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Stomatal conductance, photosynthetic rate, and pigment content in *Ruellia tuberosa* leaves as affected by coal-smoke pollution

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Abstract

Study of the effects of air pollution caused by thermal power plant emissions on some foliar traits of *Ruellia tuberosa* L. has shown that length and width of stomata, length of stomatal pore, stomatal density, photosynthetic rate, stomatal conductance and chlorophyll content were reduced in the polluted plants in pre-flowering, flowering as well as post-flowering phases of plant growth. Intercellular carbon dioxide concentration in the palisade tissue was increased at each stage of plant development. Stomatal index remained almost unchanged at the polluted site, except on the adaxial surface during the pre-flowering stage where it was higher as compared to the non-polluted plants.

Additional key words: carotenoids, chlorophyll, photosynthesis, stomatal conductance, stomatal index, stomatal morphology.

Introduction

Coal-fired thermal power stations release enormous amounts of gaseous and particulate pollutants, exerting a tremendous pressure on fauna and flora in the vicinity (Yunus and Iqbal 1996, Iqbal *et al.* 2000). Leaves experience the maximum brunt of exposure and accordingly undergo structural and functional alterations with changes in the surrounding environments. Stomatal behaviour determines the extent of absorption of pollutants by plants while the pollutants in turn influence the stomatal behaviour. Air pollution impact on plants often varies with differences in the stomatal conductance which is a function of the stomatal index, stomatal size and the extent of stomatal opening. The present study explores the effect of coal-smoke pollution on form and function of leaves of *Ruellia tuberosa* plants, with special reference to stomatal and photosynthetic responses.

Materials and methods

Ruellia tuberosa L. of the family *Acanthaceae* is an erect, annual herb or undershrub with elongated, fleshy, tuberous roots, elliptic or ovate leaves and blue violet and blue mauve flowers. The plants are propagated by cuttings or tubers or through seeds which are produced in brownish black capsules. The capsules explode on ripening and the seeds disperse all around. The herb possesses emetic properties and is employed as a substitute of ipecacuanha.

The study was carried out in the Aligarh district which lies in the north-west of Uttar Pradesh (a northern state of India) in the fertile agricultural area of the Ganga-Jamuna doab between 27° 29'N and 28° 11'N latitude and between 77° 29'E and 78° 38'E longitude. Kasimpur, a small town in this district, is about 187 meters above the sea level.

Kasimpur Thermal Power Plant, the point source of pollution in the present study, runs on the bituminous coal that has 2.92 % moisture, 22.20 % ash, 31.86 % volatile matters including 0.49 % sulphur, 5.61 % hydrogen, 5.24 %

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nitrogen, 20.23 % oxygen and 42.45 % fixed carbon on an average. The average emissions of SO_2, NO_x and CO_2 from the stacks of the power plant were 16 783, 300 667, and 6 853 814 kg h^{-1}, respectively. The soil is loam and clay-loam with a high pH and poor drainage. The climate is a dry tropical monsoon.

Sampling was made from ten plants at pre-flowering, flowering and post-flowering stages growing throughout their life at the polluted site (near Kasimpur thermal power station) and the non-polluted site (the Aligarh Muslim University Campus). Epidermal peels of fully expanded leaves, fixed in formaldehyde : acetic acid : 50 % alcohol (5:5:90), were obtained using hot nitric acid (Ghouse and Yunus 1972). Stomatal index was calculated by the formula of Salisbury (1927). Fully developed stomata were used for measurement of stomatal length, width and length of stomatal pore. Stomatal conductance, intercellular carbon dioxide concentration, and photosynthetic rate were measured using *LI-6200* Portable Photosynthesis System (*LICOR*, Lincoln, USA). The chlorophyll content was estimated according to Arnon (1949), using cold acetone and the absorbance was measured at 663, 645, 510 and 480 nm on spectrophotometer (*Beckman DU 640*, Switzerland). Chlorophylls *a* and *b*, and carotenoid contents were calculated by the formulas of Duxbury and Yentsch (1956) and MacLachlan and Zalik (1963). The data were analysed statistically to determine significance level of the variations observed.

Results

Leaves of *R. tuberosa* developed chlorotic and necrotic spots at the sites affected by coal-smoke. The number of leaves per plant increased with the plant age at both the polluted and the unpolluted sites. However, the overall number of leaves was comparatively smaller at the polluted site, the difference being statistically significant in the post-flowering stage. The leaf size was also reduced markedly at the polluted site during the whole plant life. Thus, the total leaf area per plant was conspicuously lower at the polluted site than at the unpolluted one, and so was the dry mass of the foliage (Table 1).

Table 1. Leaf features in different developmental stages of *R. tuberosa* plants growing at the polluted and the non-polluted sites. Means ± SE. n = 10 (* - significant at $P = 0.05$. ** - significant at $P = 0.01$, NS = non-significant).

Parameter	Stage	Non-polluted	Polluted	Variation [%]
Leaves per plant	pre-flowering	21.80 ± 4.26	18.60 ± 4.14	14.67^{NS}
	flowering	33.00 ± 5.97	32.80 ± 8.23	0.60^{NS}
	post-flowering	51.40 ± 10.55	35.00 ± 9.50	31.90*
Single leaf area [cm^2]	pre-flowering	26.30 ± 6.67	14.84 ± 3.84	43.57**
	flowering	28.64 ± 8.03	16.38 ± 4.51	42.80**
	post-flowering	32.88 ± 8.23	17.10 ± 4.84	47.99**
Total area of leaves [cm^2]	pre-flowering	547.38 ± 160.00	305.68 ± 90.86	44.15**
	flowering	1031.44 ± 128.73	587.66 ± 183.85	43.02**
	post-flowering	1419.56 ± 292.33	581.08 ± 197.93	59.06**
Leaf dry mass [g]	pre-flowering	1.07 ± 0.21	0.37 ± 0.06	65.42**
	flowering	1.40 ± 0.02	0.97 ± 0.02	30.71**
	post-flowering	2.69 ± 0.24	1.21 ± 0.12	55.01**

The stomata of both adaxial and abaxial epidermes increased in length and width with the age of the plant at the unpolluted site. Under pollution stress, stomata length and width were significantly smaller on both surfaces in each stage of plant growth. The difference in stomata length was maximum in flowering stage on the adaxial epidermis and post-flowering stage on the abaxial epidermis. The decrease in stomatal width on the abaxial epidermis was significant only in flowering stage. In this stage, was also the maximum decline in stomatal width on adaxial epidermis. Similarly, length of stomatal pore in both epidermes displayed an increasing trend with plant age. Pollution declined it significantly at post-flowering stage on the adaxial epidermis and at flowering and post-flowering stages on the abaxial epidermis (Table 2).

Stomatal density (S.D.) increased with plant age on both epidermes in control plants. It showed a marginal decline under the pollution stress on both epidermes except for the significant decline at the pre-flowering stage on adaxial epidermis. Per cent variation decreased consistently with

plant age in both epidermes. Stomatal index (S.I.) of both epidermal layers increased with plant age at the unpolluted site. Under pollution stress, it increased significantly in the pre-flowering stage (on adaxial surface only), and only slightly in the other stages. Per cent variation was maximum in the pre-flowering stage on both surfaces (Table 2).

Table 2. Stomatal size, density and index on leaf epidermes during different developmental stages of *R. tuberosa* plants growing in non-polluted as well as polluted areas. Means ± SE, n = 100 (* - significant at $P = 0.05$, ** - significant at $P = 0.01$, NS = non-significant).

Parameter	Stage	Adaxial epidermis non-polluted	polluted	Abaxial epidermis non-polluted	polluted
Length of stomata [μm]	pre-flowering	37.20 ± 4.05	35.00 ± 3.50*	35.10 ± 4.50	30.62 ± 4.42**
	flowering	40.63 ± 4.45	37.35 ± 4.36**	40.18 ± 4.00	35.55 ± 4.32**
	post-flowering	44.82 ± 4.41	41.40 ± 3.46**	45.00 ± 5.49	38.25 ± 3.42**
Width of stomata [μm]	pre-flowering	25.20 ± 3.73	22.95 ± 3.96*	23.85 ± 3.51	23.22 ± 3.35^{NS}
	flowering	29.07 ± 3.46	26.10 ± 4.30**	30.87 ± 3.80	25.92 ± 3.46**
	post-flowering	33.03 ± 3.95	30.00 ± 5.36**	33.75 ± 5.53	32.00 ± 3.87**
Length of stomatal pore [μm]	pre-flowering	23.72 ± 2.61	22.32 ± 3.75^{NS}	22.77 ± 3.65	22.32 ± 3.38^{NS}
	flowering	26.23 ± 3.73	25.02 ± 3.42^{NS}	26.10 ± 3.74	22.63 ± 3.60**
	post-flowering	28.17 ± 3.05	26.10 ± 3.30**	27.00 ± 3.15	25.55 ± 3.43*
Stomatal density [mm^{-2}]	pre-flowering	8.91 ± 2.42	6.77 ± 2.62*	11.05 ± 3.35	9.62 ± 3.24^{NS}
	flowering	12.47 ± 2.07	11.05 ± 3.12^{NS}	14.26 ± 2.35	12.13 ± 1.96^{NS}
	post-flowering	13.55 ± 3.27	13.19 ± 1.96^{NS}	15.50 ± 2.62	13.44 ± 2.01^{NS}
Stomatal index [%]	pre-flowering	72.44 ± 13.24	95.16 ± 14.80**	65.28 ± 11.40	57.12 ± 11.08^{NS}
	flowering	99.08 ± 17.08	103.56 ± 16.20^{NS}	88.46 ± 13.95	88.56 ± 10.15^{NS}
	post-flowering	116.56 ± 18.28	117.12 ± 19.48^{NS}	109.48 ± 15.84	108.04 ± 15.07^{NS}

Concentrations of chlorophylls *a* and *b* and carotenoids in leaves at the polluted and unpolluted sites decreased with increasing age of the plant but chlorophyll *a* showed slight deviation from this trend. The pigment content was significantly lower in each stage of plant development in the polluted zone. The differences in chlorophyll *b* decreased with plant age; the differences in chlorophyll *a* and carotenoids were greater in early stages than in the post-flowering stage (Table 3).

Stomatal conductance and net photosynthetic rate increased with plant age at the unpolluted site. Under pollution, stomatal conductance was relatively low and the difference between non-polluted and polluted plants were non-significant at pre-flowering stage but significant during the subsequent stages. The differences in photosynthetic rate were also higher in the later stages of plant life. The intercellular carbon dioxide concentration was highest in flowering stage and lowest in post-flowering stage at the non-polluted site. At the polluted site, it was increased significantly in pre-flowering and post-flowering stages and non-significantly during the flowering stage (Table 3).

Discussion

The reduced leaf area of *R. tuberosa* and lesser number of stomata reduced the volume of pollutants entering the leaves. The rate of absorption of air pollutants by the plant depends on the pollutants concentration gradient from exterior to interior of the leaf and on the stomatal conductance which play an important role in determining the impact of pollution on the plant. In the present investigation, length and width of stomata on both epidermes of the *R. tuberosa* leaf were significantly reduced under the pollution. The decrease in stomatal aperture or the stomatal closure may operate as an avoidance mechanism against inhibitory action of pollutants on photosynthesis (Iqbal *et al.* 1996). Thus, the reduced size of stomatal aperture in *R. tuberosa* should avoid its leaf damage. Similarly, Jensen and Kozłowski (1975) have demonstrated that more SO_2 is absorbed by *Fraxinus americana* leaves with large stomata and high stomatal conductance than by *Acer saccharum* leaves with small stomata and low stomatal conductance. Similar results on leaf conductance of ten species (trees and shrubs) in

Table 3. Pigment content, stomatal conductance and photosynthetic rate in leaves during the different developmental stages of *R. tuberosa* plants plants growing at the polluted and the non-polluted sites. Means ± SE, n = 10 (* - significant at $P = 0.05$, ** - significant at $P = 0.01$, NS = non-significant).

Parameter	Stage	Non-polluted	polluted	Variation [%]
Chlorophyll *a*	pre-flowering	1.04 ± 0.09	0.31 ± 0.08	70.19**
[μg g^{-1}(f.m.)]	flowering	0.86 ± 0.08	0.25 ± 0.04	70.03**
	post-flowering	0.24 ± 0.03	0.14 ± 0.02	41.66**
Chlorophyll *b*	pre-flowering	0.83 ± 0.13	0.22 ± 0.03	73.49**
[μg g^{-1}(f.m.)]	flowering	0.65 ± 0.06	0.19 ± 0.04	70.76**
	post-flowering	0.19 ± 0.04	0.10 ± 0.02	47.36**
Carotenoids	pre-flowering	0.38 ± 0.12	0.18 ± 0.08	52.63*
[μg g^{-1}(f.m.)]	flowering	0.25 ± 0.03	0.09 ± 0.03	64.00*
	post-flowering	0.17 ± 0.06	0.09 ± 0.03	47.05*
Stomatal conductance	pre-flowering	0.37 ± 0.12	0.18 ± 0.08	51.35**
[mmol m^{-2} s^{-1}]	flowering	0.42 ± 0.04	0.25 ± 0.08	40.47**
	post-flowering	1.90 ± 0.19	0.39 ± 0.05	79.47**
Net phytosynthetic rate	pre-flowering	10.32 ± 5.92	2.55 ± 1.26	75.29**
[μmol(CO_2) m^{-2} s^{-1}]	flowering	15.38 ± 1.30	7.65 ± 2.92	50.26**
	post-flowering	24.54 ± 3.31	23.21 ± 3.90	5.41^{NS}
Intercellular carbon dioxide	pre-flowering	247.20 ± 29.72	376.20 ± 22.37	52.18**
[μmol mol^{-1}]	flowering	278.51 ± 11.34	300.12 ± 73.91	7.75^{NS}
	post-flowering	14.80 ± 13.46	34.18 ± 16.23	130.94**

California have provided a good index of SO_2 uptake and resistance (Winner *et al.* 1982). Many other studies have reported variations in stomatal density (*e.g.* Khan and Khan 1994; Nighat *et al.* 1999). This study revealed that stomatal index (SI) on the adaxial leaf surface of *R. tuberosa* was greater at the polluted site; the maximum variation occurred in pre-flowering stage on both surfaces. Thus, the stomata and epidermal cells of the two surfaces seem to respond to the pollution load differentially at different stages of plant development. In the poplar clones, SI reduces under raised CO_2 in the expanding leaves of the upper plant parts (Ceulemans *et al.* 1995).

SO_2 entering in the leaves may affect the malate content (Kondo *et al.* 1984), and PEP carboxylase (Ziegler 1973, Mukerji and Yang 1974) or malate dehydrogenase (Ziegler 1974) activities which could regulate stomatal opening (Raghavendra 1980). In *R. tuberosa* leaves, stomatal conductance was significantly reduced at the polluted site, conforming to some earlier reports (Field *et al.* 1995, Kull *et al.* 1996; Kellomäki and Wang 1997) and could be cause of low photosynthetic rate (Farage *et al.* 1991). Coal-smoke pollution is known to affect photosynthesis. In the present investigation, the highest reduction (75.3 %) in the net photosynthetic rate was recorded in the pre-flowering stage. Also the intercellular CO_2 concentration was raised under pollution stress, more markedly in pre-flowering and post-flowering stages. Reduction in leaf area may be a factor contributing to the decline in photosynthesis per plant.

Leaves of *R. tuberosa* were highly sensitive to pollution load and the extent of leaf damage was closely related to stomatal responses to pollution.

The pollution load had an adverse effect on the total chlorophyll content in *R. tuberosa*. Chlorophyll *a* was maximum at flowering stage while chlorophyll *b* and carotenoids at the pre-flowering stage. Chlorophyll *b* was more severely affected than chlorophyll *a* as noticed earlier in various woody and non-woody plants (Joshi *et al.* 1993, Ajay and Subrahmanyam 1996). However, both the chlorophylls may be equally susceptible in some other species (Singh and Rao 1980, Singh *et al.* 1990a). SO_2 (Esmat 1993, Ali 1998) and O_3 (Khan and Khan 1994) inhibit chlorophyll biosynthesis. The total chlorophyll and carotenoid contents decrease in tomato leaves with increasing sodium metabisulphate ($Na_2S_2O_3$) concentration (Singh *et al.* 1990b). Chlorophyll *a* is thought to be degraded to phaeophytin under SO_2 effect by replacing Mg^{+2} ions from chlorophyll molecules. In chlorophyll *b*, SO_2 removes the phytol group of the molecule (Rao and Le Blanc 1966).

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