Exogenously-sourced ethylene increases stomatal conductance, photosynthesis, and growth under optimal and deficient nitrogen fertilization in mustard

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Received 9 August 2010; Revised 10 May 2011; Accepted 23 May 2011

Abstract

In order to ascertain the stomatal and photosynthetic responses of mustard to ethylene under varying N availability, photosynthetic characteristics of mustard grown with optimal (80 mg N kg\(^{-1}\) soil) or low (40 mg N kg\(^{-1}\) soil) N were studied after the application of an ethylene-releasing compound, ethephon (2-chloroethyl phosphonic acid) at 40 days after sowing (DAS). The availability of N influenced ethylene evolution and affected stomatal conductance and photosynthesis. The effect of ethylene was smaller under deficient N where plants contained higher glucose (Glc) sensitivity, despite high ethylene evolution even in the absence of ethephon, potentially because the plants were less sensitive to ethylene per se. Ethephon application at each level of N increased ethylene and decreased Glc sensitivity, which increased photosynthesis via its effect on the photosynthetic machinery and effects on stomatal conductance. Plants grown with sufficient-N and treated with 200 μl l\(^{-1}\) ethephon exhibited optimal ethylene, the greatest stomatal conductance and photosynthesis, and growth. These plants made maximum use of available N and exhibited the highest nitrogen-use efficiency (NUE).

Key words: Ethephon, ethylene, glucose, nitrogen-use efficiency, photosynthesis.

Introduction

Photosynthesis is the main driving force for carbon gain and crop productivity, which is controlled by several intrinsic and extrinsic factors at both the cellular and the organ levels (Lawlor, 2001). Among various factors influencing photosynthesis, plant hormones are important in the regulation of photosynthesis and growth processes (Brenner and Cheikh, 1995; Arteca, 1997). Ethylene as a plant hormone influences many aspects of plant growth and photosynthesis (Abeles et al., 1992; Fiorani et al., 2002; Pierik et al., 2006; Acharya and Assmann, 2009). Ethephon (2-chloroethyl phosphonic acid) induces ethylene release and is used commercially to hasten fruit ripening and fruit abscission, but is also known to affect several cellular, developmental, and stress response processes related to photosynthesis (Fiorani et al., 2002; Khan et al., 2008; Acharya and Assmann, 2009; Wilkinson and Davies, 2010). The involvement of ethylene in regulating stomatal and photosynthetic responses is not clear. Contradictory claims have been made on the effects of ethylene on stomatal opening and photosynthesis. Studies have shown that ethylene mediates auxin-induced stomatal opening in Vicia faba (Merritt et al., 2001) and modulates abscisic acid (ABA)-induced stomatal closure in Arabidopsis thaliana (Tanaka et al., 2005). By contrast, Pallas and Kays (1982) and Madhavan et al. (1983) have shown stomatal closure by ethylene. Desikan et al. (2006) have reported that the exogenous application of ethephon induces stomatal closure in Arabidopsis, and ethylene-mediated stomatal closure is dependent on H\(_2\)O\(_2\) generated by NADPH oxidase AtbhoF. Ethephon application has also been shown either to increase photosynthesis (Buhler et al., 1978; Grewal and Kolar, 1990; Subrahmanyan and Rathore, 1992; Grewal

Abbreviations: [CO\(_2\)], intercellular CO\(_2\) concentration; CE, carboxylation efficiency; DAS, days after sowing; Glc, glucose; g\(_s\), stomatal conductance; NUE, nitrogen-use efficiency; PN, photosynthetic rate; WUE, water use efficiency.

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et al., 1993; Khan et al., 2000; Khan, 2004b) or decrease it (Kays and Pallas, 1980; Rajala and Peltonen-Sainio, 2001; Khan, 2005). Ethylene modifies stomatal conductance and photosynthesis depending on ethylene production and sensitivity (Pierik et al., 2006).

The ethylene response also depends on the availability of mineral nutrients (Tari and Szen, 1995; Lynch and Brown, 1997; Borch et al., 1999; Kim et al., 2008; Khan et al., 2008; Garnica et al., 2009; Jung et al., 2009). Recently, Benlloch-Gonzalez et al. (2010) have reported that potassium deficiency increases ethylene production and reduces stomatal responsiveness. Nitrogen (N) availability influences photosynthesis by affecting the photosynthetic machinery (Marschner, 1995; Nobel, 2009), stomatal conductance (Chapin et al., 1988; Dodd et al., 2003; Hossain et al., 2010), and ethylene production (Lynch and Brown, 1997; Khan et al., 2008). Earlier, it was reported that ethephon application increased photosynthesis and growth of mustard under high N levels (Khan et al., 2000, 2008), but the interactive effect of ethylene and N on the photosynthetic response has not been worked out in detail. The reason for the increase in photosynthesis and growth with ethephon and N may be due to direct ethylene-mediated changes in ribulose-1,5-bisphosphate carboxylase (Rubisco), carboxylation efficiency (CE), and an indirect effect of ethylene on stomatal aperture. Therefore, the reported study was undertaken to observe the effects of ethephon-induced ethylene on stomatal conductance, photosynthesis, and growth of mustard under various N levels.

Materials and methods

Plant material and growth conditions

Seeds of mustard (Brassica juncea L. Czern & Coss.) cultivar Varuna were sown in 23 cm diameter earthen pots filled with 5 kg of reconstituted soil (sand:clay:peat; 70:20:10 by dry weight) in a greenhouse of the Botany Department, Aligarh Muslim University, Aligarh, India under natural day/night conditions with a day and night temperature of 24/18 ± 3 °C and relative humidity of 68 ± 6%. Available soil nitrate-N was 100 mg N kg⁻¹ soil. Nitrogen at a concentration of 0, 40, and 80 mg N kg⁻¹ soil was added as urea. Urea was added as the source of the added N taking into consideration that urea is hydrolysed to ammonium carbonate and then, by bacterial action, it is converted to nitrate within 1 or 2 d (Harper, 1984). A high soil phosphorus (P) status was maintained by the addition of 30 mg P kg⁻¹ soil (Harper, 1984). A high soil phosphorus (P) status was maintained by the addition of 30 mg P kg⁻¹ soil (Harper, 1984). A high soil phosphorus (P) status was maintained by the addition of 30 mg P kg⁻¹ soil (Harper, 1984). A high soil phosphorus (P) status was maintained by the addition of 30 mg P kg⁻¹ soil (Harper, 1984).

The determination of leaf area, plant dry mass, leaf N and glucose (Glc) content. The plants were uprooted; all leaves were harvested and used for leaf area determination and, subsequently, the leaves and the rest of the plant were dried separately and their combined dry mass was recorded as plant dry mass. The dried leaf was used for the determination of leaf N and Glc content.

Measurement of ethylene

Ethylene evolution was measured by cutting 0.5 g leaf material into small pieces that were placed in 30 ml tubes containing moist paper to minimize evaporation from the tissue and were stoppered with secure rubber caps and placed in the light for 2 h under the same conditions used for plant growth. Earlier experiments showed that 2 h incubation time was adequate for ethylene detection without the interference of wound-induced ethylene, which began after 2 h of leaf incubation (data not shown). A 1 ml gas sample from the tubes was withdrawn with a hypodermic syringe and assayed on a gas chromatograph (Nacon 5700, New Delhi, India) equipped with a 1.8 m Porapack N (80–100 mesh) column, a flame ionization detector, and data station. Nitrogen was used as the carrier gas. The flow rates of nitrogen, hydrogen and oxygen were 30, 30, and 300 ml min⁻¹, respectively. The detector was at 150 °C. Ethylene identification was based on the retention time and quantified by comparison with the peaks from standard ethylene concentration.

Determination of Rubisco activity

Rubisco activity was determined spectrophotometrically by monitoring NADH oxidation at 30 °C at 340 nm (Usuda, 1985). Leaf samples were homogenized in a chilled mortar with ice-cold extraction buffer containing 0.25 M TRIS-HCl (pH 7.8), 0.05 M MgCl₂, 0.0025 M EDTA, and 37.5 mg dithiothreitol (DTT). The homogenate was centrifuged at 10 000 g for 10 min. The resulting supernatant was used for the enzyme assay. The reaction mixture contained 100 mM TRIS-HCl (pH 8.0), 40 mM NaHCO₃, 10 mM MgCl₂, 0.2 mM NADH, 4 mM ATP, 0.2 mM EDTA, 5 mM DTT, and 1 U of 3-phosphoglycerate kinase. The activity was estimated after the addition of enzyme extract and 0.2 mM ribulose-1,5-bisphosphate (RuBP). Estimation of protein was done according to Bradford (1976) using bovine serum albumin as the standard.

Photosynthetic characteristics

The measurements of photosynthetic rate (Pₚₑ), stomatal conductance (gₛ), and intercellular CO₂ concentration ([CO₂]ᵢ) were done with an infrared gas analyser (Li-Cor 6200, Nebraska) at a light saturating intensity on a sunny day when the photosynthetically active radiation was ~680 μmol m⁻² s⁻¹, the air temperature was ~23 °C, and the relative humidity was ~74% on the fully expanded top leaf of the main axis of the plant. Care was taken to use leaves of the same age for the measurement of photosynthesis in control and treated plants. Carboxylation efficiency was calculated with the help of graph plotted from the values of Pₚₑ and [CO₂]ᵢ (Farquhar and Sharkey, 1982) (Fig. 1). Water use efficiency (WUE) was calculated as the ratio of photosynthesis to gₛ to avoid effects of small differences in vapour pressure between measurements (Von Cammerer and Farquhar, 1981).

Determination of glucose content

Leaf glucose content was determined with the method of Krishnaveni et al. (1984) and using glucose as the standard. Leaf extract was prepared by extracting dried leaf in 80% ethanol. The extract was heated in a water bath at 60 °C for 10 min and then cooled. The samples were then centrifuged at 1500 g for 1 min. The supernatant was used for Glc determination. The reaction mixture consisted of 25 mg O-dianisidine, 1 ml methanol, 49 ml of 0.1 M phosphate buffer (pH 6.5), 5 mg of peroxidase, and 5 mg of
The ethylene evolution of plants grown with deficient-N (0 mg N kg \(^{-1}\) soil) was higher than that of plants grown with sufficient-N (80 mg N kg \(^{-1}\) soil) or low-N (40 mg N kg \(^{-1}\) soil). The application of ethephon further increased ethylene evolution at each N level (Table 1). Maximum ethylene evolution was noted in plants sprayed with 200 \(\mu\)l \(^{-1}\) ethephon and 0 mg N kg \(^{-1}\) soil. Ethylene evolution decreased by 45.7% in plants sprayed with 200 \(\mu\)l \(^{-1}\) ethephon and grown with 80 mg N kg \(^{-1}\) soil, while a lower decrease of 37.1% was observed in plants sprayed with 200 \(\mu\)l \(^{-1}\) ethephon and grown with 40 mg N kg \(^{-1}\) soil. Moreover, the ethylene evolved with 200 \(\mu\)l \(^{-1}\) ethephon and 80 mg N kg \(^{-1}\) soil was higher than the ethylene released with the spray of 100 \(\mu\)l \(^{-1}\) ethephon and 80 mg N kg \(^{-1}\) soil.

The maximal increase in photosynthetic characteristics was noted with spraying with 200 \(\mu\)l \(^{-1}\) ethephon and plants receiving 80 mg N kg \(^{-1}\) soil (Figs 2–4). The activity of Rubisco increased with the ethephon and N treatments. The maximum increase occurred with the combined treatment of 200 \(\mu\)l \(^{-1}\) ethephon and 80 mg N kg \(^{-1}\) soil. The activity of Rubisco was enhanced by 33.3% with 200 \(\mu\)l \(^{-1}\) ethephon and 80 mg N kg \(^{-1}\) soil compared with spraying with 200 \(\mu\)l \(^{-1}\) ethephon on plants grown with 0 mg N kg \(^{-1}\) soil. The application of 200 \(\mu\)l \(^{-1}\) ethephon on plants grown with 40 mg N kg \(^{-1}\) soil could result in an increase of 14.3% in the activity of Rubisco compared with 200 \(\mu\)l \(^{-1}\) ethephon plus 0 mg N kg \(^{-1}\) soil. A lower concentration of ethephon (100 \(\mu\)l \(^{-1}\)) either with 40 or 80 mg N kg \(^{-1}\) soil proved to be less effective (Fig. 2). The application of 200 \(\mu\)l \(^{-1}\) ethephon and 80 mg N kg \(^{-1}\) soil resulted in maximal photosynthesis, \(g_s\), and \([\text{CO}_2]\) (Fig. 3A–C). Photosynthetic rate, \(g_s\), and \([\text{CO}_2]\) were enhanced by 44.4, 34.1, and 35%, respectively in 200 \(\mu\)l \(^{-1}\) ethephon and 80 mg N kg \(^{-1}\) soil over 200 \(\mu\)l \(^{-1}\) ethephon and 0 mg N kg \(^{-1}\) soil. Spraying 200 \(\mu\)l \(^{-1}\) ethephon on to plants treated with 40 mg N kg \(^{-1}\) soil could increase \(P_N\), \(g_s\), and \([\text{CO}_2]\) by 16.7, 14.3, and 18.3%, respectively compared to spraying with 200 \(\mu\)l \(^{-1}\) ethephon and treated with 0 mg N kg \(^{-1}\) soil. The similar treatment
ethephon, 80 mg N kg⁻¹ soil) increased CE (Fig. 4A) and WUE (Fig. 4B) maximally. The increases in these characteristics with 200 μl l⁻¹ ethephon and 80 mg N kg⁻¹ soil were 25.4% and 20.6% compared with 200 μl l⁻¹ ethephon and 0 mg N kg⁻¹ soil. The increases in these characteristics with 200 μl l⁻¹ ethephon and 40 mg N kg⁻¹ soil were lower.

The maximal Glc content was found in plants receiving 0 mg N kg⁻¹ soil and no ethephon. The addition of N decreased leaf Glc content, whereas the application of ethephon increased the Glc content at each level of N (Fig. 5). Plants receiving 40 mg N kg⁻¹ soil and sprayed with 200 μl l⁻¹ ethephon decreased the Glc content by 26.9% compared with 200 μl l⁻¹ ethephon and 0 mg N kg⁻¹ soil. Glc content was decreased by 34.6% with the treatments of 200 μl l⁻¹ ethephon and 80 mg N kg⁻¹ soil compared to 200 μl l⁻¹ ethephon and 0 mg N kg⁻¹ soil.

Leaf area and plant dry mass were affected maximally by spraying with 200 μl l⁻¹ ethephon when plants received 80 mg N kg⁻¹ soil (Fig. 6A, B). The plants treated with 40 mg N kg⁻¹ soil responded less to 200 μl l⁻¹ ethephon spray. Leaf area and plant dry mass were increased by 62.9% and 69.7% with the treatments of 200 μl l⁻¹ ethephon and 80 mg N kg⁻¹ soil compared to 200 μl l⁻¹ ethephon and 0 mg N kg⁻¹ soil.

Leaf area and plant dry mass were increased by 62.9% and 69.7% with the treatments of 200 μl l⁻¹ ethephon and 80 mg N kg⁻¹ soil compared to 200 μl l⁻¹ ethephon and 0 mg N kg⁻¹ soil. The plants receiving 40 mg N kg⁻¹ soil and sprayed with 200 μl l⁻¹ ethephon could increase leaf area and plant dry mass by 23.4% and 21.2%.

NUE was significantly affected by ethephon and N treatments. NUE of plants sprayed with 200 μl l⁻¹ ethephon
and 80 mg N kg\(^{-1}\) soil was about two-times higher than plants receiving 200 \(\mu l l^{-1}\) ethephon and 40 mg N kg\(^{-1}\) soil (Table 2). The effect of 100 \(\mu l l^{-1}\) ethephon plus 40 or 80 mg N kg\(^{-1}\) soil was less.

**Discussion**

Photosynthetic rate, \(g_s\), and the growth of plants responded positively and maximally to 200 \(\mu l l^{-1}\) ethephon and sufficient-N (80 mg N kg\(^{-1}\) soil). These values were lower in plants grown with 0 or 40 mg N kg\(^{-1}\) soil, but the application of ethephon (100 or 200 \(\mu l l^{-1}\)) resulted in a similar increase in photosynthesis and growth.

The enhancement in photosynthesis by ethephon application was mediated through ethylene-induced changes in various photosynthetic characteristics, and was also associated with an increase in \(g_s\) and intercellular \(\text{CO}_2\), indicating that ethylene may also have increased photosynthesis by increasing the availability of \(\text{CO}_2\). This led to the uncoupling of \(g_s\) and \([\text{CO}_2]\) both increased together whereas usually increasing \([\text{CO}_2]\) decreases \(g_s\) (Morison, 1998; Pozo et al., 2005; Levine et al., 2009; Nobel, 2009). Ethephon is a direct ethylene-releasing source when it is applied to plants and elicits responses identical to those induced by ethylene gas (Cooke and Randell, 1968; Edgerton and Blanpied, 1968). Mustard shows the typical ethylene and N responses, and

**Table 1.** Ethylene (\(\mu l m^{-2} s^{-1}\)) evolution in leaves of mustard (Brassica juncea L.) plants sprayed with 0, 100 or 200 \(\mu l l^{-1}\) ethephon at 40 DAS and grown with soil-applied 0, 40, and 80 mg N kg\(^{-1}\) soil determined at 50 DAS (\(n=6\)).

Data showing the same letter are not significantly different by LSD test at \(P<0.05\).

| N levels (mg N kg\(^{-1}\) soil) | Ethephon (\(\mu l l^{-1}\)) |
|---------------------------------|--------------------------|
|                                 | 0            | 100        | 200        |
| 0                               | 33.4±1.2 c   | 41.7±1.4 b | 51.2±2.0 a |
| 40                              | 19.5±0.8 g   | 21.0±1.0 f | 32.2±1.6 c |
| 80                              | 16.7±0.6 h   | 22.8±0.8 e | 27.8±1.2 d |

**Table 2.** N-use efficiency (mg plant N mg\(^{-1}\) N applied kg\(^{-1}\) soil) of plants sprayed with 100 or 200 \(\mu l l^{-1}\) ethephon at 40 DAS.

N-use efficiency was calculated at 50 DAS as an increase in plant N uptake at 40 and 80 mg N kg\(^{-1}\) soil over 0 mg N kg\(^{-1}\) soil (\(n=6\)). ** indicates significant \(P<0.01\) effect of ethephon over control at each N level.

| N levels (mg N kg\(^{-1}\) soil) | N-use efficiency (%) |
|---------------------------------|-------------------|
|                                 | Ethephon spray \(\mu l l^{-1}\) |
|                                 | 0             | 100       | 200       |
| 40                              | 5.9           | 7.9       | 11.6**    |
| 80                              | 11.7          | 15.8      | 27.5**    |
Ethephon application has been found to increase photosynthesis (Grewal and Kolar, 1990; Subrahmanyam and Rathore, 1992; Grewal et al., 1993; Khan et al., 2000, 2008; Khan, 2004b). Khan (2004a) showed a strong positive correlation between 1-aminoacyclopropane carboxylic acid synthase, a rate-limiting enzyme in ethylene biosynthesis, with photosynthesis of mustard cultivars differing in photosynthetic capacity.

Based on the results reported here it may be said that ethephon application to plants grown with variable N levels induced stomatal and photosynthetic responses, and there was a significant interaction between ethylene, N availability, and photosynthetic characteristics. The reports on the interaction of ethylene and N are available in the literature. Baker and Corey (1990) found equal ethylene evolution and shoot dry mass in tomato plants with urea and nitrate nutrition. Nitrogen influences the quantity, structure, and composition of the photosynthetic apparatus and hence plays a crucial role in determining the photosynthetic capacity of the plant in both natural and agricultural environments (Abrol et al., 1999; Kumar et al., 2002). In crops, Rubisco content increases linearly with N uptake and leaf N (Sage et al., 1987; Makino et al., 1997; Nakano et al., 1997).

The present work suggests that ethephon application at each level of N increases ethylene and influences stomatal, photosynthetic, and growth responses. Ethylene increased the stomatal conductance of plants, thereby increasing the diffusion of CO₂ and thus photosynthesis (Fig. 3). Ethylene can alter the rate of photosynthesis by affecting the diffusion rate of CO₂ from the atmosphere to the intercellular cavities by influencing stomatal aperture (Pierik et al., 2006; Acharya and Assmann, 2009; Wilkinson and Davies, 2010). In addition, ethylene could stimulate photosynthesis by increasing the allocation of N to the photosynthetic machinery. There is probably some signalling response chain at the guard cells that is directly affected by ethylene. The application of 200 μl L⁻¹ ethephon and sufficient-N (80 mg N kg⁻¹ soil) evolved optimal ethylene, and maximally increased stomatal conductance and photosynthetic responses.

The increase in N availability resulted in the increase in photosynthesis but decreased ethylene evolution. At 0 mg N kg⁻¹ soil, stomatal conductance and photosynthesis were low despite high ethylene evolution. It may thus be suggested that plants were less sensitive to ethylene under deficient N.

Plants grown with 0 mg N kg⁻¹ soil showed increased Glc sensitivity that resulted in the inhibition of Rubisco activity and photosynthesis. The increase in Glc content under N deficiency has been reported (Boussadia et al., 2010), which represses photosynthetic genes and enzymes (Ehness et al., 1997). High endogenous Glc concentration results in the stronger repression of Rubisco mRNA levels. Moreover, the inability to perceive ethylene results in increased sensitivity to Glc. This increased sensitivity to Glc results in negative effects on Rubisco content and photosynthetic capacity (Tholen et al., 2007). The application of ethephon to deficient-N plants increased Glc content, ethylene evolution, and photosynthesis. Endogenous Glc concentrations are often positively correlated with ethylene production in rice, and external sugar application in this species significantly stimulates ethylene production (Kobayashi and Saka, 2003). Glc sensitivity to inhibit photosynthesis in plants unable to perceive ethylene decreases with ethephon application. Seneweera et al. (2003) suggested that ethylene production promoted growth under circumstances where leaf Glc concentration was high. The developmental arrest by high sugar levels can be overcome by applying the ethylene precursor 1-aminoacyclopropane-1-carboxylic acid (Zhou et al., 1998). Ethylene reduces the negative feedback of carbohydrates on photosynthetic gene expression (Tholen et al., 2007). As the availability of N increases from 0 to 80 mg N kg⁻¹ soil, ethylene evolution and Glc content decreases. This results in reducing the negative effects of Glc on Rubisco and photosynthesis. Recently, it has been reported that the ethylene response factor TERF1 enhances Glc sensitivity in tobacco through activating the expression of sugar-related genes (Li et al., 2009).

The increase in WUE with ethephon application also supports the positive effect of ethephon on photosynthesis, especially as gs also increased at higher WUE. To maximize WUE, stomatal opening must be synchronized with the capability for CO₂ fixation (Nobel, 2009). WUE is associated with a higher Rubisco activity or rate of electron transport (Van den Boogard et al., 1995). The higher plant WUE was caused by a lower transpiration rate associated with a higher leaf area per unit plant dry mass. Maximally increased photosynthesis with 200 μL L⁻¹ ethephon and sufficient-N led to the increase in WUE. This may be due to increase in binding of CO₂ to the active site of Rubisco (Van den Boogard et al., 1995).

The greatest leaf area due to 200 μL L⁻¹ ethephon and sufficient-N increased ground cover and had an important influence on WUE, reducing soil evaporation and increasing plant dry mass accumulation. The higher leaf area is correlated with ethephon-enhanced ethylene biosynthesis. The role of low ethylene concentration in inducing leaf growth (Lee and Reid, 1997; Hussain et al., 1999; Fiorani et al., 2002; Khan, 2005) and cell expansion (Rodriguez-Pousada et al., 1993) has been reported. Tholen et al. (2004) found that ethylene-insensitive genotypes of Arabidopsis, Nicotiana tabacum, and Petunia x hybrida had no increase in total leaf area compared to normal ethylene-sensitive control plants. In addition to studies on the effects of ethylene treatment, the role of ethylene during leaf development had been confirmed physiologically using ethylene inhibitors and genetically using ethylene-insensitive mutants or transgenic plants that did not express the key enzymes of ethylene biosynthesis (Bleecker et al., 1998; Oh et al., 1999). In our earlier studies, ethephon application was found to enhance ethylene evolution and leaf area (Khan, 2004b, 2005; Khan et al., 2008).

Plants grown with deficient or low-N exhibited lower NUE than plants grown with sufficient-N. The application of ethephon on these plants increased NUE. Van Sanford et al. (1989) and Bulman and Smith (1993) found positive effects of ethephon on NUE of winter wheat and barley. Khan et al. (2008) have shown that ethylene has a role in N metabolism and increases NR activity and N content in Brassica juncea.
Ethylene has also been reported to enhance glutamine synthetase activity and mRNA level in *Hevea brasiliensis* (Pujade-Renaud et al., 1994). The low NUE of plants under low-N invested a lower amount of N to Rubisco and resulted in lower photosynthesis. Maximum NUE resulted from the application of 200 μl l⁻¹ ethephon on plants grown with sufficient-N. These plants had ethylene evolution which maximally increased photosynthesis and made maximum use of the available N due to the enhancement of vegetative growth.

In conclusion, it may be said that ethylene increases photosynthesis and growth at all levels of N (deficient and sufficient) both via an increase in carboxylation efficiency and via an increase in gs and the availability of [CO₂], thereby uncoupling the usual effect of increased [CO₂] to reduce gs. Increasing ethylene increases photosynthesis directly via Glc sensitivity, and increasing the availability of [CO₂], and this occurs at all levels of N, although less sensitively at deficient N where stress ethylene production may have reduced gs and photosynthetic machinery sensitivity to ethylene although this remains to be tested.

References

Abeles FB, Morgan PW, Saltveit ME. 1992. *Ethylene in plant biology*, 2nd edn. San Diego: Academic Press.

Abrol YP, Chatterjee SR, Kumar PA, Jain V. 1999. Improvement in nitrogen use efficiency: physiological and molecular approaches. *Current Science* 76, 1357–1364.

Acharya BR, Assmann SM. 2009. Hormone interactions in stomatal function *Plant Molecular Biology* 69, 451–462.

Arteca RN. 1997. Plant growth substances: principles and applications. Delhi: CBS Publishers and Distributors.

Baker AV, Corey KA. 1990. Ethylene evolution by tomato plants receiving nitrogen nutrition from urea. *Horticultural Science* 25, 420–421.

Benlloch-González M, Romera J, Cristescu S, Harren F, Fournier JM, Benlloch M. 2010. K⁺ starvation inhibits water-stress-induced stomatal closure via ethylene synthesis in sunflower plants. *Journal of Experimental Botany* 61, 1139–1145.

Bleecker AB, Estelle MA, Somerville C, Kende H. 1998. Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science* 241, 1086–1089.

Borch K, Bouma TJ, Lynch JP, Brown KM. 1999. Ethylene: a regulator of root architectural responses to soil phosphorus availability. *Plant, Cell and Environment* 22, 425–431.

Boussadja O, Steppeb K, Zagalaïd H, Hadjc SBE, Braham A, Lemeurb R, Labeeke MCV. 2010. Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars ‘Meski’ and ‘Koroneiki’. *Scientia Horticulturae* 123, 336–342.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein–dye binding. *Analytical Biochemistry* 72, 248–254.

Brenner ML, Cheikh N. 1995. Hormones in photosynthetic partitioning and seed filling. In: Davies PJ, ed. *Plant hormones*. Dordrecht: Kluwer, 649–670.

Buhler B, Drumm H, Mohr H. 1978. Investigations on the role of ethylene in phytochrome-mediated photoporphogenesis. II. Enzyme levels and chlorophyll synthesis. *Planta* (Berlin) 142, 119–122.

Bulman P, Smith DL. 1993. Yield and grain protein response of spring barley to ethylene and triadimefon. *Crop Science* 33, 798–803.

Chapin FS, Walter CHS, Clarkson DT. 1988. Growth response of barley and tomato to nitrogen stress and its control by abscisic acid water relations and photosynthesis. *Planta* 173, 352–366.

Cooke AR, Randall DI. 1968. 2-Haloethanephosphonic acids as ethylene releasing agents for the induction of flowering in pineapples. *Nature* 218, 974–975.

Desikan R, Last K, Harrett-Williams R, Tagliavac C, Harter K, Hooley R, Hancock JT, Neil SJ. 2006. Ethylene-induced stomatal closure in *Arabidopsis* occurs via AtRbohF-mediated hydrogen peroxide synthesis. *The Plant Journal* 47, 907–916.

Dodd IC, Tan LP, He J. 2003. Do increases in xylem sap pH and/or ABA concentration mediate stomatal closure following nitrate deprivation? *Journal of Experimental Botany* 54, 1281–1288.

Edgerton LJ, Blanpied GD. 1968. Regulation of growth and fruit maturation with 2-chloroethanephosphonic acid. *Nature* 219, 1064–1065.

Ehness R, Ecker M, Godt DE, Roitsch T. 1997. Glucose and stress independently regulate source and sink metabolism and defense mechanisms via signal transduction pathways involving protein phosphorylation. *The Plant Cell* 9, 1825–1841.

Farquhar GD, Sharkey TD. 1982. Stomatal conductance and photosynthesis. *American Review of Plant Physiology* 33, 317–345.

Fiorani F, Bogemann GM, Visser EJW, Lambers H, Voesenek LACJ. 2002. Ethylene emission and responsiveness to applied ethylene vary among *Poa* species that inherently differ in leaf elongation rates. *Plant Physiology* 129, 1382–1390.

Garnica M, Houdusse F, Yvin JC, Garcia-Mina JM. 2009. Nitrate supply induces changes in polyamine content and ethylene production in wheat plants grown with ammonium. *Journal of Plant Physiology* 166, 363–374.

Grewal HS, Kolar JS. 1990. Response of *Brassica juncea* to chlorocholine chloride and ethrel sprays in association with nitrogen application. *Journal of Agriculture Science* 114, 87–91.

Grewal HS, Kolar JS, Cheema SS, Singh G. 1993. Studies on the use of growth regulators in relation to nitrogen for enhancing sink capacity and yield of gobhi-sarson (*Brassica napus*). *Indian Journal of Plant Physiology* 36, 1–4.

Harper JE. 1984. Uptake of nitrogen forms by roots and leaves. In: Hauck RD, ed. *Nitrogen in crop production*. American Society of Agronomy, 165–170.

Hossain MD, Musa MF, Talib J, Jol H. 2010. Effects of nitrogen, phosphorus and potassium levels on kenaf (*Hibiscus cannabinus L.*) growth and photosynthesis under nutrient solution. *Journal of Agricultural Science* 2, 49–57.
Hussain A, Black CR, Taylor IB, Roberts JA. 1999. Soil compaction: a role for ethylene in regulating leaf expansion and shoot growth in tomato. Plant Physiology 121, 1227–1237.

Jung Ji-Yul, Shin R, Schachtman DP. 2009. Ethylene mediates responses and tolerance to potassium deprivation in Arabidopsis. The Plant Cell 21, 607–621.

Kays SJ, Pallas JE. 1980. Inhibition of photosynthesis by ethylene. Nature 385, 51–52.

Khan NA. 2004a. Activity of 1-aminocyclopropane carboxylic acid synthase in two mustard (Brassica juncea L.) cultivars differing in photosynthetic capacity. Photosynthetica 42, 477–480.

Khan NA. 2004b. An evaluation of the effects of exogenous ethephon, an ethylene releasing compound, on photosynthesis of mustard (Brassica juncea) cultivars that differ in photosynthetic capacity. BMC Plant Biology 4, Article No. 21.

Khan NA. 2005. The influence of exogenous ethylene on growth and photosynthesis of mustard (Brassica juncea) following defoliation. Scientia Horticulturae 105, 499–505.

Khan NA, Lone NA, Samiuulah. 2000. Response of mustard (Brassica juncea L.) to applied nitrogen with or without ethrel spray under non-irrigated conditions. Journal of Agronomy and Crop Science 184, 63–66.

Khan NA, Mir MR, Nazar R, Singh S. 2008. The application of ethephon (an ethylene releaser) increases growth, photosynthesis and nitrogen accumulation in mustard (Brassica juncea L.) under high nitrogen levels. Plant Biology 10, 534–538.

Kim Hye-Ji, Lynch JP, Brown KM. 2008. Ethylene insensitivity impedes a subset of responses to phosphorous deficiency in tomato and petunia. Plant, Cell and Environment 31, 1744–1755.

Kobayashi H, Saka H. 2003. Relationship between ethylene evolution and sucrose content in excised leaf blades of rice. Plant Production Science 3, 398–403.

Krishnaveni S, Balasubramanian T, Sadasivam S. 1984. Sugar distribution in sweet stalk sorghum. Food Chemistry 15, 229–232.

Kumar PA, Parry MAJ, Mitchell RAC, Ahmad A, Abrol YP. 2002. Photosynthesis and nitrogen use efficiency. In: Foyer CH, Noctor G, eds. Photosynthetic nitrogen assimilation and associated carbon and respiratory metabolism. Advances in photosynthesis and respiration, Vol. 12. Dordrecht: Springer, 23–34.

Lawlor DW. 2001. Photosynthesis. Oxford: Bios Scientific Publishers, 309–351.

Lee SH, Reid DM. 1997. The role of endogenous ethylene in the expansion of Helianthus annuus leaves. Canadian Journal of Botany 75, 501–508.

Levine LH, Richard JT, Wheeler RM. 2009. Super-elevated CO₂ interferes with stomatal response to ABA and night closure in soybean (Glycine max). Journal of Plant Physiology 166, 903–913.

Li A, Zhang Z, Wang Xue-Chen, Huang R. 2009. Ethylene response factor TERF1 enhances glucose sensitivity in tobacco through activating the expression of sugar-related genes. Journal of Integrative Plant Biology 51, 184–193.

Lindner RC. 1944. Rapid analytical methods for some of the more common inorganic constituents of plant tissues. Plant Physiology 19, 70–89.

Lynch JP, Brown KM. 1997. Ethylene and plant responses to nutritional stress. Physiologia Plantarum 100, 613–619.

Madhavan S, Chrimonski A, Smith BN. 1983. Effect of ethylene on stomatal opening in tomato and carnation leaves. Plant and Cell Physiology 24, 569–572.

Makino A, Sato T, Nakano H, Mae T. 1997. Leaf photosynthesis, plant growth, and nitrogen allocation in rice under different irradiances. Planta 203, 390–398.

Marschner H. 1995. Mineral nutrition of higher plants. New York: Academic Press, 229–299.

Merritt F, Kemper A, Tallman G. 2001. Inhibitors of ethylene synthesis inhibit auxin-induced stomatal opening in epidermis detached from leaves of Vicia faba L. Plant and Cell Physiology 42, 223–230.

Morison JIL. 1998. Stomatal response to increased CO₂ concentration. Journal of Experimental Botany 49, 442–452.

Nakano H, Makino A, Mae T. 1997. The effect of elevated partial pressures of CO₂ on the relationship between photosynthetic capacity and N content in rice leaves. Plant Physiology 115, 191–198.

Nobel PS. 2009. Physicochemical and environmental plant physiology, 2nd edn. San Diego: Academic Press.

Oh SA, Park JH, Lee GL, Pack KH, Park SK, Nam HG. 1999. Identification of three genetic loci controlling leaf senescence in Arabidopsis thaliana. The Plant Journal 12, 527–535.

Pallas JE, Kays SJ. 1982. Inhibition of photosynthesis by ethylene: a stomatal effect. Plant Physiology 70, 598–601.

Pierik R, Tholen D, Poorter H, Visser EJW, Voosenk LACJ. 2006. The jayus face of ethylene: growth inhibition and stimulation. Trends in Plant Sciences 11, 176–183.

Pozo AD, Pérez P, Morcuende R, Alonso A, Carrasco-Martinez R. 2005. Acclimatory responses of stomatal conductance and photosynthesis to elevated CO₂ and temperature in wheat crops grown at varying levels of nitrogen supply in a Mediterranean environment. Plant Science 169, 908–916.

Pujade-Renaud V, Clement A, Perrot-Rechenmann C, Prevot JC, Chrestin H, Jacob JL, Guern J. 1994. Ethylene induced increase in glutamine synthetase activity and mRNA levels in Hevea brasiliensis latex cells. Plant Physiology 105, 127–132.

Rajala A, Peltonen-Sainio P. 2001. Plant growth regulator effects on spring cereal root and shoot growth. Agronomy Journal 93, 936–943.

Rodriguez-Pousida RA, De Rycke R, Dedondar A, Van Caeneghem W, Engler E, Van Montagu M, Van Der Straeten D. 1993. The Arabidopsis 1-aminocyclopropane-1-carboxylate synthase gene 1 is expressed during early development. The Plant Cell 5, 897–911.

Sage RF, Pearcy RW, Seemann JR. 1987. The nitrogen efficiency of C₃ and C₄ plants. Ill. Leaf nitrogen effect on activity of carboxylating enzymes in Chenopodium album L. and Amaranthus retroflexus L. Plant Physiology 85, 355–359.
| Author(s)                  | Year   | Title                                                                 | Journal/Volume | Pages                  |
|---------------------------|--------|-----------------------------------------------------------------------|----------------|------------------------|
| Seneweera S, Aben SK, Basra AS, Jones B, Conroy JP. | 2003   | Involvement of ethylene in the morphological and developmental response of rice to elevated atmospheric CO2 concentrations. | Plant Growth Regulation 39 | 143–153               |
| Subrahmanyam D, Rathore VS. | 1992   | Influence of ethylene on carbon-14 labeled carbon dioxide assimilation and partitioning in mustard. | Plant Physiology and Biochemistry 30 | 81–86               |
| Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S. | 2005   | Ethylene inhibits abscisic acid-induced stomatal closure in Arabidopsis. | Plant Physiology 138 | 2337–2343            |
| Tari I, Szen L.            | 1995   | Effect of nitrite and nitrate nutrition on ethylene production by wheat seedlings. | Acta Phytopatholica et Entomologica Hungarica 30 | 99–104               |
| Tholen D, Voesenek LACJ, Poorter H. | 2004  | Ethylene insensitivity does not increase leaf area or relative growth rate in Arabidopsis thaliana, Nicotiana tabacum and Petunia × hybrid. | Plant Physiology 134 | 1803–1812           |
| Tholen D, Pons TL, Voesenek LACJ, Poorter H. | 2007  | Ethylene insensitivity results in the down-regulation of Rubisco expression and photosynthetic capacity in tobacco. | Plant Physiology 144 | 1305–1315            |
| Usuda H.                  | 1985   | The activation state of ribulose 1,5-bisphosphate carboxylase in maize leaves in dark and light. | Plant and Cell Physiology 91 | 455–463             |
| Van den Boogard R, Kostadirova S, Veneklaas EJ, Lambers H. | 1995   | Association of water use efficiency and nitrogen use efficiency with photosynthetic characteristics of two wheat cultivars. | Journal of Experimental Botany 46 | 1429–1438            |
| Van Sanford DA, Grove JH, Grabau J, MacKown CT. | 1989   | Ethephon and nitrogen use in winter wheat. | Agronomy Journal 81 | 951–954               |
| Von Cammerer S, Farquhar GD. | 1981  | Some relationship between the biochemistry of photosynthesis and the gas exchange of leaves. | Planta 153 | 376–387               |
| Wilkinson S, Davies WJ.   | 2010   | Drought, ozone, ABA and ethylene: new insights from cell to plant to community. | Plant, Cell and Environment 33 | 510–525             |
| Zhou L, Jang J-C, Jones TL, Sheen J. | 1998  | Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. | Proceedings of the National Academy of Sciences, USA 95 | 10294–10299          |

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