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RESEARCH ARTICLE

EFFECT OF ETHREL SPRAY AND NITROGEN ON GROWTH, PHOTOSYNTHESIS, CARBOXYLATION EFFICIENCY AND WATER –USE EFFICIENCY OF MUSTARD (*BRASSICA JUNCEA* L.)

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ABSTRACT

India depends more than any other nation on groundwater depletion for food security and must seek ways to increase crop water-use efficiency (WUE). One approach is to increase plant photosynthetic capacity and transpiration ration (TR). Our objective was to measure the effects of ethephon and N on photosynthesis and related physiological traits of Indian mustard (*Brassica juncea* L. Czern and Coss, cv. Alankar). Field experiments were conducted in Aligarh, India under irrigated and non-irrigated conditions. Four levels of N (0 to 80 kg ha^{-1}) and three levels of ethrel (0 to 200 µl l^{-1}) were used. Ethrel and N nearly always had additive effects on LAI and plant dry matter (PDM) in both irrigated and non-irrigated experiments. Similarly, most gas exchange traits responded linearly to ethrel and N addition. At 80 days after sowing (DAS), photosynthesis increased from 16.5 to 25.7 µmol m^{-2} s^{-1} due to N application, and from 19.5 to 24.7 µmol m^{-2} s^{-1} due to ethrel application. TR increased from 36 to 45 µmol mol^{-1} due to N, and from 36 µmol mol^{-1} to ~43.5 µmol mol^{-1} due to ethrel. The highest combination of ethrel and N gave TR values of 51 µmol mol^{-1}. Carboxylation efficiency (CE) was nearly constant among treatments at ~0.095 µmol m^{-2} s^{-1} per µmol mol^{-1} increase of [CO_2]. However all points above the 0.95 confidence level of the regression curve belonged to the 200 µl l^{-1} ethrel treatment, and all below to the 0 µl l^{-1} ethrel treatment, suggesting lower photorespiration and CO_2 compensation point for ethrel treated plants. Results are consistent with reports that ethrel affects several cellular processes related to photosynthesis. Ethrel spray, either alone or in combination with N application, may provide a potential management tool for increasing water use efficiency (WUE) in India and other water-limited regions of the world.

INTRODUCTION

India depends more than any other nation on the unsustainable depletion of groundwater for its food security (Postel, 2000). Confronted with increasing population, decreasing land availability, and growing competition for fresh water, India and other water-limited countries of the world must seek ways to increase crop water-use efficiency (WUE) (Unger and Howell, 1999). One of the several approaches to increasing WUE (Stone, 1975; Sinclair *et al.,* 1984; Unger *et al.,* 2006) is to increase photosynthetic capacity. Various plant growth regulators, including absicic acid and indole-3-acetic acid, have been used to increase photosynthesis (Makeev *et al.,* 1992; Zerbe and Wild, 1981; Arteca and Dong, 1998; Foroutan–pour *et al.,* 1997; Child *et al.,* 1985; Liu *et al.,* 1993; Yang *et al.,* 1994). The growth regulator ethephon (2-chloroethyl phosphonic acid), which induces ethylene release, is commercially used to hasten ripening and fruit abscission (Warner and Leopold, 1969 Jana and Kabir, 1991; Joshi *et al.,* 1987).

However, it also affects several cellular, developmental and stress-response processes related to photosynthesis (Buehler *et al.,* 1978; Dolan, 1997; Esashi, 1991. Balota *et al.,* 2004). Foliar application of ethephon has been observed to increase photosynthetic rate in some crops (Pua and Chi, 1993) but reduce it in others (Pallas and Kays, 1982). Varied results have been reported for Indian mustard [*Brassica juncea* (L.) Czern.], which is an economically important crop in Utter Pradesh, Punjab, Haryana, and other Indian states. Subrahmanyam and Rathore (1992a, b) found that exogenous application of

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ethephon to mustard reduced photosynthesis by as much as 18%. On the other hand, Khan (1996, 1998) and Khan *et al.* (2000); Khan *et al.* (2007); Mir *et al.* (2008); Mir *et al.* (2009 a,c); Lone *et al.* (2010) reported that ethephon enhanced mustard photosynthesis under both irrigated and non-irrigated conditions.

Crop nitrogen status also affects photosynthetic rate (Dhoble, 1998; Boltan and Brown, 1980; Nova and Loomis 1981; Wong *et al.,* 1995; Sinclair, 1990), because N is a major constituent of chlorophyll (Pham *et al.,* 1981), thylakoid proteins, and many enzymes of the photosynthetic carbon reduction cycle (Evans and Seemann, 1989). For some species, the rate of photosynthesis is linearly related to leaf nitrogen content (Sage *et al.,* 1987; Evans, 1989; Nobel, 1999). Leaves with greater N content utilize high photon flux densities more effectively (Field, 1983; DeJong and Doyle, 1985). For example, Varade *et al.* (1995) increased the rate of photosynthesis by 43% in soybean (*Glycine max* L.) by adding nitrate. Nitrogen application can also enhance soil water extraction and increase water use (Saran and Girri, 1990, Vyas *et al.,* 1995; Thakral *et al.,* 1997; Zaman and Choudhari 1998; Dodd, 2001).

Although previous studies have examined the agronomic effects of ethephon and N application on Indian mustard, to our knowledge none has examined their individual and combined effects on photosynthesis and related gas exchange parameters. The objective of this study was to observe the effects of ethephon and N application on photosynthesis and related physiological traits including dry matter production, leaf area index (LAI), carboxylation efficiency (CE) and transpiration ratio (photosynthesis/stomatal conductance) of Indian mustard grown under irrigated and non-irrigated conditions.

MATERIAL AND METHODS

Two field experiments were conducted during the winter season of 2005-2006 at the Experiment Farm of Aligarh Muslim University, Aligarh, India. Aligarh is located ~145 km southeast of New Delhi in Uttar Pradesh (latitude 27°52'N, longitude 78°51'E, 188 m asl). It has a semi-arid, subtropical environment characterized by hot and dry summers, and cold winters. Mean annual rainfall is ~840 mm, with 85% of the annual total received from June to September. Soils at the Experiment Farm have sandy loam texture, a pH of 8.0 to 8.2 in 1:2 soil/water mixture, electrical conductivities of 0.41 mmhos cm^{-1} and available nitrogen of ~200 kg ha^{-1} (Mir, 2002).

Experiment 1 was carried out under irrigated conditions and Experiment 2 under non-irrigated conditions following pre-planting irrigation. Two factorial experiments were carried out, each using a completely randomized block design with three replications. Nitrogen (four levels) and ethephon (three levels) were experimental factors. Individual plot size was 2 m x 5 m. Prior to planting, plots in both irrigated and non-irrigated experiments were pre-irrigated at a rate of 20 l m^{-2}. Irrigated plots were watered a second time at 50 days after sowing (DAS) with an additional 20 l m^{-2}. Seeds of the mustard cultivar Alankar were sown at a rate 10 kg ha^{-1}. At seedling establishment, a distance of 0.30 m between rows and 0.15 m between the plants in each row was maintained. Nitrogen was applied at sowing in the form of urea at the rate of 0, 40, 60, and 80 kg N ha^{-1}. At 60 DAS (at flowering stage), ethephon was applied at concentrations of 0, 100, and 200 μl l^{-1} and rates of 600 l ha^{-1} with 0.5% teepol (a surfactant). In the control treatment, plants were sprayed with an equal amount of deionised water and 0.5% teepol. The concentration of ethephon and time of application were based on earlier findings (Khan et al 2000, Lone 2001). Additional cultural details were given by Mir (2002).

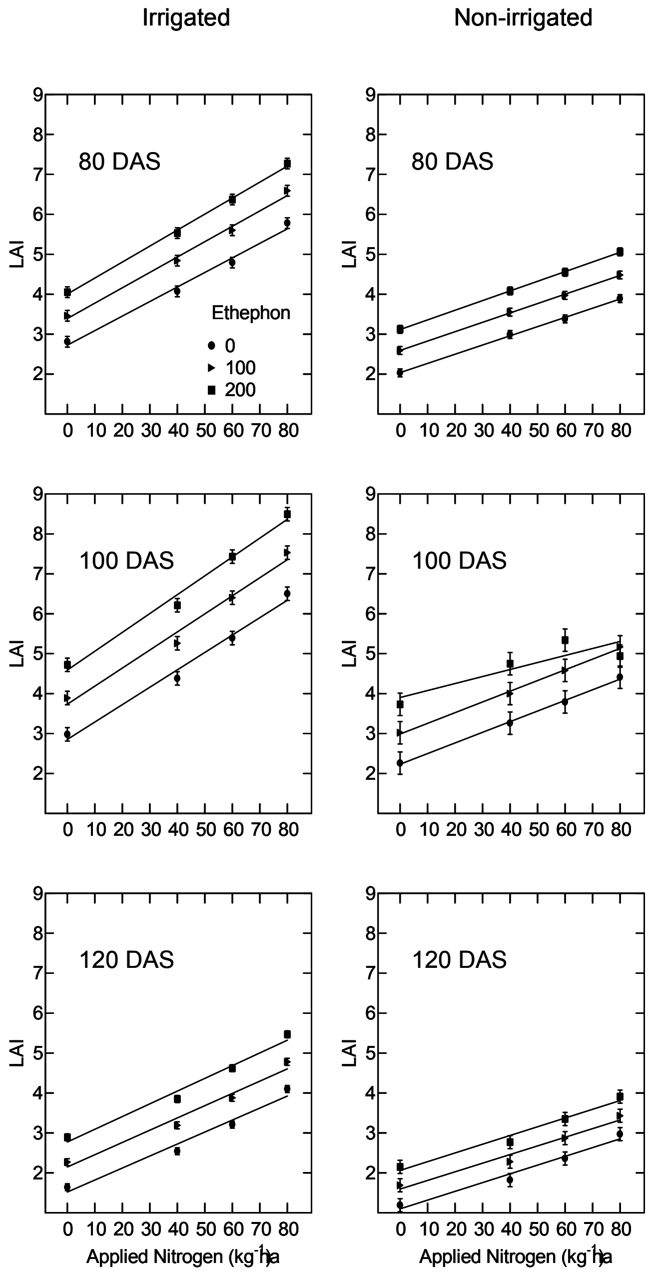

Figure 1. Effects of nitrogen and ethrel application on leaf area index (LAI) of Indian mustard at three dates.

At 80 DAS (pod fill), 100 DAS (pod maturity) and 120 DAS (harvest), five plants were removed from each plot. Leaves were removed and dried at 70 °C and weighed. Before drying, several leaves from each plant were individually traced on paper, and the area of each leaf pattern was measured using a planimeter. Leaf area of the entire plant was determined by multiplying leaf mass times the mean ratio of leaf area to mass determined for the subset of leaves. Leaf area index (LAI) was

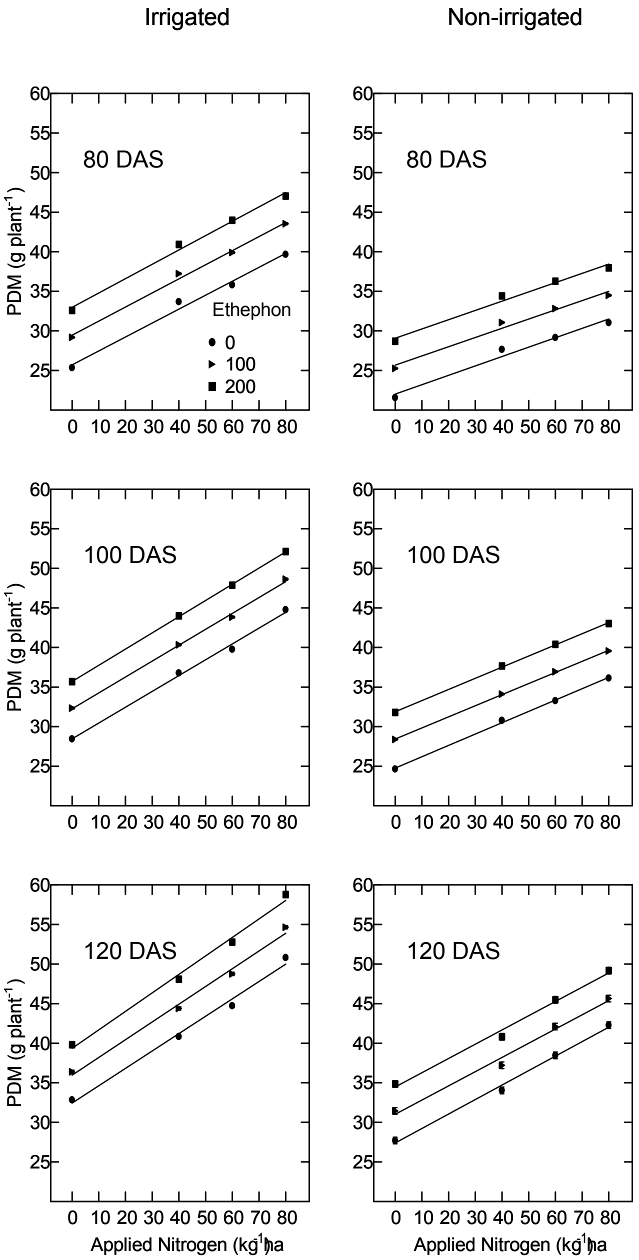

Figure 2. Effects of nitrogen and ethrel application on plant dry matter (PDM) of Indian mustard at three dates.

determined by dividing mean leaf area per plant by ground area (Watson, 1958).

At 80 and 100 DAS, photosynthesis, stomatal conductance (g_s), and internal CO_2 concentration ($[CO_2]$) were measured in on the uppermost fully expanded leaf on the main stem of two plants in each plot using a LICOR-6200 portable photosynthesis system (LICOR, Lincoln, Nebraska, USA). Measurements were taken from 1100 to 1200 hours, when photosynthetically active radiation was ~1250 μ mol m^{-2} s^{-1}, air temperature was ~23 ºC, and relative humidity was ~72%. Carboxylation efficiency (CE) was calculated from initial slopes of CO_2 assimilation rate (A) plotted against $[CO_2]$. Transpiration ratio (TR) was expressed as the ratio of A to gs to avoid effects of small differences in vapor pressure between measurements (Von Cammerer and Farquhar, 1981). Analysis of variance was made using nitrogen and ethrel as treatment factors with SYSTAT v. 11.0 (SYSTAT

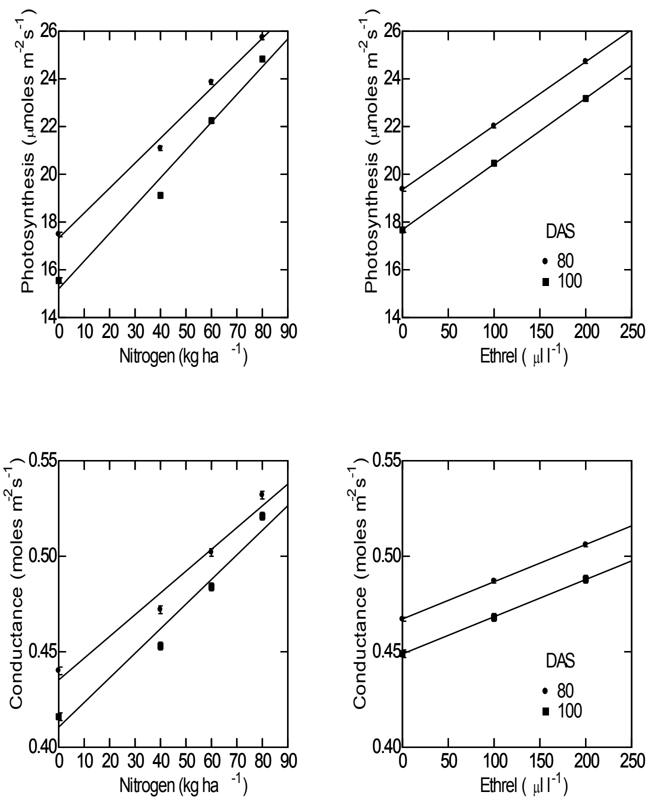

Figure 3. Photosynthesis and stomatal conductance of irrigated Indian mustard as affected by nitrogen and ethrel application on two measurement dates.

Software Inc., San Jose, CA, U.S.A.). Linear regression was also done with SYSTAT.

RESULTS AND DISCUSSION

The effects of ethrel and N addition were nearly always additive for LAI (Fig. 1) and plant dry matter (PDM) (Fig. 2) under both irrigated and non-irrigated conditions. The only time there was a statistical interaction between ethrel and N was for LAI at 100 DAS, when there was a slight decrease in LAI for the highest nitrogen/ethrel combination under non-irrigated conditions, and for PDM at 80 and 100 DAS under irrigated conditions. The highest LAI values were observed at pod maturity (100 DAS) under both irrigated and non-irrigated conditions. Depending upon the treatment, irrigation increased PDM by ~10-25%. In most cases, a pronounced linear response was apparent to nitrogen and ethrel, suggesting that mustard may have continued to respond to higher application rates of both.

Under irrigated conditions, there was no significant interaction between N and ethrel on mustard photosynthesis or stomatal conductance for either measurement date (Fig. 3). Photosynthesis increased from ~16.5 µmol m^{-2} s^{-1} at 0 kg N ha^{-1} to ~25.7 µmol m^{-2} s^{-1} at 80 kg N ha^{-1} on 80 DAS, and from ~15.5 µmol m^{-2} s^{-1} at 0 kg N ha^{-1} to ~24.5 µmol m^{-2} s^{-1} at 80 kg N ha^{-1} on 100 DAS. Ethrel increased photosynthesis on 80 DAS from ~19.5 µmol m^{-2} s^{-1} at 0 µl l^{-1} to ~24.7 µmol m^{-2} s^{-1} at 200 µl l^{-1}, and on 80 DAS from ~17.8 µmol m^{-2} s^{-1} at 0 µl l^{-1} to ~23.0 µmol m^{-2} s^{-1}. Both photosynthesis and conductance responded linearly to both N and ethrel application, suggesting as did LAI and PDM data (Fig. 1 and 2) that additional response might be expected from higher application rates.

The main effects of ethrel and nitrogen on [CO_2] under irrigated conditions are plotted separately for 80 DAS because there was no interaction between them, but all treatments were graphed together for 100 DAS because of a small but statistically significant interaction (Fig. 4). At 80 DAS, [CO_2] increased from ~275 to 360 µmol mol^{-1} as N application increased from 0 to 80 kg ha^{-1}, and from 305 to 345 µmol mol^{-1} as ethrel concentration increased from 0 to 200 µl l^{-1}. At 100 DAS, [CO_2] increased from its lowest concentration of ~245 µmol mol^{-1} for the 0 N, 0 ethrel treatment to ~375 µmol mol^{-1} for the 80 kg ha^{-1} N, 200 µl l^{-1}ethrel treatment. For both measurement dates, stomatal conductance increased from ~0.425 mol m^{-2} s^{-1} at 0 kg ha^{-1} N to ~0.530 mol m^{-2} s^{-1} at 80 kg ha^{-1} N, and from~0.455 mol m^{-2} s^{-1} at 0 µl l^{-1} ethrel to ~0.480 mol m^{-2} s^{-1} at 200 µl l^{-1}ethrel.

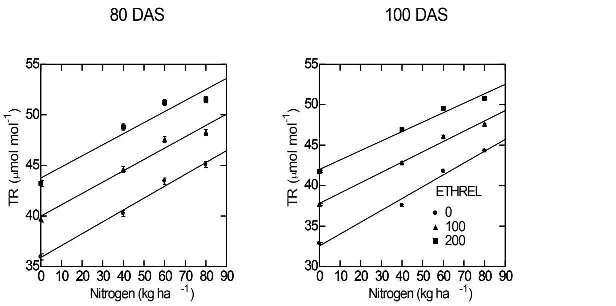

Figure 4. Transpiration ratio of irrigated Indian mustard as affected by nitrogen and ethrel application on two measurement dates.

Although in most cases the effects of ethrel and N were additive for photosynthesis and stomatal conductance, there was a statistical interaction of the two factors on TR (Fig. 4). Nitrogen alone increased TR from ~36 µmol mol^{-1} at 0 kg ha^{-1} N to ~45 µmol mol^{-1} at 80 kg ha^{-1} N at 80 DAS, and from ~32.5 µmol mol^{-1} at 0 kg ha^{-1} N to ~44 µmol mol^{-1} at 80 kg ha^{-1} N at 100 DAS. Ethrel alone increased TR from ~36 µmol mol^{-1} at 0 µl l^{-1} ethrel to ~43.5 µmol mol^{-1} at 200 µl l^{-1} ethrel at 80 DAS, and from ~32.5 µmol mol^{-1} at 0 µl l^{-1} ethrel to ~43.5 µmol mol^{-1} at 200 µl l^{-1} at 100 DAS. The highest TR values of ~51 µmol mol^{-1} were observed for the combined highest N and ethrel application rates. We arbitrarily fitted a linear model to the nitrogen response curve for the 200 µl l^{-1} ethrel treatment, but TR in that treatment appears to perhaps to be asymptotically approaching an upper value of ~53 µ mol mol^{-1}.

Based on the linear response of A to [CO_2], photosynthesis did not appear to be light or CO_2-saturated (Fig. 5). Despite N and ethrel effects on both [CO_2] and photosynthesis, the slope of their relation, CE, was relatively consistent constant among treatments (Fig. 4). For both 80 and 100 DAS, linear regression gave CE values indicated an increase of ~0.095 µmol m^{-2} s^{-1} per µmol mol^{-1} increase of [CO_2]. However, for both measurement dates, all points above the 0.95 confidence level of the regression curve belonged to the 200 µl l^{-1} ethrel treatment, and all those below to the 0 µl l^{-1} ethrel treatment. This implies that curves for ethrel-treated plants would have a higher y-intercept value, which has been associated with photorespiration, and a lower [CO_2] value for A=0, which has been associated with the CO_2 point (Robertson, 2007). This would be consistent with reports that ethrel affects several cellular, developmental and stress-response processes related to photosynthesis (Buehler *et al.,* 1978; Dolan, 1997; Esashi, 1991. Balota *et al.,* 2004).

Gas-exchange data for non-irrigated plots was fairly similar to those for irrigated plots, with some minor differences. In contrast to irrigated conditions, there was a nitrogen/ethrel interaction effect on photosynthesis on

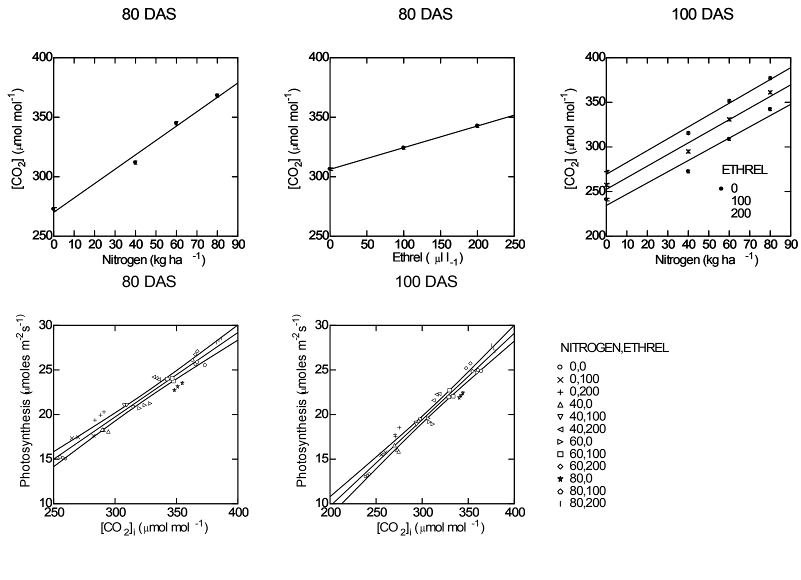

Figure 5. Effect of Nitrogen and ethrel application on internal CO_2 concentration ([CO_2]) and carboxylation efficiency (CE) of irrigated Indian mustard on two measurement dates. CE is the slope of photosynthesis (A) vs. [CO_2] relation. Regression equations are A=0.095[CO_2]-8.68 (r^2=0.945) for 80 DAS, and A=0.097[CO_2]-9.67 (r^2=0.957) for 100 DAS.

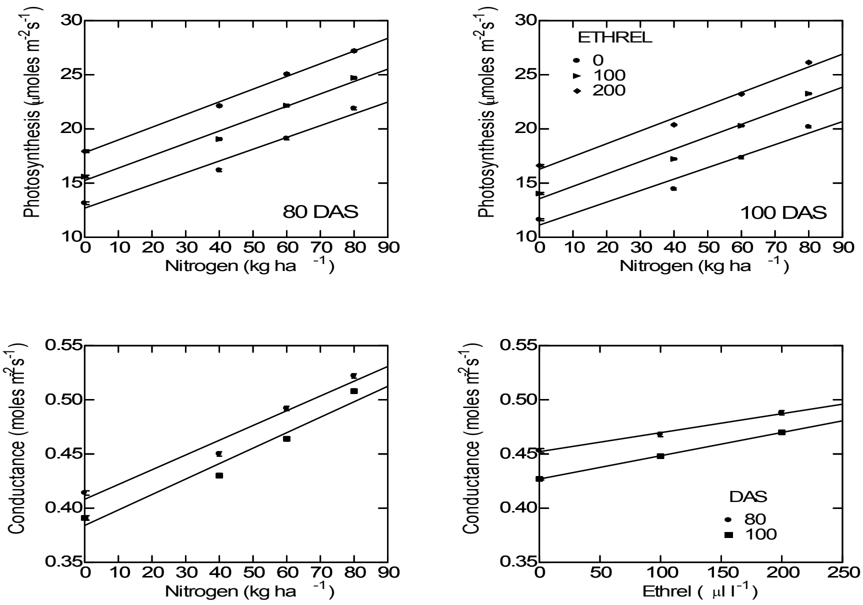

Figure 6. Photosynthesis and stomatal conductance as affected by nitrogen and ethrel application on two dates in non-irrigated Indian mustard.

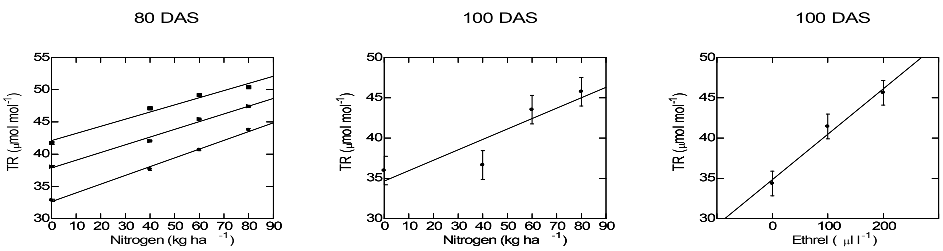

Figure 7. Transpiration ratio of non-irrigated Indian mustard as affected by nitrogen and ethrel application on two dates.

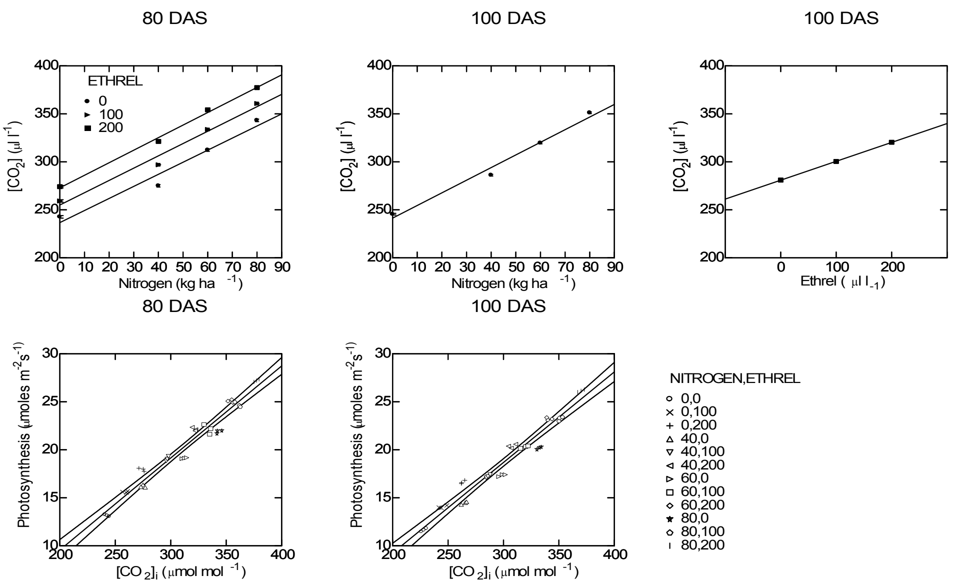

Figure 8. Effect of nitrogen and ethrel application on internal CO_2 concentration ($[CO_2]$) and carboxylation efficiency (CE) of non-irrigated Indian mustard on two measurement dates. CE is the slope of the photosynthesis (A) vs. $[CO_2]$ relation. Regression equations are $A=0.096[CO_2]-9.62$ ($r^2=0.956$) for 80 DAS, and $A=0.094[CO_2]-9.63$ ($r^2=0.953$) for 100 DAS.

both measurement days (Fig. 6). Nonetheless, the overall general response was approximately linear to both N and ethrel. For example, under both irrigated and non-irrigated experiments, photosynthesis increased by ~10 µmol m^{-2} s^{-1} when N application increased from 0 to 80 kg ha^{-1}, and stomatal conductance increased by ~10 mol m^{-2} s^{-1}. The maximum values for photosynthesis and conductance for "non-irrigated" plants were similar to those of irrigated plants, suggesting that there was little water stress at the time of measurement.

While ethrel and N had interactive effects on TR of irrigated mustard on both dates of measurement (Fig. 4), interactive effects were only significant at 80 DAS for non-irrigated mustard (Fig. 7). But overall trends were similar. For example, at 100 DAS, both N application alone and ethrel application alone increased TR from ~34 to ~46 µmol mol^{-1}, which are similar to values for irrigated mustard. Additionally, at the highest N (80 kg ha^{-1}) and ethrel (200 ml l^{-1}) applications, TR asymptotically approached a value of ~51 µmol mol^{-1}, similar to that for irrigated mustard. Similar TR values also suggest that non-irrigated mustard was under little mustard stress, as partial stomatal closure due to water stress is often associated with increased TR (Nobel, 1999; Unger *et al.,* 2006).

Finally, while N and ethrel had no interactive effects on [CO_2] of irrigated mustard on 80 DAS but did on 100 DAS, the opposite was true for non-irrigated mustard (Fig. 7). But as for other gas exchange traits, the interaction was small even though it was statistically significant. At 100 DAS, from 0 to 80 kg ha^{-1} N, [CO_2] increased from ~245 to ~350 µmol mol^{-1}, which is a lower range than values observed for irrigated mustard. From 0 to 200 µl l^{-1} ethrel, [CO_2] increased from 275 to 320 µmol mol^{-1} which is also a lower range than for irrigated mustard. However, the increase in [CO_2] from 245 µmol mol^{-1} in the 0 ethrel, 0 N treatment to 375 µmol mol^{-1} in the 80 kg ha^{-1} N, 200 µl l^{-1} ethrel treatment was very similar to increases in the irrigated mustard.

The linear response of A to [CO_2] in non-irrigated mustard was similar to irrigated mustard (Fig. 8). Once again, the slope of the relation between [CO_2] and photosynthesis, CE, was relatively consistent constant among treatments (Fig. 4), with the same value of ~0.095 µmol m^{-2} s^{-1} per µmol mol^{-1} increase of [CO_2]. However, once again all points above the 0.95 confidence level of the regression curve belonged to the 200 µl l^{-1} ethrel treatment, and all those below to the 0 µl l^{-1} ethrel treatment, once again suggesting a lower rate of respiration for the higher ethrel rates, and a lower CO_2 compensation point, consistent with reports that ethrel affects several processes related to photosynthesis.

The linear response that we observed of photosynthesis to N application is consistent with observation of many other authors, e.g. Sage *et al.* (1987), Evans (1989), Evans and Seemann (1989), and Nobel (1999). Ethrel spray at a concentration of 200 µl l^{-1} spray has been shown by other authors to increase LAI (Grewal and Kolar, 1990; Vansanford *et al.,* 1989; Khan, 1986) and photosynthetic rate (Suberahmanyam and Rathore, 1992 a; Pua and Chi, 1993; Khan *et al.* (2000); Khan *et al.* (2007); Mir *et al.* (2008); Mir *et al.* (2009 a,c); Lone *et al.* (2010) in combination with applied N, but to our knowledge this is the first report of increased photosynthesis at lower ethrel concentrations and independent of N application. Grewal and Kolar (1990) and Grewal *et al.* (1993) attributed increased photosynthesis from ethrel to increased chlorophyll per unit of leaf area, similar to the effects of N (Evans and Terrshimas, 1988, Sage *et al.,* 1990, Liu and Dickman, 1992). Khan *et al.* (2000) and Mir *et al.* (2009 b) found that plants sprayed with 200 µl l^{-1} ethrel accumulated higher K concentration in the plant and in stomatal guard cells, which they believed provided sufficient osmotic potential to increase turgor and therefore stomatal opening for CO_2 and water vapor diffusion (Jensen and Tophoj, 1985; Tanguilag *et al.,* 1987; Thakral *et al.,* 1997). The increase in TR with both N and ethrel application may due to increased binding of CO_2 and less binding of O_2 to the active site of Rubisco as CO_2 concentration increased. Since CE was relatively unchanged by N and ethrel application, there is little evidence that Rubisco activity increased due to ethrel or N application (Vanden Boagrad *et al.,* 1996 a,b), although nutrients have also been shown to influence TR (Payne *et al.,* 1992; Bruck *et al.,* 2000). Based on the trends in Figs.5 and 8 of ethrel-treated plants having higher y-intercepts and lower values of [CO_2] at y=0 irrespective of N application, the influence of ethrel on photorespiration and CO_2 compensation point merit further study.

In conclusion, our study provides evidence that ethrel spray application, either alone or in combination with N application, increases yield, LAI, and TR of Indian mustard, and may therefore constitute a potential management tool for increasing water use efficiency (WUE) in India and other water-limited regions of the world. Since ethrel increased WUE independent of N, further studies may be warranted using ethrel and other forms of N than urea to determine an optimally economic management of WUE.

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