

## Seasonal variations in adaptational strategies of *Beta vulgaris* L. plants in response to ambient air pollution: Biomass allocation, yield and nutritional quality

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**Abstract:** Seasonal variations and effects of ambient air pollutants on palak (*Beta vulgaris* L. var. Allgreen) plants were investigated with respect to root, shoot length, number of leaves plant<sup>-1</sup>, leaf area and root and shoot biomass at a suburban site situated in dry tropical area of India, experiencing elevated levels of ambient air pollutants. Air monitoring data showed that mean concentrations of SO<sub>2</sub> and NO<sub>2</sub> were higher during winter, whereas O<sub>3</sub> was the major air pollutant during summer. Plants grown in non filtered chambers showed stunted growth, reductions in biomass and yield and modification in biomass allocation pattern as compared to those grown in charcoal filtered air. Magnitudes of changes in various parameters were more in summer than winter season. Nutritional quality of palak was also negatively affected in non filtered chambers during winter but there was no significant change in nutritional quality during summer season. Biomass allocation pattern revealed that during summer photosynthate allocation to roots reduced with consequent increment in leaf weight ratio, which helped in sustaining nutritional quality of palak even after more yield reductions in NFCs as compared to FCs. This study depicts the variations in adaptational strategies of palak in two different seasons.

**Resumen:** Se investigaron las variaciones estacionales y los efectos de los contaminantes ambientales del aire sobre plantas de palak (*Beta vulgaris* L. var. Allgreen) en términos de la raíz, la longitud del vástago, el número de hojas por planta, el área foliar y la biomasa de la raíz y del vástago, en un sitio suburbano de la región tropical seca de la India sujeto a niveles elevados de contaminantes ambientales del aire. Los datos de monitoreo del aire mostraron que las mayores concentraciones medias de SO<sub>2</sub> y NO<sub>2</sub> se presentaron durante el invierno, mientras que el O<sub>3</sub> fue el principal contaminante del aire en el verano. Las plantas que crecieron en cámaras sin filtros mostraron un crecimiento achaparrado, reducciones en la biomasa y en el rendimiento, y una modificación en el patrón de asignación de biomasa, en comparación con las que crecieron en aire filtrado con carbón. La magnitud de los cambios en varios parámetros fue mayor en el verano que en el invierno. La calidad nutricional del palak también fue afectada negativamente en las cámaras no filtradas durante el invierno, pero no hubo cambios significativos en la calidad nutricional durante el verano. El patrón de asignación de biomasa reveló que durante el verano se redujo la asignación de fotosintatos a las raíces, con un consecuente incremento en el cociente de peso foliar, lo cual contribuyó a mantener la calidad nutricional del palak aun después de más reducciones del rendimiento en presencia de NFCs en comparación con los FCs. El estudio ilustra las variaciones en las estrategias adaptativas del

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palak en dos estaciones contrastantes.

**Resumo:** As variações sazonais e efeitos dos poluentes aéreos no ar nas plantas de beterraba (*Beta vulgaris* L. var. Allgreen) foram investigadas em relação à raiz, comprimento do lançamento, número de folhas.planta<sup>-1</sup>, área folhear e biomassa da raiz e do lançamento numa estação suburbana situada numa área tropical seca na Índia e experimentando elevados níveis de poluentes na atmosfera. Os dados da monitorização atmosférica mostraram que as concentrações médias de SO<sub>2</sub> e NO<sub>2</sub> eram elevadas durante o inverno, enquanto o O<sub>3</sub> era o principal poluente atmosférico durante o verão. As plantas crescendo em câmaras não filtradas apresentaram crescimento definhado, reduções na biomassa e rendimento, bem como modificações no padrão de afectação da biomassa quando comparadas com aquelas vegetando em atmosfera filtrada por carvão. A dimensão das mudanças nos vários parâmetros foram maiores no verão do que no inverno. A qualidade nutricional da beterraba foi também negativamente afectada em câmaras não filtradas durante inverno mas não apresentaram mudanças significativas de qualidade nutricional durante o verão. O padrão de afectação da biomassa revelou que durante o verão a afectação dos fotossintetizados às raízes reduziu-se com um aumento conseqüente do ratio de peso da folha, o que ajudou a sustentar a qualidade nutricional da beterraba mesmo depois de maior redução do rendimento na NFCs quando comparada com o lote FCs. Este estudo evidencia as variações nas estratégias adaptativas da beterraba em duas estações diferentes.

**Key words:** Biomass allocation, NO<sub>2</sub>, nutritional quality, O<sub>3</sub>, open top chambers, SO<sub>2</sub>, yield.

## Introduction

Ambient air pollutants are known to adversely affect the growth and economic yield of several crops all over the world (Agrawal *et al.* 2003; Ariyaphanphitak *et al.* 2005; Heck *et al.* 1988; Ishii *et al.* 2004; Jagar *et al.* 1994; Kobayashi *et al.* 1995; Wang *et al.* 2007). The principal gaseous atmospheric pollutants worldwide are SO<sub>2</sub>, NO<sub>x</sub> and O<sub>3</sub>. All these gases are phytotoxic to varying extents, singly and in combination. Earlier, air pollution impacts characteristically caused severe, but localized effects close to emission sources in urban and industrialized areas. Air pollution problems have now assumed regional significance. Effects of air pollutants like SO<sub>2</sub>, NO<sub>x</sub> and O<sub>3</sub> have been described in terms of inhibition in physiological processes (Agrawal *et al.* 2003; Verma *et al.* 2000), alteration in metabolic functions and enzyme activities (Loewus *et al.* 1990; Okpodu *et al.* 1996), nutrient uptake (Agrawal & Verma 1997) and suppression of growth and yield (Agrawal *et al.* 2003; Agrawal *et al.* 2006; Biswas *et al.* 2008; Tiwari *et al.* 2006).

The problem of air pollution assumes greater importance in developing countries, which are rapidly industrializing. Significant yield reductions in

different crops have been recorded in China (Wang & Mauzerall 2004; Wang *et al.* 2007, 2008), India (Rai *et al.* 2007; Singh *et al.* 2009; Tiwari *et al.* 2006) and Pakistan (Wahid 2006a & b). Reduction of 45.32 and 38.68 %, respectively were recorded in root and shoot biomass of carrot plants in open top chambers under 8 h mean concentrations of 37.45 ppb SO<sub>2</sub>, 38.72 ppb NO<sub>2</sub> and 38.37 ppb O<sub>3</sub> at a suburban site of Varanasi, India (Tiwari *et al.* 2006). In a similar experimental setup, Wahid (2006a) recorded yield reductions varying from 13 to 44 % as compared to charcoal filtered chambers, in four barley cultivars at 8 h mean O<sub>3</sub>, NO<sub>2</sub> and SO<sub>2</sub> concentrations of 71, 30 and 16 ppb, respectively in the outskirts of Lahore, Pakistan. In a gradient study, yield losses of 30 to 50 % were recorded in wheat, depending upon the distance from coal fired power plants (CFPP) where the main pollutant was SO<sub>2</sub> (Agrawal 2005). Agrawal *et al.* (2003) reported 34 % reduction in yield of mungbean at a site experiencing 6 h average O<sub>3</sub> concentration of 55 ppb. Singh *et al.* (2009) reported yield reduction of 16.4 % in mustard at 12 h average O<sub>3</sub> concentration ranging from 41.65 to 54.2 ppb. Significant reductions in yield of *Vigna radiata* (Agrawal *et al.* 2003), *Beta vulgaris* (Singh *et al.* 2005) and

*Triticum aestivum* (Agrawal *et al.* 2004) have been reported at higher concentrations of ambient SO<sub>2</sub>, NO<sub>2</sub> and O<sub>3</sub> using a transect approach.

National ambient air quality data have shown that pollutant concentrations in ambient air are increasing. Survey conducted by Central Pollution Control Board (CPCB), New Delhi in 2004 showed that annual mean SO<sub>2</sub> concentrations varied from 5 to 7.6 ppb in different parts of the country from 2000 to 2003. Annual mean NO<sub>2</sub> concentrations showed variations from 13.8 to 34.4 ppb from 2000 to 2003. Data from CPCB further showed that 24 h average concentration of SO<sub>2</sub> and NO<sub>2</sub> varied from 2.3 to 5.7 ppb and from 36.6 to 57.7 ppb, respectively in 2005 (CPCB 2005) and 2.6 to 13.3 ppb and 12 to 103 ppb, respectively across the country in February 2007 (CPCB 2007). Studies conducted in Varanasi during 1989 - 2001 showed that SO<sub>2</sub> concentrations varied from 5.3 to 55 ppb and NO<sub>2</sub> from 8.5 to 82 ppb (Trivedi *et al.* 2003). In the recent years, increasing concentrations of O<sub>3</sub>, a secondary pollutant, have been reported throughout the country. In India, high O<sub>3</sub> concentrations have been reported from several parts of the country like Trivandrum and Pune (Saraf & Beig 2004), Delhi (Jain *et al.* 2005; Saraf & Beig 2004) and Varanasi (Tiwari *et al.* 2008). Twelve hourly mean monthly O<sub>3</sub> concentrations varied from 45 to 62 ppb during summer and 28.5 to 44 ppb during winter season from 2002 to 2006 at the experimental site (Tiwari *et al.* 2008).

Several techniques such as gradient analysis, use of antiozonant like ethylenediurea (EDU) as a tool and air filtration have been adopted to analyze the impact of ambient air pollution on crop yield. Use of open top chambers (OTCs) is a widely accepted technique to estimate the yield losses of crops by filtering the pollutant gases from the chambers. Several recent studies in Asia such as with barley (Wahid 2006a) and wheat (Wahid 2006b) at Lahore, Pakistan, wheat (Rai *et al.* 2007) and carrot (Tiwari *et al.* 2006) at Varanasi, India and Chinese cabbage in China (Yang *et al.* 2006) have reported significant growth and yield reductions in plants growing in non filtered air as compared to those in charcoal - filtered air.

The objective of the present investigation was to assess the seasonal variations in growth and yield responses of a leafy vegetable (*Beta vulgaris* L. var. Allgreen) under ambient air pollutant concentrations using open top chambers with emphasis on biomass allocation pattern and nutritional quality. Palak is a nutritious vegetable rich in iron and grown throughout the year. Significant varia-

tions in air pollutant concentrations have been recorded throughout the year at the experimental site and hence pattern of pollutant combinations may play an important role in modifying the plant response in two seasons. The present study has focused on different biomass allocation strategies adapted by the plants growing in two different seasons with significant variations in ambient air pollutant concentrations.

## Material and methods

### *Study area*

The study was conducted at Susuwahi, a suburban area of Varanasi, located in the Eastern Gangetic Plains of Indian subcontinent at 25° 14' N latitude, 82° 03' E longitude and 76.19 m above mean sea level. The climate of the area is tropical monsoonic with distinct summer (March to June), winter (November to February) and rainy (July to October) seasons. Susuwahi is located south of the city, about 6 km from the city centre. The present experiment was carried out during summer (April-May) and winter (November - December) seasons of the year 2005. Variations in climatological data during the different study periods are shown in Table 1.

### *Experimental design*

Six OTCs were installed at the experimental site. Chambers were 1.8 m in height and 1.5 m in diameter, fabricated according to the design of Bell & Ashmore (1986). A detailed description of the OTC design is given by Tiwari *et al.* (2006). Each chamber was attached to a high speed blower that ensured three air changes per minute around the inner perimeter of the chamber. This type of OTCs have been extensively used in air filtration studies in a number of locations in south east England, India and Pakistan over the last decade (Rai *et al.* 2007; Singh *et al.* 2009; Tiwari *et al.* 2006; Wahid 2006a&b). Three of these chambers were equipped with charcoal filters and were treated as filtered chambers (FCs) and other three were non filtered chambers (NFCs). All the chambers were provided with prefilters made up of non woven polyester to remove dust entering in the air flow. During the experimental period, the OTCs were ventilated continuously by passing air through filters. Temperature and relative humidity were 0.1 to 0.2 °C and 2 to 4 %, respectively more in the chambers as compared to outside. The light intensity in the chambers was 95 % of the ambient level.

**Table 1.** Variations in the climatological conditions during the study period.

Season	Total rainfall (mm)	Mean monthly temperature (°C)		Mean monthly relative humidity (%)		Average sunshine hours (h)
		Maximum	Minimum	Maximum	Minimum	
Winter	00	28.8	14	86.25	41	8
Summer	00	40.8	25.5	50.5	21	9.1

Field was prepared using standard agronomic practices. Seeds of palak (*Beta vulgaris* L. var. All-green) were hand sown in rows in OTCs. Treatment was started after germination of the plants and was continued till the final harvest. Subsequent thinning was done manually and the field was maintained in well watered condition. The experimental design was a randomized block design with three OTCs each for filtered and non filtered treatments.

#### *Air pollution monitoring*

Eight hourly monitoring for SO<sub>2</sub> and NO<sub>2</sub> concentrations was done at the experimental site using portable gas sampler following wet chemistry methodologies. Monitoring was done between 8.00 to 16.00 h IST thrice a week. The sampling probe was placed successively in FCs and NFCs in the centre just above the crop canopy. The height of the sampling probe was adjusted to crop height accordingly. SO<sub>2</sub> was estimated by the method of West & Gaeke (1956), NO<sub>2</sub> by Merryman *et al.* (1973) and O<sub>3</sub> by Photometric Ozone Analyzer (Model 400A, API, Inc., USA).

#### *Plant sampling and analysis*

Morphological parameters like root and shoot length, number of leaves plant<sup>-1</sup> and leaf area were estimated at the end of the vegetative growth (45 days after germination, DAG). Leaf area was measured using a leaf area meter (Model 3100, LICOR, Inc., Lincoln, NE, USA). For biomass determination, monoliths containing a single plant were washed and root and shoots separated and oven dried at 80 °C till constant weight. Dry weights of the plant parts are expressed as g plant<sup>-1</sup>. Yield was calculated by the fresh weight of the edible part (shoot) of the plant. For all the measurements, 10 plants per chamber were taken. To assess the biomass allocation, growth indices like relative growth rate (RGR), net assimilation rate (NAR), specific leaf weight (SLW), specific leaf area (SLA), leaf weight ratio (LWR), root shoot

ratio (RSR), etc. were calculated from biomass data using the formulae modified by Hunt (1982).

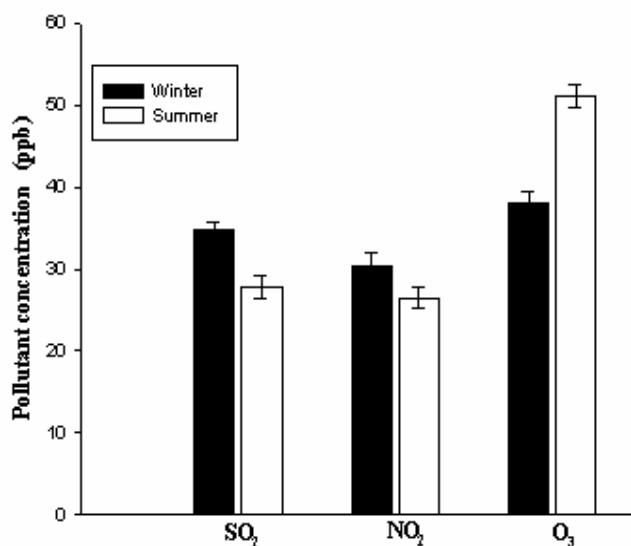
For nutrient analysis, oven dried samples of final harvest were ground in a stainless steel grinder and passed through a 0.5 mm sieve. For determination of Na<sup>+</sup>, Ca<sup>2+</sup>, K<sup>+</sup>, Mg<sup>2+</sup> and Fe<sup>2+</sup> contents, digestion of powdered samples was done by the method given by Allen *et al.* (1974). The nutrients in the digested material were determined using Atomic Absorption Spectrophotometer (Model 2380, Perkin- Elmer, USA).

#### *Statistical analysis*

All statistical analysis was done by using SPSS/PC programme (version 11). Mean and standard error of the replicates was calculated through one way analysis of variance (ANOVA). The significance of difference between treatments was calculated by student t test. Two way analysis of variance (ANOVA) was conducted on root and shoot length, number of leaves plant<sup>-1</sup>, area, biomass and yield.

## **Results and discussion**

Air monitoring conducted at the experimental site showed distinct seasonal variations in pollutant concentrations during the experimentation year (Fig. 1). Mean monthly concentrations (8 h) of pollutants in NFCs were 34.79 ppb for SO<sub>2</sub>, 30.37 ppb for NO<sub>2</sub> and 38.15 ppb for O<sub>3</sub> during winter season. During summer, however, the same were 27.79, 26.46 and 51 ppb, respectively (Fig. 1). As compared to NFCs the concentrations of pollutants in FCs were reduced by 84.5, 85 and 92.3 % during winter and 82.4, 84.4 and 94 % during summer for SO<sub>2</sub>, NO<sub>2</sub> and O<sub>3</sub>, respectively. Pollutant concentrations in NFCs indicate that the concentrations of SO<sub>2</sub> and NO<sub>2</sub> were higher during winter whereas O<sub>3</sub> was higher during summer. In winter, due to low wind speed, pollutants are not dispersed effectively and a large proportion remained confined near their sources of origin. During



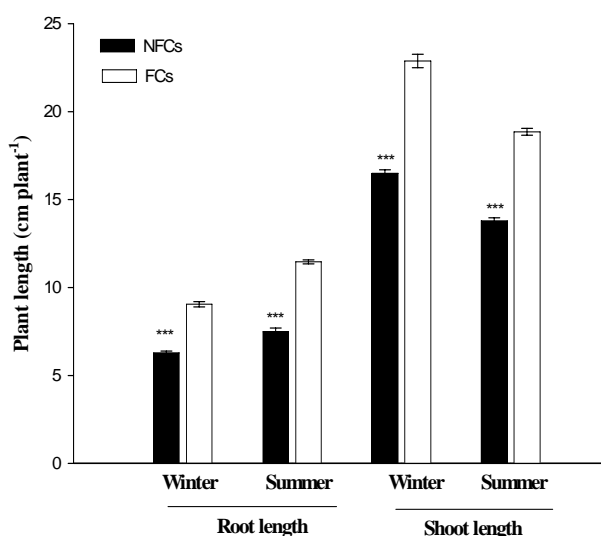
**Fig. 1.** Variations in the pollutant concentrations during growth period of winter and summer palak at the experimental site (Mean  $\pm$  1SE).

summer, high wind speed carried out the pollutants to far off places in prevailing wind directions. O<sub>3</sub> is an important secondary pollutant formed from precursor gases (NO<sub>x</sub> and VOC) emitted mainly from transport sector. High temperature, high light intensity and long sunshine hours are ideal conditions for O<sub>3</sub> formation. These conditions are frequently available during summer, which explain the prevalence of high O<sub>3</sub> concentrations in summer. During winter season, sunlight duration and intensity are low, as such lower O<sub>3</sub> concentrations were recorded. Such seasonal variations in O<sub>3</sub> concentrations were reported earlier from different parts of the country (Agrawal *et al.* 2003; Jain *et al.* 2005; Saraf & Beig 2004; Tiwari *et al.* 2008).

Variations in NO<sub>2</sub> concentrations can be explained by the variability in the concentration of O<sub>3</sub>. As NO<sub>2</sub> act as a precursor in O<sub>3</sub> formation, it is rapidly utilized for O<sub>3</sub> formation and, therefore, NO<sub>2</sub> concentrations are low during summer. These conditions reverse during winter, thus NO<sub>x</sub> is comparatively higher.

The air quality data indicate that the concentrations of SO<sub>2</sub>, NO<sub>2</sub> and O<sub>3</sub> in combination may cause adverse impacts on growth and yield of plants during winter (Fig. 1). However, during summer season, O<sub>3</sub> may have a larger impact on growth and yield of plants as compared to other two pollutants. Palak plants growing in non filtered chambers (NFCs) showed reductions in all the morphological parameters as compared to those in

filtered chambers (FCs) in both winter and summer seasons. Significant reductions, of 21 and 15.9 %, were recorded in the root length of palak grown in NFCs as compared to those in FCs during winter and summer seasons, respectively (Fig. 2). Shoot length of the plants in NFCs decreased significantly by 27.8 and 26.8 %, respectively during winter and summer seasons as compared to those in FCs (Fig. 2). Two way ANOVA test showed that root and shoot length varied significantly due to season, treatment and season x treatment interaction (Table 2).



**Fig. 2.** Variations in root and shoot length of winter and summer palak grown in non filtered (NFCs) and filtered (FCs) chambers. Bars with asterisks show significant difference at  $p < 0.05$ .

Reductions in plant length under elevated pollutant concentrations have been shown in several studies (Adaros *et al.* 1991a&b; Heggstad & Lee 1990; Ishii *et al.* 2004; Rai *et al.* 2007; Tiwari *et al.* 2006). Significant reduction of 25.4 % was recorded in the plant length of palak experiencing ambient concentrations of 13.3, 31 and 55.7 ppb of SO<sub>2</sub>, NO<sub>2</sub> and O<sub>3</sub>, respectively, at a site near the present experimental site, during summer, using a gradient approach of yield estimation (Agrawal *et al.* 2003). In the present study, the reduction was 15.9 % in plant length in NFCs as compared to those in FCs. The lower magnitude of reduction in length can be explained by higher mean O<sub>3</sub> concentration (55.7 ppb) recorded by Agrawal *et al.* (2003) as compared to the present study (51 ppb). Saitanis & Karandinos (2002) recorded reduction

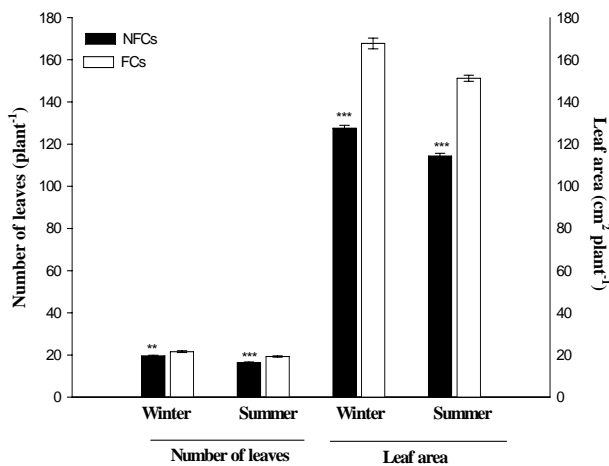
**Table 2.** Levels of significance (Two way ANOVA) for growth, biomass and yield of palak plants.

Parameters	Season	Treatment	Season x treatment
Root length (cm)	47.45***	122.21***	21.20**
Shoot length (cm)	54.89***	108.54***	29.28*
Number of leaves (plant <sup>-1</sup> )	24.51**	94.25**	17.52**
Leaf area (cm <sup>2</sup> )	29.56***	104.38***	20.75**
Root biomass (g plant <sup>-1</sup> )	9.21 <sup>NS</sup>	112.47***	18.46**
Shoot biomass (g plant <sup>-1</sup> )	9.05 <sup>NS</sup>	94.28**	22.57***
Yield (g plant <sup>-1</sup> )	6.54 <sup>NS</sup>	101.55***	24.84***

\*\*\* = p<0.001; \*\* = p<0.01; \* = p<0.05; <sup>NS</sup>= Not significant

of 37 % in plant length of *Nicotiana tabacum* L. var. Bel W3 exposed to 90 ppb O<sub>3</sub> for 8 h d<sup>-1</sup> for 20 days.

Number of leaves plant<sup>-1</sup> showed significant decrements of 9.25 and 14.8 %, respectively in plants grown in NFCs as compared to those in FCs during winter and summer seasons (Fig. 3). Reduction in number of leaves may be due to decreased leaf production and enhanced rate of senescence under increased levels of ambient pollutants particularly SO<sub>2</sub> and O<sub>3</sub>. Significant reductions in the number of leaves were reported in tolerant and sensitive lines of *Hordeum vulgare* L. cv. Maris Mink upon fumigation with a mixture of SO<sub>2</sub> and



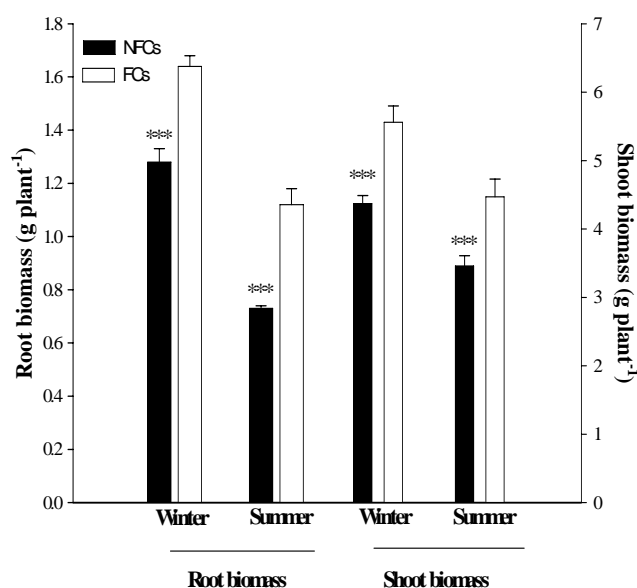
**Fig. 3.** Variations in number of leaves and leaf area of winter and summer palak grown in non filtered (NFCs) and filtered (FCs) chambers. Bars with asterisks show significant difference at p<0.05.

NO<sub>2</sub> (100 + 100 ppb) for 22 days (Kasana & Lea 1994). Ishii *et al.* (2004) also recorded significant reductions in number of leaves plant<sup>-1</sup> in *Oryza sativa* var. MR84 and MR185 exposed to 55 and

81.7 ppb O<sub>3</sub> (8 h mean) for 147 days.

Leaf area also decreased significantly by 23.9 and 24.39 % in the plants grown in NFCs as compared to those in FCs in winter and summer seasons, respectively (Fig. 3). Two way ANOVA showed that leaf area and number of leaves varied significantly due to season, treatment and season x treatment interaction (Table 2). Reduction in leaf area was more than reduction in number of leaves in plants grown in NFCs as compared to those in FCs. Keutgen *et al.* (2005) also reported more reduction in leaf area of strawberry than number of leaves plant<sup>-1</sup> when grown at 78 ppb O<sub>3</sub>. It has been established that cell elongation in plants is more sensitive to stresses than cell division (Horst & Nelson 1979). When plants are exposed to pollution stress, leaf elongation ceases and carbohydrate reserves are utilized in the production of new leaves. It was found that plants grown in NFCs utilized more biomass for production of new leaves compared to leaf expansion. This resulted in lower magnitude of reduction in number of leaves plant<sup>-1</sup> as compared to leaf area. Reductions of 41 and 12 % in leaf area and number of leaves respectively, were recorded in a sensitive variety of *Nicotiana tabacum* L. var. Bel W3 exposed to 90 ppb O<sub>3</sub> at 8 h day<sup>-1</sup> for 20 days (Saitanis & Karandinos 2002).

Root and shoot biomass showed significant reductions in plants grown in NFCs as compared to those in FCs during both summer and winter seasons. Root biomass decreased by 22 and 35 % in NFCs as compared to FCs during winter and summer seasons, respectively (Fig. 4). Shoot biomass reduced significantly by 21.5 and 22.6 % in NFCs as compared to FCs during winter and summer seasons, respectively (Fig. 4). These results show that the root fraction of the plants was more severely damaged than the top fraction under pollution stress. Reduction in root growth may be ascribed to reductions in available C from photo-



**Fig. 4.** Variations in root and shoot biomass of winter and summer palak grown in non filtered (NFCs) and filtered (FCs) chambers. Bars with asterisks show significant difference at  $p < 0.05$ .

synthesis (Saxe 1991), an increased C demand for aboveground repair or replacement mechanisms (Kelly *et al.* 1993) and/or by impaired phloem functioning (Spence *et al.* 1990). Adverse effects of air pollutants on biomass of several crops have been reported widely (Keutgen *et al.* 2005; Paludan - Muller *et al.* 1999; Tiwari *et al.* 2006).

Root shoot ratio (RSR) of summer palak significantly decreased by 20 %, whereas the decrease was insignificant in winter palak grown in NFCs as compared to FCs (Table 3). This differential response can be ascribed to high  $O_3$  concentrations during summer. Plants grown at elevated levels of  $O_3$  have been shown to retain their photosynthate in leaves, instead of allocating to below ground parts of the plants (Cooley & Manning 1987; Grantz *et al.* 2006; McCrady & Anderson 2000).

Relative growth rate (RGR) and net assimilation rate (NAR) of the plants grown in NFCs were lower than those in FCs (Table 3). Reductions in RGR can be attributed to reduced rate of photosynthesis in plants due to reduced leaf number and area. In the present study, RGR decreased by 10.7 and 18.5 %, respectively in winter and summer seasons in plants of NFCs as compared to those in FCs. Significant reductions in NAR further suggest adverse impact on the assimilation rate of the

plants under ambient pollutant concentrations. Reduction in RGR was also reported in *Daucus carota* L. var. Pusa Kesar grown in NFCs (37.45 ppb  $SO_2$ ; 38.72 ppb  $NO_2$  and 38.37 ppb  $O_3$ ) as compared to those in FCs (Tiwari *et al.* 2006).

Higher leaf weight ratio (LWR) in the plants during summer in NFCs as compared to FCs suggests partitioning of a large amount of fixed carbon into leaf growth to enable the plants to overcome the adverse impacts of ambient air pollutants. In winter, however, no significant variation was recorded in LWR of palak grown in FCs and NFCs (Table 3). Specific leaf weight (SLW) reduced significantly with a subsequent increase in specific leaf area (SLA) in winter palak of NFCs as compared to those in FCs (Table 3). Increase in SLA with a simultaneous decrease in SLW suggests production of thinner leaves under air pollution stress as more biomass was utilized in the production of new leaves.

$Na^+$ ,  $Ca^{2+}$ ,  $K^+$ ,  $Mg^{2+}$  and  $Fe^{2+}$  contents decreased significantly by 14, 17.6, 14.6, 15 and 24.8 %, respectively in palak grown in NFCs as compared to those in FCs (Table 4). Nutrient contents of summer palak did not show significant variations between NFCs and FCs (Table 4). These observations suggest that although the ambient pollutants caused more yield reductions in summer palak, the plants were able to retain their nutritional quality. This could be explained by higher leaf weight ratio of summer palak grown in NFCs as compared to FCs. Rajput & Agrawal (2004) reported reductions in K and Ca contents of pea seeds under elevated concentrations of  $SO_2$ ,  $NO_2$  and  $O_3$  during a transect study in Varanasi. Significant reductions in nutrient contents in both root and shoot fractions of carrot plants under ambient pollutant concentrations of 37.45, 38.72 and 38.37 ppb  $SO_2$ ,  $NO_2$  and  $O_3$ , respectively were reported (Tiwari *et al.* 2006).

Yield is the most important economic factor of agricultural and horticultural crops. In the present study, yield decreased significantly by 23.9 and 28.6 %, respectively in winter and summer palak in NFCs as compared to FCs (Fig. 5). Two way ANOVA showed that yield varied significantly due to season, treatment and season x treatment interaction suggesting significant seasonal impact of air pollutants on yield of the plants. Studies conducted in Pakistan showed yield reductions in the range of 13 to 44 % in barley at 71 ppb  $O_3$ , 30 ppb  $NO_2$  and 16 ppb  $SO_2$  (Wahid 2006a) and 18 to 43 % in wheat grown at 72 ppb  $O_3$ , 28 ppb  $NO_2$  and 15 ppb  $SO_2$  (Wahid 2006b). Agrawal *et al.* (1983 a & b)

**Table 3.** Variations in growth indices of palak plants grown in filtered (FCs) and non filtered (NFCs) chambers during winter and summer seasons (Mean  $\pm$  1SE).

Growth indices	Winter		Summer	
	NFCs	FCs	NFCs	FCs
Relative growth rate (g g <sup>-1</sup> day <sup>-1</sup> )	0.025*** $\pm$ 0.0003	0.028 $\pm$ 0.0002	0.022*** $\pm$ 0.00008	0.0277 $\pm$ 0.00006
Net assimilation rate (g cm <sup>-2</sup> day <sup>-1</sup> )	0.0030*** $\pm$ 0.00005	0.034 $\pm$ 0.00006	0.0027*** $\pm$ 0.00004	0.0029 $\pm$ 0.00003
Specific leaf weight (cm <sup>2</sup> g <sup>-1</sup> )	0.029*** $\pm$ 0.0006	0.033 $\pm$ 0.0008	0.028 <sup>NS</sup> $\pm$ 0.0004	0.027 $\pm$ 0.0003
Specific leaf area (g cm <sup>-2</sup> )	34.86*** $\pm$ 0.77	30.52 $\pm$ 0.70	35.64 <sup>NS</sup> $\pm$ 0.64	36.16 $\pm$ 0.43
Leaf weight ratio (g g <sup>-1</sup> )	0.773 <sup>NS</sup> $\pm$ 0.003	0.775 $\pm$ 0.004	0.81*** $\pm$ 0.003	0.78 $\pm$ 0.003
Root shoot ratio (g g <sup>-1</sup> )	0.294 <sup>NS</sup> $\pm$ 0.006	0.291 $\pm$ 0.007	0.229*** $\pm$ 0.004	0.275 $\pm$ 0.005

Level of significance for the differences between NFCs and FCs: \*\*\* = p<0.001; \*\* = p<0.01; \* = p<0.05; <sup>NS</sup>= Not significant.

**Table 4.** Variations in nutrient contents (mg g<sup>-1</sup>) in the shoots of palak plants grown in filtered (FCs) and non filtered (NFCs) chambers during winter and summer seasons (Mean  $\pm$  1SE).

Nutrients	Winter		Summer	
	NFCs	FCs	NFCs	FCs
Mg	19.47*** $\pm$ 0.44	22.94 $\pm$ 0.62	18.30 <sup>NS</sup> $\pm$ 1.44	17.78 $\pm$ 2.73
Na	40.36* $\pm$ 0.39	46.90 $\pm$ 0.54	41.59 <sup>NS</sup> $\pm$ 1.22	43.34 $\pm$ 1.59
Ca	7.04* $\pm$ 0.19	8.55 $\pm$ 0.25	7.06 <sup>NS</sup> $\pm$ 0.58	7.57 $\pm$ 0.58
Fe	2.09** $\pm$ 0.07	2.78 $\pm$ 0.10	1.83 <sup>NS</sup> $\pm$ 0.14	1.77 $\pm$ 0.27
K	40.95*** $\pm$ 0.37	47.46 $\pm$ 0.54	39.31 <sup>NS</sup> $\pm$ 1.29	42.15 $\pm$ 1.08

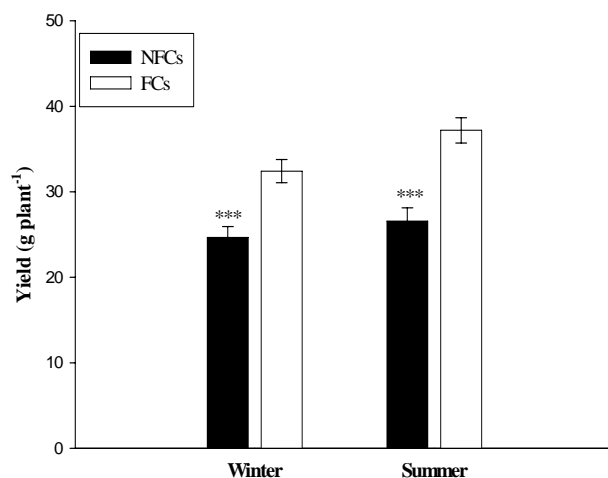
Level of significance for the differences between NFCs and FCs: \*\*\* = p<0.001; \*\* = p<0.01; \* = p<0.05; <sup>NS</sup>= Not significant.

showed that O<sub>3</sub> and SO<sub>2</sub> in combination produced synergistic negative influence on the yield of rice, common millet and brinjal plants as compared to individual pollutants. Transect study conducted by Agrawal *et al.* (2003) along varying pollution gradient showed significant reduction of 34 % in the yield of mungbean at a suburban site of Varanasi experiencing 13 ppb SO<sub>2</sub>, 31 ppb NO<sub>2</sub> and 57 ppb O<sub>3</sub>. Rajput & Agrawal (2004) observed

reductions of 15.3 and 37.8 %, respectively in the weight of seeds plant<sup>-1</sup> of pea grown at two suburban sites at ambient pollutant concentrations.

The variations in response of palak observed during two different seasons can be explained by the pollutant concentrations existing during the two growth periods. The average concentration of O<sub>3</sub> was much higher (51 ppb) during summer as compared to winter (38 ppb). The reduction in yield





**Fig. 5.** Variations in yield of winter and summer palak grown in non filtered (NFCs) and filtered (FCs) chambers. Bars with asterisks show significant difference at  $p < 0.05$ .

of palak was higher during summer (28.6 %) as compared to winter (23.9 %).  $O_3$  appears to play the most significant role in causing yield reduction in palak during summer. During winter, combinations of  $SO_2$  and  $NO_2$  along with  $O_3$  may have participated in causing reductions in growth and yield of palak.

### Conclusions

The present study clearly indicates that ambient concentrations of gaseous pollutants  $SO_2$ ,  $NO_2$  and  $O_3$  have detrimental effects on growth, biomass accumulation and yield of palak. The magnitude of response, however, varied between winter and summer seasons. Air quality data recorded at the experimental site showed that concentrations of  $SO_2$  and  $NO_2$  were comparatively higher during winter than summer, whereas  $O_3$  was higher during summer than winter. Concentration of  $O_3$  was highest among the three air pollutants during both the seasons, but the variations in different pollutant concentrations were more pronounced in summer.  $O_3$  seems to play a more significant role in causing greater yield loss of palak during summer season. Biomass and yield reductions were higher in summer than in winter, but plants retained the nutritional quality even when grown in polluted environment of NFCs during summer. Lower root shoot ratio and higher leaf weight ratio in summer palak in NFCs as compared to FCs helped the

plants to sustain their nutritional quality, even though the yield was reduced.

Increasing  $O_3$  concentration is becoming a threat to the already deteriorating air quality of developing countries. Several model based studies indicate a further increase in  $O_3$  concentrations in the coming years, which may have damaging effects on the already declining crop yields in different parts of the world. Because sources of  $O_3$ , primarily  $NO_x$  and HCs are transported from urban and industrial areas over long distances along with the prevailing wind direction to agricultural areas, crop yield loss assessments are also required in rural areas supporting agriculture.

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