

# **Use of ethylenediurea (EDU) as a research tool in assessing the impact of ambient ozone on plants**

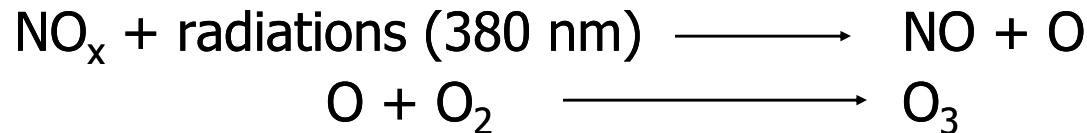
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Varanasi-221 005, India

# Troposphere Ozone

- **Ozone, a major secondary air pollutant**
- **Meteorological conditions such as high temperature and high light intensity favor O<sub>3</sub> formation due to long range transport of precursors**

# Formation of tropospheric Ozone

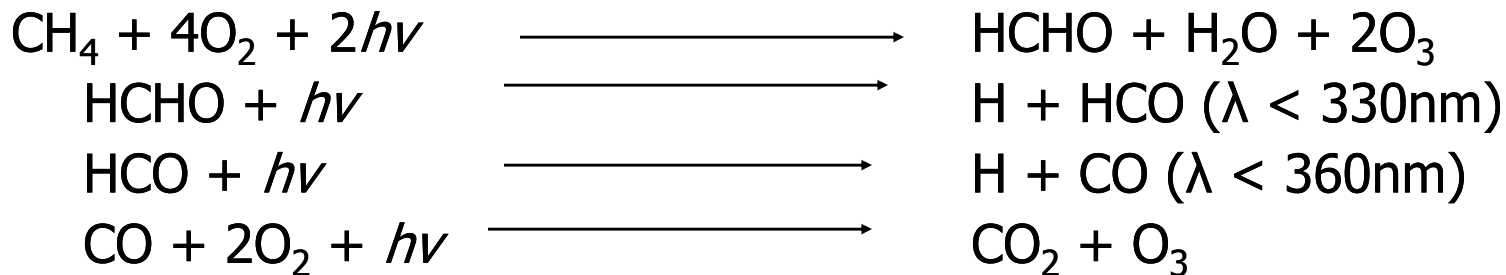
Ozone from  $\text{NO}_x$



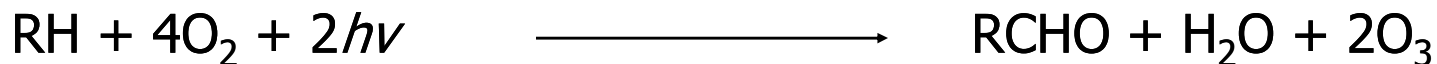
Ozone from carbon monoxide



Ozone from methane



Ozone from methane hydrocarbons



# Impact of ozone on plants

## Higher levels of ozone cause:

- Foliar injury
- Accelerate senescence
- Decrease plant growth
- Alter plant metabolism
- Reduce crop yield

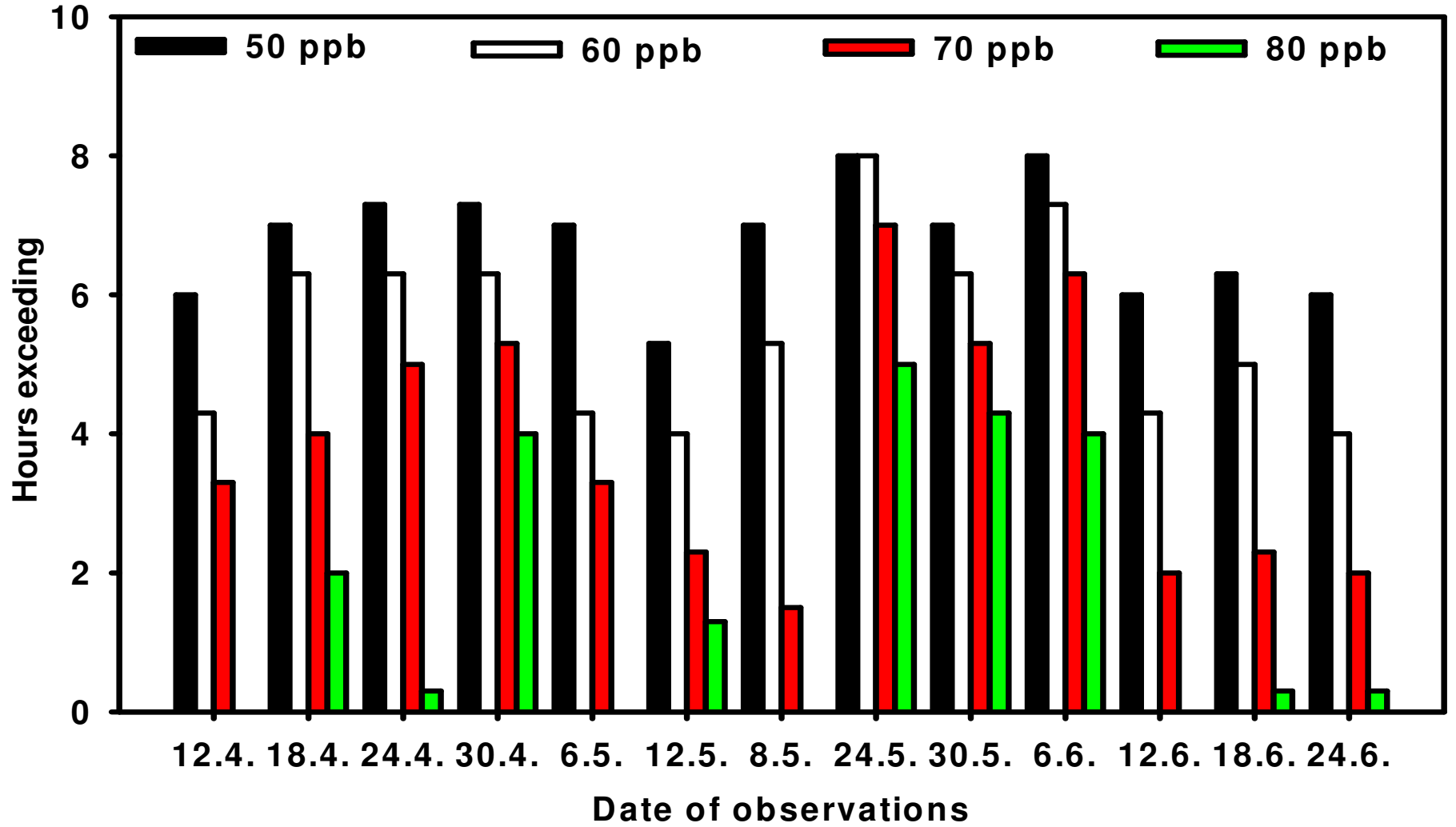
# Assessment of O<sub>3</sub> injury on plants using EDU

- **Ethylenediurea (N-[2-(2-oxo-1-imidazolidinyl) ethyl]- N' phenylurea; EDU)**
- **Synthetic chemical**
- **Provides protection to wide range of plants from O<sub>3</sub> injury without confounding effects of its own**
- **Allows assessment of yield losses**

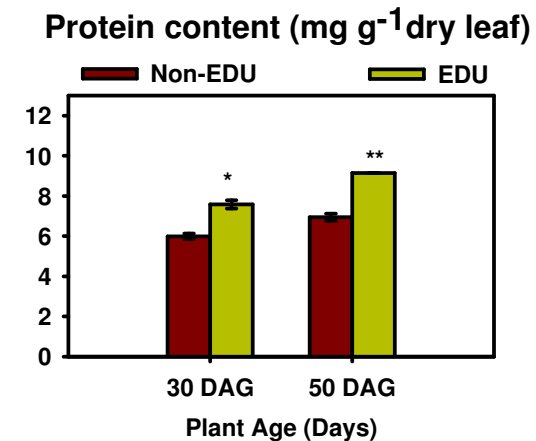
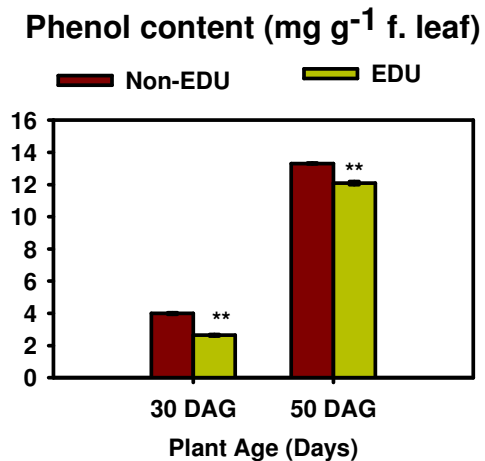
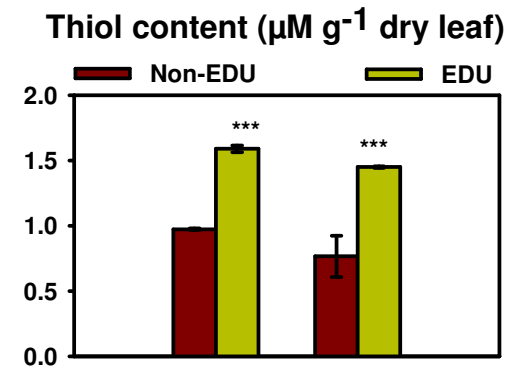
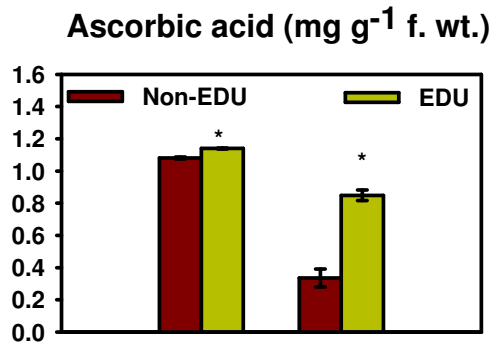
# **EDU as a tool to assess ozone injury on mungbean plants**

- **EDU solution (400 ppm) @ 100 ml plant<sup>-1</sup> as soil drench, one week after seedling emergence, at interval of 10 days up to 70 days**
- **Mean ozone concentration 60 ppb**

**O<sub>3</sub> CONCENTRATION EXCEEDING 50, 60, 70 and 80 ppb  
DURING THE EXPERIMENTAL PERIOD (April to June 2006)**

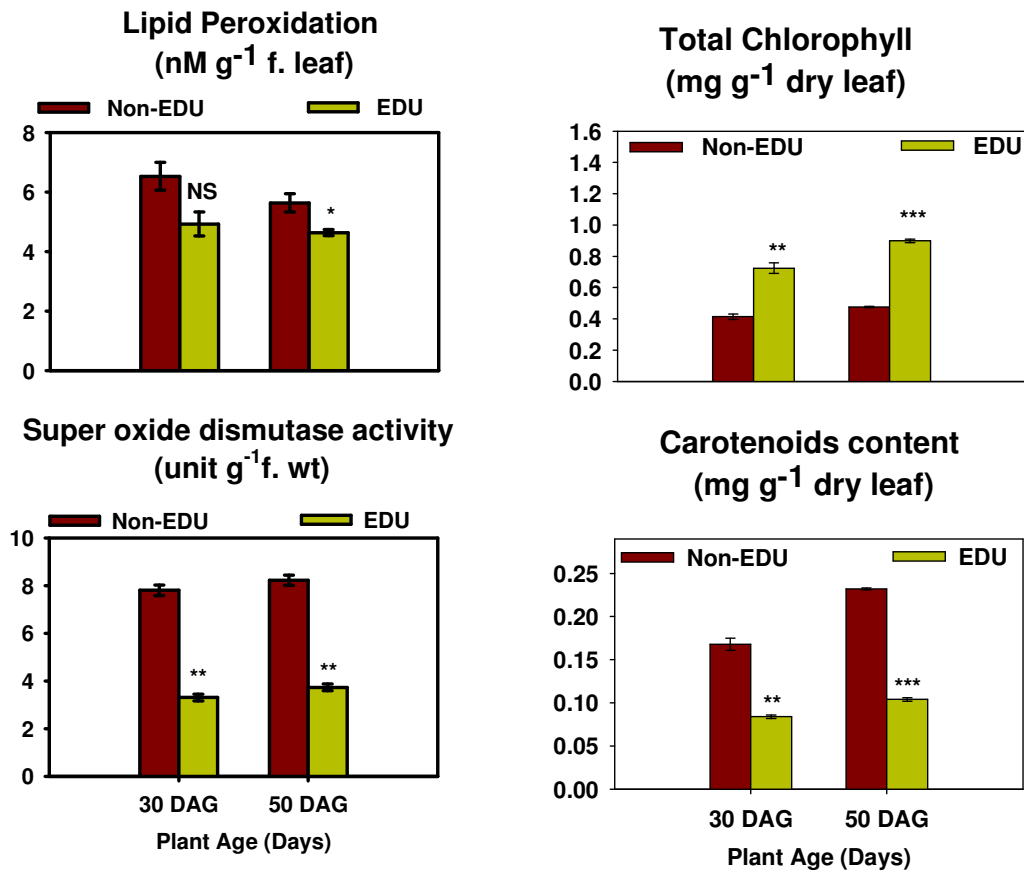


# Effect of EDU treatment on ascorbic acid, phenol, thiol and protein contents of mungbean plants

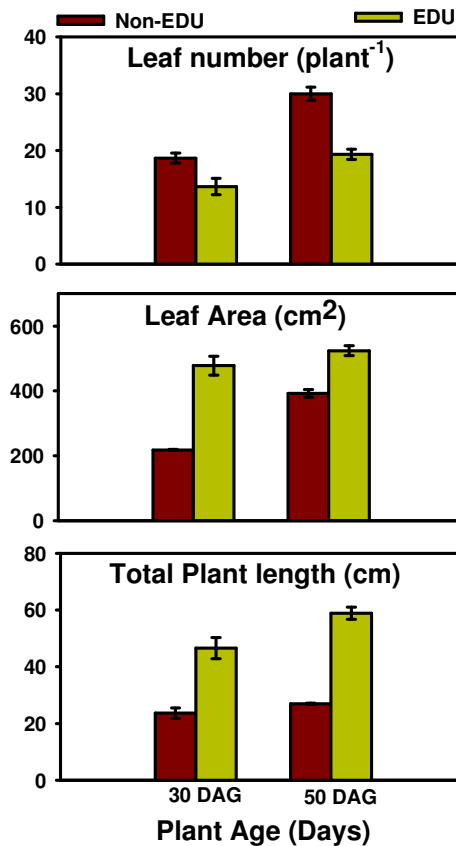




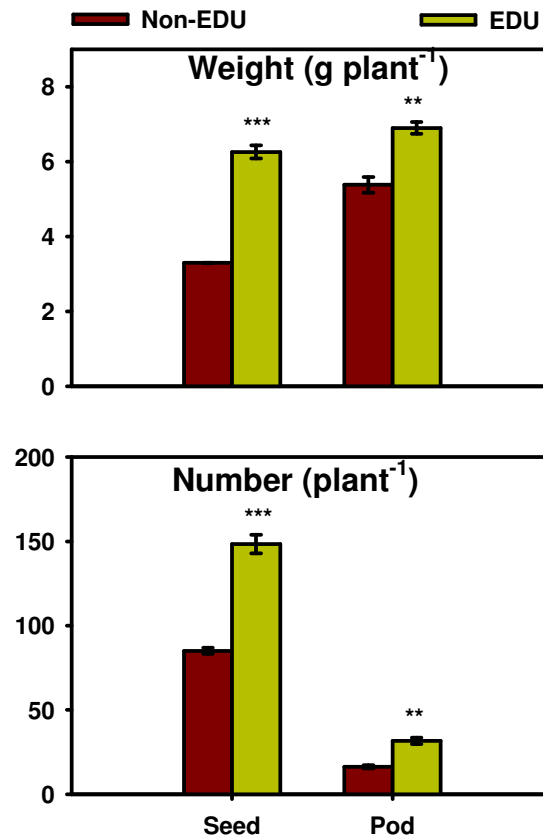
# Effect of EDU treatment on LPO, SOD and photosynthetic pigments of mungbean plants



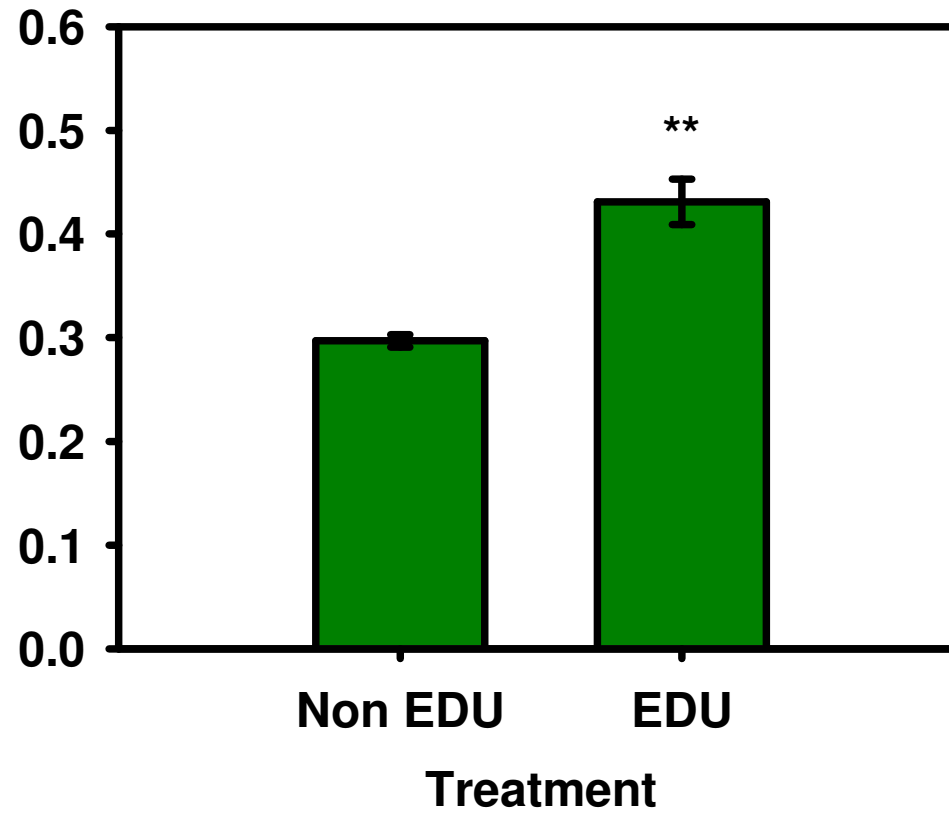
# Effect of EDU treatment on Leaf number, leaf area and total plant length of mungbean plants



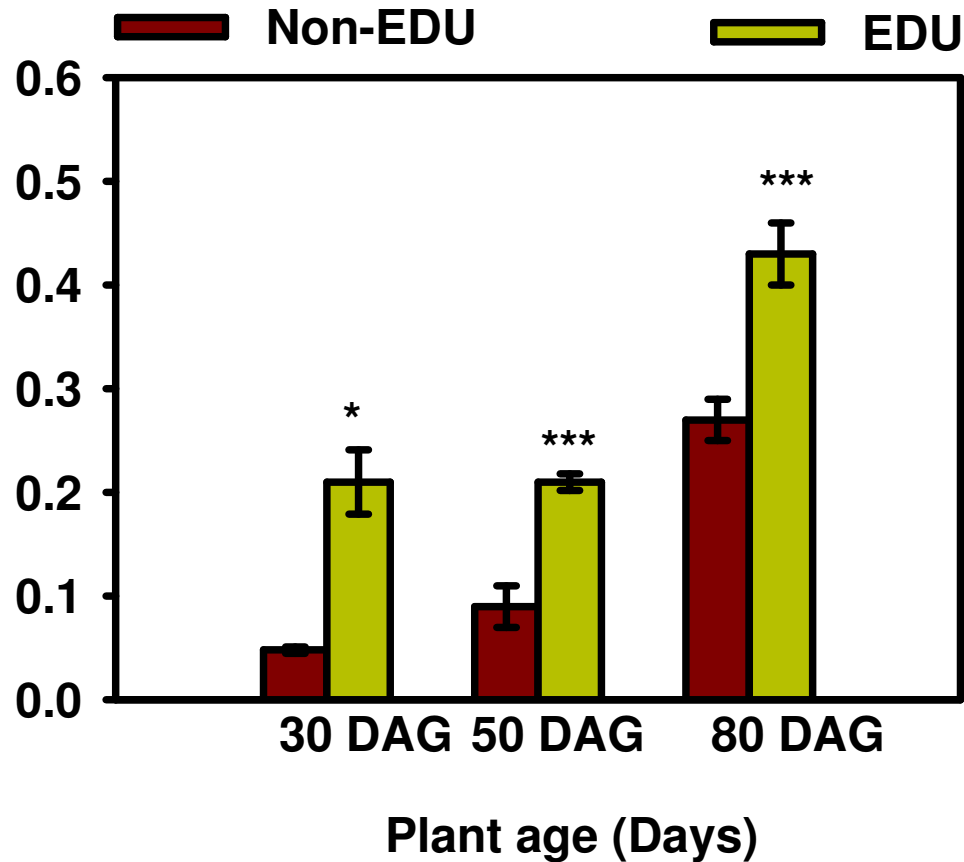
# Effect of EDU treatment on weight and number of seeds and pods of mungbean plants



**Effect of EDU treatment on harvest index ( $\text{g g}^{-1}$ )  
of mungbean plants**



# Age wise effect of EDU treatment on root shoot ratio ( $\text{g g}^{-1}$ ) of mungbean plants



## **Effect of EDU treatment on yield parameters of mungbean plants**

<b>Parameter</b>	<b>Non – EDU</b>	<b>EDU</b>
<b>Seed wt. (g plant<sup>-1</sup>)</b>	<b>3.30</b>	<b>6.26 (47%)</b>
<b>Pod wt. ( g plant<sup>-1</sup>)</b>	<b>5.38</b>	<b>6.90 (22%)</b>
<b>No. of seeds (plant<sup>-1</sup>)</b>	<b>85</b>	<b>148 (42%)</b>
<b>No. of pods (plant<sup>-1</sup>)</b>	<b>16</b>	<b>31 (48%)</b>
<b>Yield (g m<sup>-2</sup>)</b>	<b>223.12</b>	<b>432.65 (48%)</b>





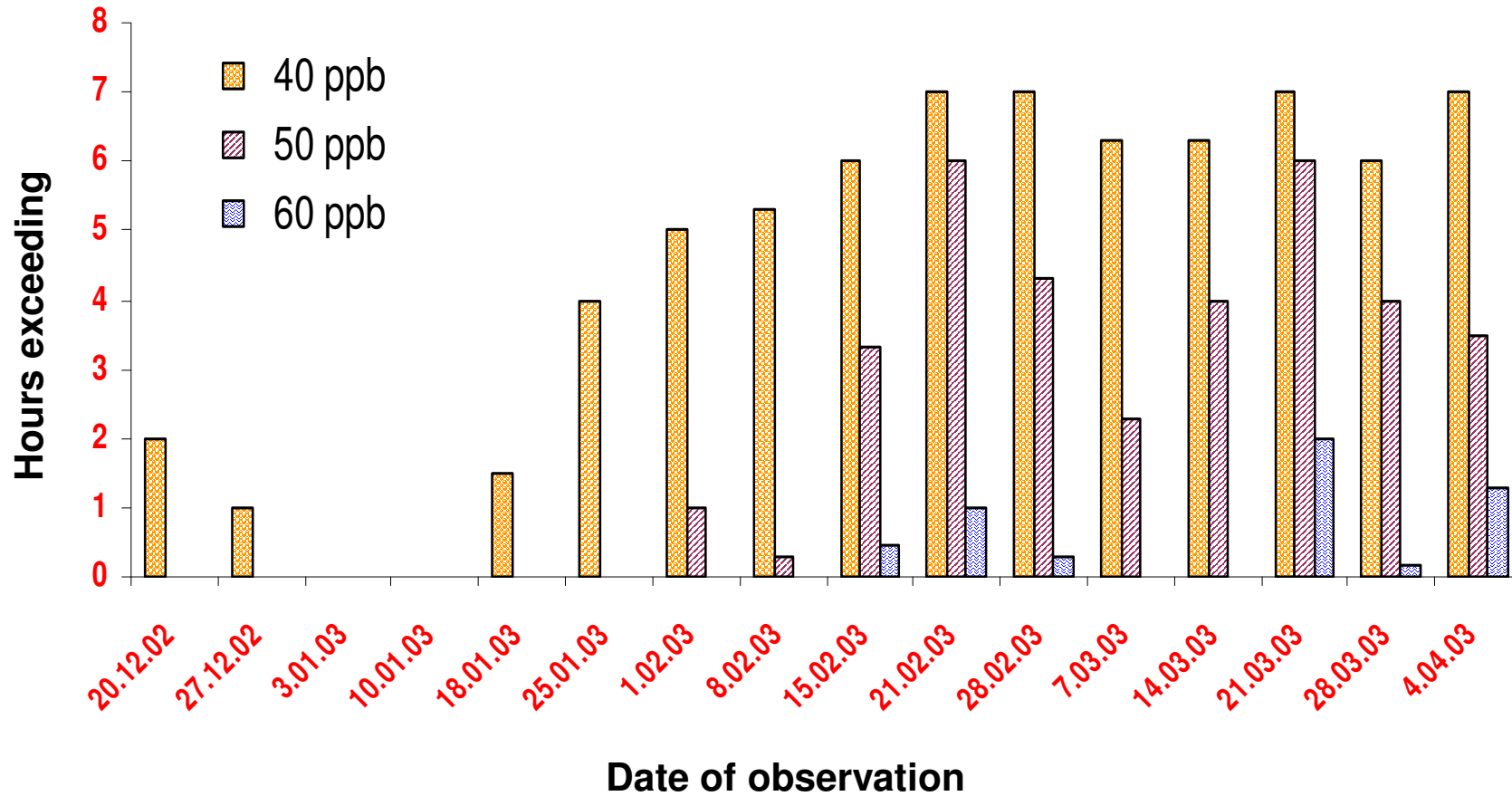




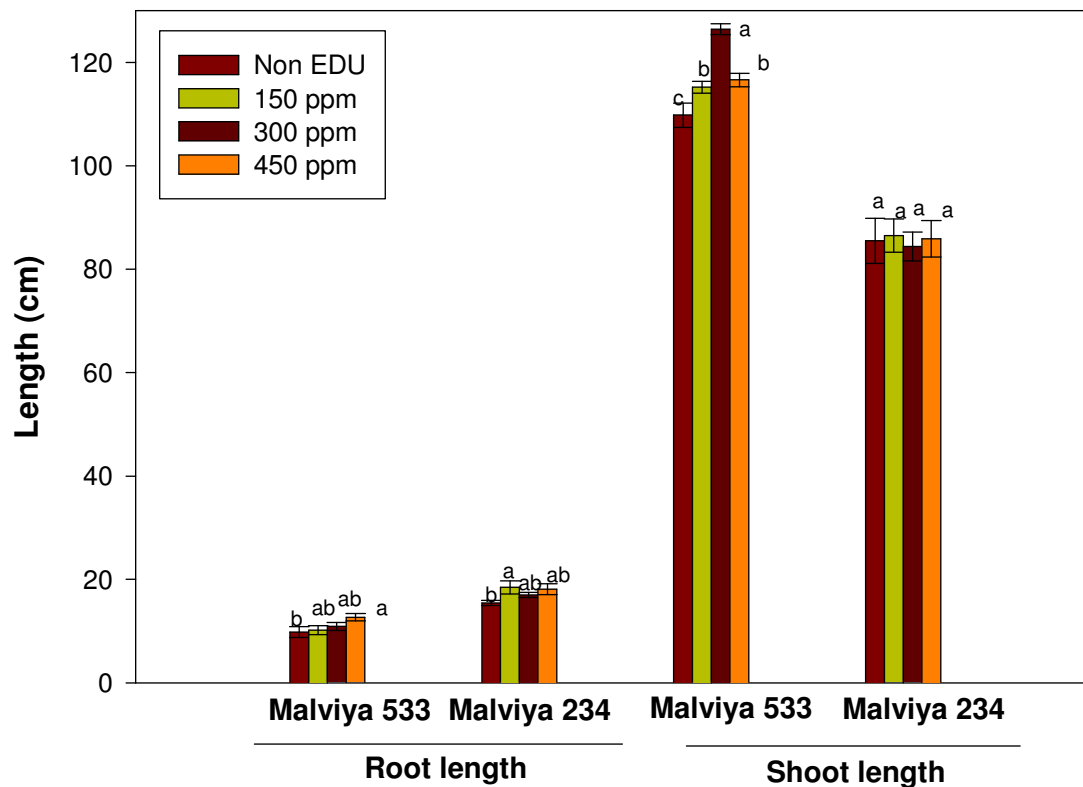
# **EDU as a tool to assess ozone injury on wheat plants**

- **EDU (150, 300 and 450 ppm) at 10 days interval after germination up to 100 days age**
- **Mean ozone concentration 43 ppb**

# O<sub>3</sub> CONCENTRATIONS EXCEEDING 40, 50 AND 60 ppb DURING THE EXPERIMENTAL PERIOD



### Variations in root and shoot length of two wheat cultivars at different levels of EDU treatments at 60 days age



## Selected morphological characteristics of two cultivars of wheat at different EDU treatments at 60 DAG (Mean $\pm$ 1SE)

Cultivars/ treatment	No of tillers (plant <sup>-1</sup> )	No of leaves (plant <sup>-1</sup> )	No of standing dead (plant <sup>-1</sup> )	Leaf area (cm <sup>2</sup> )
<b>Malviya 533</b>				
<b>Control</b>	<b>9.00<sup>b</sup></b> $\pm$ 0.31	<b>13.40<sup>a</sup></b> $\pm$ 2.50	<b>47.80<sup>a</sup></b> $\pm$ 2.35	<b>44.05<sup>a</sup></b> $\pm$ 3.10
<b>EDU 150 ppm</b>	<b>13.6<sup>a</sup></b> $\pm$ 1.40	<b>16.20<sup>a</sup></b> $\pm$ 1.15	<b>42.80<sup>b</sup></b> $\pm$ 3.36	<b>53.77<sup>a</sup></b> $\pm$ 3.99
<b>EDU 300 ppm</b>	<b>10.4<sup>ab</sup></b> $\pm$ 2.31	<b>16.40<sup>a</sup></b> $\pm$ 1.56	<b>35.20<sup>c</sup></b> $\pm$ 5.35	<b>55.78<sup>a</sup></b> $\pm$ 4.04
<b>EDU 450 ppm</b>	<b>14.4<sup>a</sup></b> $\pm$ 0.81	<b>12.20<sup>a</sup></b> $\pm$ 3.91	<b>31.0<sup>c</sup></b> $\pm$ 2.42	<b>54.18<sup>a</sup></b> $\pm$ 2.46
<b>Malviya 234</b>				
<b>Control</b>	<b>7.20<sup>b</sup></b> $\pm$ 1.06	<b>11.60<sup>a</sup></b> $\pm$ 1.43	<b>37.40<sup>a</sup></b> $\pm$ 7.63	<b>43.24<sup>a</sup></b> $\pm$ 3.10
<b>EDU 150 ppm</b>	<b>12.6<sup>a</sup></b> $\pm$ 1.80	<b>17.00<sup>a</sup></b> $\pm$ 2.75	<b>20.00<sup>c</sup></b> $\pm$ 2.84	<b>46.69<sup>a</sup></b> $\pm$ 0.86
<b>EDU 300 ppm</b>	<b>10.8<sup>ab</sup></b> $\pm$ 1.98	<b>19.00<sup>a</sup></b> $\pm$ 2.36	<b>31.80<sup>b</sup></b> $\pm$ 3.90	<b>47.00<sup>a</sup></b> $\pm$ 2.60
<b>EDU 450 ppm</b>	<b>9.20<sup>ab</sup></b> $\pm$ 1.24	<b>15.40<sup>a</sup></b> $\pm$ 2.40	<b>15.80<sup>c</sup></b> $\pm$ 1.39	<b>42.12<sup>a</sup></b> $\pm$ 1.81

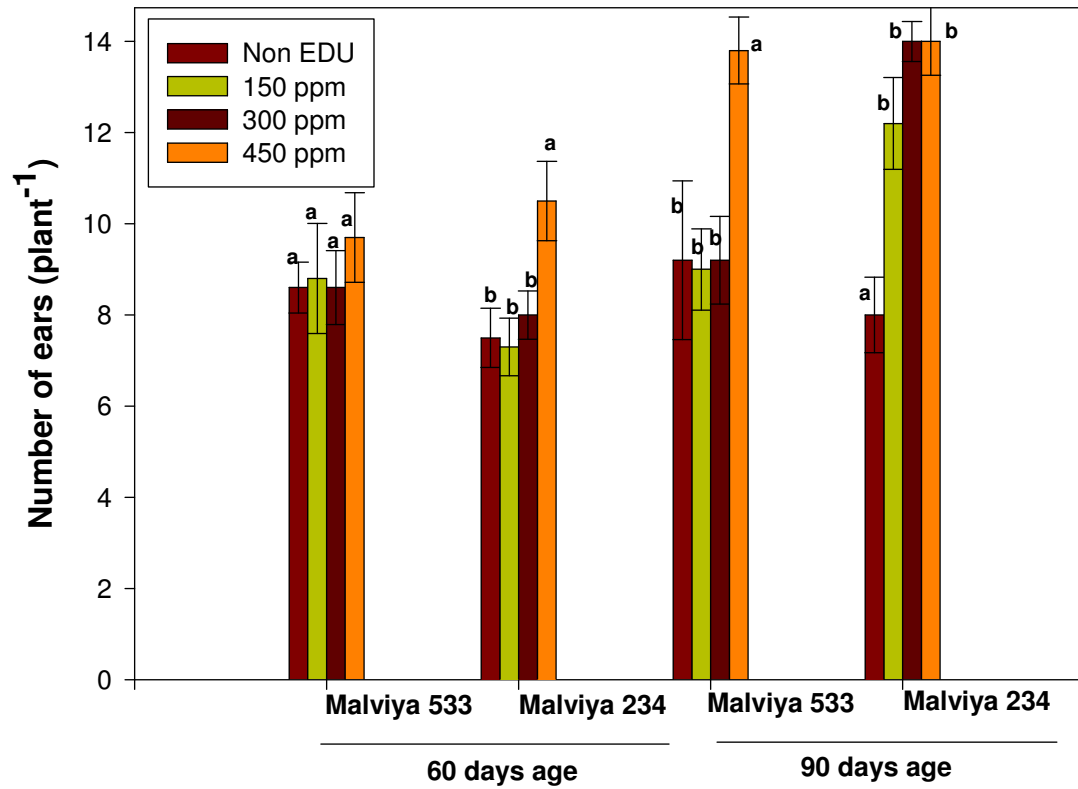
Within each grouping, values not followed by same letter are significantly different at  $p < 0.05$

## Component wise biomass accumulation (g plant<sup>-1</sup>) of two wheat cultivars at different EDU treatments at 60 DAG (Mean ± 1SE)

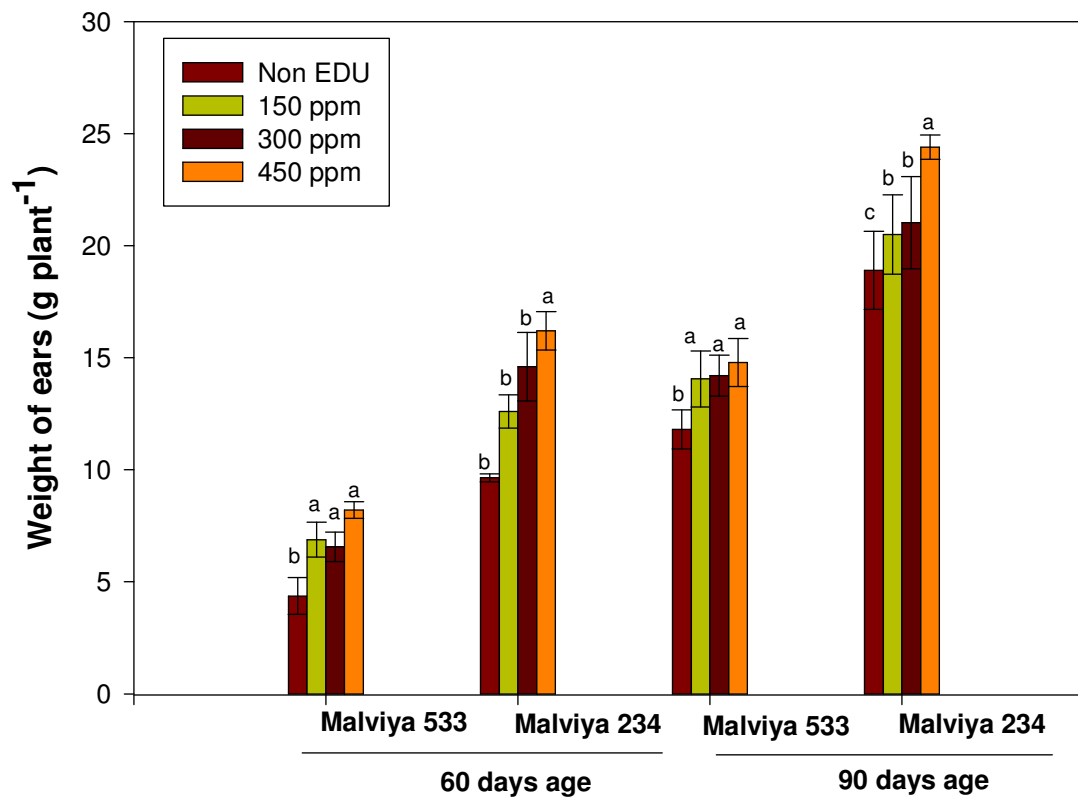
Cultivar / treatment	Root	Shoot	Leaf	Standin g dead	Ear	Total
<b>Malviya 533</b>						
<b>Control</b>	<b>5.45<sup>b</sup></b>	<b>12.05<sup>b</sup></b>	<b>1.39<sup>b</sup></b>	<b>3.96<sup>a</sup></b>	<b>4.37<sup>b</sup></b>	<b>25.58<sup>a</sup></b>
	<b>± 0.46</b>	<b>± 1.78</b>	<b>± 0.24</b>	<b>± 2.01</b>	<b>± 0.82</b>	<b>± 2.30</b>
<b>EDU 150 ppm</b>	<b>6.31<sup>b</sup></b>	<b>14.40<sup>ab</sup></b>	<b>2.19<sup>a</sup></b>	<b>2.32<sup>a</sup></b>	<b>6.88<sup>a</sup></b>	<b>32.12<sup>ab</sup></b>
	<b>± 0.72</b>	<b>± 0.81</b>	<b>± 0.35</b>	<b>± 0.18</b>	<b>± 0.78</b>	<b>± 2.21</b>
<b>EDU 300 ppm</b>	<b>6.71<sup>b</sup></b>	<b>18.20<sup>a</sup></b>	<b>2.41<sup>a</sup></b>	<b>2.04<sup>a</sup></b>	<b>6.56<sup>a</sup></b>	<b>35.92<sup>bc</sup></b>
	<b>± 0.59</b>	<b>± 2.39</b>	<b>± 0.10</b>	<b>± 0.30</b>	<b>± 0.65</b>	<b>± 3.64</b>
<b>EDU 450 ppm</b>	<b>8.71<sup>a</sup></b>	<b>18.99<sup>a</sup></b>	<b>2.03<sup>a</sup></b>	<b>3.12<sup>a</sup></b>	<b>8.20<sup>a</sup></b>	<b>41.08<sup>c</sup></b>
	<b>± 0.70</b>	<b>± 2.15</b>	<b>± 0.18</b>	<b>± 0.41</b>	<b>± 0.37</b>	<b>± 2.23</b>
<b>Malviya 234</b>						
<b>Control</b>	<b>2.83<sup>b</sup></b>	<b>9.54<sup>b</sup></b>	<b>1.46<sup>a</sup></b>	<b>2.51<sup>a</sup></b>	<b>9.64<sup>c</sup></b>	<b>25.10<sup>a</sup></b>
	<b>± 0.53</b>	<b>± 0.29</b>	<b>± 0.07</b>	<b>± 0.52</b>	<b>± 0.18</b>	<b>± 0.82</b>
<b>EDU 150 ppm</b>	<b>5.55<sup>a</sup></b>	<b>14.40<sup>a</sup></b>	<b>2.12<sup>a</sup></b>	<b>2.14<sup>ab</sup></b>	<b>12.60<sup>b</sup></b>	<b>37.19<sup>b</sup></b>
	<b>± 0.43</b>	<b>± 0.97</b>	<b>± 0.25</b>	<b>± 0.13</b>	<b>± 0.74</b>	<b>± 1.92</b>
<b>EDU 300 ppm</b>	<b>3.63<sup>b</sup></b>	<b>14.10<sup>a</sup></b>	<b>1.83<sup>a</sup></b>	<b>1.62<sup>a</sup></b>	<b>14.60<sup>a</sup></b>	<b>36.31<sup>b</sup></b>
	<b>± 0.31</b>	<b>± 1.66</b>	<b>± 0.24</b>	<b>± 0.12</b>	<b>± 1.53</b>	<b>± 0.88</b>
<b>EDU 450 ppm</b>	<b>5.35<sup>a</sup></b>	<b>15.92<sup>a</sup></b>	<b>1.86<sup>a</sup></b>	<b>1.46<sup>b</sup></b>	<b>16.20<sup>a</sup></b>	<b>40.85<sup>b</sup></b>
	<b>± 0.56</b>	<b>± 1.41</b>	<b>± 0.31</b>	<b>± 0.11</b>	<b>± 0.86</b>	<b>± 2.00</b>

Within each grouping, values not followed by same letter are significantly different at p < 0.05

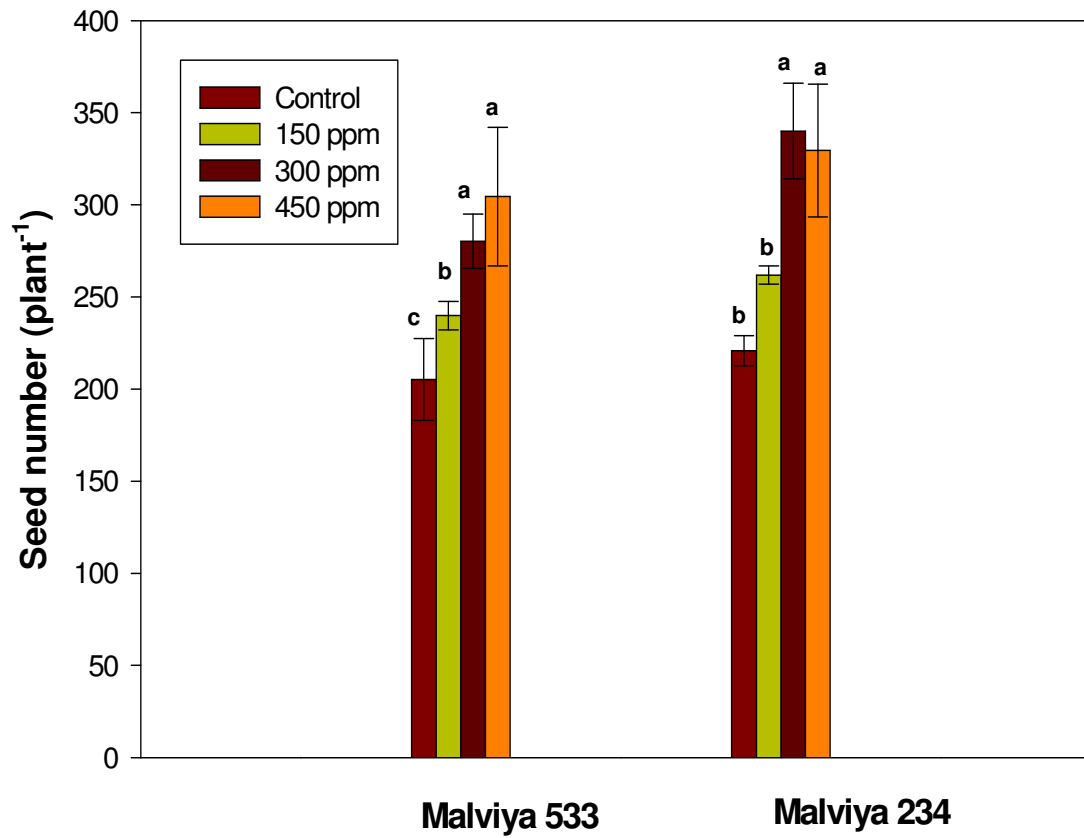
### Agewise changes in the ear number of two wheat cultivars at different levels of EDU treatments



### Agewise changes in ear weight of two wheat cultivars at different levels of EDU concentrations

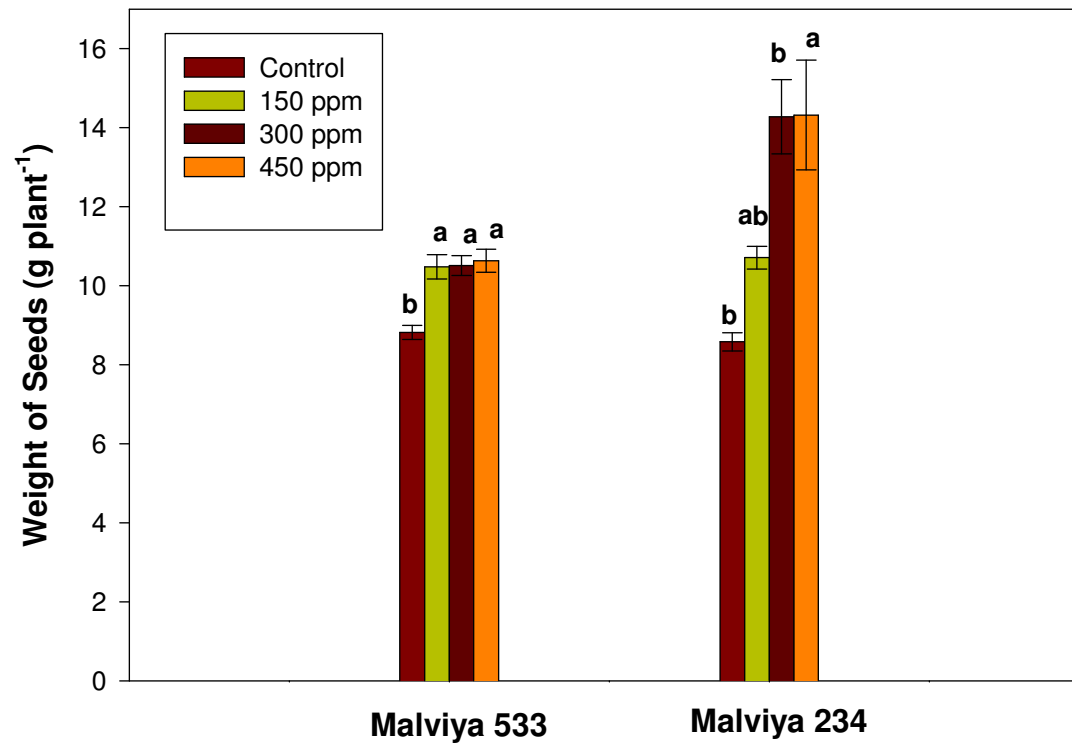


Variations in seed number of two wheat varieties at different EDU concentrations

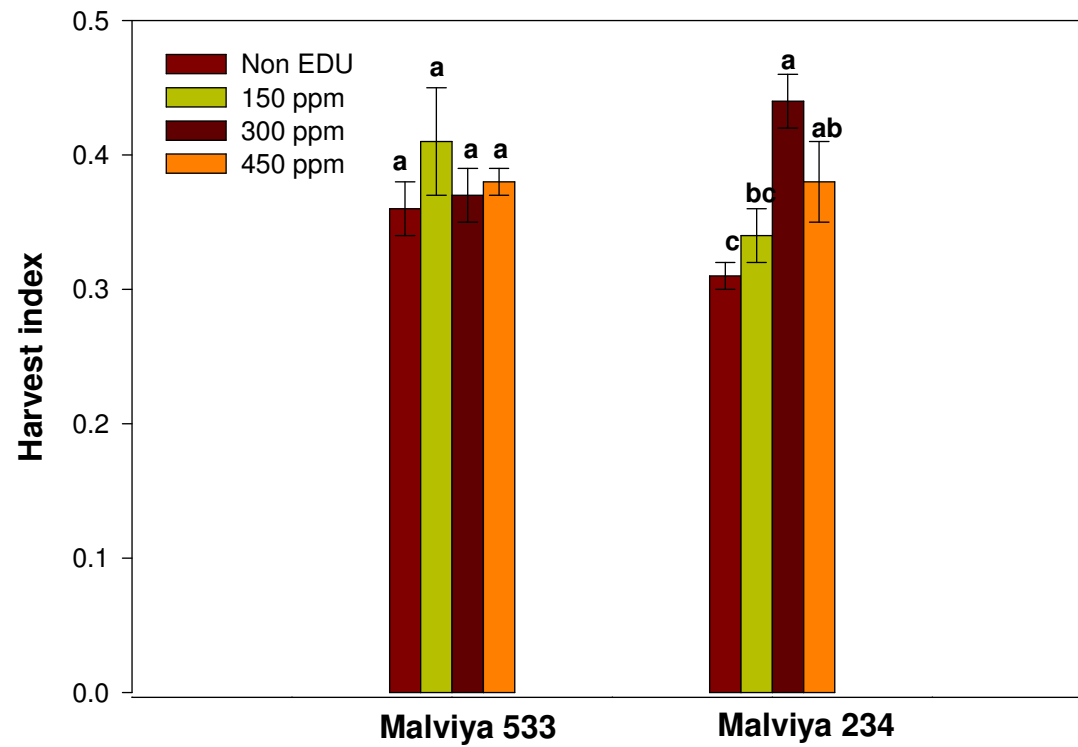




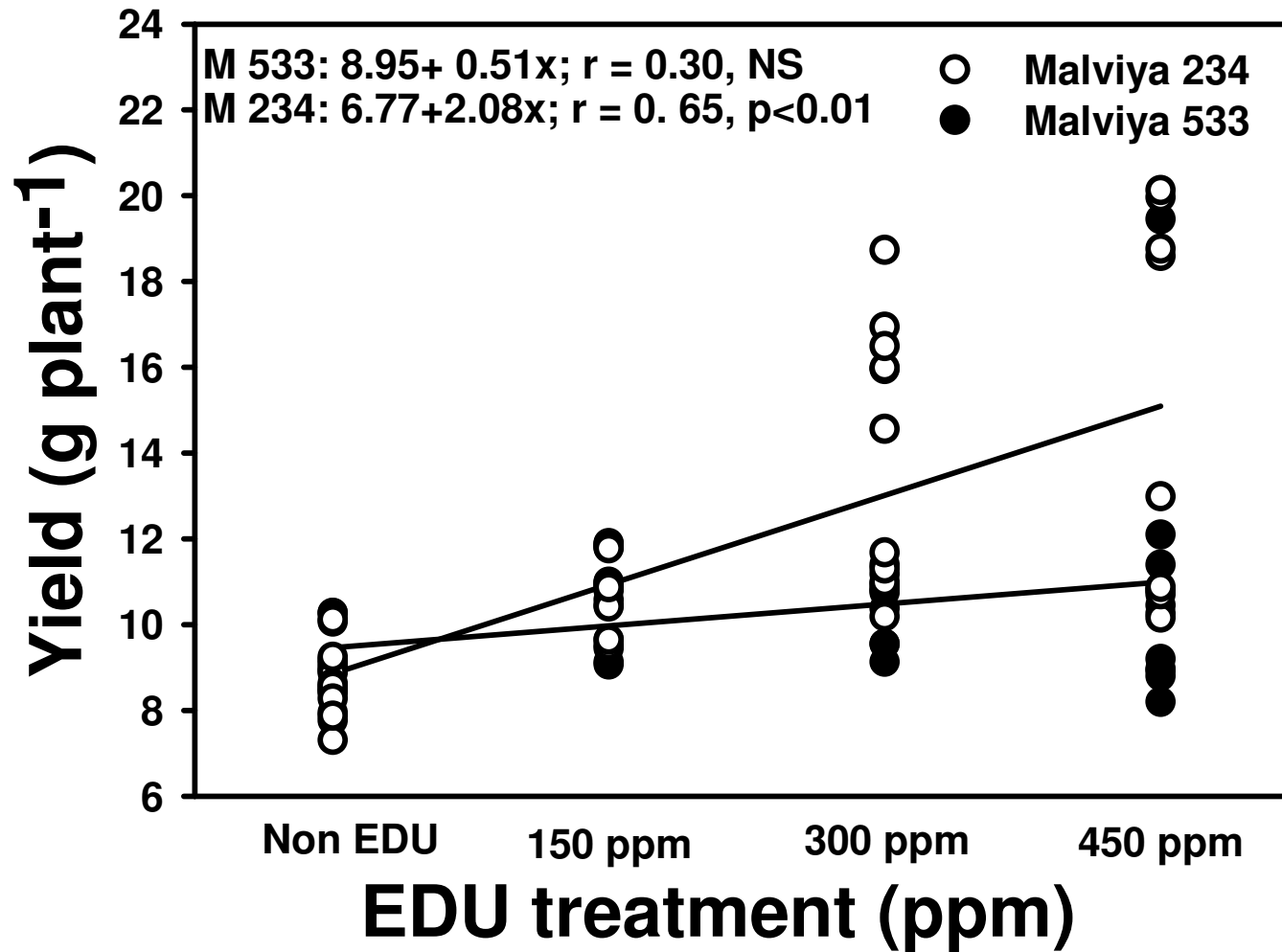
### Variations in the seed weight of two wheat varieties at different EDU concentrations



### Variations in harvest index of the two wheat cultivars at different EDU concentrations



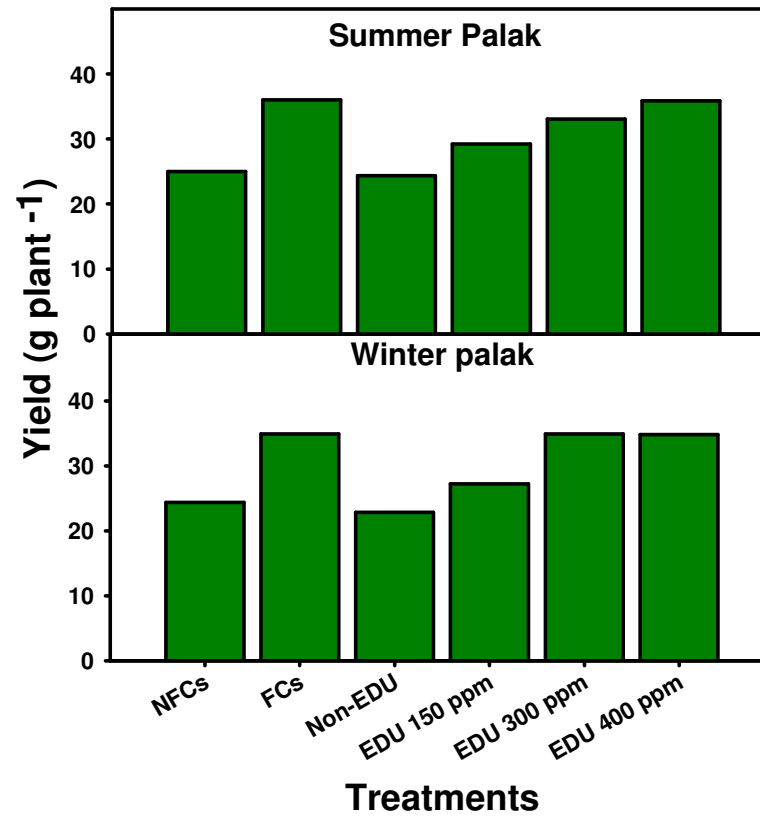
## Relationship between concentrations of EDU and yields of wheat cultivars



**Percent increment in yield (g plant<sup>-1</sup>) of selected crops upon EDU treatment in ambient air**

<b>Site</b>	<b>Percent increment</b>		
	<b>Wheat</b>	<b>Mung</b>	<b>Pea</b>
<b>Reference area</b>	<b>0.2</b>	<b>0.6</b>	<b>0.5</b>
<b>Industrial and urban area</b>	<b>4.2</b>	<b>3.6</b>	<b>4.9</b>
<b>Periurban area</b>	<b>14.2</b>	<b>14.0</b>	<b>18.8</b>
<b>Urban area</b>	<b>6.4</b>	<b>8.9</b>	<b>13.2</b>
<b>Rural area</b>	<b>18.9</b>	<b>19.2</b>	<b>29.8</b>

## Comparison of yield of palak grown in filtered and non-filtered chambers and at different EDU treatments



# Mechanism of action

- **Biochemical or biophysical or both**
- **Detoxification of O<sub>3</sub> in apoplastic region of cells, not working directly as antioxidant**
- **Maintenance of higher levels of cellular antioxidants associated with protection during O<sub>3</sub> stress**

# Conclusions

## **In EDU –treated mung bean plants:**

- **Maintained higher levels of pigments, protein and ascorbic acid contents**
- **Reduction in free radical generation and associated enzyme activities**
- **Enhancement in plant height, leaf area, biomass accumulation and yield**

## **In EDU-treated wheat plants:**

- **Effect on plant growth varied with cultivars, growth stage and concentrations of EDU**
- **Increase in root and shoot lengths, number of tillers plant<sup>-1</sup> and total biomass**
- **Increase in weight of ears plant<sup>-1</sup> and weight and number of seeds plant<sup>-1</sup>**
- **No significant variation in harvest index for cv M533 but significant variation in cv M234 more sensitive to ozone**
- **Higher magnitude of protection to yield as compared to growth parameters (high concentration of O<sub>3</sub> during anthesis period)**
- **Higher magnitude of protection to sensitive as against resistant cultivar**

## **In field studies:**

- **Greater protection in the rural areas having higher ozone levels compared to urban areas having relatively lower averages of ozone**
- **Greater protection during summer as compared to winter season**

# General Conclusions

- **EDU can be successfully used for assessing O<sub>3</sub> induced changes in plants under ambient field conditions**
- **EDU can be used as a tool in biomonitoring programme to map O<sub>3</sub> injury in plants especially in developing countries (major constraints: continuous electricity and non-availability of monitoring equipments)**



THANK YOU