CONTENTS

DISCUSSION

5.1 Introduction ........................................ 100
5.2 Comparison of cultivars ............................... 101
5.3 Effects of ethrel, a ethylene releasing compound .. 103
  5.3.1 Growth characteristics ............................ 103
  5.3.2 Physiological characteristics ..................... 106
  5.3.3 Biochemical characteristics ....................... 110
    5.3.3.1 Nitrogen use efficiency ..................... 112
  5.3.4 Yield characteristics ............................. 113
  5.3.5 Quality characteristics .......................... 115
5.4 Conclusion ........................................... 116
5.5 Future prospects ..................................... 116
CHAPTER-5

DISCUSSION

5.1 Introduction

The efforts to increase land productivity have increased due to expanding food demand because of population explosion and decreasing land-man ratio. In post-independent India a new surge began in the shape of well known ‘Green Revolution’, which became meaningless after five decades due to non-sustainable food production for the ever-increasing population. The task of Green Revolution was achieved through the use of chemical fertilizers, one of the several factors. With this chemical fertilizer tool, although food grain production in the country was achieved but its use also resulted in problems of soil salinization, ground water pollution, nutrient imbalance, emergence of new pest diseases and environmental degradation. The problem further aggravates by the constant rise in population, which is estimated that by 2020, India’s population is likely to be around 1.3 billion. In addition to this, All India Yield Indices of Major Crops is found to be stagnating or decreasing due to stagnating yield and decreasing land-man ratio. This has exerted a massive pressure for managing the available resources in such a way as to be benefited maximally through environmentally sustainable techniques. Nitrogen is one of the mineral nutrient elements of prime importance in increasing crop productivity. It has a well-established role in the crop plants and is taken up in large quantity by plants. It is estimated that terrestrial plants assimilate approximately 1.4 gigatons of nitrogen annually and about 90 to 95% of the total in form of mineral nitrogen (Marschner, 1986). Although more input of nitrogen increases growth and yield of the crops, but its excessive use causes environmental degradation phenomenon like eutrophication of the resources.

Therefore, an approach is to be explored which minimizes the use of nitrogen without decreasing the growth and yield of crops. In this context, use of plant hormones may prove its potential as it has been found to enhance
growth and productivity of the crop plants (Leopold and Kriedmann, 1979; Khan, 1996a; Singh, 1996; Khan et al., 1997; Khan et al., 2000; 2001). Of several naturally occurring phytohormones, ethylene influences about all aspects of plant growth and development (Mattoo and White, 1991; Abeles et al., 1992) as well as the induction of some plant defence responses (Boller, 1991). Ethylene produced in trace amounts elicits many physiological responses, acting at a concentration as low as 0.01μL/L (Reid, 1987). Ethylene releasing compounds are applied to cereal crops to prevent lodging, thereby reducing yield losses and deterioration of grain quality (Foster et al., 1991; Moes and Stobbe, 1991a, b), and to mustard for increasing yield (Khan, 1996b; Khan et al., 2000).

Keeping in view the above facts, five field trials were conducted on mustard (Brassica juncea L. Czem & Coss.) on the following lines:

1. To study the effect of ethrel spray on growth, physiological, biochemical, yield and quality characteristics of mustard cultivars under irrigated and non-irrigated conditions.

2. To study the effect of ethrel spray on mustard grown with nitrogen levels under irrigated and non-irrigated conditions for nitrogen use efficiency and yield of the crop.

3. To test the hypothesis that ethylene has a central role in mediating plant responses with the use of ethylene action inhibitor silver thiosulphate.

The results obtained in Chapter 4 are discussed below:

5.2 Comparison of Cultivars

In Experiment 1 and 2, Alankar and PBM16 cultivars of mustard (Brassica juncea L.) were used as experimental material. The rationale for use of the two cultivars is given in Materials and Methods section. The two cultivars responded similarly to ethrel application, giving maximal response with 200μL/L ethrel concentration. Both the cultivars showed inhibitory response to spray of ethrel higher than 200μL/L concentration.
For almost all growth characteristics, Alankar registered higher values than PBM16. Increase in inter-nodal length in Alankar was reflected in taller plants than PBM16. Leaf growth (area) in Alankar was more than PBM16 because of massive and irreversible expansion of daughter cell by meristematic divisions (Fig. 4). Also, Alankar showed higher inherent efficiency of retaining more moisture content in leaf tissues than PBM16. This was reflected in higher values for leaf fresh weight, leaf turgid weight and leaf relative water content (Tables 19, 47, 20, 48, 21, 49). Since leaf expansion is associated with trapping of solar energy and production of dry matter, therefore, Alankar registered higher dry weight (Fig. 6), and per cent distribution of dry weight towards leaf and pod (on emergence) was more in Alankar than PBM16. Thus, the efficiency of Alankar for translocating photoassimilates from leaf (source) to pod (sink) was greater than PBM16.

Alankar with large canopy structure, helped in efficiently receiving photosynthetically active radiation resulting in higher rate of net photosynthesis (Fig. 5) than PBM16. Higher leaf surface area as mentioned earlier was due to enhanced number of cell division and cell expansion. Increase in internal CO₂ concentration and stomatal conductance were reflected in more carboxylation efficiency, photosynthetic water use efficiency and water use efficiency in Alankar (Tables 26, 54, 27, 55, 28, 56).

The high efficiency of Alankar for conversion of biological matter to seed resulted in higher number of pods and seed yield (Fig. 7). The main contributing factor for increase in seed yield was pod number and seed number and 1000 seed weight was found almost equal in the two cultivars. Together with higher seed yield, oil yield of Alankar was more because of higher oil content than PBM16 (Tables 32, 60, 33, 61). It may be mentioned here that Alankar is the most suited cultivar grown in the region and surpassed other cultivars so far released. The cultivar was found equally suitable for irrigated and non-irrigated conditions.
Fig. 4. Effect of ethrel spray on leaf area of Alankar and PBM16 cultivars of mustard (*Brassica juncea* L.)
Fig. 5. Effect of ethrel spray on net photosynthetic rate of Alankar and PBM16 cultivars of mustard (Brassica juncea L.)
Fig. 6. Effect of ethrel spray on dry weight of Alankar and PBM16 cultivars of mustard (Brassica juncea L.)
Fig. 7. Effect of ethrel spray on seed yield of Alankar and PBM16 cultivars of mustard (*Brassica juncea* L.)
5.3 Effects of ethrel, a ethylene releasing compound

It is mentioned in Material and Methods section that Experiment 1 and 3 were conducted under irrigated conditions and Experiment 2 and 4 under non-irrigated conditions. The response of the crop to ethrel and nitrogen application was similar under these conditions of availability of water. This was possibly due to the fact that mustard is a harder crop as moisture availability is concerned and may be said as drought tolerant. Moreover, monsoon showers during the month of December, when there is a higher need of moisture for reproductive parts formation, meet the water requirement. Therefore, the crop behaved similarly under irrigated and non-irrigated conditions, although with a difference in the absolute values for the plant characteristics. In the following pages, attempt has been made to discuss the results obtained in experiments with an assumption that similar phenomenon occurred due to the effect of ethrel or nitrogen under irrigated and non-irrigated conditions.

5.3.1 Growth characteristics

Spray of 200μL/L ethrel increased growth characteristics maximally in Experiments 1-4. Further increase in ethrel concentration (400 to 800μL/L) proved inhibitory. Plant heights in Experiment 1 and 2 were increased by 14.9 and 16.2% respectively due to 200μL/L ethrel application. Such increasing effects of ethrel are basically on internode growth of plants (Sisler and Yang, 1984). Contrasting effects of ethylene sources on plant height have been reported in different plant species. For example, increased plant height in cauliflower (Jana and Kabir, 1991) due to ethrel application has been found. Contrarily, higher concentrations of ethrel/ethephon reduced plant height in sunflower (Sauerbrey et al., 1988), winter wheat (Van Sanford et al., 1989), radish (Vreugdenhil and Harro, 1989), lupin (Ortuno et al., 1993), arabidopsis seedlings (Smalle et al., 1997), barley (Sanvicente et al., 1999) linseed (Leitch and Kuat, 1999) and barley, oat and wheat (Rajala and Peltonen-Sainio, 2001). Wherever reduction of plant height was observed, this was due to use of higher
concentrations of ethrel. In this study also, higher concentrations of ethrel (400–800μL/L) reduced plant height. These conflicting observations involved several mechanisms. Firstly, ethylene is found to promote the reorientation of cortical microtubules, thereby possibly controlling elongation (Shibaoka, 1994). Secondly, a role for ethylene has been suggested in regulating the expression of cell wall peroxidase involved in the control of the wall extensibility and cell growth (Ridge and Osborne, 1971). Regulating the levels of peroxidase activity by suitable concentration of ethrel possibly influenced the direction of growth of active tissues and organs. This effect of ethrel led to the emergence and formation of leaves with enhanced total leaf area of plant. Lower concentration of ethrel (200μL/L) produced maximum leaf area, giving 27.0 and 35.7% increases over control in Experiment 1 and 2 respectively and higher concentration reduced the leaf area (Tables 8, 36, Fig 4). Similar observation has been reported by Lee and Reid (1997) in sunflower. Ivensh and Kreicbergs (1992), in cereal seedlings, reported that leaf emergence is associated with a peak of ethylene evolution. A similar phenomenon has been observed in Arabidopsis and burst of ethylene was accompanied by an increased expression of the ACC synthase gene 1, a gene suggested to be involved in the control of cell expansion (Rodrigues-Pousida et al., 1993). Thus, the induction of ethylene biosynthesis as in this may be associated with leaf emergence or in the control of cell expansion. This is also supported from the studies on ethylene-insensitive mutants, which have a large rosette than the wild type (Ecker, 1995) resulting from cell enlargement (Hua et al., 1995). Leaf fresh weight, leaf turgid weight, leaf relative water content and plant dry weight were significantly increased by 200μL/L ethrel (Tables 19, 47, 20, 48, 21, 49, 12, 40) because of observed increase in water use efficiency and photosynthetic water use efficiency in this ethrel treatment (Tables 27, 55, 28, 56). These aspects of efficiency parameters have been discussed in the following pages under a separate heading Physiological characteristics (Section 5.3.2).
It was found that the distribution of dry weight (on per cent basis) towards pods was higher in 200\mu L/L ethrel-sprayed plants. The dry weight thus accumulated was efficiently translocated to pod causing increase in per cent pod dry weight (Tables 18, 46). Linear regression analysis for various growth parameters with seed yield for Experiments 1–4 (Tables 167–178) also confirms the contribution of growth characteristics in yield.

Experiments 3 and 4 were conducted to assess the efficacy of foliar spray of ethrel on plants grown with nitrogen levels under irrigated (Experiment 3) and non-irrigated conditions (Experiment 4). Nitrogen is a major limiting nutrient for most plant species (Greenwood, 1982). Acquisition and assimilation of nitrogen is second in importance only to photosynthetic carbon assimilation for plant growth and development (Heickel, 1980; Araus et al., 1993; Anten et al., 1995; Arthamawar et al., 1996). Non-availability of nitrogen in Experiment 3 and 4 affected growth characteristics (Tables 72–86, 105–119). The best dose of nitrogen was 80kg N/ha and nitrogen level lower than this showed poor growth. At low (0 to 60kg N/ha) nitrogen level, the N absorbed by the roots was utilized for protein synthesis from reserve root carbohydrates and supply of N to the top of plants was limited affecting the growth of the shoot. The lesser N content present in the plant (Tables 98, 131, 99, 132) also show that at lower levels of basally applied N, the plant growth was poor and not benefited much.

In this study the growth response of the plant was maximum with application of 80kg N/ha together with spray of 200\mu L/L ethrel. The values obtained for this combination were maximum and higher than any other combinations of nitrogen and ethrel concentration (Fig. 8–15). The positive effect of 200\mu L/L ethrel spray together with 80kg N/ha is attributed to the changes in ethylene evolution (discussed in section 5.3.2). The ethylene evolution is reported to be increased by excessive ammonia accumulation (Corey et al., 1987; Arshad and Frenkenberger, 1991) and can be induced by
Fig. 8. Effect of ethrel spray on leaf area of cultivar Alankar of mustard (Brassica juncea L.) grown with different levels of nitrogen.
Fig. 9. Effect of ethrel spray on net photosynthetic rate of cultivar Alankar of mustard (Brassica juncea L.) grown with different levels of nitrogen.
Fig. 10. Effect of ethrel spray on dry weight of cultivar Alankar of mustard (Brassica juncea L.) grown with different levels of nitrogen.
Fig. 11. Effect of ethrel spray on 1-aminocyclopropane-1-carboxylic acid (ACC) of cultivar Alankar of mustard (Brassica juncea L.) grown with different levels of nitrogen.
Fig. 13. Effect of ethrel spray on ethylene evolution of cultivar Alankar of mustard (*Brassica juncea* L.) grown with different levels of nitrogen.
urea fertilization (Barker and Cory, 1990). Thus, the suitable N availability led to tissue ammonia accumulation and increased ethylene evolution, which triggered many physiological effects and enhanced growth of plants. The obtained dry mass increase in plant (Fig. 10) and its higher distribution in leaf than in stem and pod were because of better management of available photosynthates and source-sink relation. These results were observed in the form of per cent leaf, stem and pod dry weights (Tables 81, 114, 82, 115, 83, 116), specific leaf weight (Tables 76, 109) and enhanced leaf area (Tables 73, 106).

The contribution of leaf dry mass to total dry mass was found to be relatively higher for nitrogen dose 80kg N/ha and sprayed with 200μL/L ethrel than any other treatments. As the growth progressed pod dry mass increased. This was due to remobilization of dry matter from source (leaves) to sink (pods) under the influence of balanced nutrient regime. The balanced nutrient profile helped in maintaining maximum growth characteristics. Together with this, spraying of ethrel also helped the plant to produce signals for better utilization of nutrients (Graan and Boyer, 1990; Zhang and Davies, 1990; Davies and Zhang, 1991; Nilsen and Orcutt, 1996).

The altered structure of canopies in relation to leaf size and leaf area index were helpful in improving solar energy harvesting ability of the leaves as evident from increased plant dry matter production (Tables 77, 110; Fig. 10). Earlier reports from the author’s laboratory have shown that spray of 200μL/L of ethrel on mustard plants enhanced dry matter through increase in leaf area index (Khan, 1998; Khan et al., 2000) of mustard grown under non-irrigated conditions. These studies were, however, limited to the use of only a single ethrel concentration and few plant characteristics were studied.

5.3.2 Physiological characteristics

A variety of plant physiological processes at the biochemical and whole plant level are the driving force behind biomass production. Canopy
photosynthesis, integrated over a whole growing season, and whole plant pattern of photoassimilates partitioning is more important physiological determinant of biomass production and crop yield. Rate of photosynthesis is responsive to number of factors, like canopy structure, interception of solar radiation, stomatal conductance, carbon dioxide concentration and levels of ethylene. The degree to which photosynthesis responds to exogenous levels of ethylene is not well understood.

Rates of photosynthesis were increased by 23.7, 27.2, 27.7 and 31.3% with 200μL/L ethrel over control in Experiments 1, 2, 3 and 4 respectively. This ethrel concentration has shown to increase leaf area and leaf area index (discussed in Growth characteristics section). Further, ethrel induced leaf area showed a strong correlation with ethrel-induced photosynthesis (Fig. 21A). Ethrel-induced photosynthesis exhibited strong relationship with ethrel-induced carboxylation efficiency (Fig. 21B). Observations of Buehler et al. (1978); Grewal and Kolar (1990) and Grewal et al. (1993) have shown the increase in photosynthesis with ethrel due to increase in chlorophyll per unit of leaf area. The increase in photosynthesis with ethrel has also been reported by Subrahmanyam and Rathore (1992a), Pua and Chi (1993) and Khan et al. (2000). Retaining higher leaf area index in ethrel-treated plants helped in an increase in photosynthesis. However, at 100d, inspite of increase in leaf area, the photosynthesis rate declined because of mutual shading of leaves. Ethrel concentrations higher than 200μL/L in Experiments 1 and 2 decreased the photosynthesis. Inhibition of photosynthesis in these experiments was because of higher amount of ethylene evolution due to 400–600μL/L ethrel application. Such conditions of inhibition of photosynthesis by ethylene have also been suggested by Kays and Pallas Jr (1980) and Rajala and Peltonen-Sainio (2001). This is possible that threshold value for ethylene with 200μL/L ethrel was comparable to that which elicits the ethylene induced hormonal responses.
In the reported observation, a correlation between ethrel-induced photosynthesis and stomatal conductance (Fig. 22B) suggests that differences in stomatal conductance contributed significantly to the variation in photosynthesis. Analysis of A/Ci values (carboxylation efficiency) also suggests non-stomatal limitation to photosynthesis. The correlation studies showed that variation in photosynthetic capacity (carboxylation efficiency) accounted for the differences in photosynthetic rate (Fig. 21B) more so than differences in stomatal limitation. This view is again strengthened by the observed relationship of carboxylation efficiency with photosynthetic water use efficiency (Fig. 22A). Vanden Boogard et al. (1995) reported a correlation between photosynthetic water use efficiency with rubisco activity and photosynthetic water use efficiency as a measure of rubisco activity. Water use efficiency measured from the data on biomass and transpiration rate showed that it was increased maximally with 200μL/L ethrel. The effect of ethrel on water use efficiency was through maintenance of turgor potential and stomatal movement. At maturity, high water use efficiency was caused by lower transpiration rate associated with a high leaf area per unit plant weight, also observed by Vanden Boogard (1996a, b). Similarly, nutrients have also been shown to have a role in water use efficiency of plants (Payne et al., 1992; Bruck et al., 2000). Ethylene-enhanced net photosynthetic rate helped in an increase in plant dry weight, therefore photosynthetic water use efficiency showed relationship with plant dry weight (Fig. 23A–B).

Contrasting reports on exogenously applied ethylene sources on transpiration rate appear in the literature. Transpiration is reportedly non responsive to ethylene in both herbaceous and woody species (Aharnoi, 1978; Pallaghy and Raichke, 1972; Johnson, 1984). In contrast, significant responses in transpiration are shown in several herbaceous species (Govindarajan and Pooviah, 1982; Kays and Pallas Jr, 1980; Pallas Jr and Kays, 1982). Increase in carboxylation efficiency and photosynthesis was related to the increase in
intercellular carbon dioxide concentration by ethrel. At later maturity stages decrease in photosynthesis was related to resistance to CO₂ diffusion and was stomatal limitation. Moreover, Mattoo and White (1991) reported that CO₂ could promote or inhibit ethylene evolution, depending on the tissue concentration. On the same lines Dharan et al. (1981), Kao and Yang (1982) and Grodzinski et al. (1982) reasoned that inhibition of ethylene evolution resulted from a decrease in internal CO₂ concentration and regulated photosynthesis. In this study, 200µL/L ethrel significantly favoured ethylene evolution that fundamentally influenced the central regulatory system. Further, increase in ethrel concentration (400 or 600µL/L) possibly promoted ethylene evolution to inhibit the physiological and biochemical processes. It seems probable that there is some requirement of ethylene for optimum response. Low and high concentrations represent the two ends of an optimum curve, like that for many hormones, promoting at low concentration and inhibiting at high. There is, thus, an interrelation between CO₂ metabolism and ethylene evolution, which controls other biochemical and physiological changes. Ethylene evolution was linked with activity of ACC oxidase. It was possible that stimulatory effect of ethrel on ACC oxidase might involved not only on enhancement of the activity but also its synthesis and degradation, thereby, resulting in higher ACC dependent ethylene evolution. It may be emphasized that ethrel application promoted ACC dependent ethylene evolution (Tables 96, 129).

Mattoo et al. (1997) observed that autocatalytic effect of ethylene was physiologically achieved by an enhancement in the activities of both ACC synthase and ACC oxidase. It may be reiterated that the high conversion rate of ACC to ethylene permitted to influence the fundamental control system. The concentration of ethylene with spray of 200µL/L ethrel showed most suitable concentration for plant growth and development. The concentration of ethylene
with 100 μL/L ethrel remained below a critical threshold and higher concentration than 200 μL/L possibly exceeded threshold values of ethylene.

In Experiment 5, application of 200 μL/L ethrel was used to promote ethylene evolution together with 1 mM of silver thiosulphate (STS) to block the action of ethylene. The objective was to test the hypothesis that ethylene has a central role in mediating plant responses, reported in Experiment 1–4.

The hypothesis that ethylene was responsible for the observed enhancement in plant characteristics with 200 μL/L ethrel was substantiated by this experiment (Experiment 5) where ethrel and STS were supplied.

It was discussed in preceding pages that 200 μL/L ethrel increased ethylene evolution to optimum concentration causing maximum increase in growth and physiological characteristics. Limiting the action of ethylene with STS reduced the plant characteristics. Thus, a reduction of growth and physiological rates was achieved by blocking the ethylene action (Tables 147–166). As STS-treated plants exhibited values lower than the plants treated with water (control), it is possible that the intrinsic ethylene biosynthesis was affected (Fig. 16–18).

5.3.3 Biochemical characteristics

Nitrogen related characteristics were studied in Experiment 3 and 4. Nitrogen status of a plant in a particular spatial or temporal zone exhibits the nutritional requirement of the crop during that period in that part of plant phase. In different water availability regime, the nutrient availability differed, and plant respond to the changing environment by proliferating the roots, which develop deep into the soil layers in search of nutrients and water. Thus, the amounts of nitrogen that can be transported to the shoots depend on the capacity of the roots to absorb nitrogen from the soil and transport them to the transpirational stream. The concentration of nitrogen in growth media exerts a considerable influence on growth and mineral composition of crop plants (Kurvitis and Kirkby, 1980; Gashew and Mugwira, 1981; Ansari, 1990;
Fig. 16. Effect of ethrel spray (E: 0 or 200 µL/L) or silver thiosulphate (S: 1mM) on leaf area of Alankar cultivar of mustard (Brassica juncea L.)
Fig. 17. Effect of ethrel spray (E: 0 or 200 µL/L) or silver thiosulphate (S: 1mM) on net photosynthetic rate of Alankar cultivar of mustard (Brassica juncea L.)
Fig. 18. Effect of ethrel spray (E: 0 or 200 µL/L) or silver thiosulphate (S: 1mM) on dry weight of Alankar cultivar of mustard (*Brassica juncea* L.)
Fig. 19. Effect of ethrel spray (E: 0 or 200 μL/L) or silver thiosulphate (S: 1mM) on seed yield of Alankar cultivar of mustard (*Brassica juncea* L.)
Jeschke et al., 1992) and affects the relation uptake of cations and anions by plants (Kirkby, 1981; Lovatt, 1986).

In the present study, nitrogen content was found to increase with increasing doses of nitrogen. At initial growth stage, nitrogen content increased by 8.6 and 11.5% in Experiment 3 and Experiment 4 respectively with 80kg N/ha applied as compared to 0kg N/ha. The increase in the nitrogen content at early growth stage coincides with the increasing rate of nitrate reductase activity (Tables 97, 130). Nitrogen accumulation was found to be well coordinated with supply of nitrogen. It is believed that availability of a given nutrient may interact with the uptake of other nutrients and thus making the uptake more complex (Amoruwa et al., 1987; Marschner, 1986). The response of accumulation to nitrogen deprivation (0kg N/ha) was negligible for nitrogen content at 80d sampling. The possible explanations are that there was a tendency for the root system to have more proliferation in the soil to meet the plant nutrient demand or it became larger relative to the shoot during nitrogen deprivation. Ethylene regulated differentiation in trachied (Zobel and Roberts, 1978), root elongation (Koning and Jackson, 1979), adventitious root formation (Liu et al., 1990) and root/shoot ratio (Rajala and Peltonen-Sainio, 2001) have been observed. In a study on mustard, Khan et al. (1996a) reported increase in nitrate reductase activity with GA₃ application. Enhancement of nitrate reductase activity by GA₃ or GA₃ + cytokinin in tobacco leaves has been observed by Roth-Bejerano and Lips (1970). The accumulation of nitrogen was associated with increased photosynthetic rate, which resulted in higher dry matter accumulation. Together with this other possibility is of accumulation of other cations (preferably potassium), which helped in maintaining the rate of photosynthesis by improving the relative water content of the leaf through osmotic adjustment. In a study on mustard, Khan et al. (2000) reported that potassium accumulation increased with nitrogen supply, which caused increase in stomatal conductance, photosynthetic rate and dry matter accumulation.
Regarding this trial, it has been reported that this was a preliminary trial. Accumulation of potassium in guard cells provides the necessary amount of solute for developing the water potential gradient required for water movement into the guard cells for stomatal opening necessary for photosynthesis (Jensen and Tophøj, 1985; Tanguilag et al., 1987 and Thakral et al., 1997). The findings also encourage the view that there is some form of co-regulating of the nutrients accumulation in mustard, which may akin to that described for their accumulation (Vyas et al., 1995; Khan et al., 1997; Zaman and Choudhri, 1998).

5.3.3.1 Nitrogen use efficiency

Nitrogen use efficiency has been defined as seed yield with per unit of available N (soil N + fertilizer N). It has two components, nitrogen uptake efficiency (plant N per unit of soil + fertilizer N) and nitrogen utilization efficiency (seed yield per unit of N in plant). A product of these two components results in nitrogen use efficiency (Moll et al., 1982; Prasad et al., 2000). In the present research, maximal use efficiency of N was found with 200μL/L ethrel and 80kg N/ha (Tables 179–184; Fig. 20). Nitrogen applied in sub-optimal level (40 or 60kg N/ha), the use efficiency was less. At optimal N (80kg N/ha) use efficiency was higher and application of 200μL/L ethrel on plants receiving 80kg N/ha enhanced use efficiency further. It may be mentioned here that treatment 80kg N/ha and 200μL/L ethrel enhanced top growth of plants maximally, which puts demand on soil N to meet the growing need of the shoot. Increased nitrate reductase activity in leaves with 80kg N/ha and ethrel (200μL/L) also supports the incorporation of nitrogen in the shoot. Moreover, plant N was translocated to seed N reflecting more of its translocation on application of ethrel finally increasing the seed yield. Moreover, nitrogen application results in enhanced ethylene evolution, which is reported to increase by excessive ammonia accumulation (Corey et al., 1987; Arshad and Frenkenberger, 1991) and can be induced by urea fertilization.
Fig. 20. Effect of ethrel spray on N uptake, N use and N utilization efficiency of cultivar Alankar of mustard (Brassica juncea L.) grown with different levels of nitrogen.
Fig. 21. The relationship between (A) ethrel induced changes in leaf area and ethrel induced changes in net photosynthetic rate ($P_N$) and (B) ethrel induced changes in carboxylation efficiency (CE) and ethrel induced changes in net photosynthetic rate ($P_N$). The per cent increase in values from 200 µL/L ethrel over control of the cultivars PBM16 and Alankar in Experiments 1 and 2 were used for these correlation studies. ** significant at $P = 0.01$. 
Fig. 22. The relationship between (A) ethrel induced changes in photosynthetic water use efficiency (PWUE) and ethrel induced changes in carboxylation efficiency (CE) and (B) ethrel induced changes in stomatal conductance ($g_s$) and ethrel induced changes in net photosynthetic rate ($P_N$). The per cent increase in values from 200 μL/L ethrel over control of the cultivars PBM16 and Alankar in Experiments 1 and 2 were used for these correlation studies. * significant at $P = 0.05$, ** $P = 0.01$. 
Fig. 23. The relationship between (A) ethrel induced changes in dry mass (DM) and ethrel induced changes in net photosynthetic rate ($P_N$) and (B) ethrel induced changes in dry mass (DM) and ethrel induced changes in photosynthetic water use efficiency (PWUE). The per cent increase in values from 200 μL/L ethrel over control of the cultivars PBM16 and Alankar in Experiments 1 and 2 were used for these correlation studies. ** significant at $P = 0.01$. 

$r = 0.891^{**}$

$r = 0.874^{**}$
Availability of N leads to tissue ammonia accumulation and increased ethylene formation (Feng and Barker, 1992, 1993). Nitrogen use efficiency with fertilizer N has also been reported by Gajri et al. (1993), Feiz et al. (1994), Gardner et al. (1994) and Sowers et al. (1994). The effect of GA₃ on the nitrogen use efficiency has been reported from the author’s laboratory but the effect of ethrel on nitrogen use efficiency has not been reported earlier.

5.3.4 Yield characteristics

Seed yield of a crop may depend on the vegetative growth of the crop because photosynthesizing sites have determinant role in producing the photosynthates. Yield is the final manifestation of several intricate morphological and physiological traits that initiate at germination and terminates at harvest. Yield is dependent on the maintenance of an array of metabolic processes including photosynthesis and hormonal status. There is increasing evidence for metabolic growth regulator effects on various crops.

In fact, leaf size constitutes the canopy structure and is actively involved in interception of solar radiation and in contribution of photoassimilates to the developing pods. Two sequential steps are necessary for a mustard plant to produce pods, a sink of pollinated pods capable of further development must be created and this must be supplied with photosynthates over the subsequent period of development. Thus, seed yield at harvest may be determined either by the seed capacity established at pollination or by the quantity of photosynthate made available between pollination and maturity. There is a positive feed back cycle between photosynthetic products, growth and leaf area. The effectiveness of this cycle is increased under favourable conditions. The CO₂ enrichment through increase in leaf canopy may increase plant growth by stimulating photosynthetic rate and thereby accelerating the cycle (Rogers et al., 1996).

The aim of the Experiments 1 and 2 was find out suitable concentration of ethrel spray on mustard and thereby, if possible to increase seed yield. It was
found that 200μL/L ethrel spray contributed for enhancement of pod number by 11.9 and 14.6% over control in Experiment 1 and 2 respectively (Tables 31, 59). Primarily, the pod number enhancement contributed to increase of 32.3 and 40.5% seed yield in these experiments. Moreover, increase in number of seeds per pod (14.5% in Experiment 1 and 11.8% in Experiment 2) and slight increase in 1000 seed weight also helped in increasing the seed yield. Increase in seed yield resulted in an increase of 38.8 and 48.3% oil yield in Experiment 1 and Experiment 2 respectively (Tables 32, 60, 33, 61).

In Experiment 3 and 4, it was found that there was differential response of 200μL/L ethrel spray for the yield when the plants were grown with nitrogen deficient (0kg N/ha) to sufficient nitrogen (80kg N/ha). This combination of treatments improved the seed yield by increasing the proportion of the reproductive tissues (inflorescence and/or pods) to total plant dry matter (biological yield). There was 8.9 and 10.5% increase in pod number and 21.0 and 26.4% increase in seed yield in Experiments 3 and 4 respectively (Tables 100, 133, 101, 134). The increase in yield was directly related to the increase in sink capacity. Ethrel application enhanced the incorporation of soil nitrogen (Section 5.3.3), which was manifested in the better vegetative growth (Section 5.3.1) and better partitioning into reproductive parts, evident from increased pod number and harvest index. Linear regression analysis for growth and yield attributing characteristics with seed yield in Experiment 1–4 also confirms the view (Tables 167–178). The beneficial effects of ethrel have also been studied by many research scientists including Dah nous et al. (1982), Leary and Oplinger (1983), Wiersma et al. (1986), Joshi et al. (1987), Singh et al. (1987), Ramos et al. (1989), Singh and Kumar (1991), Bulman and Smith (1993a, b), Grewal et al. (1993) and Kasele et al. (1995) in several crop species.

Nitrogen yield potential provides a measure of nitrogen partitioning potential from vegetative parts to seeds. In Experiment 3 and 4, nitrogen yield potential was enhanced by 32.3 and 35.4% respectively due to 200μL/L ethrel
spray (Tables 102, 135). Increase in seed yield has also resulted in increase in oil yield by 30.6 and 36.7% in Experiments 3 and 4 respectively.

5.3.5 Quality characteristics

For assessing oil quality, oil content, acid, iodine and saponification values of oil were determined. It may be added that low acid and iodine values are considered good for oil quality and denote good keeping and easy hydrogenation. High saponification value is good for digestibility and soap making quality.

Ethrel application increased the oil content. The ethrel (ethylene) might have increased the supply carbon to lipid synthesis by induction of a specific transporter and played a role in determining the amount of oil. There is possibility of ethrel (ethylene) affecting acetyl CoA carboxylase. Other workers have also shown that an additional carbon input to lipid synthesis contribute to the oil formation (Post-Beittenmiller et al., 1992; Hunter and Ohlrogge, 1998; Kozaki and Sasaki, 1999; Sawage and Ohlrogge, 1999). Leitch and Kuat (1999) demonstrated role of ethylene applied as ethrel in fatty acid synthesis, increasing the oleic acid in linseed oil. In the present study increase in iodine and saponification values indirectly provide evidence of increase in short chain unsaturated fatty acid. However, the possibility of ethylene affecting acetyl CoA carboxylase and to the flux of carbon to oil in developing embryos of oilseed modifying fatty acid composition needs to be investigated.

Application of nitrogen had not any significant effect in altering oil content of the seed in the present study. However, reports are available for reduced oil content due to N fertilization (Smith et al., 1988; Gendy and Marquard, 1989; Khan et al., 1990; Pinkerton, 1991; Samiullah et al., 1991; Asare and Scarisbrick, 1995). Inspite of non-significant effect on oil content, oil yield was significantly enhanced by ethrel (200μL/L) and 80kg N/ha due to increase in seed yield.
5.4 Conclusion

This has been shown in the study, reported in the thesis, that manipulating the quantity of ethylene alters the leaf expansion and total plant leaf area. Specifically, low concentration of ethylene releasing compound ethrel (200μL/L) stimulated leaf area and higher concentration of ethrel reduced the leaf area. Other effects of ethylene were documented in stomatal and non-stomatal limitations to carboxylation and photosynthesis, which resulted in higher biomass production. In experiments conducted with doses of nitrogen and ethrel, the stimulatory effects of ethylene on nitrate reductase activity and plant nitrogen use efficiency were seen. Enhanced plant top growth due to ethrel (200μL/L) exerted demand on the root for higher utilization of available soil-N.

Nitrogen use efficiency became high and nitrogen accumulation enhanced in the plant above ground part. Nitrogen was incorporated into macromolecules and reflected in higher dry matter accumulation. Efficient partitioning of dry matter resulted in increased seed yield and seed yield potential of the plants.

5.5 Future prospects

The role of plant hormones in growth and development is well known. However, the action of hormones is brought about by producing its effect on other hormones and also controlling the biosynthesis of other hormones. For example, it has been shown that ethylene inhibits GA induced elongation and GA reverses the effects of ethylene. Similarly, the action of ABA inhibits endogenous production of ethylene, but not that formed from ACC and regulates stomatal movements. ABA inhibits the formation of ethylene that is induced by IAA. These aspects of hormonal control over the action of ethylene need to be investigated. It is proposed to work out the level of GA and ABA to have in depth understanding on the regulatory role of ethylene in photosynthesis and biomass accumulation.