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PHYSIOLOGICAL AND YIELD RESPONSES OF PEA PLANTS TO AMBIENT AIR POLLUTION

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SUMMARY

The study aimed to assess the effect of ambient air pollution on physiological as well as yield characteristics of pea plants grown at different sites in the periurban area of Varanasi city. Assessment of selected paramenters such as photosynthesis rate, stomatal conductance, transpiration rate, water use efficiency, pigment concentrations, yield and quality of seeds were made in relation to ambient concentrations of SO_2, NO_2 and O_3. The ambient levels of all the pollutants were lowest at site 1 (BHU), and hence this site was treated as reference site for comparing the response pattern observed at other sites. Photosynthetic rate and water use efficiency declined at sites 2 to 6 in response to ambient levels of pollutants. Stomatal conductance did not correspond directly to the lowering of photosynthesis. Total chlorophyll content and yield were less at sites 2 to 6 compared to reference site 1. Seed quality in terms of metabolite and nutrient contents also deteriorated at sites receiving higher pollution load. The study indicated that ambient air pollution has potential negative impact on physiological characteristics of pea plants, which led to lower yield and inferior quality of seeds at sites in periurban areas experiencing higher levels of pollutants.

***Key words*: Ambient air quality, photosynthesis, seed quality, stomatal conductance, yield, water use efficiency.**

INTRODUCTION

Air pollution has become a serious environmental stress to crop plants due to increasing industrialization and urbanization during last few decades. Urban air pollution may have direct impact on crop production in periurban and adjacent rural areas due to dispersion of pollutants emitted in the cities in different directions along the wind. The gaseous air pollutants known to cause adverse impact on crop production are sulphur dioxide (SO_2), nitrogen oxides (NO_x) and ozone (O_3) (Agrawal 2000). Emissions of these pollutants are increasing in India due to massive increase in urbanization and consequent increases in number of vehicles and small-scale industries in cities. Laboratory experiments have shown that the gaseous pollutants cause deleterious effect on physiological and biochemical characteristics of plants. In ambient condition, combinations of various pollutants in different proportions may cause greater impact on carbon fixation and hence on yield (Heck *et al*. 1988). The present study reports the results of pot experiments conducted in ambient condition in periurban area of Varanasi city to assess the impact of air pollutants generated in the city on physiological characteristics, yield and seeds quality of pea (*Pisum sativum*) plants.

MATERIALS AND METHODS

Six sites were selected in the outskirts of Varanasi city (25º14'N latitude, 82º03 E longitude and 76.19m

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above sea level) in agricultural area for the experiments. The sites were selected in all the directions from the city on the basis of some specific activities (Table 1). The plants were grown in earthen pots (30 cm diameter) filled with soil prepared at one place by mixing garden soil and farm yard manure in 3:1 ratio. Three seeds of *P. sativum* L. var. Arkel were sown at equal distance and after germination, plants were thinned to one per pot. Just after germination, twenty pots were transferred to each site. During growing season of pea the average mean monthly maximum temperature ranged from 21.1 to 28.4ºC and minimum from 9.2 to 16.5ºC. The mean monthly maximum humidity ranged from 83.6 to 86.6% and minimum from 47.8 to 68.8% and wind direction was mostly westerly or north westerly.

Air quality monitoring was done by using handy gas samplers two times in a week for 6 hrs daily for SO_2, NO_2 and O_3 using wet chemical methods at all sites after transfer of germinated plants up to their maturity. SO_2, NO_2 and O_3 were scrubbed separately in tetrachloromercurate, NaOH (0.1N) and buffered KI (0.1N), respectively. These absorbing solutions were further analysed colorimetrically for SO_2 (West and Gaeke 1956), NO_2 (Merrymann *et al*. 1973) and O_3 (Byers and Saltzman 1958) pollutants. Physiological characteristics such as photosynthesis rate, stomatal conductance, transpiration rate and internal CO_2 concentrations on fully expanded third leaflet from apex were measured with the help of LICOR Portable Photosynthetic system (Model 6200 LICOR, Lincoln, NE, USA) at ambient climatic conditions. The measurements were made on cloud free days between 9:00 and 11:00 h on three randomly selected plants at each site. The water use efficiency (WUE) was calculated as a ratio of photosynthesis rate to transpiration rate. The total chlorophyll and carotenoids were extracted through 80% acetone and contents were calculated by using the formulae of Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively.

Yield was calculated by weighing seeds plant^{-1}. Twenty replicates were used for yield measurement. The seed quality was assessed with respect to different pools of carbohydrates, proteins, potassium (K), calcium (Ca), sulphate-sulphur (SO_4^{2-} S) and nitrogen (N) contents. The reducing sugar was determined by using the method of Somogyi Nelson (Herbart *et al*. 1971) and total soluble sugar and starch by following phenol/H_2SO_4 colorimetric assay (Dubois *et al*. 1956). Protein content was estimated by Lowry *et al*. (1951). For K and Ca analysis seeds were powdered and digested in mixtures of ternary acid ($HClO_4$, HNO_3 and H_2SO_4 in 5:1:1 ratio) following the method described by Allen *et al*. (1974). Digested samples were analyzed for K and Ca content with the help of Atomic Absorption Spectrophotometer (Model-2380, Perkin Elmer, U.S.A). The method of Rossum and Villaruz (1961) was used for the estimation of SO_4^{2-} S. Total N was estimated by using the Gerhardt Automatic N analyzer (Germany).

Table 1. Characterization of experimental sites situated around Varanasi City.

Site	Direction and distance from the city centre	Site no.	Comments
B.H.U.	South 5.8 km	1	Residential localities, official buildings, plantations, gardens, cultivated lands and minor roads
Govt. Ag. Farm	West 5.2 km	2	Large cultivated land, some intersections, vehicle stops, small scale industries, 50 m from NH2 highway
Phulwaria	North West 5.5 km	3	Near railway tracks and cantonment area, plantations, residential localities, 50 m from railway track
Shivpur	Norht West 6.8 km	4	High Density population, small scale industries and busy roads
Sarnath	North East 6.9 km	5	Medium density population, agricultural field, dense vegetation and tourist spot
Ramnagar	Sourth East 5.7 km	6	Residential localities and markets

The data were analysed statistically following analysis of variance (ANOVA) and Duncans multiple range tests. The correlation coefficients were also determined between some of the parameters.

RESULTS AND DISCUSSION

The air quality monitoring data showed variations in concentrations of SO_2, NO_2 and O_3 at different sites situated around Varanasi city. The study sites can be ranked from maximum to minimum concentration for SO_2 as site 3>2>6>4>5>1, for NO_2 4>6>3>2>5>1 and for O_3 6>5>4>3>2>1 (Fig. 1). The highest concentration of SO_2 was found at site 3 which is situated near railway tracks and medium density residential localities from where domestic emissions are the main source of SO_2. It was followed by site 2 having small scale industries and some road intersections. Some transport agencies are also located there which allow parking of vehicles. Site 5 located far from the city center having medium density population, agricultural field and dense vegetation showed lower pollutant levels than other sites. Site 1 having official buildings, good plantation, large canopies absorbing the pollutants resulted in lowest concentration of all the gaseous pollutants. NO_2 concentration was found maximum at site 4 where small scale industries mostly of saree printing, dense population and busy roads are the sources of NOx. However, O_3 concetration was found maximum at site 6 having residential localities and markets. It may be because during winter season wind flows from north west to south east directions hence whole city plume went to south east direction and thus accumulation of O_3 precursors led to maximum O_3 formation at site 6.

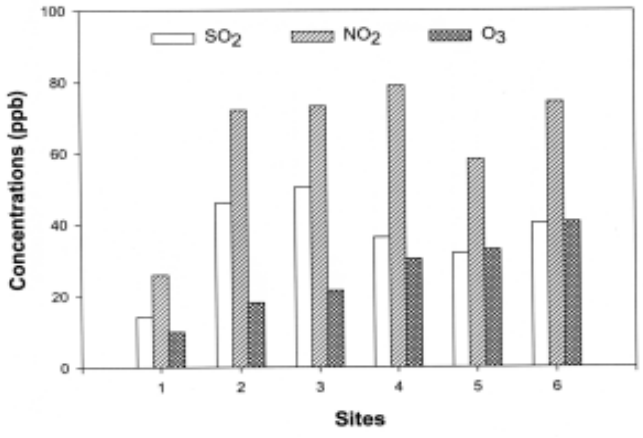

Fig. 1. Mean air pollutant concentrations (ppb) at different sites around Varanasi city.

Pea plants showed significant difference in photosynthesis rate (Pn) among sites. Pn declined by 33.8, 50.6, 60.4, 21.7 and 56.9%, respectively at sites 2 to 6 as compared to that of site 1 (Table 2). Pn was found maximum at site 1 and minimum at sites 4 and 6. Significant negative correlations between Pn and individual concentrations of pollutants were observed. The correlation coefficient values were highest for O_3 ($r = -0.87$; $p<0.01$) followed by SO_2 ($r = -0.79$; $p<0.01$) and NO_2 ($r = -0.66$; $p<0.01$). Gaseous pollutants have been shown to inhibit photosynthesis rate depending upon exposure dose and species involved (Darrall 1989). In the present study, a variety of gaseous pollutants were present, hence, a combined impact may have reduced the threshold concentration for individual pollutants. Gaseous pollutants enters into the foliage through stomata, hence, pattern of stomatal opening and closing under pollutant stress are important in affecting CO_2 uptake. The stomatal conductance (Cs), however, did not show a specific pattern in response to pollutant levels at different sites. Cs declined by 17.1 and 39%, respectively at sites 3 and 4 and increased by 7.3, 31.7 and 19.5% respectively at sites 2, 5 and 6 (Table 2). The sites showing reduction in stomatal conductance also showed higher internal CO_2 concentration than that observed at other sites. It is due to build up of internal CO_2 in leaves, which occurs due to impairment of photosynthetic fixation of CO_2 (Mckee *et al*. 1995).

Transpiration rate increased by 60, 40, 60 and 40%, respectively at sites, 2, 4, 5 and 6 while there was no significant change at site 3 (Table 2). This trend may be explained on the basis of variations in pollutant combinations at different sties. O_3 is known to reduce transpiration in plants (Sanders *et al*. 1992), but in presence of SO_2 and NO_2 transpiration rate increased even at site 6 experiencing high O_3. WUE showed significant decrease in pea plants at all sites compared to reference site 1 (Table 2). The reduction in WUE may be attributed to both lowering of Pn and increase in transpiration rates at sites experiencing high pollution load. SO_2 at concentrations of 60 ppb for 8 h d^{-1} for 70 days is shown to reduce WUE in soybean plants due to increase in transpiration rate because photosynthesis did not differ significantly (Deepak and Agrawal 2001).

Table 2. Site wise changes in the physiological characteristics of *P. sativum* plants (Mean ± SE).

Sites	Physiological characteristics				
	Photosynthesis (μmol CO_2 $m^{-2}s^{-1}$)	Internal CO_2 concentration (μmol mol^{-1})	Stomatal conductance (cm s^{-1})	Transpiration (mmol H_2O $m^{-2}s^{-1}$)	Water use efficiency (μmol CO_2/mmol H_2O)
1	11.51[a]	283.90[c]	0.41[d]	0.005[c]	2.30[a]
	±0.41	±7.37	±0.01	±0.0001	±0.08
2	7.62[c]	275.83[c]	0.44[c]	0.008[a]	0.95[bc]
	±0.09	±1.17	±0.01	±0.00003	±0.01
3	5.68[d]	360.80[a]	0.34[e]	0.005[c]	1.14[bc]
	±0.38	±6.48	±0.01	±0.0001	±0.07
4	4.56[d]	323.20[b]	0.25[f]	0.003[d]	1.29[b]
	±0.64	±15.22	±0.01	±0.0002	±0.29
5	9.01[b]	279.10[c]	0.54[a]	0.008[a]	1.13[bc]
	±0.43	±3.32	±0.01	±0.00003	±0.05
6	4.96[d]	320.53[b]	0.49[b]	0.007[b]	0.71[e]
	±0.19	±1.73	±0.01	±0.0001	±0.03

Within each grouping, values not followed by the same letter are significantly different at $p \leq 0.05$.

Total chlorophyll was maximum at site 1 and minimum at site 2 (Fig. 2). The correlation coefficients between total chlorophyll and individual pollutants showed most significnat negative impact of SO_2 ($r = -0.78$; $p<0.01$) followed by NO_2 ($r = -0.74$; $p<0.01$) and O_3 ($r = -0.47$; $p<0.05$). A decrease in chlorophyll has often been suggested as an indicator of SO_2 and O_3 injury (Agrawal and Agrawal 1989). It has been suggested that polluting gases such as SO_2, NO_x and O_3 produce oxyradicals in reactions with cell components (Shimazaki *et al*. 1980). These radicals cause widespread damage to membranes and associated molecules including the chlorophyll pigments (Sakaki *et al*. 1983). Pollutants have been shown to reduce the synthesis of chlorophyll and enhance degradation of chlorophyll (Sandelius *et al*. 1995). Carotenoid content was significantly higher at site 1 compared to other sites (Fig. 2). More than additive reductions in carotenoid content of a variety of crop plant due to SO_2 and O_3 exposure have been reported (Agrawal 1985). Carotenoids protect chlorophyll from photoxidative destruction (Siefermann-Harms 1987).

Weight of seeds $plant^{-1}$ was found maximum at site 1 and minimum at site 6 (Fig. 3). As compared to site 1,

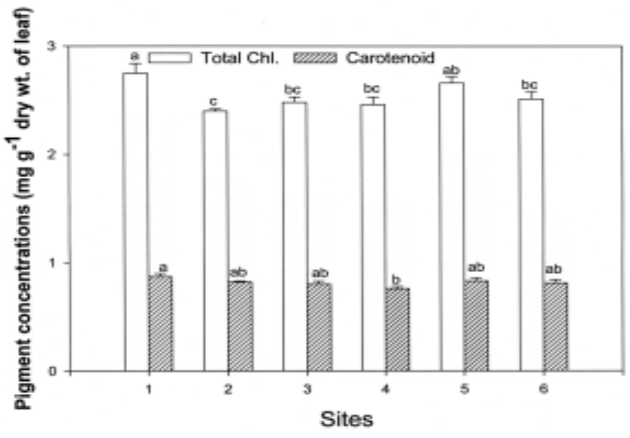

Fig. 2. Changes in pigment concentrations of pea plants grown at different sites around Varanasi city. For each parameter values not followed by the same letter are signficantly different at $p < 0.05$.

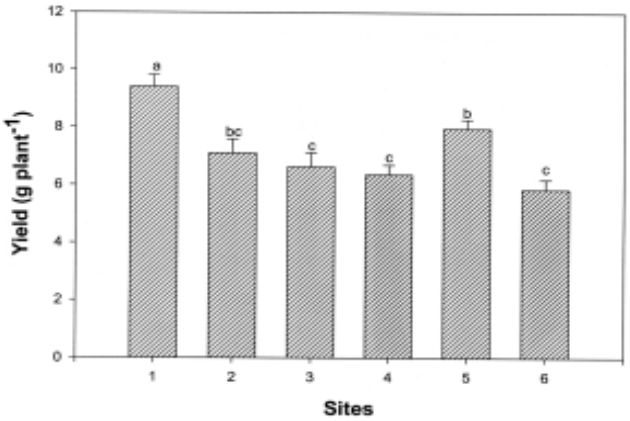

Fig. 3. Changes in yield of pea plants kept at different sites around Varanasi city. Values not followed by the same letter are significantly different at $p < 0.05$.

Table 3. Site wise changes in the seed characteristics of *P. sativum* plants (Mean ± SE).

Sites	Metabolites (mg g^{-1} dw)				Nutrients (mg g^{-1} dw)			
	TSS	Red sugar	Starch	Protein	K	Ca	SO_4^{2-} S	Nitrogen
1	2.85[a]	24.60[a]	311.33[a]	287.67[a]	10.98[a]	0.76[a]	2.40[d]	13.86[a]
	±0.01	±0.34	±8.51	±11.35	±0.11	±0.01	±0.20	±0.39
2	2.35[c]	23.00[b]	275.67[bc]	247.67[bc]	9.81[b]	0.65[bc]	5.90[a]	13.05[ab]
	±0.01	±0.56	±8.25	±11.85	±0.12	±0.01	±0.11	±0.51
3	2.22[d]	22.18[bc]	266.67[dc]	235.00[bc]	9.77[b]	0.63[cd]	5.70[a]	12.48[ab]
	±0.02	±0.10	±6.76	±10.41	±0.11	±0.01	±0.05	±0.36
4	2.12[e]	21.93[bc]	254.33[c]	225.33[bc]	9.75[b]	0.60[bc]	5.60[a]	11.85[bc]
	±0.01	±0.05	±7.75	±14.15	±0.12	±0.01	±0.11	±0.43
5	2.58[b]	23.01[b]	285.00[b]	260.33[ab]	9.98[b]	0.68[b]	3.90[c]	13.28[ab]
	±0.01	±0.53	±7.64	±10.17	±0.07	±0.02	±0.06	±0.42
6	1.99[f]	21.24[c]	250.67[c]	217.00[c]	9.18[c]	0.57[e]	5.10[b]	10.87[c]
	±0.03	±0.11	±6.36	±11.50	±0.11	±0.01	±0.06	±0.52

Within each grouping, values not followed by the same letter are significantly different at $p \leq 0.05$.

weight of seeds $plant^{-1}$ reduced by 24.5, 29.5, 32.2, 15.3 and 37.8%, respectively at sites 2, 3, 4, 5 and 6. The seed weight showed significant negative correlation with individual pollutants. The correlation coefficient value was highest with O_3 ($r = -0.71$; $p<0.01$) followed by SO_2 ($r = -0.66$; $p<0.01$) and NO_2 ($r = -0.52$; $p<0.01$). According to Heggested and Lesser (1990), O_3, SO_2 and NO_2 individually and in combination have reduced the yield of many crop plants. Yield losses have often been attributed to reductions in photosynthetic activity and assimilate supplies to support reproductive growth and seed development (Balaguer *et al*. 1995, Pleijel *et al*. 1998).

The disturbed physiological activities of plants at sites receiving higher pollutant levels not only led to lower weight of seeds but also deterioration of quality in terms of reductions in carbohydrate pools, protein and nutrient contents. The sites can be ranked from maximum to minimum with respect to above seed quality as 1>5>2>3>4>6 (Table 3). Sulphate-S content was, however, maximum at site 2 experiencing maximum SO_2 concentration. Sulphur accumulation in plants is suggested to be a reliable measure of SO_2 contamination in air (Agrawal 2000).

The study clearly indicates that the ambient air pollutants in the periurban area of Varanasi have potential negative effect on physiological characteristics, which have led to yield reductions of pea plants. Quality of seeds also deteriorated due to air pollutants.

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