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Reduction of Climate Change Damage Using Chemical Foliar Spray Treated Broad Bean Plants Grown at a Rural Site in Egypt

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LONG-TERM changes in the Earth's climate and temperature patterns are one of the environmental threats that face our planet today. Broad bean plants (*Vicia faba* L. cv. Baladey) were grown at a temperate rural site in Sharkia, Egypt. Plants were grown in 12-open top chambers (OTCs) and 6-plots. Half of OTC's and plots are treated with ethylenediurea (EDU). Ethylene-diurea was used as a antiozonant factor. The air quality treatments consisted of charcoal filtered (CF) air, nonfiltered (NF) air and ambient air (AA). The EDU (300 ppm) used as foliar spray for seed germination and before the growth stages of the plants. Air pollutants monitoring data clearly showed high concentrations especially ozone (O_3) with a mean value ranging between 39 to 56 ppb. Ethylene-diurea treatment caused significant increases in various growth parameters and total biomass accumulation in NF and AA environments. Weight of seeds plant^{-1} was higher by 40 to 50% in NF/300-EDU compared to 0-EDU-treated plants. Seed quality showed improvement for nutritional parameters such as sugars, proteins and K under 300-EDU treatment and thus enhanced the Quality Response Index (QRI) of seeds. This study concluded that 300-EDU caused retention of more biomass in leaves during vegetative period and transported more photosynthates towards reproductive parts, which resulted into yield enhancement. Also, EDU helped in identifying the species susceptibility to climate change stress and therefore is very useful as monitoring tool to assess the impact of ambient air on plants under natural field conditions particularly in areas that have a moderate exposure to O_3.

Keywords: Growth, Biomass, EDU, O_3, Beans, Seed quality index.

Impacts of climate change are well known, more efforts are needed to assess and estimate their socio-economic implications; costing of the impacts will highlight the natural development of climate change as contrasted to a more narrow environmental issue and thus attracts the attention of economists and development planners (IPCC, 2007A). Most adaptation efforts will take place at the local and sub-national levels and more additional efforts are needed to inform domestic audiences (e.g. mayors and local communities) about the impacts of climate change. More efforts are necessary to reduce the impacts of climate change (IPCC, 2007B).

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In spite of getting some benefits as a result of climate change such as extended growing seasons or more moderate temperatures in some areas, the overall effects are likely to be harmful (IPCC, 2007A, B). For example sea-level rise, as a result of climate change, could lead to the loss of many coastal wetlands, and entire island nations could disappear. Changes in the quality and availability of water resources could occur and worsen conflicts over water use and plant production (IPCC, 2007A). Healthy forests could be greatly reduced as the range of tree species shifts. Additionally, humans could suffer from increases in the spread of infectious diseases, heat-related deaths, and air pollution (IPCC, 2007B).

Numerous investigators showed that chronic, whole growth season or whole life cycle exposures to polluted air can result in losses of marketable yield in crops and reductions in growth and productivity of species (McGrath, 2000; Kanoun, *et al.*, 2001 and Ali, *et al.*, 2002). Ali (2003) proved that ambient concentrations of O_3 are only present at levels, which have been reported to have significant detrimental negative effects on commercial yield and biological parameters of great importance at rural sites. on the other hand, beans could be used as indicator for pollutants (Ali, 1993; Guidi *et al.*, 2000; Kanoun, *et al.*, 2001; Madkour and Laurence, 2002).

Chemical antiozonant are used to protect plants against O_3 damage (Kuehler and Flaglar, 1999; Ribas and Penuelas, 2000). N-(-2 (2-oxo-1-imidazolidinyl) ethyl)-N'-phenylurea or ethylenediurea (EDU) is a well known antiozonant against damaging effects of O_3 (Agrawal *et al.*, 2004; Tiwari *et al.*, 2005; Agrawal *et al.*, 2005; Singh and Agrawal, 2009). The O_3 injury and senescence can be retarded by retreating plants with EDU (Rudell *et al.*, 2011). Singh and Agrawal (2009) found increase in biomass and yield of five cultivars of wheat (*Triticum aestivum* L. cv. HUW234, HUW468, HUW510, PBW343 and Sonalika) upon application of 400 ppm EDU under field conditions. In another study, application of 500 ppm EDU increased the test weight of three wheat cultivars between 13.8 to 23.9% at a suburban site (Agrawal *et al.*, 2004). Higher root and shoot biomass was observed in tomato (*Lycopersicon esculentum* L.) when treated with 400 ppm EDU (Singhal et al., 2013). However, Szantoi *et al*. (2009) noticed significant negative effects on root and total biomass, but not on leaf biomass of cut leaf coneflower (*Rudbeckia laciniata* L.) on application of 200, 400 and 600 ppm EDU.

Higher activities of certain scavenger enzymes along with several antioxidants could be the agents that protect plants against O_3 (Liu *et al.*, 2015; Poljšak, and Fink, 2014). Roshchina and Roshchina (2013) reported that catalse and peroxidases can act to regulate injurious oxyradical and peroxyl concentrations in cells to determine equilibrium rates. Superoxide dismutase extracted from EDU-treated and EDU-untreated controls had the same activity as that extracted from EDU-treated plants after fumigation with O_3 and this further the earlier suggestion of Bennett *et al.* (1984) that EDU protection is a biochemical rather than biophysical. EDU prevent the loss of glutathione reductase in ozonated leaves and retained its concentration as high as control plants.

Experiment conducted to examine the use of foliar spray of the anti-ozonant ethylene diurea (EDU) is able to reduce ozone (O_3) damage in bean plants. Growth parameters and total biomass of beans during all growth stages are used as indicators of treatments.

Material and Methods

Study site

The study area performed at the agricultural farm located in the eastern of Egypt at 30^0 $22^{'}$ N latitude, 32^0 $15^{'}$ E longitude at sea level. The experiment carried out between the months of December, 2010 to May, 2011. The soil at the study site was sandy loam in texture (sand 45%, silt 28% and clay 27%) with pH 7. This site surrounded by a 2-m high-screen from all sides to protect the plants in the AA-plots and in the OTC’s against wind damage. Major air pollutants sources are absent in the vicinity of the site. The soil was covered with anti-rooting plastic to suppress weed growth. The weeds were daily controlled by hand removal. The roots that protruded from the plots also removed periodically.

Experimental design

Broad beans (*Vicia faba* L. Baladey) grew in 12-OTCs and 6-plots. The OTCs are designated as in Heagle et al., 1973 with 2-m diameter and 2-m height. The 6-plots are 2-m length and 2-m width. Half of OTCs and plots were treated with EDU. The plant samples grew in a split-plot arrangement. Air quality treatments are CF, NF and AA. Seeds sowed in OTCs and plots at the beginning of December, 2010 using standard agronomic practices. Fifty healthy of equal size broad bean seeds planted in each OTCs, and similarly sowed outdoors under a transparent plastic roof as ambient air treatment (AA). The plants grew in rows 0.6 m apart and spaced 0.1 m apart within the rows. There were eight rows of plants per chamber or plot. Recommended doses of fertilizers N, P and K as urea, single super phosphate and muriate of potash (20:30:30) added during the preparation of the field respectively. Plants thinned to one plant every 15 cm. Plots and OTC's irrigated from time to time to maintain uniform soil moisture.

Application of EDU made as a foliar spray. Three plots and 6- OTCs treated with 300 ppm EDU (300- EDU) received 100 ml plant^{-1} and other nine control plots and OTCs (0-EDU) received 100 ml plant^{-1} distilled water. EDU solution was freshly prepared for each time using distilled water. Applications were done four times at seed germination and before all growth stages (vegetative, flowering, pod formation) between 9:00-10:00 hr. The leaf samples collected from all treatments a week after each EDU applications. One more leaf samples collection was done at the time of harvesting. A sample consisted of terminal leaflets.

Air analysis

Seven hour O_3, NO_2, CO and SO_2 concentrations monitored and analyzed, using AQM-version 60 ambient air analyzer (AEROQUAL, Air quality Monitor, New Zealand) during the entire experimental period.

Plant analysis

The recorded growth parameters were done for root and shoot lengths, number of nodules, number of leaves and leaf area. For biomass determination, plant parts separated and oven dried at 80°C to constant weight. Dry weights of plant parts were taken. Different growth indices such as Net assimilation rate (NAR), Leaf area ratio (LAR), Specific leaf area (SLA) calculated by formulae modified by Tessmer *et al*. (2013) which were given as under.

$$\text{Net Assimilation Rate } (g/cm^2/d) = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{\ln L_{A2} - \ln L_{A1}}{L_{A2} - L_{A1}}$$

$$\text{Leaf area ratio } (cm^2/g) = \frac{L_{A2} - L_{A1}}{W_2 - W_1} \times \frac{\ln W_2 - \ln W_1}{\ln_{A2} - \ln_{A1}}$$

$$\text{Specific leaf area } (cm^2/g) = \frac{L_A}{L_W}$$

$$\text{Specific leaf weight } (g/cm^2) = \frac{L_W}{L_A}$$

where: W_1 and W_2 are lowest and highest dry weight at time T_1 and T_2; L_A is leaf area; L_W is leaf weight; A_1 and A_2 are lowest and highest leaf area.

Seed morphology

Final harvest was done at late pod-fill and different yield parameters were assessed. Ten plants were sampled from each replicate. Number and weight of plant pods, number of plant seeds pod^{-1}, number and weight of plant seeds plant^{-1}, weight of above ground plant parts and weight of 100 seed (test weight) recorded. Harvest index (HI) was calculated as the ratio of weight of seeds plant $^{-1}$ and total above ground biomass of the plant (Yang and Zhang, 2010).

Seed composition

Oven dried seed samples were ground in a stainless steel grinder and passed through a 2.0 mm sieve. The powdered samples used for analysis of sodium, phosphorous, potassium, calcium, protein, reducing sugar and non-reducing sugar Total Na, P, Ca and K contents determined by digestion of powdered seed samples using the method given by Gibson (2014). K, Ca, Na content in digested material determined with the help of Atomic Absorption Spectrophotometer (Model 2380, Perkin-Elmer, USA). Total P in the filtrate estimated using the method from Conklin (2014). Protein content is measured by the method of Jagow (2013). Reducing sugar and total sugar content are determined by using the methods of Somogyi-Nelson (Chandraju *et al.*, 2014) and Rogerio-Candelera (2014), respectively. Non-reducing sugar calculated by subtracting amount of total sugars from reducing ones. The seed quality index (SQ_I) used to evaluate the best minimum data set expressed of seed quality response against air pollutants vs EDU stress.

Statistical analysis

Treatment means compared statistically by using the statistical package SPSS software (SPSS Inc., Version 16.0). All the growth and biomass data were analyzed by three-way ANOVA to test the individual and interactive effects of growth stages, EDU and air quality treatments. Yield parameters analyzed by two-way ANOVA to test the individual and interactive effect of treatment and growth stages.

Result and Discussions

Climate variables

The minimum monthly temperature varied from 9 to 22 ^{o}C and maximum temperature from 17 to 32 ^{o}C and was highest in May. Total rainfall during the study period was 599.6 mm, with maximum recorded in January being 225.8 mm. Maximum monthly relative humidity ranged from 51 to 58%. Wind speed was highest during April and May, 2011(Figure 1a, 1b, 1c, 1d).

Air monitoring data reveals that there was a constant increase in O_3 concentration during the study period *i.e.* from December, 2010 to May, 2011. Ozone concentration ranged from 39 to 56 ppb and was starting higher during the reproductive phase of plant life (March to May) (Fig. 2). Maximum monthly SO_2 concentrations ranged from 10 to 16 ppb. Levels of NO_2 were started highest during April and May, 2011(Fig. 2). The mean monthly CO concentrations varied from 15 to 25 ppb.

Countries like Egypt have suitable climatic conditions favoring the formation of ground level O_3 (Tiwari *et al.*, 2006). Warm climatic condition, high light intensity, high temperature and long sunshine hours provided conductive conditions for high concentrations of O_3 during the study period. Rai and Agrawal (2008) reported an increasing trend of ambient O_3 in warm conditions ranged between 30.5 to 45.4 ppb at a rural site of Varanasi. Also, average mean O_3 concentration varied between 24.1 to 43.8 ppb in rainy season from 2002 to 2006 in suburban areas of Varanasi (Tiwari *et al.*, 2008). Significant positive correlations between mean maximum temperature and O_3 concentrations were also shown (Tiwari *et al.*, 2008). Wahid *et al.* (2001) reported a mean O_3 concentration of 48 ppb at a rural roadside area of Pakistan during August-October, 1996. Beig *et al.* (2007) also reported lower O_3 concentrations (17.5 to 25 ppb) in rainy season as compared to summer and winter seasons during 2003-2004 in a semi-urban site of Pune, India. High mean O_3 concentrations varying from 39 to 49 ppb during July to October, 2002 were reported from an urban site of New Delhi (Jain *et al.*, 2005).

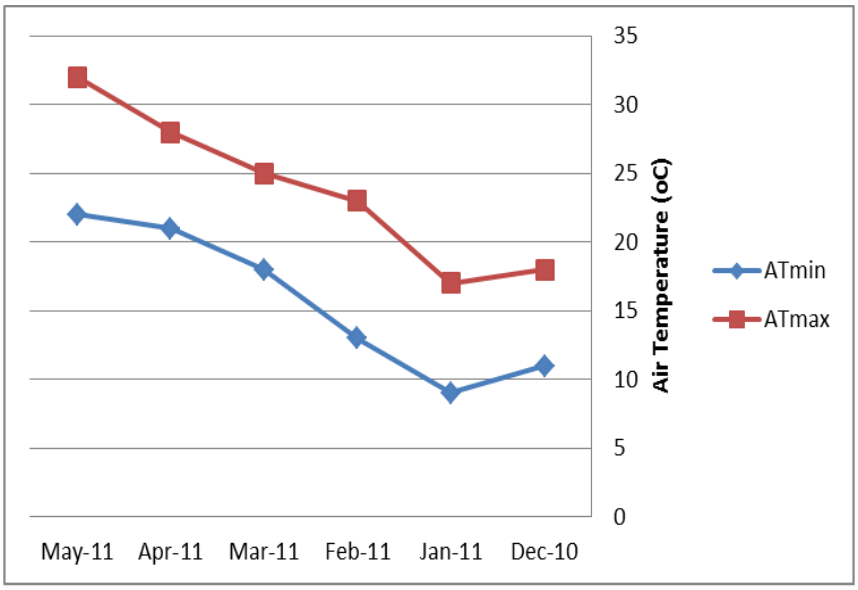

Fig. 1a. Mean values for monthly average air temperature of a rural site, Egypt during 2010-2011.

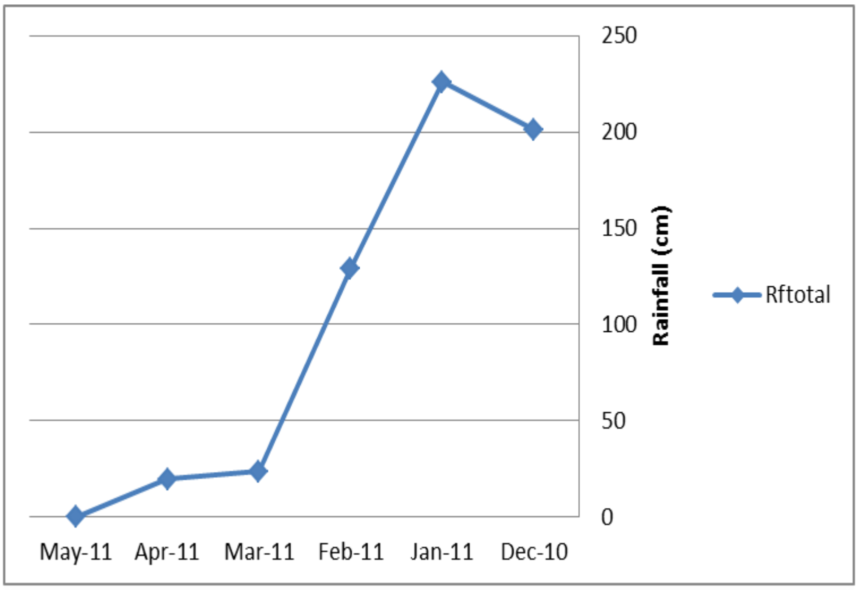

Fig. 1b. Mean values for monthly average total rainfall of a rural site, Egypt during 2010-2011.

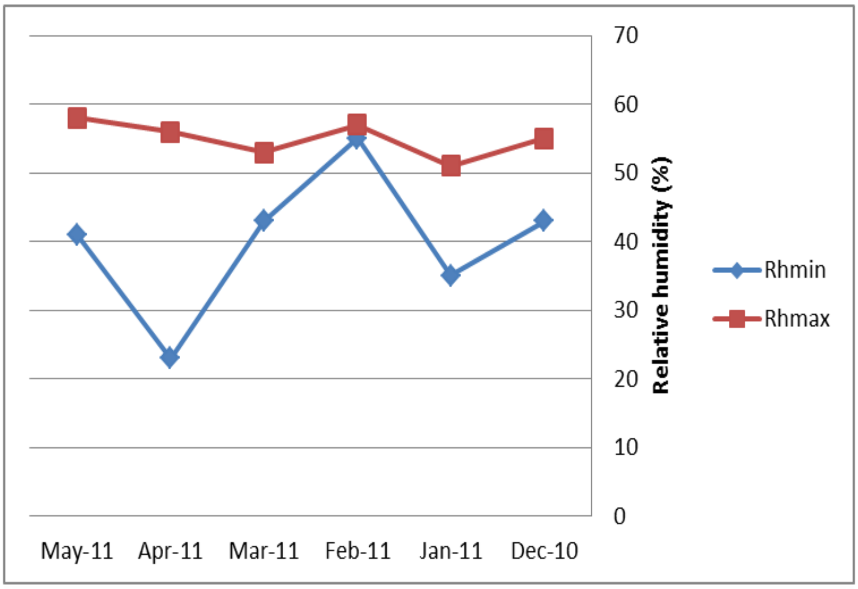

Fig. 1c. Mean values for monthly average relative humidity of a rural site, Egypt during 2010-2011.

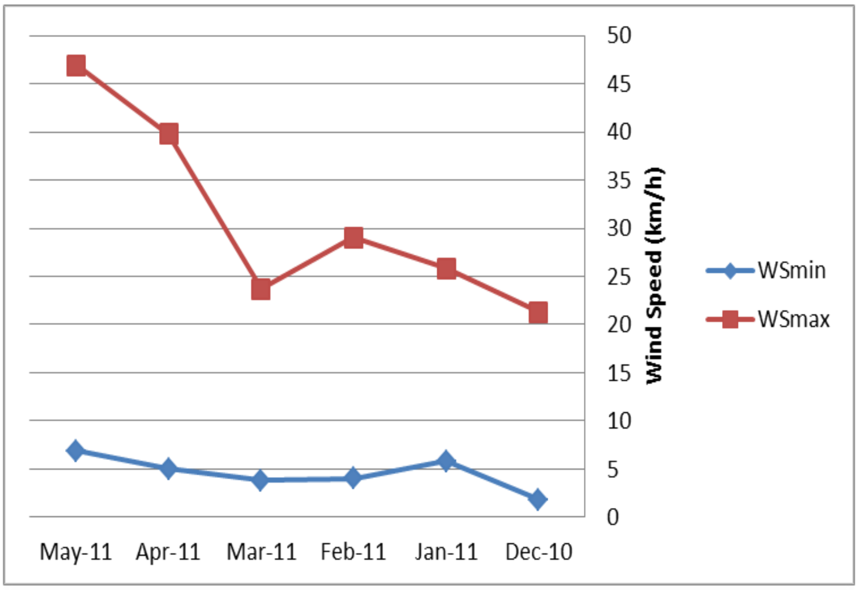

Fig. 1d. Mean values for monthly average wind speed of a rural site, Egypt during 2010-2011.

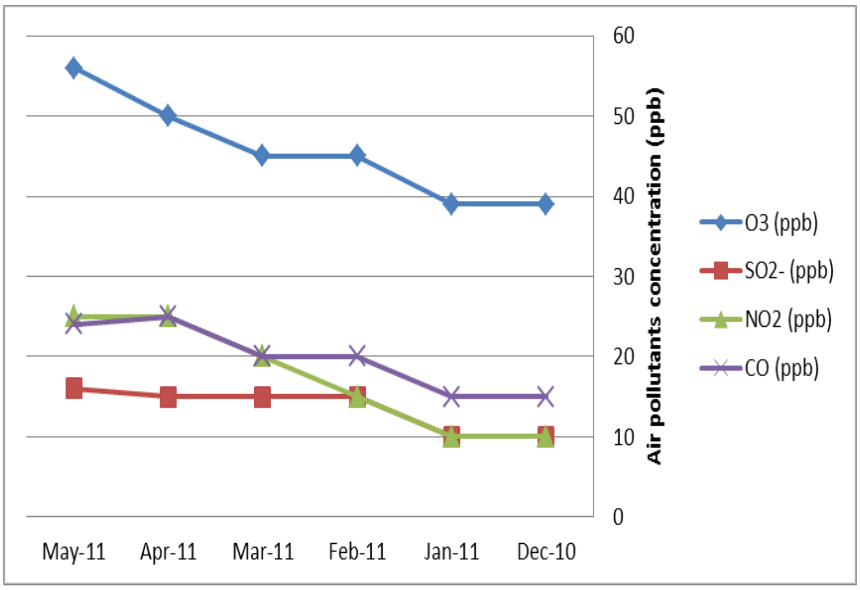

Fig. 2. Mean values for air pollutants (O_3, SO_2, NO_2 and CO) of a rural site, Egypt during 2010-2011.

Growth responses

Treatment EDU could positively changed the appearance of bean plants. Significant increments were observed in shoot and root lengths of EDU-treated plants of NF and AA but more in shoot length (Table 1). The increments in shoot length were 98.3 and 93.3 cm in NF/300-EDU and AA/300-EDU, respectively. Number of leaves increased significantly in both NF/300-EDU and AA/300-EDU, as compared to control ones CF/0-EDU and CF/300-EDU (Table 1). Number of leaves was higher by 9.67 and 12.04% in EDU-treated NF and AA, respectively as compared to non-treated ones.

Effective increase of bean biomass to EDU foliar spray recorded. Root, stem and leaf biomass significantly increased in beans treated with EDU. Root, stem and leaf biomass higher by 6.52, 3.18 and 3.86% in NF/300-EDU, respectively as compared to their respective controls at NF/0-EDU. Highly significant variations noticed due to all the factors for LAR, NAR, SLA and SLW (Table 1). Significant increase in shoot biomass observed in EDU-treated beans as compared to non-EDU-treated ones (Table 1). Root biomass significantly reduced only in NF/300-EDU as compared to CF/300-EDU. Significant increments also observed in leaf biomass of EDU-treated AA (35.16%) as compared to non-EDU-treated plants at AA plots. Total biomass increased significantly in NF and AA at all sampling upon 300-EDU treatment as compared to controls. Significant variations in root and shoot biomass observed due to all the factors and their interaction, however for leaf and total biomass it varied significantly due to all the factors except treatment

(Table 1). Increased biomass allocation took place towards shoot and leaf portions of EDU-treated plants as compared to root. However, in all the three air treatments, major biomass accumulated in leaf portion in control as well as in EDU treated plants (Table 1).

TABLE 1. Responses of broad beans growth parameters to ambient air ± EDU during complete pod-fill at a rural site in Egypt.

Treatments/ Growth parameters	CF/ 0-EDU	CF/ 300-EDU	NF/ 0-EDU	NF/ 300-EDU	AA/ 0-EDU	AA/ 300-EDU	LSD $P<0.05$
Shoot length (cm)	106.3±0.3	105.3±0.2	88.3±0.6	98.3±0.2	85.3±0.2	93.3±0.2	8.2
Root length (cm)	6.3±0.4	8.8±0.2	4.4±0.5	5.3±0.2	4.3±0.5	5.1±0.2	3.8
Leaves no.	36.3±0.5	36.8±0.1	28.3±0.5	31.3±0.1	26.3±0.5	29.9±0.2	2.2
Leaf area (cm^2)	9.3±0.6	11.3±0.4	6.6±0.4	8.8±0.3	5.9±0.4	9.1±0.2	3.5
Root biomass (g)	13.3±0.2	13.3±0.3	11.5±0.5	12.3±0.4	11.1±0.3	12.1±0.5	2.2
Stem biomass (g)	96.6±0.4	97.3±0.5	91.3±0.5	94.3±0.5	89.3±0.3	90.3±0.5	4.4
Leaf biomass (g)	26.3±0.4	28.1±0.4	22.4±0.5	23.3±0.6	21.3±0.3	23.5±0.6	1.6
Total biomass (g)	136.2±1.0	138.7±1.2	125.2±1.5	129.9±1.5	121.7±0.9	125.9±1.6	4.4
SLA (cm^2/g)	6.9±0.7	6.9±0.5	5.5±0.4	6.3±0.4	6.1±0.4	6.3±0.3	1.1
SLW (g/cm^2)	8.8±0.5	8.9±0.2	6.3±0.2	6.7±0.5	6.1±0.3	6.5±0.5	3.5
NAR (g/cm^2/d)	36.3±0.8	37.3±0.3	21.2±0.2	23.5±0.2	19.3±0.8	21.3±0.4	3.2
LAR (cm^2/g)	56.3±0.8	57.3±0.3	51.2±0.2	58.5±0.2	55.3±0.8	56.3±0.4	3.2

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, NAR = Net assimilation rate, LAR = Leaf assimilation rate, SLA = Specific leaf area, SLW = Specific leaf weight.

Wahid *et al.* (2001) reported an increment of 20.7% in shoot length of EDU-treated (400 ppm) soybean (*Glycine max* L.) grown at a roadside rural area have mean O_3 concentration of 48 ppb. Significant increment of 19.7% in shoot length of palak (*Beta vulgaris* L.). All green plants varied in response to 300 ppm EDU at O_3 concentrations from 52 to 73 ppb at a suburban site of Varanasi (Tiwari and Agrawal, 2009). Increment of 22.8% in shoot length of mungbean at 50 DAG recorded upon 400 ppm EDU treatment in an area experiencing O_3 concentration between 52.9 to 64.5 ppb (Singh *et al.*, 2010). Number of leaves and leaf area showed increments in Barkha and Shekhar treated with EDU. Induction of these parameters showed that EDU helped in cell proliferation as it consists of phenylurea as an important component of its structure. Earlier studies also suggested cytokinin like activities of EDU, such as prevention of chlorophyll degradation, protein and RNA synthesis, cell proliferation, etc. (Whitacre, 2014). Singh *et al.* (2010) reported significant increase in leaf area of EDU-treated mungbean at elevated O_3 concentration at both the ages of sampling. Wahid *et al.* (2001) found an increment of 44% in number of leaves of EDU-treated soybean plants grown in a rural area of Pakistan. Agrawal *et al.* (2005) found an increase of 21.9% in number of leaves in *Vigna radiata* due to application of 500 ppm EDU as soil drench at 60 DAG. Study conducted under ambient O_3 concentration in field conditions showed increments of 28 and 27.6% in number of leaves and leaf area, respectively of *B. vulgaris* plants upon EDU treatment (Tiwari and Agrawal, 2009). The present study also showed that EDU is helpful in increasing the nodulation in roots of test plants. Agrawal *et al.* (2005) also noticed an increment of 71.4% in root nodules of mungbean after EDU treatment. However, exposure of O_3 was found to decrease nodulation in soybean plants (Gillespie *et al.*, 2011).

The protective influence of EDU was further supported by enhancement in biomass accumulation of EDU-treated plants over non-EDU-treated ones. Significant increments reported in root, shoot and total dry weight of turnip and radish plants after EDU treatment of 500 ppm in an area having high mean O_3 concentration (66.9 ppb) (Hassan *et al.*, 2013). In mungbean plants, root, shoot and total dry weight increased significantly after EDU application (Agrawal *et al.*, 2005). Recently, Singh and Agrawal (2009) noticed increments in root, shoot, leaves and total dry weight of five wheat cultivars treated with 400 ppm EDU in a suburban area having mean O_3 concentration ranging between 35.3 to 54.2 ppb.

Present study clearly shows that application of EDU higher biomass retained in leaves and shifted more towards reproductive parts under O_3 stress with simultaneous decline in root and stem biomass. Fiscus *et al.* (2012) also reported modification in biomass partitioning due to EDU treatment (relative biomass increased in leaves and decreased in pods). Net carbon exchange rate (NCER) and carbohydrate status of the tissues reflected the protective effects of EDU against O_3 (Fiscus *et al.*, 2012). Szantoi *et al.* (2009) reported significant decline in root and total biomass with increasing EDU levels on exposure to elevated O_3 as compared to non-EDU treated ones.

The ANOVA test showed complete significant variations in shoot and root biomass for all factors. Highly significant variations in shoot and root lengths for all the factors except treatment for shoot and root length to growth stages (GS) × air treatment (AT) × EDU (Table 2). Some significant variations exhibited in leaves number and leaf area in relation to all factors.

In general, specific leaf area (SLA) decreased in EDU-treated beans at both NF and AA which increased significantly at 300 EDU applications (Table 1). Only significant decrease in SLA noticed in NF/0-EDU as compared to CF's (20.29%). Also, a similar trend was observed for specific leaf weight (SLW). Net assimilation rate (NAR) insignificantly increased in EDU-treated plants of *Vicia faba* L. cv. Baladey during both the sampling of NF and AA in comparing to non-EDU, whereas a significant decrement was noticed in EDU-treated plants of NF and AA as compared to CF controls by 41.60 and 46.83 %, respectively. Significant increase of leaf area ratio (LAR) in during treated broad beans with EDU being 12.48%, however, insignificant increase observed during EDU treated plants of AA plots (Table 1).

Growth indices reflect the photoassimilate partitioning in plants. In this study, SLA and SLW showed an opposite trend of response under EDU treatment. Increase in SLW denotes more leaf biomass accumulation due to leaf thickness, which is an adaptation to reduce influx of O_3 to leaf interior. NAR represents the photosynthetic efficiency of a plant showing the dry matter accumulation per unit leaf area per unit time.

TABLE 2. Levels of significance for various growth parameters of broad beans treated with ambient air and EDU as obtained by three way ANOVA test.

Growth parameters	Growth stages (GS)	Air treatments (AT)	EDU	GS/AT	AT/EDU	GS/EDU	GS/AT/EDU
Shoot length (cm)	***	*	**	*	***	*	NS
Root length (cm)	*	**	*	*	*	***	NS
Leaves no.	***	NS	***	NS	***	NS	***
Leaf area (cm^2)	NS	NS	NS	*	NS	***	NS
Root biomass (g)	**	***	***	***	***	*	**
Stem biomass (g)	***	*	**	*	*	***	**
Leaf biomass (g)	**	**	*	***	***	*	**
NAR ($g/cm^2/d$)	**	NS	***	NS	*	***	*
LAR (cm^2/g)	NS	***	**	***	***	NS	***
SLA (cm^2/g)	NS	**	NS	**	NS	*	NS
SLW (g/cm^2)	NS	***	NS	***	NS	***	*

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, Level of significance: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant, NAR = Net assimilation rate, LAR = Leaf assimilation rate, SLA = Specific leaf area, SLW = Specific leaf weight.

Higher NAR values in EDU-treated plants suggests higher photosynthetic fixation in the foliage. However, LAR did not vary significantly between EDU-treated and non-treated plants. Agrawal *et al.* (2005) reported higher value of NAR in EDU-treated mungbean as compared to control ones. Increase in NAR was also found in EDU-treated wheat cultivars (Singh and Agrawal, 2009). However, Singh *et al.* (2010) did not find any significant effect of EDU treatment on SLA and SLW of mungbean plants.

Three way ANOVA test showed that NAR and SLW varied significantly due to all the individual factors and interactions of GS × EDU, AT × GS and AT × GS × EDU (Table 2). LAR significantly affected by growth stages, air treatments, EDU. Significant effect was observed in SLA due to growth stages, air treatments and interaction of AT × GS, AT × EDU and AT × GS × EDU (Table 2). Significant variations recorded in LAR, NAR, SLA and SLW for air treatments, growth stage x air treatments, growth stage x EDU and GS × AT × EDU factors.

Seed responses

Ethylene diurea application positively modified the yield parameters of bean plants. Weight of pods plant^{-1} and weight of seeds plant^{-1}, increased significantly in beans treated with EDU as compared to their respective controls (Fig. 3). However, number of seeds pod^{-1} and number of seeds plant^{-1} did not vary significantly in any of the test NF and AA (Table 3). Significant decrement observed in number of pods plant in non-EDU treatments. Weight of seeds plant^{-1} increased by 34.05 and 32.37% in NF and AA, respectively when treated with EDU as compared to respective non-EDU-treated ones. Harvest index increased significantly by 20.69, 22.64 and 43.64% in EDU treated CF, NF and AA, respectively as compared to their control counterparts.

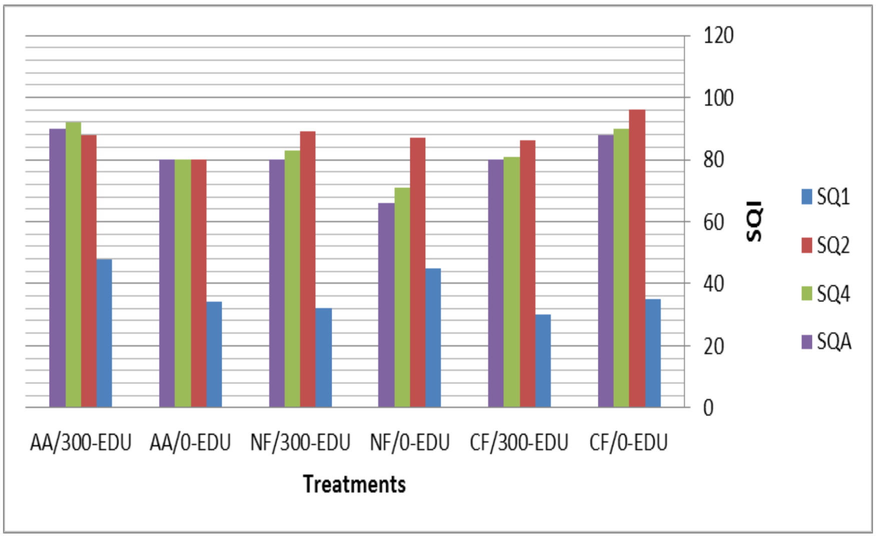

Fig. 3. Calculated seed quality index (SQ_I) of broad beans treated with ambient air and EDU based on minimum data set of seed content.
CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea. SQ1 = Seed quality index based on seed protein content; SQ2 = Seed quality index based on seed sugars; SQ4 = Seed quality index based on elemental composition; SQA = Seed quality index based on all seed composition.

The results of this experiment clearly demonstrated the negative effects of ambient O_3 on the yield of broad beans and application of EDU modified various yield parameters. Wahid *et al.* (2001) found insignificant increments in number of pods plant, 1000 seed weight (test weight), number of seeds pod^{-1} and seed weight plant in EDU-treated soybean plants. Hassan (2006) also found increments of 40 and 47% in tuber weight and 43 and 42% in number of tubers at two sites of Egypt having mean O_3 concentration of 81 and 98 ppb, respectively after treatment with 300 ppm EDU. In an experiment conducted on two bean cultivars S156 (O_3-sensitive) and R123 (O_3-tolerant) at 300 ppm EDU, above ground biomass, pod and seed weight increased significantly in S156 whereas a reverse trend was observed for R123 (Elagoz and Manning, 2002). Singh *et al.* (2010) reported significant increment of 32.8% in weight of seeds plant in 400 ppm EDU-treated mungbean plants as compared to non-EDU-treated ones. Yield losses in cereals due to O_3 may be ascribed to reductions in photosynthate allocation to grains (Jackson and Black, 2013), leading to reduction.

TABLE 3. Responses of broad beans yield parameters to ambient air ± EDU during complete pod-fill at a rural site in Egypt.

Treatments/ Yield parameters	CF/ 0-EDU	CF/ 300-EDU	NF/ 0-EDU	NF/ 300-EDU	AA/ 0-EDU	AA/ 300-EDU	LSD P<0.05
Pod no./plant	6.7±0.2	6.8±0.4	5.7±0.3	5.5±0.8	5.1±0.6	5.6±0.2	1.2
Seed no./pod	8.8±0.3	8.9±0.5	6.5±0.5	7.3±0.4	6.1±0.3	7.1±0.2	2.2
Seed no./plant	77.7±0.3	78.7±0.5	65.7±0.3	74.7±0.5	66.3±0.6	70.6±0.5	8.1
Pod wt./plant (g)	21.3±0.4	22.2±0.5	18.9±0.5	23.9±0.6	19.3±0.3	23.5±0.3	2.1
Seed wt./plant (g)	19.9±0.3	20.3±0.5	12.2±0.3	18.5±0.2	11.7±0.6	17.3±0.4	3.1
Pod length (cm)	8.8±0.8	7.3±0.5	6.2±0.2	8.5±0.2	5.9±0.4	6.9±0.7	1.5
Harvest index	4.6±0.3	5.8±0.5	4.1±0.3	5.3±0.4	3.1±0.4	5.5±0.3	1.2

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea,

Statistical analysis showed significant variations in weight of seeds plant, weight of pod plant^{-1} and pod length due to air treatments, EDU singly and interaction of both (Table 4). Significant effect of EDU and AT × GS's observed on number of pods plant and above ground biomass, on harvest index due to treatment and growth stages and on number of seeds plant^{-1} due to EDU. However, insignificant variation on number of seeds pod^{-1} was recorded (Table 4).

TABLE 4. Levels of significance for various yield parameters of broad beans treated with ambient air and EDU as obtained by three way ANOVA test.

Yield parameters	Growth stages (GS)	Air treatments (AT)	EDU	GS/AT	AT/EDU	GS/EDU	GS/AT/EDU
Pod no./plant	**	***	***	*	*	**	*
Seed no./pod	NS	NS	NS	NS	NS	NS	NS
Seed no./plant	***	***	***	**	**	**	*
Pod wt./plant (g)	NS	***	*	***	***	NS	***
Seed wt./plant (g)	***	NS	NS	NS	NS	NS	NS
Pod length (cm)	**	***	***	*	*	**	*
Harvest index	***	**	***	**	**	**	**

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, Level of significance: * = p < 0.05; ** = p < 0.01; *** = p < 0.001; ns = not significant.

Seed quality index

Minimum data set seed quality properties is used for calculation of seed quality index (SQ_I). These properties include reducing and non-reducing sugars, total protein, Na, K, Ca and P. Based on one or more properties, SQ_I was determined. All the test bean plants showed a significant decrease in reducing sugar content in seeds (p<0.01) of plants treated with EDU (Table 5). Non-sugar content decreased significantly in all treated with EDU (p<0.001) CF, NF and

AA. Significant change in protein content observed only in AA, which showed significant reduction ($p<0.01$) in EDU-treated plants (18.02). Na and P insignificantly decreased whereas K and Ca increased significantly in all the EDU-treated plants (Table 5).

TABLE 5. Responses of broad beans seed quality to ambient air ± EDU during complete pod-fill at a rural site in Egypt.

Treatments/ Seed content	**CF/ 0-EDU**	**CF/ 300-EDU**	**NF/ 0-EDU**	**NF/ 300-EDU**	**AA/ 0-EDU**	**AA/ 300-EDU**	**LSD P<0.05**
Reducing Sugar (mg g^{-1})	22.3±0.0	16.5±0.3	18.5±0.0	13.5±0.4	16.7±0.5	11.8±0.5	5.3
Non-reducing sugar mg g^{-1}	116.3±0.2	112.4±0.1	100.3±0.1	96.6±0.1	99.8±0.4	86.8±0.1	6.4
Protein (mg g^{-1})	86.3±0.2	67.6±0.2	56.7±0.4	51.5±0.4	56.6±0.1	46.4±0.1	7.2
Sodium (mg g^{-1})	6.3±0.1	6.7±0.1	4.8±0.2	3.6±0.1	4.5±0.1	3.5±0.5	1.4
Phosphate(mg g^{-1})	0.33±0.0	0.29±0.1	0.24±0.1	0.23±0.0	0.26±0.4	0.26±0.2	3.5
Potassium (mg g^{-1})	6.09±0.0	6.61±0.0	5.8±0.2	8.4±0.1	6.3±0.2	9.8±0.22	3.0
Calcium (mg g^{-1})	4.11±0.0	4.71±0.3	3.41±0.1	8.11±0.2	3.81±0.0	9.14±0.6	2.1

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea.

The lower carbohydrate levels were a result of reductions in photosynthesis rates caused by chronic O_3 exposures, the utilization of photosynthate in repair processes for cellular components damaged by the toxic products of O_3, and enhanced aging of leaves due to O_3 exposures (Campbell *et al.*, 2007; Singh *et al.*, 2015). Chronic exposure to O_3 affects photosynthesis processes in the following ways: damage to cellular proteins in membranes which cause leakage of ions and fluids that result in reduced stomatal conductance; damage to enzymes including rubisco; reductions in chlorphyll contents and leaf area expansion during canopy development, thereby reducing the canopy photosynthetic capacity (Momen *et al.*, 2013).

The general patterns in leaf carbohydrates in the plants during podfill in response to air quality treatments typically paralleled those for leaf photosynthesis and grain yields (Momen *et al.*, 2013). The stimulation of leaf carbohydrate and pigment contents by the EDU treatment, compared to the CF and NF + non-EDU or AA + non-EDU treatments, provide a rational explanation for the counteracting effects commonly reported for grain yields in C_3 plants in response to elevated atmospheric O_3 in combination with moderate exposures to EDU levels. The number of pods (Ali, 2003) and sink capacity (Campbell *et al.*, 2007) established during pod set are likely closely linked to photosynthate levels in the plants. Likewise, seeds per plant and seed wt. 100^{-1}, the primary components of grain yield per plant, all parallel the leaf carbohydrate results for the air quality treatments (Momen *et al.*, 2013; Campbell *et al.*, 2007). However, considering that the carbohydrate levels in the EDU treatment were consistently lower than that found in the CF treatment, these results confirm suggestions that exposure to chronic high O_3 (*i.e.*, 80 ± 5 nl O_3 l^{-1}), even in the presence of levels of EDU, will likely limit C_3 plants from attaining their maximum potential benefits regarding yields (Momen *et al.*, 2013; Ainsworth *et al.*, 2012). As a consequence, efforts to limit or reduce atmospheric O_3 concentrations as EDU levels raise in the future should be maintained and strengthened, especially in developing countries, in order to promote high levels of productivity in C_3 crops to feed an expanding world population.

Agrawal *et al.* (2005) and Singh *et al.* (2010) reported an increment of 14.0 and 20.71% at 400 and 500 ppm EDU, respectively in mungbean. Contents of amino acids, total sugar, starch and K increased whereas protein, reducing sugar, N and P decreased in seeds of plants treated with EDU as compared to non-EDU-treated plants. These changes reflect the indirect effects of ambient O_3 levels on overall metabolism of plant leading to modifications in seed quality. A negative correlation between cereal grain yield (GY) and grain protein concentration (GPC) has been previously reported (Hancock, 2012). This phenomenon is called 'growth dilution' which occurs due to accumulation of more non-nitrogenous compounds in the seeds. A similar correlation of GY and GPC was reported by Wanga *et al.* (2012) for soybean and Pleijel *et al.* (1999) for wheat.

In the present study, EDU induced the accumulation of non-nitrogenous compounds *i.e.* total sugar and starch. EDU treatment helped the plants to undergo better growth through overcoming the oxidative stress, thus investing more in carbohydrate production. De Temmerman *et al.* (2007) also recorded 9% reduction in the sugar contents of sugar beet at 60 ppb O_3 exposed. Significant decrease in reducing sugar content was noticed under present investigation in all the cultivars under EDU treatment. Pell and Pearson (1984) reported an increase in reducing sugar content of potato under acute exposure of 200 ppb O_3 for 3hr at fortnightly intervals over 3 months. Increase in amino acid content in seeds of Shekhar correlates well with the decrease in protein content of these plants. Increase in concentration of N and amino acids for lima beans under O_3 exposure was reported (Cui *et al.*, 2014).

Calculation of seed quality index (SQ_I) is to understand the impact of ambient O_3 on major nutritional parameters of seeds showed large variations between the test beans (Fig. 3). The percentage of each indicator parameter was different, but their added values reflect the final sensitivity index. The lower value of SQ_I reflects its resistance towards O_3 whereas the higher values reflect their sensitiveness towards O_3. The SQ_I in this study which used as an integration of the effect of O_3 and EDU on sugar, protein and metal contents may reflect the sensitivity of seed quality against O_3 stress. The SQ_1 showed higher response in AA/300-EDU, while SQ_2 showed lower response in AA/0-EDU. However, SQ_4 and SQ_A showed higher response in AA/300-EDU only (Fig. 3). The AA/300-EDU with higher positive value of SQ_I was more sensitive than other treatments. Zu *et al.* (2004) also calculated the SQ_I value for 10 different cultivars of wheat to assess the sensitivity of grain quality of wheat to enhanced UV-B radiation.

Conclusions

Climate change injury and senescence can be retarded by retreating plants with chemical antiozonant. The results obtained during the present study showed that ambient O_3 concentrations were high enough to cause significant reductions in crop yield of broad bean plants. The EDU in foliar spray application can modify the biomass allocation pattern and thus helped the plants to overcome the effect of O_3 in terms of quantity as well as quality of the seeds. In this study, harvest index (HI) increased significantly with EDU. Increase in HI of EDU-treated plants reflects that EDU helped the plants to overcome the oxidative stress caused by O_3 thus partitioning more proportion of photosynthates towards the reproductive parts. In some cases, no impact of EDU treatment with respect to various growth and yield parameters hence denotes its highest resistance against O_3 levels.

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الحد من أضرار التغير المناخي باستخدام الرش الكيميائي لأوراق نبات الفول النامية بموقع ريفي في مصر

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تعتبر التغييرات المناخية أحد الأنماط الحرارية التي تهدد كوكب الأرض على المدى الطويل. ولدراسة هذا الموضوع تم زرعة نباتات الفول في موقع ريفي في محافظة الشرقية، بمصر لتقييم استجابته لنوعيات الهواء المختلفة ومدى فاعلية الرش بمادة الإثيلين ثنائي اليوريا (EDU) على خفض ذلك التأثير الضار للتغير المناخي. وقد زرعت النباتات في ١٢ صوبة بلاستيكية مفتوحة من الأعلى. واشتملت نوعية معاملات الهواء على كل من: عدد ستة معاملات الفحم المرشح للهواء (CF) ، وعدد ستة معاملات هواء غير مرشح (NF) و عدد ستة قطع معرضة للهواء الطبيعي (AA) مباشرة. وتم تقسيم جميع المعاملات نصفين. بحيث يكون هناك ثلاث معاملات بمادة الإثيلين ثنائي اليوريا و ثلاث معاملات بدونها. وقد تم إضافة مادة الإثيلين ثنائي اليوريا (٣٠٠ جزء في المليون) كرش ورقي عند مراحل: انبات البذور (ظهور الاوراق الفلقية) وقبل مراحل نمو النبات الثلاث الخضري والازهار والاثمار. أظهرت بيانات رصد ملوثات الهواء بشكل واضح أن التركيزات العالية خصوصا لغاز الأوزون تراوحت قيمتها المتوسطة بين ٣٩-٥٦ جزء في البليون. تسببت المعالجة بمادة الإثيلين ثنائي اليوريا زيادات كبيرة في مقاييس النمو المختلفة وتراكم الكتلة الحيوية الكلية في بيئات NF وAA. وأيضاً كان وزن البذور لكل نبات أعلى بنسبة تتراوح ٤٠ إلى ٥٠٪ في معاملة EDU / NF مقارنة مع النباتات التي لم تعامل بمادة EDU. وأظهرت جودة محتوى البذور تحسين في المحتوى الغذائي مثل السكريات والبروتينات والبوتاسيوم تحت تأثير المعاملة بمادة الإثيلين ثنائي اليوريا مما عزز مؤشر استجابة جودة البذور (QRI). وخلصت هذه الدراسة إلى أن مادة الإثيلين ثنائي اليوريا تسبب المزيد من الحماية للكتلة الحيوية في الأوراق خلال فترة النمو الخضري ونقلها بفاعلية أكثر نحو الأجزاء التكاثرية، مما يؤدى إلى تعزيز الانتاجية في محصول نبات الفول. أيضا، ساعدت مادة الإثيلين ثنائي اليوريا في تحديد قابلية الأنواع للإجهاد الناتج عن التغير المناخي ، وبالتالي من المفيد جدا عن رصد وتقييم تأثير الهواء الملوث على النباتات (في الظروف الميدانية الطبيعية خاصة) أن تجرى الدراسة في المناطق ذات الظروف المناخية المعتدلة والتي لا ترتفع فيها تركيزات غاز الأوزون عن المعدلات الطبيعية بشكل هائل.