichome density were lower in contrast to epidermal cell density and stomatal frequency which increased with increasing acidity. Decreases in chlorophyll (Chl), carotenoid (Car), and starch contents in cv. ADT-1 at pH 2.5 were observed after 5 showers, while in cv. CO-5 decreases were noted only after 10 showers. In contrast to total sugar levels, the protein content of cv. CO-5 was augmented significantly after simulated acidic rain (SAR) treatment.

Additional key words: carotenoids; chlorophyll; leaf wettability; mesophyll volume; plant height; starch; stomatal index; sugars; *Vigna radiata*.

Introduction

Instances of acidic precipitations have been reported from several developing countries like India and China, and the potential for wide-spread acidification is further building up (Khemani *et al.* 1985, Rodhe 1989). In this context, it has become imperative to screen the performance of local crop plants and forest species under the prevailing tropical conditions as most of the literature pertains to temperate countries. A systematic research programme was initiated in this laboratory to assess the performance of some crop plants against SAR. As a necessary prelude to studies on photosynthesis and yield, effects on early plant growth and foliar structural characteristics were investigated.

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Materials and methods

Seeds of green gram [*Vigna radiata* (L.) 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 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). They were irrigated with borewell-water daily just adequately to keep the soil moist.

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, double distilled water was adjusted to pH 5.5, 4.0, or 2.5. H_2O adjusted to pH 7.0 by adding one or two drops of 0.1 M NaOH served as control.

Fifteen-day-old plants at two-leaf stages with primary leaf (PL) and the emerging first trifoliate leaf (TF1) were exposed to SAR of 6 min duration each for 10 d. About 200 cm^3 of water adjusted to pH 7.0, 5.5, 4.0, or 2.5 was applied to the top of the plant using a rain-generating device after Kohno and Kobayashi (1989). The rain drop size ranged from 0.35 to 1.35 mm diameter, at a flow rate of 7.8 mm^3 per h. No efforts were made to prevent the 'run off' to soil. TF1 from treated plants were sampled for analysis. Morphometric measurements and biochemical estimations were made at stages 1 and 2 corresponding to 20 and 30 DAS, respectively. At stage 1, plants received 5 showers whereas at stage 2 they received 10 showers followed by 5 non-rainy days allowing recovery, if any. Chlorophylls (Chl $a+b$) were estimated following the method of Shoaf and Lium (1976) by extracting the pigments into dimethyl sulphoxide (DMSO) at 60 °C in dark for 1 h, and the absorbance was measured at 645 (Chl b) and 663 nm (Chl a) with DMSO as blank. Carotenoids (Car) were determined following Ikan (1969) with absorbance values measured at 480 nm.

The total soluble protein content was estimated by the method of Lowry *et al.* (1951) from fresh leaves. The contents of total soluble sugars (Dubois *et al.* 1956) and starch (McCready *et al.* 1950) were estimated from the dried leaf tissues.

Stomatal/epidermal characteristics were observed from the leaf impressions on polystyrene plastic films. Stomatal counts were made randomly on five selected regimes on the adaxial side. Stomatal index was calculated following Salisbury (1928). Stomatal and cell sizes were measured using micrometers.

Mesophyll thickness was measured from 10 µm thick leaf sections using an ocular micrometer, and from these measurements mesophyll volume was calculated (Patterson *et al.* 1978).

The leaf surface contact angle (LSCA) was measured on the adaxial surface of freshly detached leaves. Drops of H_2O (5 mm^3) were placed on areas free of larger veins, and the profiles of droplets were projected onto a wall using a microprojector and traced on a graph paper. Contact angle was measured as the angle between air, liquid, and leaf surface as suggested by Fogg (1947).

Water holding capacity (WHC) of the leaves as a measure of leaf wettability was determined following Haines *et al.* (1985). The primary and first trifoliate leaves of each cultivar were sprayed with distilled water for 20 s from 1 m above. The droplets (0.34 to 1.40 mm diameter) on the adaxial surface were blotted onto a filter paper and weighed. Leaf areas were determined using a *Systronics* leaf-area meter. The difference between the wetted and dried filter paper gave the amount of water retained on the surface of the leaf.

Table 1. Changes in growth characteristics of green gram cultivars ADT-1 and CO-5 exposed to simulated acidic rain (SAR). Linear dimensions in [cm], fresh masses in [g]. Differences in a row followed by different letters are significant according to Tukey's HSD multiple range test at 5 % level (n = 10). Stages 1 and 2 correspond to 5 and 15 d after initiating the SAR treatment, respectively.

	Cultivar	Stage 1				Stage 2			
		7.0	5.5	4.0	2.5	7.0	5.5	4.0	2.5
Root length	ADT-1	7.20^a	6.22^b	6.05^b	5.73^b	7.73^a	7.23^a	6.67^b	6.13^b
	CO-5	8.54^a	8.50^a	8.67^a	8.09^b	10.50^a	10.38^a	10.50^a	9.56^b
Shoot length	ADT-1	8.57^a	7.52^b	6.47^c	6.27^c	10.58^a	8.55^b	8.13^b	8.59^b
	CO-5	11.90^{ab}	12.50^b	11.60^{ab}	11.24^a	12.60^a	13.12^a	12.65^a	12.50^a
Plant height	ADT-1	15.77^a	13.74^b	12.52^c	12.00^c	18.31^a	15.78^b	14.80^b	14.72^b
	CO-5	20.44^a	21.00^a	20.27^a	19.33^a	23.10^a	23.50^a	23.15^a	22.06^a
Root fr. mass	ADT-1	0.11^a	0.09^{bc}	0.09^{bc}	0.08^c	0.16^a	0.16^a	0.14^b	0.13^b
	CO-5	0.18^a	0.16^b	0.15^b	0.15^b	0.39^a	0.37^a	0.33^b	0.21^c
Shoot fr. mass	ADT-1	0.42^a	0.46^a	0.42^a	0.44^a	1.10^a	1.09^a	1.10^a	1.11^a
	CO-5	0.55^a	0.52^a	0.52^a	0.54^a	1.24^a	1.22^a	1.25^a	1.02^b
Plant fr. mass	ADT-1	0.53^a	0.55^a	0.51^a	0.52^a	1.26^a	1.25^a	1.24^a	1.24^a
	CO-5	0.73^a	0.68^a	0.67^a	0.69^a	1.63^a	1.59^a	1.58^a	1.23^b

The plants were uprooted at specified stage, and growth parameters such as root and shoot length and plant height were determined. The plant parts were weighed in an *Anamed* digital balance for fresh mass. They were then dried in an oven at 80 °C for 48 h and weighed again for dry mass determination.

For statistical analysis, the values were analysed by Tukey's multiple range test (TMRT) at 5 % level of significance (Zar 1984).

Results and discussion

Our experiments confirm the detrimental nature of rainfall acidity at pH 2.5 in line with most studies from the northern hemisphere (Evans 1988). Inhibitions in root and shoot elongation were significant in cv. ADT-1, but the adverse effect was of lower order in cv. CO-5. This trend was further evident in the whole plant height. Treated plants of cv. ADT-1 were shorter by *ca.* 13-25 % while the height of cv. CO-5 was not reduced significantly (Table 1). Decreased seedling growth and reduced biomass were generally observed in plants exposed to SAR of pH 3.0 or below (Ferenbaugh 1976, Neufeld *et al.* 1985, Kohno and Kobayashi 1989, Muthuchelian *et al.* 1995). In several studies, however, SAR failed to evoke a consistent trend, and stimulations, inhibitions or no effects were observed (Shriner 1978, Shriner and Johnston 1981, Evans *et al.* 1982).

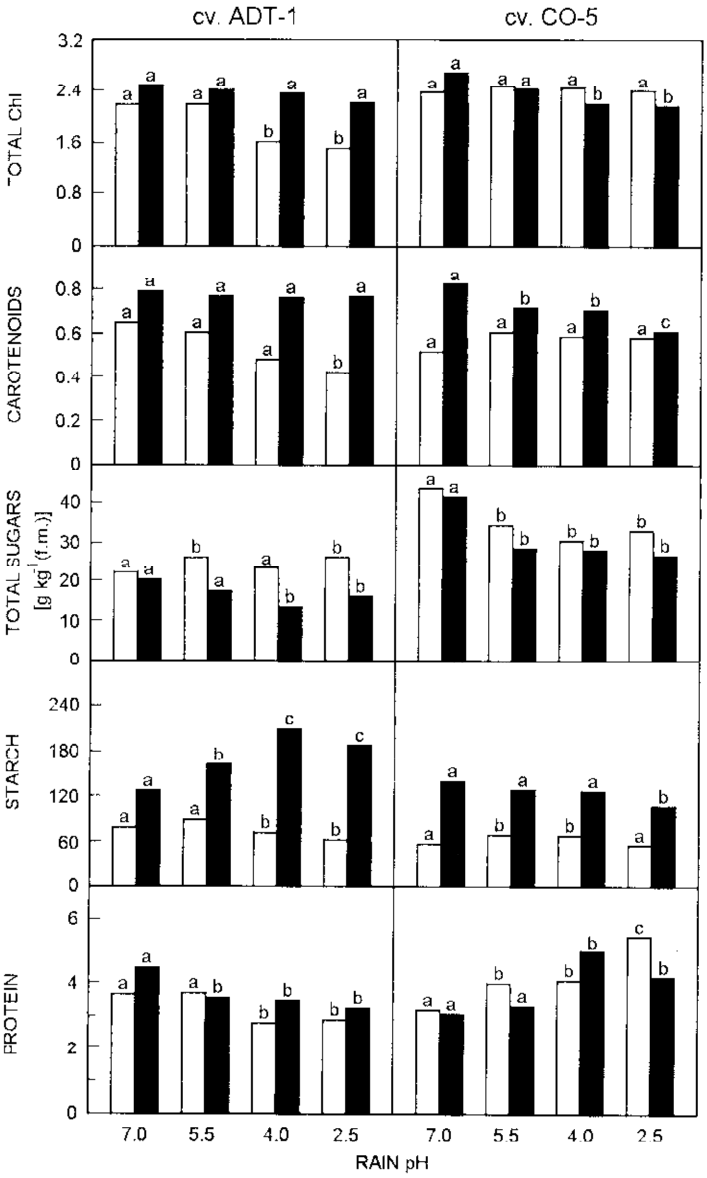

Fig. 1. Effects of SAR on total chlorophyll (Chl), carotenoids, sugar, starch, and protein contents in green gram plants. Values are means of 10 determinations at □ stage 1, or ■ stage 2. Bars sharing the same letters in the respective stages are not significant according to Tukey's HSD multiple range test at 5 % level of significance.

The two green gram cultivars had reduced root biomass especially after SAR treatment of pH 2.5. Insignificant increases in shoot biomass masked the inhibitions at whole plant level (Table 1). Lee *et al.* (1981) found an increase in the shoot biomass of alfalfa at pH 3.5 and 4.0 (pH 5.6 was control) but not in clover; root biomass of alfalfa was the highest at pH 3.5. Evans *et al.* (1983) also observed a slight stimulation of plant biomass after acidic rain upto pH 4.0 in several crop plants. Increased shoot dry mass of soybean was recorded by Norby *et al.* (1985) at pH 3.4 without a corresponding increase in roots. Our results conform to the growth and biomass trends observed in soybean by Kohno and Kobayashi (1989) who used an acid mixture similar to our one.

A decrease in Chl ($a+b$) content was an immediate response of cv. ADT-1 at pH 2.5 but it was nullified subsequently in stage 2 (Fig. 1). In contrast, cv. CO-5 which showed little changes in stage 1, had lower Chl content at pH 4.0 and 2.5 during the post-treatment period. A similar trend prevailed also in the Car contents (Fig. 1). Though decreases in Chl ($a+b$) consequent to SAR have been reported for beans (Ferenbaugh 1976, Hindawi *et al.* 1980), cowpea and black gram (Muthuchelian *et al.* 1993), apples (Sharma *et al.* 1994), Chl content was increased in soybean (Irving and Miller 1980). No significant changes were observed by Dixon and Kuja (1995).

In the present study, a consistent reduction in protein content was noted for cv. ADT 1 only, whereas the trend for cv. CO-5 indicated the reverse (Fig. 1). The cv. ADT-1 seems to have finally benefitted from the acidic showers as the starch content was increased at pH 5.5, 4.0, and 2.5 by 28, 60, and 72 %, respectively (stage 2). In contrast, the cv. CO-5 had significantly less starch at pH 2.5. But for a marginal increase (stage 1) in cv. ADT-1, the total sugar contents were always less than in the control (stage 2 for cv. ADT-1, and stages 1 and 2 for cv. CO-5). Such decreases are supported by the findings of Ferenbaugh (1976) in beans and Muthuchelian *et al.* (1993) in cowpea and black gram. The latter authors have further found a decreased content of soluble protein after acid rain.

Table 2. Leaf wettability (water-holding capacity, WHC) [g(H_2O) m^{-2}] and leaf contact angles [°] of green gram cv. ADT-1 and CO-5 prior to simulated acidic rain (SAR) application. Leaves were showered for 20 s with distilled water from 2 m above. Values are mean ± SE of 10 determinations.

Cultivar	Leaf	Contact angle	WHC
ADT-1	primary	79.5	78.0 ± 2.4
	first trifoliate	82.0	72.9 ± 5.6
CO-5	primary	81.5	71.5 ± 5.1
	first trifoliate	81.7	71.3 ± 5.4

Surface characteristics which contribute to the wettability of leaves, retention of rain drops, and penetration into the underlying cells may be critical for determining the relative susceptibilities of the cultivars. A zero contact angle indicates extreme wettability while 180° indicates almost no wettability. Calculating the leaf surface contact angle (LSCA) and water-holding capacity as a measure of wettability has been advocated by Evans (1979) and Haines *et al.* (1985). The latter paper describes a positive correlation between the LSCA and WHC on one hand, and the susceptibilities of six crop species of SAR on the other. The two cultivars have similar contact angles and WHCs, though cv. ADT-1 appears to be more prone to SAR damage (Table 2). The possibility that these differences, though marginal, could have subtly regulated the penetration and subsequent impact on cellular metabolic events cannot be gainsaid.

Of the stomatal characteristics (Table 3), stomatal size was affected only slightly but the stomatal index (SI) was lowered significantly at pH 4.0 and 2.5 in cv. ADT-1 and only at pH 2.5 in cv. CO-5. Yet, the frequency of stomata increased in all SAR-treated leaves. On a unit area basis, the density of epidermal cells was higher at pH 2.5. The thickness and volume of the mesophyll were also suppressed by SAR. Increasing the acidity of rain also decreased the trichome density (Table 3). Sharma *et al.* (1994) found a decrease in the SI in SAR-showered *Prunus persica* leaves along with a parallel decrease in stomatal frequency. Since the stomata per leaf area are precociously determined in early stages of ontogeny (Ciha and Brun 1975), a compressed leaf with the same number of cells and stomata would result in higher stomatal frequency, unless the leaves were at their primordial stages when they received SAR.

Table 3. Changes in anatomical features of green gram cv. ADT-1 and CO-5 exposed to simulated acidic rain (SAR) of pH 7.0, 5.5, 4.0, and 2.5. Lengths and breadths in [μm], stomatal, epidermal cell, and trichome numbers in [mm^{-2}]. Mesophyll thickness [μm] multiplied by 10 gives mesophyll volume [cm^3 m^{-3}]. Values in a row followed by different letters are significantly different according to Tukey's HSD multiple range test at 5 % level (n = 10).

	ADT-1				CO-5			
	7.0	5.5	4.0	2.5	7.0	5.5	4.0	2.5
Stomatal index	14.49^{a}	14.03^{a}	13.61^{a}	13.84^{a}	14.58^{a}	14.52^{a}	13.87^{ab}	13.05^{b}
length	26.14^{a}	26.95^{a}	25.24^{a}	22.59^{b}	24.75^{a}	24.99^{a}	24.95^{a}	24.01^{a}
breadth	15.51^{a}	14.46^{b}	14.70^{b}	15.09^{a}	14.72^{a}	14.35^{a}	14.54^{a}	14.65^{a}
number	124^{a}	132^{ab}	127^{a}	138^{b}	126^{a}	126^{a}	125^{a}	139^{b}
Epidermal cell length	72.77^{a}	72.52^{a}	66.40^{b}	61.25^{b}	76.20^{a}	71.54^{a}	72.52^{a}	61.50^{b}
breadth	39.45^{a}	34.79^{a}	36.75^{a}	28.86^{b}	41.90^{a}	40.18^{a}	40.67^{a}	33.81^{b}
number	732^{a}	809^{b}	806^{b}	859^{c}	738^{a}	742^{a}	776^{b}	926^{c}
Trichome number	5.0^{a}	4.8^{a}	4.3^{ab}	4.0^{b}	5.0^{a}	4.7^{ab}	4.5^{ab}	4.1^{b}
Mesophyll thickness	166^{a}	148^{b}	136^{c}	127^{c}	149^{a}	128^{b}	110^{c}	99^{c}

Our experiments confirm that the two cultivars of green gram differ in the sensitivities to SAR despite very little differences in the surface characteristics. The variations in the metabolic effects could be, as suggested by Thomas *et al.* (1944), a reflection of the pH-stat mechanism or buffering capacity of cells, which are cultivar specific and inherited.

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