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Use of ethylene diurea (EDU) in assessing the impact of ozone on growth and productivity of five cultivars of Indian wheat (*Triticum aestivum* L.)

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Abstract Increase in concentrations of tropospheric ozone (O_3) is one of the main factors affecting world agriculture production. Tropical countries including India are at greater risk due to their meteorological conditions (high solar radiation and temperature) being conducive to the formation of O_3. The most effective anti-ozonant chemical is *N*-[2-(2-oxo-1-imidazolidinyl) ethyl]-*N*-phenylurea or ethylene diurea (EDU). Due to its specific characteristics, EDU has been used in the field as a phytomonitoring agent to assess crop losses due to O_3. Field experiments were conducted on five local cultivars of wheat (*Triticum aestivum* L. cv HUW234, HUW468, HUW510, PBW343, and Sonalika) grown under natural field conditions in a suburban area of Varanasi, Uttar Pradesh, India during December 2006 to March 2007 to determine the impact of O_3 on their growth and yield characteristics. Mean monthly O_3 concentrations varied between 35.3 ppb and 54.2 ppb at the experimental site. EDU treatment positively affected various growth and yield parameters with difference between cultivars. EDU-treated plants showed increase in shoot and root length, leaf area, absolute growth rate, relative growth rate, and net primary productivity, indicating O_3 induced suppression in growth. EDU treatment was highly significant in different cultivars for total biomass and test weight but not for harvest index. Yield per plant was higher by 25.6%, 24%, 20.4%, 8.6%, and 1.9% in EDU-treated cultivars HUW468, Sonalika, HUW510, HUW234, and PBW343, respectively, than non-EDU-treated ones. These results clearly indicate the sensitivity of all the wheat cultivars to ambient levels of O_3 with cv HUW468 appearing to be most sensitive. The present study also supports the view that EDU has great potential in alleviating the unfavorable effects of O_3 and can be effectively used as a monitoring tool to assess growth and yield losses in areas experiencing elevated concentrations of O_3.

Keywords EDU · Ozone · Growth · Test weight · Wheat · Yield

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Introduction

Tropospheric O_3 has become a great problem in many developing countries including India due to rapid urbanization and industrialization (Wang et al. 2007a, b; Rai et al. 2007). Tropical countries like India and China are at greater risk due to meteorological conditions which favor the formation of O_3. Ozone concentrations have increased considerably during the last century throughout the world.

Tropospheric O_3 is one of the most potent phytotoxic gases affecting agriculture in India. Increased concentrations have an adverse effect on plant metabolism, cause leaf injury, reduce photosynthesis, and accelerate premature senescence and overall reduction in growth (Mudd 1996; Del Valle-Tascon and Carrasco-Rodriguez 2004). O_3 enters plant leaves through the stomata and generates various reactive oxygen species such as hydrogen peroxide, hydroxyl radicals, superoxide, and singlet oxygen (Mudd 1982, 1996; Langebartels et al. 2002). These free radicals interfere with the normal functioning of the plant system and cause deleterious effects on growth and yield of various important crop plants. Marshall et al. (1998) published a report entitled "A hidden threat to food production, air pollution and agriculture in the developing world". This important report highlighted the current and future significance of air pollution as a global threat to agricultural productivity and identified parts of Asia as being at particular risk from crop losses due to O_3.

The solution to this problem to avoid crop losses due to tropospheric O_3 could be solved by (1) application of some protective chemicals and (2) selection of suitable resistant cultivars of crops to protect them from O_3 injury. Several O_3 protectants were used earlier like fungicides, insecticides and herbicides, plant growth regulators, extracts of plants, dust, mechanical barriers, and antioxidants such as ethylene diurea (EDU) by various workers (e.g., Pandey and Agrawal 1993; Agrawal et al. 2005; Blum and Didyk 2007). Several experiments suggest that the application of these chemical protectants against O_3 might be used to assess O_3 effects on crops under field conditions but EDU ([*N*-(2-(2-oxo-1-imidazolidinyl) ethyl]-*N*-phenyl urea) is considered to be the best one against O_3 injury (Agrawal et al. 2003; Tiwari et al. 2005).

EDU is systemic and is not redistributed to new tissues, so repeated application of EDU is required to maintain protection. It appears to be specific for the suppression of O_3 injury, having no effects on PAN or SO_2 injury (Cathey and Heggestad 1982a, b; Lee et al. 1992). EDU has significant anti-ozonant effects on growth and yield of different crop plants (Hassan et al. 1995; Wahid et al. 2001; Agrawal et al. 2005) and has been shown to suppress O_3 injury in different plants grown in different parts of the world. Examples of plants where EDU has been used to assess O_3 injury include soybean (Wahid et al. 2001), radish, turnip (Hassan et al. 1995), mung bean (Agrawal et al. 2005), wheat (Tiwari et al. 2005), tomato (Varshney and Rout 1998), rice, and wheat (Wang et al. 2007a).

Triticum aestivum L. (wheat) is one of the major staple crops grown all over India and is sensitive to O_3. Our study was aimed at evaluating the impact of ambient O_3 on five different cultivars of wheat (HUW234, HUW468, HUW510, PBW343, and Sonalika) to determine intraspecific responses to O_3 by using EDU to determine effect on various growth and yield parameters under natural field conditions in a suburban area of Varanasi city, Uttar Pradesh, India. This study may be helpful in screening various wheat cultivars for their tolerance against O_3 injury and yield loss in areas experiencing elevated concentrations of O_3.

Materials and methods

Experimental site

The experimental site is located at the agriculture farm, Banaras Hindu University (BHU) (25°14′N latitude; 82°03′E longitude), a suburban area of Varanasi located in the eastern Gangetic plains of the Indian subcontinent and 76.19 m above mean sea level. The experiment was carried out between December 2006 and March 2007. The mean monthly minimum temperature varied from 7.8 to 12.0°C and mean monthly maximum temperature from 24.2 to 27.7°C. Total rainfall during the wheat growth period was 105.3 mm with the maximum recorded during February. Maximum monthly relative humidity ranged from 71.5% to 84.5%. Sunshine hours ranged from 6.8 to 9.2 from December to March (Table 1). Soil at the study site was a sandy loam (sand 45%, silt 28%, and clay 27%) with pH of 7.26.

Table 1 Meteorological data of the experimental site during the study period

Month/year	Total rainfall (mm)	Temperature (°C)		Relative humidity (%)		Wind speed ($km\ h^{-1}$)	Sunshine (h)
		Max	Min	Max	Min		
December 2006	0	25.8	9.9	84.5	41.3	3.1	7.2
January 2007	0	24.2	7.8	79.0	35.2	3.3	7.7
February 2007	99.8	24.4	12.0	82.5	54.5	3.7	6.8
March 2007	5.5	27.7	10.7	71.5	45.0	4.4	9.2

Plant material

Five wheat cultivars were studied: HUW234, HUW468, HUW510, PBW343, and Sonalika. HUW234 is an outstanding cultivar for the late sown conditions of the north eastern plain zone and was developed from the cross HUW12*2/CPAN 1966. It is a double gene dwarf variety, having early maturity, a club-shaped spike, and amber hard grains, with a life span of 120 days and 4.5 tons ha^{-1} yield. HUW468 is a double dwarf wheat cultivar produced by crossing CPAN 1962/Toni/Lira/Prl′ s′. It has amber, hard, and medium bold shining grains, with a life span of 125 days and 5.5 tons ha^{-1} yield. HUW510 is a late sown, double dwarf wheat with amber bold grains with a life span of 120 days and yield of 4.5 tons ha^{-1}. PBW343 was developed at CIMMYT, Mexico. It is a single dwarf, long duration line which takes around 130 days for proper expression and yield of 4.0–5.0 tons ha^{-1}. Its pedigree is ND/VG 9144/KAL/BB/3/YCO″ s′/4/VEE## S″. Sonalika was the first cultivar that ushered in the Green Revolution in India. It has a shorter life span and double dwarf nature with yield of 3.5–4.5 tons ha^{-1}. Its pedigree is II54.388/AN/3/4T54/NIOB/LR. All the test wheat cultivars are widely grown locally, high yielding, and disease resistant. These cultivars were tested for their sensitivity to O_3 in order to recommend resistant varieties to avoid the negative effects of O_3 on yield.

Ambient ozone monitoring

Eight hourly O_3 monitoring was done using an O_3 analyzer (Model 400A, API, Inc., USA) from 9:00 to 17:00 twice weekly until plants were mature. Air samples were collected with the help of a Teflon tube (0.35 cm diameter) placed above the canopy of the wheat plants.

Plant cultivation

Wheat grains of all the five cultivars were sown in 30 plots of $1 \times 1\ m^2$ (six plots for each cultivar) on 7th December 2006 using standard agronomic practices. Each plot had 30 plants with a distance of 15 cm between them. Recommended dose of fertilizers (120, 60, and 40 kg ha^{-1} N, P, and K as urea, single superphosphate, and muriate of potash, respectively) were added during the preparation of the field. Plots were irrigated from time to time to maintain uniform soil moisture.

EDU application

Three plots of each cultivar were treated with 400 ppm EDU and the other three with same amount of deionized water to maintain an equal water regime in both treatments. EDU solution was freshly prepared each time using deionized water and applied as a soil drench (100 ml $plant^{-1}$) 10 days after germination (DAG) between 9:00 and 10:00 h at intervals of 12 days to 82 DAG.

Plant sampling and analysis

Random samplings of plants were done at 25, 50, and 75 DAG for various growth analyses. Plant samples were analyzed for root length, number of leaves, tillers and ears, and leaf area. Leaf area was measured using a portable leaf area meter (Model LI-3000, LI-COR, Inc., USA). For biomass determination, plants were oven dried (80°C) to constant weight. Various growth indices were calculated using the standard formulae of Hunt (1982). Final harvesting was done at 125 DAG for all cultivars. Different yield parameters, such as number and weight of ears per plant, number of grains per ear, number and weight of grains per plant, weight of above ground parts, and test weight (1,000 seed weight), were recorded. Harvest index (HI) was calculated as the ratio of economic yield (weight of grains per plant) to total above ground biomass of wheat plant.

Statistical analysis

Data were analyzed using a *t* test and two- and three-way ANOVA tested through SPSS software (SPSS Inc., version 10.0) to assess the level of significance of quantitative changes due to EDU treatment in different parameters in different samplings.

Results

Ambient concentrations of O_3 varied during the growth period of the wheat. In December 2006, the mean concentration of O_3 was 35.3 ppb which increased to 54.2 ppb in March 2007 (Fig. 1).

Shoot and root lengths increased in all cultivars treated with EDU compared with non-treated ones at all the ages (Table 2). At 75 DAG, there was an increase of 12.8% and 8.0%, 15.2% and 27.2%, 17.3% and 12.7%, 9.2% and 12.5%, and 2.3% and 6.3% in shoot and root lengths of cv HUW 234, HUW 468, HUW 510, PBW 343, and Sonalika, respectively. Numbers of tillers and leaves were also higher in all cultivars treated with EDU than non-treated plants at all ages except for cv Sonalika where these parameters showed a decline at 50 and 75 DAG (Table 2). Number of leaves increased by 41.5%, 29.4%, 28.5%, and 23.7% in cv HUW468, PBW343, HUW510, and HUW234, respectively, after EDU treatment, while it decreased by 14.3% at 75 DAG in cv Sonalika. Numbers of standing dead leaves are higher in non-EDU-treated plants of all the cultivars of wheat as compared to EDU-treated ones (Table 2). Statistical analysis showed significant variations in shoot length due to age, treatment ($p < 0.001$), cultivar, and interactions of A × C ($p < 0.01$) and A × T ($p < 0.05$) (Table 3). Root length varied significantly due to all the factors except in the interactions between C × T and A × C × T. Number of tillers and leaves also varied significantly due to all the factors, except the interaction of A × T (Table 3). Leaf area increased in all cultivars treated with EDU at all the ages (Table 2). Maximum percent increase in leaf area was recorded by 52% in cv HUW 468 due to EDU treatment. Highly significant variations were found for leaf area due to all the factors and their interactions (Table 3). Fresh weight of shoot and leaves also increased in EDU-treated plants of all cultivars at all sampling ages. Fresh weight of shoot and leaves increased by 53.7% and 48.3%, 53.5% and 41.8%, 19.1% and 18.9%, 19.2% and 15.7%, and 10.5% and 2.9% in EDU-treated plants of cv HUW234, HUW468, HUW510, PBW343, and Sonalika, respectively, at 50 DG (Fig. 2).

Biomass accumulation showed an increasing trend in all the EDU-treated wheat cultivars. Total plant biomass was higher by 54.5%, 47.6%, 31.3%, 10.9%, and 2.6% in EDU-treated plants of cv HUW234, HUW468, HUW510, PBW343, and Sonalika, respectively than non-EDU-treated ones at 50 DAG (Fig. 2). Dry weight of leaves and shoots were also higher in all the cultivars at all the ages in EDU-treated plants than non-EDU-treated ones. Leaf dry weight was higher by 54.3%, 40.0%, 28.5%, 17.5%, and 10.0% in EDU-treated cv HUW234, HUW468, HUW510,

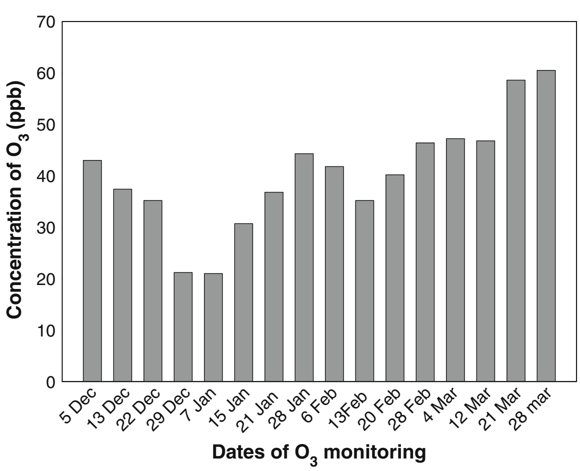


Fig. 1 Ozone concentration during the study period

Table 2 Effect of EDU treatment on root length, shoot length, number of tillers, number of leaves, leaf area, and number of standing deads of different wheat cultivars (mean ± 1 SE)

Plant age/cultivar	Shoot length (cm)		Root length (cm)		No. of tillers	
	Non-EDU	EDU	Non-EDU	EDU	Non-EDU	EDU
25 DAG						
HUW234	26.5750 ± 1.9964	33.4750 ± 0.66128*	8.8000 ± 0.2901	9.6000 ± 0.6868^{NS}	4.2500 ± 0.6292	6.000 ± 0.7071^{NS}
HUW468	23.5500 ± 1.1836	27.6250 ± 0.6969*	8.7000 ± 0.5115	8.8250 ± 0.5662^{NS}	4.2500 ± 0.2500	7.0000 ± 0.4083*
HUW510	25.4750 ± 0.3092	30.5750 ± 1.096*	10.250 ± 0.7696	10.400 ± 0.6646^{NS}	5.5000 ± 0.2887	6.250 ± 0.2500
PBW343	28.1000 ± 1.3644	30.0000 ± 1.3360^{NS}	8.9750 ± 0.7543	9.7000 ± 0.9083^{NS}	4.0000 ± 0.5773	6.2500 ± 0.6292*
Sonalika	25.1500 ± 2.4133	26.0250 ± 1.0078^{NS}	8.7500 ± 0.4518	9.8500 ± 0.2102^{NS}	4.5000 ± 0.9574	7.000 ± 0.4082^{NS}
50 DAG						
HUW234	53.6000 ± 3.4017	62.7250 ± 2.1219*	16.9750 ± 1.4608	17.500 ± 0.3162^{NS}	11.7500 ± 0.2500	18.0000 ± 0.7071*
HUW468	50.4000 ± 2.0441	59.2000 ± 3.1920^{NS}	15.5250 ± 0.8260	17.650 ± 1.0021^{NS}	12.7500 ± 0.8539	19.2500 ± 1.031*
HUW510	57.8500 ± 1.7225	58.5000 ± 2.3843^{NS}	15.3750 ± 1.0160	15.650 ± 0.9665^{NS}	11.7500 ± 0.6292	13.2500 ± 0.8539*
PBW343	57.250 ± 3.1978	61.0500 ± 4.9084***	13.1250 ± 0.2754	16.1500 ± 0.2754**	13.5000 ± 1.1902	14.250 ± 1.7500^{NS}
Sonalika	56.6250 ± 4.9084	62.2000 ± 2.9735^{NS}	13.2500 ± 1.2128	14.8750 ± 0.6860^{NS}	20.0 ± 1.9579	16.500 ± 0.8660^{NS}
75 DAG						
HUW234	84.3250 ± 3.2745	96.7500 ± 2.6043^{NS}	18.5250 ± 3.2745	20.3250 ± 2.6043^{NS}	12.7500 ± 0.4787	21.7500 ± 1.3229*
HUW468	75.2500 ± 3.4517	88.8250 ± 2.3722*	16.9750 ± 0.2780	23.3250 ± 0.5186**	16.2500 ± 2.0966	17.500 ± 1.0408^{NS}
HUW510	69.7000 ± 1.8650	84.3750 ± 3.1354**	16.6250 ± 0.6787	19.0500 ± 1.2017^{NS}	19.0 ± 1.2910	22.5000 ± 1.2583*
PBW343	76.2000 ± 3.2179	84.0000 ± 4.5100^{NS}	15.4500 ± 0.6500	17.6750 ± 0.9810^{NS}	11.7500 ± 0.4787	16.0000 ± 1.4720*
Sonalika	87.6500 ± 4.3456	89.7750 ± 3.0883^{NS}	17.1000 ± 0.3873	18.2500 ± 0.7730^{NS}	20.0 ± 2.5000	12.000 ± 0.9129^{NS}

Table 2 (continued)

Plant age/cultivar	No. of leaves		Leaf area (cm^2)		No. of standing deads	
	Non-EDU	EDU	Non-EDU	EDU	Non-EDU	EDU
25 DAG						
HUW234	14.0 ± 1.3540	20.7500 ± 2.1360^{NS}	96.5000 ± 4.0517	161.7500 ± 12.0442**	–	
HUW468	16.7500 ± 1.5478	21.2500 ± 1.3149^{NS}	73.7500 ± 8.7785	124.7500 ± 10.1602*	–	
HUW510	16.0 ± 1.0801	18.0000 ± 0.4082^{NS}	78.7500 ± 6.2899	118.7500 ± 2.4622*	–	
PBW343	14.7500 ± 1.3149	21.2500 ± 2.3229*	82.2500 ± 7.7177	124.7500 ± 5.3131*	–	
Sonalika	15.5000 ± 1.1902	23.5000 ± 2.0616^{NS}	85.2500 ± 6.7992	127.0000 ± 4.1433^{NS}	–	
50 DAG						
HUW234	44.6667 ± 2.7285	72.2500 ± 6.1152*	841.7000 ± 33.3400	1,455.4975 ± 122.3631**	9.0 ± 0.5774	4.5000 ± 1.041**
HUW468	51.0 ± 2.1602	73.7500 ± 4.4230*	803.2200 ± 28.8314	1,290.4300 ± 68.9449**	11.2500 ± 0.8539	8.2500 ± 2.056^{NS}
HUW510	40.7500 ± 2.0567	53.0000 ± 1.0801**	742.7750 ± 62.4249	1,034.3675 ± 21.8092*	8.2500 ± 1.4930	7.2500 ± 1.1815^{NS}
PBW343	54.7500 ± 3.0653	59.7500 ± 7.1923^{NS}	921.2200 ± 70.2589	1,095.7275 ± 100.6230^{NS}	4.5000 ± 0.9574	3.2500 ± 0.2500^{NS}
Sonika	73.5000 ± 5.3774	59.5000 ± 2.7415*	1,074.6425 ± 34.8958	1,127.1250 ± 13.6706^{NS}	13.7500 ± 2.8687	7.0000 ± 2.7386^{NS}
75 DAG						
HUW234	33.7500 ± 0.8539	44.2500 ± 1.4361^{NS}	701.2500 ± 18.8431	725.2500 ± 36.7976^{NS}	25.5000 ± 3.7969	21.7500 ± 1.5478^{NS}
HUW468	39.7500 ± 2.0565	68.0000 ± 3.3665*	738.2500 ± 31.3884	1,543.0000 ± 109.4859*	20.2500 ± 7.2154	19.0000 ± 1.9738^{NS}
HUW510	37.5000 ± 1.0408	52.5000 ± 4.3493**	525.2500 ± 25.8759	1,007.7500 ± 22.4365**	20.7500 ± 2.217	9.2500 ± 1.5000**
PBW343	34.7500 ± 1.6008	49.2500 ± 5.3600*	598.5000 ± 35.1319	1,004.2500 ± 28.9032**	14.2500 ± 1.8875	12.0000 ± 0.9129^{NS}
Sonika	42.0 ± 3.1358	36.0000 ± 4.5644^{NS}	572.2500 ± 18.8254	804.5000 ± 26.0176**	14.2500 ± 1.7078	13.0000 ± 1.4720^{NS}

Level of significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ^{NS}not significant

Table 3 Significance level for various growth parameters of wheat plants as obtained by three-way ANOVA test

Parameters	Age (A)	Cultivar (C)	Treatment (T)	Age × cultivar (A × C)	Age × treatment (A × T)	Cultivar × Treatment (C × T)	Age × cultivar × treatment (A × C × T)
Shoot length	***	**	***	**	*	NS	NS
Root length	***	***	***	**	**	NS	NS
No. of tillers	***	**	***	***	NS	***	***
No. of leaves	***	***	***	***	NS	***	***
Fresh wt. of shoot	***	***	***	***	**	NS	NS
Fresh wt. of root	***	*	NS	NS	NS	*	*
Fresh wt. of leaves	***	***	***	***	***	***	***
Leaf area	***	***	***	***	***	***	***
Dry wt. of shoot	***	***	***	***	***	***	***
Dry wt. of root	***	***	**	***	*	***	***
Dry wt. of leaves	***	***	***	***	***	**	***
Total biomass	***	***	***	***	***	***	***
No. of standing dead	***	**	**	**	NS	NS	NS
Relative growth rate (RGR)	***	NS	NS	***	*	NS	NS
Leaf wt. ratio (LWR)	***	***	NS	**	NS	NS	NS
Net assimilation rate (NAR)	***	NS	NS	*	NS	NS	NS
Leaf area ratio (LAR)	***	**	*	***	NS	NS	NS
Specific leaf area (SLA)	***	**	*	NS	NS	NS	NS
Specific leaf weight (SLW)	***	**	NS	NS	NS	NS	NS
Root shoot ratio (RSR)	***	NS	***	*	NS	NS	NS
Absolute growth rate (AGR)	**	NS	**	**	NS	NS	NS
Net primary productivity (NPP)	***	***	***	***	***	NS	*

Level of significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; *NS* not significant

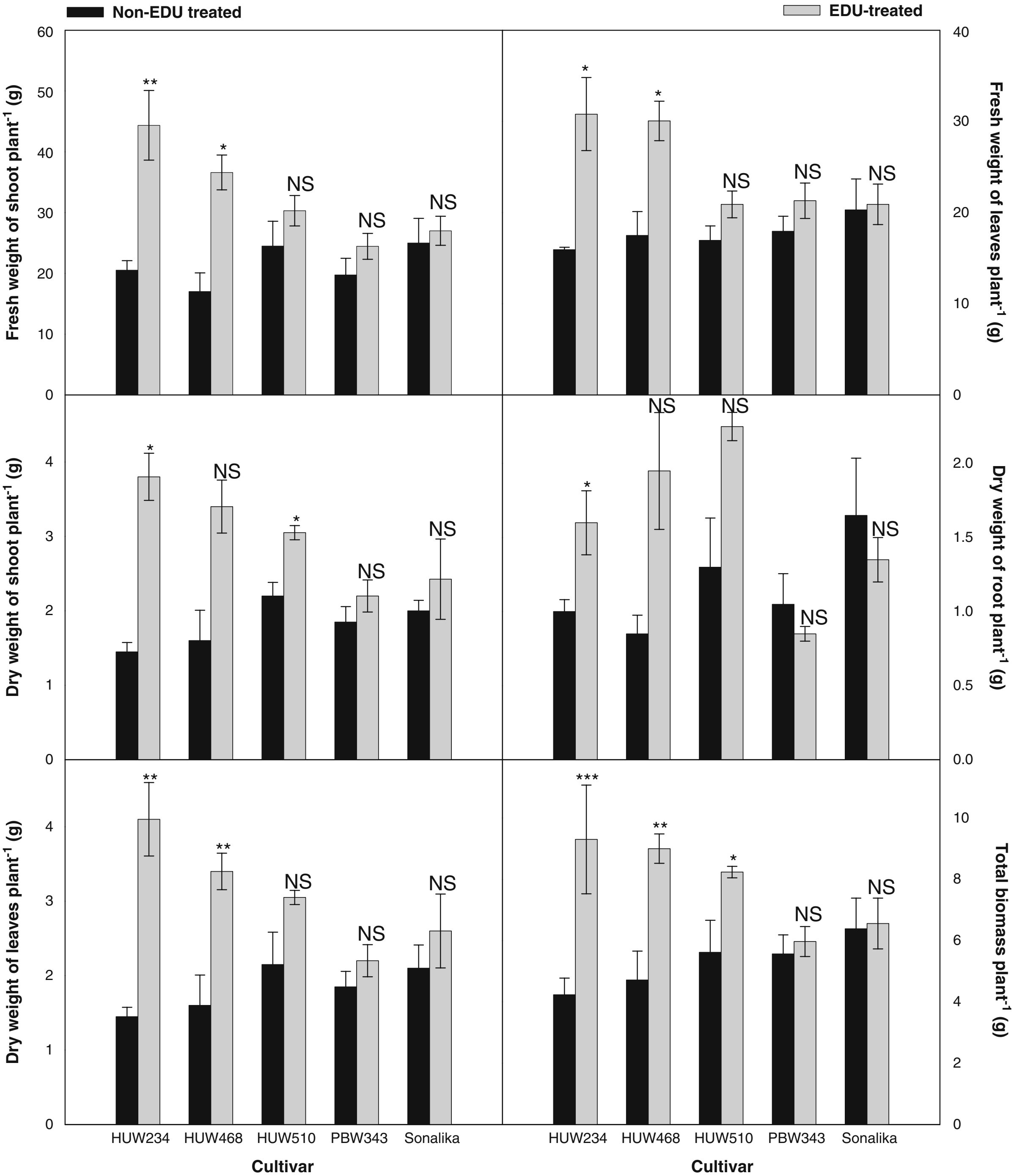
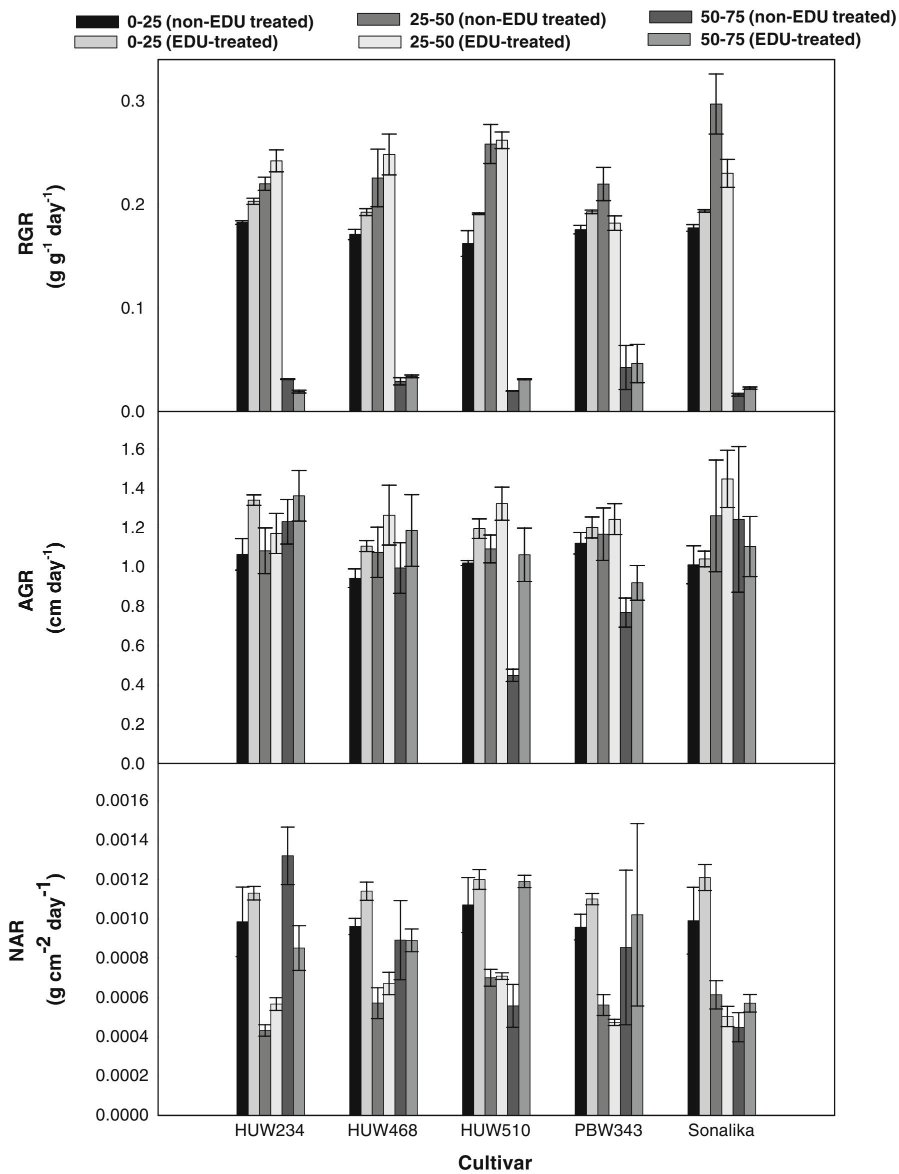


Fig. 2 Effect of EDU application on fresh weight of shoot and leaves, dry weight of root, shoot, leaves, and total biomass at 50 DAG (*bars* represent mean ± 1 SE; level of significance difference $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; NS not significant)

PBW343, and Sonalika, respectively than non-treated ones at 50 DAG (Fig. 2). Total biomass, leaf dry weight, and shoot and root dry weights were significantly higher for EDU-treated plants in all cultivars due to all the factors and their interactions (Table 3).

AGR increased in all five cultivars at all ages in EDU- treated plants compared with non-treated ones, except for cv Sonalika where it declined during 50–75 DAG (Fig. 3). AGR showed increment of 57.7% in EDU-treated plants of cv HUW510 as compared to control ones, this being the maximum increase in AGR among all the test cultivars. AGR varied significantly due to age, treatment, and due to interaction of A × C ($p < 0.01$) (Table 3). Higher relative growth rate (RGR) values were observed in EDU-treated plants of cv HUW468 and HUW510 as compared to non-EDU-treated ones at all age intervals, whereas it decreased during 50–75 DAG in cv HUW234 and 25–50 DAG in cv PBW343 and Sonalika (Fig. 3). RGR was higher by 10.0%,

Fig. 3 Effect of EDU application on RGR, AGR, and NAR of different cultivars of wheat (*bars* represent mean ± 1 SE)



11.2%, 15.1%, 8.9%, and 8.4% in EDU-treated plants of cv HUW234, HUW468, HUW510, PBW343, and Sonalika, respectively, as compared to control ones during 0–25 DAG. Significant variations were noticed due to age and interactions between A × C ($p < 0.001$) and A × T ($p < 0.05$) (Table 3). Net assimilation rate (NAR) increased during 0–25 and 25–50 DAG in cv HUW234 and HUW468 but decreased during 50–75 DAG, whereas in cv HUW510 it showed increments at all age intervals. It decreased in cv PBW343 and Sonalika during 25–50 DAG but increased during other age intervals (Fig. 3). NAR increased maximally by 53.1% in EDU-treated plants of cv HUW 510 as compared to non-EDU-treated ones during 50–75 DAG. Significant effects on NAR were observed due to age ($p < 0.001$) and also due to interaction between A × C ($p < 0.05$) (Table 3).

Leaf area ratio (LAR) and specific leaf area (SLA) decreased in EDU-treated plants in all cultivars at 25 and 50 DAG as compared to non-EDU-treated ones, whereas LAR increased in cv HUW468, PBW343, and Sonalika and SLA increased in cv HUW468 and HUW510 at 75 DAG (Fig. 4). LAR varied significantly due to age, cultivar, treatment, and interaction of A × C (Table 3). Significant differences were found for SLA due to age ($p < 0.001$), cultivar ($p < 0.01$), and treatment ($p < 0.05$) (Table 3). Specific leaf weight (SLW) increased in all the EDU-treated cultivars as compared to non-EDU-treated plants

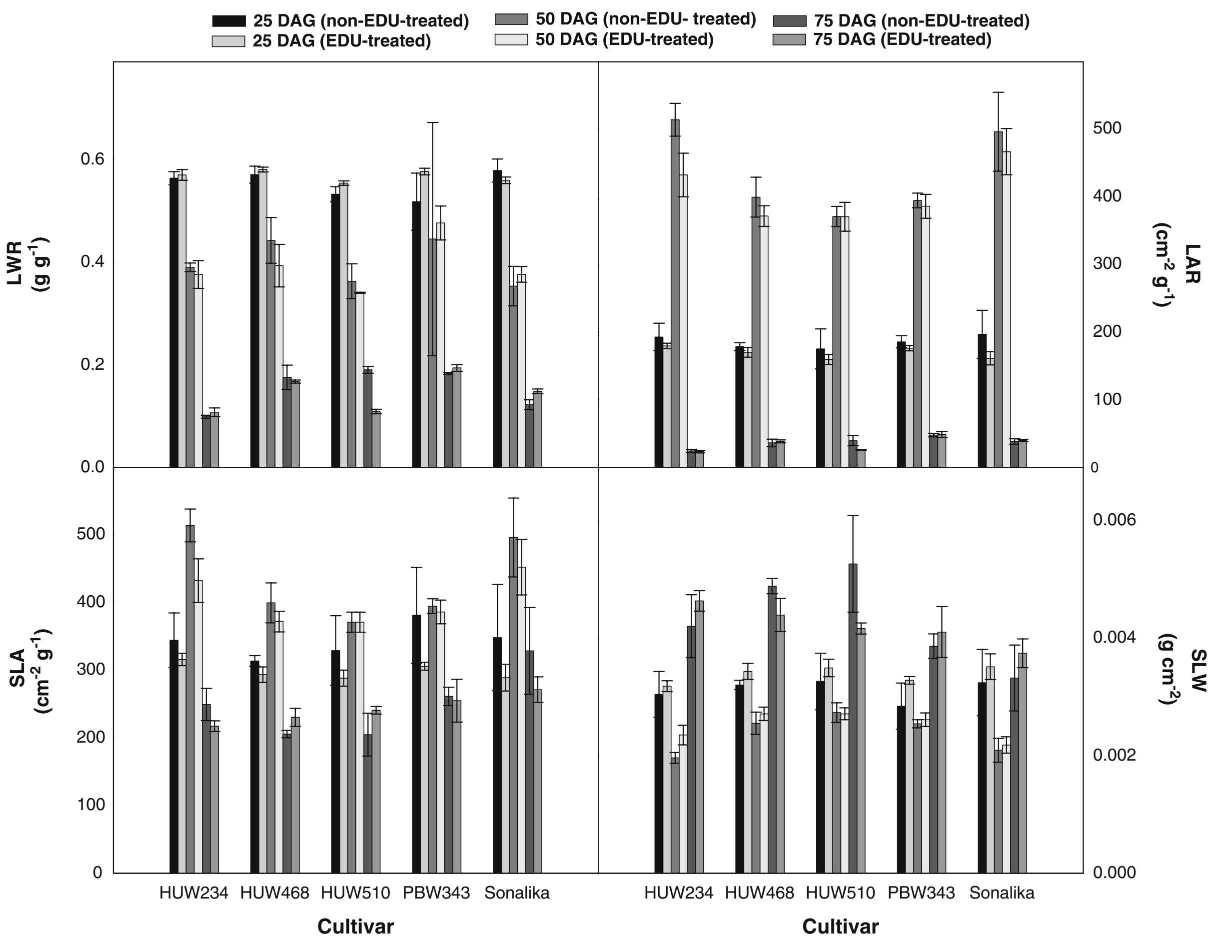


Fig. 4 Effect of EDU application on LAR, LWR, SLW, and SLA of different cultivars of wheat (*bars* represent mean ± 1 SE)

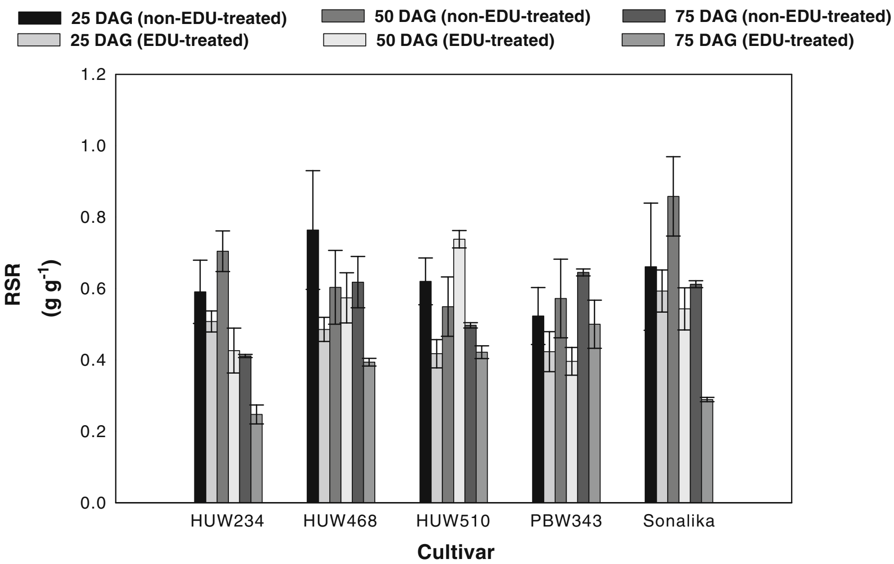


Fig. 5 Effect of EDU application on RSR of different cultivars of wheat (*bars* represent mean ± 1 SE)

at all ages except in cv HUW468 where it declined at 75 DAG and cv HUW510 showed a decrease at 50 and 75 DAG. SLW increased by 4.4%, 6.7%, 6.6%, 13.4%, and 7.7% in EDU-treated plants of cv HUW234, HUW468, HUW510, PBW343, and Sonalika, respectively when compared to non-EDU-treated ones at 25 DAG. SLW varied significantly due to age ($p < 0.001$) and cultivar ($p < 0.01$) (Table 3) (Fig. 4). Leaf weight ratio (LWR) increased in EDU-treated plants at 25 and 75 DAG in cv HUW234 but decreased at 50 DAG (Fig. 4). LWR was higher in EDU-treated plants of cv HUW468 and HUW510 only at 25 DAG and subsequently declined (Fig. 4). LWR increased at all ages in cv PBW343, whereas it declined in cv Sonalika at 25 DAG in EDU-treated plants as compared to non-EDU-treated ones and increased in later samplings (Fig. 4). LWR increased maximally by 17.7% in EDU-treated plants of cv Sonalika at 75 DAG when compared to other test cultivars. Significant differences were found for LWR due to age, cultivar ($p < 0.001$), and interaction of A × C ($p < 0.01$) (Table 3). Root: shoot ratio (RSR) decreased in all cultivars at all ages in EDU-treated plants compared with non-EDU-treated ones except for cv HUW510 which showed a higher value of RSR at 50 DAG (Fig. 5). RSR varied significantly due to age, EDU treatment ($p < 0.001$), and A × C ($p < 0.05$) (Table 3). Net primary productivity (NPP) increased by 3.2%, 45.5%, 51.8%, 40.0%, and 24.2% in EDU-treated plants of cv HUW234, HUW468, HUW510, PBW343, and Sonalika, respectively than non-EDU-treated ones at 75 DAG. Variations in NPP were significant due to age, cultivar, treatment, and interaction of A × C, A × T ($p < 0.001$) and interaction of A × C × T ($p < 0.05$) (Table 3).

EDU exhibited positive effect on plant productivity and various yield parameters of all cultivars. Number of ears per plant increased in EDU-treated plants of cv HUW234 and HUW468 but such effects were not observed for other cultivars (Fig. 6). Weight of ears per plant also increased in EDU-treated plants as compared to non-EDU-treated ones in all cultivars and varied significantly due to EDU treatment ($p < 0.01$) (Fig. 6) (Table 4). Cultivar HUW468 showed a maximum increase of 24.4% for ear weight per plant after EDU treatment. Higher number of grains per ear was observed in EDU-treated plants in all the cultivars and variations were highly significant due to all the factors ($p < 0.001$) (Table 4) (Fig. 6). Higher yield (weight of grains per plant) was ob-

Fig. 6 Effect of EDU application on various yield parameters of different cultivars of wheat (*bars* represent mean ± 1 SE; level of significance difference $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; ^{NS}not significant) ▶

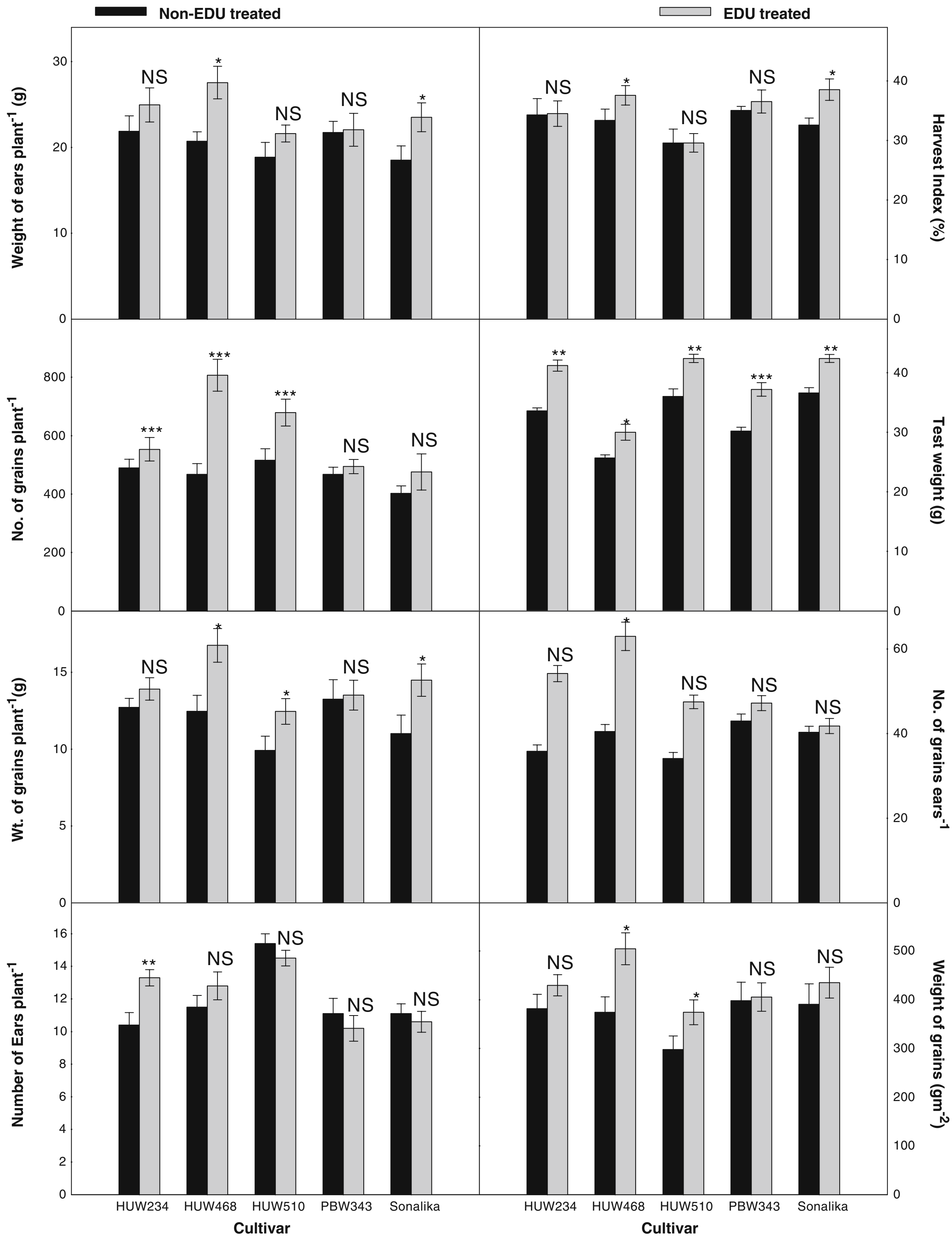

Non-EDU treated
EDU treated
Weight of ears plant-1 (g)
Harvest Index (%)
No. of grains plant-1
Test weight (g)
Wt. of grains plant-1(g)
No. of grains ears-1
Number of Ears plant-1
Weight of grains (gm-2)
NS
*
**
***
HUW234
HUW468
HUW510
PBW343
Sonalika
Cultivar

Table 4 Significance level for various yield parameters of wheat plants as obtained by two-way ANOVA test

Parameters	Cultivar (C)	Treatment (T)	Cultivar × treatment (C × T)
No. of ears per plant	***	NS	NS
Wt. of ears per plant (g)	NS	**	NS
No. of grains per ear	***	***	***
No. of grains per plant	*	**	**
Wt. of grains per plant (g)	*	***	NS
Wt. of above ground biomass (g)	NS	*	NS
Harvest index (HI)	**	NS	NS
Test weight (g)	**	**	NS
Weight of grains (g m^{-2})	*	*	NS

Level of significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; *NS* not significant

served in EDU-treated plants in all the cultivars of wheat. Maximum increase in yield was recorded in cv HUW468 (25.6%), whereas the least increase was recorded (1.9%) in cv PBW343. It varied significantly due to cultivar ($p < 0.05$) and EDU treatment ($p < 0.001$) (Table 4). Harvest index (HI) was also higher for all the EDU-treated cultivars but significant variation was noticed for cv HUW468 and Sonalika (Fig. 6). HI increased by 11.2% and 15.4% in EDU-treated plants of cv HUW468 and Sonalika as compared to non-EDU-treated ones. Test weight (1,000 seed weight) was higher by 18.8%, 18.4%, 15.1%, 14.3%, and 13.7% in EDU-treated wheat cultivars PBW343, HUW234, HUW510, HUW468, and Sonalika, respectively (Fig. 6). Test weight of wheat plants varied significantly due to cultivar and EDU treatment ($p < 0.01$) (Table 4). Total yield (g m^{-2}) was enhanced by 11.2%, 25.8%, 20.5%, 1.9%, and 10.2% by EDU application in cultivars HUW234, HUW468, HUW510, PBW343, and Sonalika, respectively when compared to non-EDU-treated ones. EDU-treated plants of cv HUW468 showed maximum increments of various yield parameters as compared to other EDU-treated cultivars, whereas the least increase in yield parameters was recorded for cv PBW343.

Discussion

The results showed higher concentrations of O_3 at the experimental site, ranging between 35.3 ppb and 54.2 ppb (December 2006–March 2007). O_3 concentration was lower in early stages of plant growth (December–January) and increased during the reproductive phase (anthesis period) of wheat plants (February–March). Agrawal et al. (2004) also reported higher concentrations of O_3 in summer (34.6 ppb) than winter (22.5 ppb) in an urban area of Allahabad city located in the eastern Gangetic plains of India. Higher O_3 concentration often exceeding 40 ppb levels for several hours during February and March was reported in an agricultural area of Varanasi (Tiwari et al. 2005). Recently, Rai et al. (2007) have also reported a higher mean concentration of O_3 (48 ppb) in Varanasi in March 2005. The mean concentration of O_3 recorded in the present study was lower than the concentration reported by Wahid (2006) from Lahore (Pakistan) but higher for the same period in the studies of Tiwari et al. (2005) and Rai et al. (2007). Wang et al. (2007b) showed that mean O_3 concentration was higher than 56 ppb during growth of wheat crop when examining of data collected from different locations of China and suggested that it may cause crop losses.

EDU has been used as a protectant from O_3 injury since 1978 and a protective effect of EDU against O_3 was reported by several workers (Carnahan et al. 1978; Archambault et al. 2000; Agrawal et al. 2005; Tiwari et al. 2005; Wang et al. 2007a). Results of the present study showed that EDU improved various morphological parameters of all five wheat cultivars under ambient concentration of O_3. Shoot length showed significant increase in cv HUW234, HUW468, and HUW510, whereas non-significant differences were noticed in cv PBW343 and Sonalika as compared to non-EDU-treated plants. Agrawal et al. (2005) found an increase of 12.3% in shoot length in *Vigna radiata* plants due to application of 500 ppm EDU as soil drench. Tiwari et al. (2005) also reported significant increase in plant height at 300 ppm EDU concentration in wheat cv M533. In another study, increase in shoot length was observed in *Fraxinus excelsior* L. due to application of 450 ppm EDU by gravitational trunk infusion but the increase was not significant (Paoletti et al. 2008). Other growth characteristics like number of tillers, number of leaves, and leaf area also increased significantly in wheat cv HUW234, HUW468, HUW510, and PBW343, whereas non-significant difference was noticed in cv Sonalika as compared to their control plants. Agrawal et al. (2004) also observed 12%, 17%, and 9.6% increase in number of leaves due to EDU treatment of 500 ppm in three wheat cultivars. Increment in leaf area was also found in soybean due to EDU treatment (Wahid et al. 2001). However, there are also studies showing that the number of leaves did not vary significantly due to EDU treatment (Tonneijck and Didyk 1997; Agrawal et al. 2003; Tiwari et al. 2005). Recently, Szantoi et al. (2007) reported a significant change in number of leaves over time due to application of EDU in *Echinacea purpurea* (purple coneflower) plants.

Total biomass, shoot, and leaf dry weight was higher in all the cultivars of wheat treated with EDU but significant differences were found only in cv HUW234, HUW468, and HUW510. EDU treatment was highly significant for leaf, root, and shoot dry weight leading to a higher biomass in EDU-treated plants of *Phaseolus vulgaris* L. (Brunschon-Harti et al. 1995). Tonneijck and Didyk (1997) reported a significant increase in leaf dry weight in *Trifolium subterraneum* plant treated with EDU in the Netherlands. In another study, EDU application suppressed the effects of O_3 on shoot dry weight and total biomass completely and root dry weight partially in turnip and radish (Hassan et al. 1995). Tiwari et al. (2005) reported significant increase in root, shoot, leaf dry weight, and total biomass production of wheat plants. Agrawal et al. (2005) also showed an increase by 24% in total biomass in mung bean plants due to EDU treatment. In *E. purpurea*, 100 and 200 ppm treated plants produced more above ground biomass than non EDU-treated plants (Szantoi et al. 2007). Varshney and Rout (1998) found that increased ambient O_3 level decreased shoot and root biomass of *Lycopersicon esculentum* whereas EDU-treated plants had higher biomass than non-treated ones.

EDU application had a positive effect on various other growth indices of the wheat test cultivars. Higher values for AGR, LWR, SLW, NAR, and RGR were found in EDU-treated cultivars of wheat over non-treated ones. EDU treatment helped the plants to accumulate more biomass and also to overcome the negative effects of O_3. NAR represents the increase in plant dry weight per unit of assimilatory surface per unit time and was higher in EDU-treated plants. EDU treatment also helps the plant to increase their assimilatory surface. Agrawal et al. (2005) found higher value for RGR, AGR, and NAR in EDU-treated mung bean plants with application of 500 ppm EDU as a soil drench. Higher RGR was also observed in snap bean plants due to application of EDU (Gillespie et al. 1998). The present study showed that root:shoot ratio (RSR) was lower in EDU-treated plants than non-EDU-treated ones in all the test cultivars which showed more allocation of biomass in shoots than roots in EDU-treated plants. Wahid et al. (1995) reported a decrease in RSR of plants growing in a filtered air open top chamber compared with a non-filtered air chamber and open plots in two different rice cultivars. It has already been reported by several workers that filtered air open top chambers had 86–92% less O_3 than non-filtered chambers and open plots (Wahid et al. 1995; Tiwari et al. 2006; Wahid 2006; Rai et al. 2007).

EDU application increased various yield attributes in the form of number of grains per plant, weight of grains per ear, weight of grains (g m^{-2}), and harvest index (HI) in all cultivars. Test weight increased significantly in EDU-treated plants in all the cultivars as compared to non-EDU-treated ones. Wang et al. (2007a) also found a significant increase in various yield parameters of wheat after EDU application but not in case of rice plants. They showed that wheat yield (g $plant^{-1}$) increased by 3.4% at 150 ppm EDU, 12.7% at 300 ppm, and 7.1% at 450 ppm EDU used as soil drench. In another study, EDU application increased test weight of three different cultivars of wheat by 13.8% to 23.9% (Agrawal et al. 2004). A study conducted by Hassan (2006) in an Egyptian field also found a significant increase of tuber weight of potato after EDU treatment.

Several studies made by different workers showed that O_3 exposure reduced photosynthetic rate might be due to changes in photophosphorylation, electron transport system, and carbon fixation. Reduction in quantity of an important photosynthetic enzyme, ribulose bisphosphate/oxygenase, is often associated with the reduction of photosynthetic rates of plants exposed to O_3 (Dann and Pell 1989; Pell et al. 1992). Secondly, O_3 is a potent oxidant which generates reactive oxygen species after entering into the cell via the stomata (Mehlhorn et al. 1990) which initiates lipid peroxidation, causing destruction in the membranes (Heath 1988). It is believed that EDU treatment prevents loss of leaf lipid peroxidation and thus helps in membrane protection (Whitaker et al. 1990). EDU is known to maintain higher levels of various antioxidants in the apoplastic region and reduces the adverse effect of O_3 (Hassan 2006), consequently helping plants in development and production. EDU can persist in plants for 10 days or even more (Regner-Joosten et al. 1994). Accelerated senescence is a well-known phenomenon occurring due to O_3 stress. Various reports showed that EDU possesses 'antisenescent' properties (Brunschon-Harti et al. 1995; Tiwari et al. 2005). The present study also supported the view of other workers that EDU treatment delays the senescence in all the test cultivars of wheat. It has been suggested that this is due to the amount of urea in EDU molecule, acting as a foliar fertilizer (Manning et al. 2003). Our results showed that numbers of standing dead leaves were fewer in EDU-treated plants suggesting the possible role of EDU as an antiozonant in delaying the senescence (Lee et al. 1981; Brunschon-Harti et al. 1995). Tiwari et al. (2005) also reported lower numbers of standing dead leaves in EDU-treated cultivars of wheat.

In the present investigation, different growth and yield parameters of all the wheat cultivars did not show a similar response to EDU application. From this study, we concluded that all the cultivars are sensitive to O_3 with cv HUW468 proving to be the most sensitive, showing significant reductions in various growth and yield parameters. EDU treatment positively affected wheat cv HUW468 in terms of total yield (g m^{-2}) followed by cv HUW510, HUW234, Sonalika, and PBW343. Agrawal et al. (2004) also reported greater sensitivity of wheat cv HUW468 in terms of total yield as compared to other cultivars (HUW234 and HD2329). Significant difference was also found in HI for cv HUW468 and Sonalika due to EDU treatment but not for other cultivars. This showed the positive effect of EDU on reproductive as well as vegetative parts of cv HUW234, HUW510 and PBW343 whereas more photo-assimilates were translocated to reproductive parts than to vegetative parts of cv HUW468 and Sonalika. We also agree with the findings of Wang et al. (2007a) that, while comparing the response of crops to ambient O_3, one must consider evaluating the cultivar responses within species and no generalization just by species alone.

The present study clearly showed that application of EDU alleviated the negative impact of O_3 on all wheat varieties of India. EDU protected the plants from the damaging effects of O_3 by modifying various plant processes, thus the growth characteristics and biomass allocation, leading to yield enhancement successfully. Thus, EDU can be used as a tool to determine the location and magnitude of crop losses due to O_3. Among the test cultivars of wheat, HUW468, being most sensitive, could be used for biomonitoring O_3 effects and PBW343 can be recommended for cultivation in areas experiencing elevated concentrations of O_3.

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