Growth and Metabolism of Mustard (*Brassica juncea*)

following Defoliation and Nitrogen Treatment

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The present thesis comprises six chapters. In Chapter 1, the importance of the problem and justification for the study undertaken were emphasized.

Chapter 2 is the review of literature. It deals with the relevant literature on the aspects of defoliation and nitrogen. The chapter has been divided in sections and sub-sections for better understanding of the results of other researchers in this field of study.

Chapter 3 describes the details of the material used in the study and methodology adopted to determine various characteristics recorded in the three experiments. Relevant information on the experimental design, agro-climatic conditions, location of the study and environmental conditions during the data sampling times has been mentioned.

Chapter 4 includes the results on crop responses to treatments in
the three experiments. The results have been statistical analyzed and significance at $P<0.05$ was determined. The treatment means have been separated by the calculation of least significant difference. The results found significant have been given in this chapter.

In Chapter 5, significant results have been discussed in the light of observations recorded and supported with the earlier findings, if available on the subject. Possible explanations of the data obtained have also been included to reach a conclusion. This chapter was followed by bibliography.

In the following pages a brief account of the importance of the study undertaken, results obtained, conclusion and proposed future research are given.

**Importance of the study undertaken**

Mustard (*Brassica juncea* L. Czern & Coss.) is an important oilseed crop grown in the tropical and sub-tropical regions of the world. It is cultivated for human consumption as condiment and spices, as fodder and for seed. *Brassica* accounts for approximately 10% of the total world oilseed production and 15% of the total vegetable oil production (Downey and Rimmer, 1993). Mustard plant is characterized by large number of broad, oblong-shaped leaves in lower layers (Weiss, 1983). Such leaves contribute to the development of supra-optimal leaf area with accompanying self shading and by other leaves within the
plant axis. They are poorly illuminated, therefore, are less efficient in photosynthate production. Later, at maturity, these leaves are shed (Plate 1A, B). It has been reported earlier that defoliation brings about changes in growth, photosynthesis and carbon reserve remobilization (Ericsson et al., 1980; Mc Naughton, 1983; Foggo, 1996; Bruening and Egli, 1999; Collin et al., 2000). In Northern India defoliation has been practiced from time immemorial to use leaves for condiment and spices, and for fodder without knowing the effect on the physiology and productivity of the crop. Earlier research has shown that removal of such leaves modulates growth and assimilates balance in mustard (Khan, 2002; 2003; Khan et al., 2002a, b). In the present study the effect of defoliation of 50% leaves on lower layers of plant either at 40 (pre-flowering) or 60 (post-flowering) days after sowing was studied on growth, physiology and assimilate balance of the plant. Moreover, studies were also conducted to find out the effect of defoliation of such 50% leaves at 40 days after sowing, on the basis of comparison of stage of defoliation, of the plants treated with single or split application of soil-applied N. Experiments were conducted in completely randomized design.

Determinations and Results

Standard plant cultivation practices and analyses procedures were adopted. Plants were sampled at 40 (pre-flowering), 60 (post-flowering),
Plate 1: (A) mustard field and (B) Leaves in lower layers of axis of mustard (*Brassica juncea* L.).
80 (pod-fill) and 100 (pod-maturity) days after sowing to record growth, photosynthetic and biochemical characteristics, N assimilation and ethylene biosynthesis. At 120 days after sowing (maturity), yield characteristics were noted. Growth characteristics were: leaf number per plant, leaf area per plant, fresh and dry masses per plant, distribution of dry mass in leaf, stem and pod, leaf mass ratio, stem mass ratio, leaf area ratio, crop growth rate, relative growth rate and unit leaf rate. Photosynthetic characteristics were: chlorophyll content, carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO₂ concentration and intrinsic water-use efficiency. N assimilation was assessed in terms of the activities of nitrate reductase, nitrite reductase and glutamine synthetase, N concentration and content. Biochemical characteristics were: Plant carbon, carbohydrate and protein concentrations and carbon: nitrogen ratio. ACC synthase activity and ethylene evolution were noted for ethylene biosynthesis. At harvest, yield characteristics were: pod number per plant, seed number per pod, 1000 seed weight, seed yield, biological yield and harvest index.

Experiment 1 (2000-2001) was conducted to assess the effects of removal of 50% leaves on lower layers of mustard (Brassica juncea L.) plant axis either at 40 (pre-flowering stage) or 60 (post-flowering stage) DAS on growth, photosynthetic and biochemical characteristics, N assimilation, ethylene biosynthesis at 40 (pre-flowering). 60 (post-
flowering), 80 (pod-fill) and 100 (pod-maturity) DAS. Yield characteristics were determined at 120 (harvest) DAS. The design of the experiment was randomized block design. Growth characteristics were: leaf number per plant, leaf area per plant, fresh and dry masses per plant, distribution of dry mass in leaf, stem and pod, leaf mass ratio, stem mass ratio, leaf area ratio, crop growth rate, relative growth rate and unit leaf rate. Photosynthetic characteristics were: chlorophyll content, carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO₂ concentration and intrinsic water-use efficiency. N assimilation was assessed in terms of the activities of nitrate reductase, nitrite reductase and glutamine synthetase, concentration and content of N. Biochemical characteristics were: Plant carbon, carbohydrate and protein concentrations and carbon: nitrogen ratio. ACC synthase activity and ethylene evolution were noted for ethylene biosynthesis. At harvest, yield characteristics were: pod number per plant, seed number per pod, 1000 seed weight, seed yield, biological yield and harvest index.

Defoliation at 40DAS was found superior than 60DAS time of defoliation or no defoliation control, and enhanced the characteristics maximally. The number of leaves and leaf area increased following defoliation, maximum being with 40DAS time of defoliation because of emergence of new leaves on the upper axis of the plant. The plants
defoliated at 60DAS showed lesser increase in leaf number. Thus, the increase in photosynthesizing surface area of high photosynthetic capacity young leaves led to increase in carboxylation rate (carbonic anhydrase activity and intrinsic water-use efficiency). This resulted in increased plant dry mass. The other effects were manifestation of more N assimilation due to increased demand of young leaves enhancing photosynthetic rate. The growth of leaves was found associated with a optimal ethylene requirement, which was fulfilled by the activity of ACC synthase activity. The greatest beneficial effects of defoliation at 40DAS over 60DAS or no defoliation were due to improved source-sink relationship, as seen in increase in per cent pod dry mass and seed yield in plants defoliated at 40DAS. The defoliation at 60DAS also improved the characteristics but to a lesser degree than defoliation at 40DAS.

Experiment 2 (2001-2002) was conducted based on the findings of Experiment 1. The aim of the experiment was to study the effect of defoliation of 50% leaves on lower layers at 40DAS (based on the findings of Experiment 1) in mustard (Brassica juncea L.) plants treated with soil-applied 0, 60, 100 and 150kg N/ha on the plant characteristics at the sampling times similar to that described for Experiment 1. The design of the experiment was randomized block design.

The defoliated plants treated with 150kg N/ha registered significantly superior values compared to any other N application rate.
Growth, photosynthetic and biochemical characteristics, N assimilation, ethylene biosynthesis and yield characteristics were maximal when the defoliation was done and plants received 150kg N/ha. N application at 150kg N/ha met the plants requirement after defoliation to grow faster and assimilate N at increased rates and enhance the characteristics maximally. Any other N application rate did not suffice the plants requirement to grow after defoliation and, therefore, was not as effective as 150kg N/ha in increasing the plant characteristics. The overall good performance of defoliated plants treated with 150kg N/ha in growth and photosynthetic characteristics, N assimilation and improved sink capacity led to increase in seed yield.

Experiment 3 (2002-2003) was conducted to assess the effects of defoliation of 50% lower leaves on mustard (Brassica juncea L.) plants treated with N as single or split doses. Nitrogen was applied as single dose of 150kg N/ha or this was split in two doses, i.e. 100kg N at the time of sowing and 50kg N/ha as top dressing at 40DAS [BN100+N50(40d)] or 60DAS [BN100+N50(60d)]; 75kg N/ha at the time of sowing and 75kg N/ha as top dressing at 40DAS (BN75+N75(40d)) or 60DAS [BN75+N75(60d)]. The design of the experiment, determinations and sampling times were similar as in earlier experiments. N application in split dose of 150kg N/ha as 100kg N/ha at the time of sowing and 50kg N/ha at 60DAS [BN100+N50(60d)] proved
superior over single N application or any other N rate applied as split dose at 40 or 60DAS. N applied as [BN100+N50(60d)] to defoliated plants increased growth, photosynthetic and biochemical characteristics, N assimilation maximally that led to maximal increase in seed yield. The maximal response of defoliated plants to supplemental soil N treatment at 50kg N/ha at 60DAS was due to the fact that at post-flowering stage (60DAS) plants require an additional N for the sink strength and increased plant metabolism. It also avoided loss of N through leaching and volatilization processes that may occur if the N was given at 40DAS because this is the time of irrigation in mustard. Plants treated with one-time N (150kg N/ha) at the time of sowing did not utilize N beyond its requirement and, therefore, N possibly remain in the soil and might have lost. Similarly, split doses of N (100kg N/ha at the time of sowing and 50kg N/ha at 40DAS or 75kg N/ha at the time of sowing and 75kg N/ha at 40 or 60DAS) also proved ineffective in producing maximum effects.

Defoliation and N application significantly affected yield characteristics. Number of pods per plant, number of seeds per pod, 1000-seed weight, seed yield, biological yield and harvest index were found greatest in defoliated plants treated with BN100+N50 (60d).

The present chapter is followed by an up-to-date bibliography of the literature cited in the text.
Conclusion

From the overall study following conclusions may be drawn:

1. Defoliation of 50% leaves on lower layers of mustard plant at 40DAS increased plant dry mass and seed yield in comparison to control due to compensatory regrowth capacity of the plant.

2. A lesser decrease in plant dry mass and seed yield occurred when defoliation was done at 60DAS.

3. Growth, photosynthetic, biochemical and yield characteristics were found higher in plants defoliated at 40DAS.

4. Defoliation at 40DAS increased N assimilation maximally.

5. Plants responded better to soil-applied N after defoliation.

6. The amount and timing of N application after defoliation has an important influence in augment growth and productivity of crop.

7. Single application of soil-applied N (150kg N/ha) proved inferior in comparison to split application of N to soil (100kg N/ha at the time of sowing and 50kg N/ha at 60DAS).

8. Split N fertilization rate ((100kg N/ha at the time of sowing and 50kg N/ha at 60DAS) enhanced growth, photosynthetic, biochemical and yield characteristics maximally.

9. N assimilation increased maximally in plants treated with 100kg N/ha at the time of sowing and 50kg N/ha at 60DAS.

10. The management of regrowth after defoliation was possibly
brought about by the signals produced in plants as ethylene.

11. The role of ethylene has been suggested in every aspect of plant growth and development, besides its involvement in producing signals to control abscission. It may be researched in future to correlate mechanical defoliation with abscission.

12. Efforts should be focused on finding a strategy that causes early senescence of leaves on the lower layers.

13. An ideotype that shed lower leaves at early stage of plant cycle may help in maximum use of soil-applied N and break the yield plateau in mustard.

Future Research

Defoliation in mustard (Brassica juncea) is an age-old practice. The leaves of the plants are in use for human consumption as a condiment and spices. The implications of defoliation on overall performance of the crop were not known. The study reported in the thesis shows that defoliation of 50% leaves in the lower layers at early stage of plant growth (pre-flowering stage) is benefited for crop growth and productivity. Moreover, defoliated plants make efficient use of soil-applied nitrogen. The physiological phenomenon underlying the control of regrowth of plants after defoliation was not known. However, the factors that control abscission in plants are well documented. The approach to link mechanical defoliation with abscission would be
advantageous in the control of defoliation in mustard. The identification of molecule that provides signals for the defoliation would have an impact on the development of ideotype that losses lower leaves at early stage of development. In this regard it may be said that growth regulators are important messengers in deciphering the described results. For example, ethylene is a gaseous growth hormone involved in a diverse array of cellular developmental and stress-related processes in plants. Future studies that utilize modern technologies to answer age-old questions will provide valuable insights into the role of ethylene in the development of plant form. Ethylene-induced leaf abscission induces cellulolytic enzymes that break down cell walls in the abscission zone. Ethylene-induced abscission is associated with the expression of polygalacturonase and ends β-1, 4 glucan hydrolase in the vicinity of the distal abscission zone. Examination of the expression of the protein during the induction of defoliation in mustard would be an important aspect of study. Developing of antibodies against the protein might also help to answer the phenomenon underpin the defoliation in mustard.

Examination of the processes in *Arabidopsis* in which there is a wealth of genetic, molecular and cellular tools available will be available for future advances in this area.

Further, it may also not be ruled out that ethylene may not act as a single developmental switch setting a cascade in motion, but may act as
inducer whose presence is required for long periods of time. Therefore, study of interaction of ethylene with other plant hormones may also lead to some fruitful conclusion.

References


