STUDIES ON PLANT SIGNALS AND GROWTH RESPONSES TO HERBIVORY AND ITS SIMULATION

ABSTRACT

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ABSTRACT

Studies on plant signals and growth responses to herbivory and its simulation

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Abstract of the thesis, submitted to the Aligarh Muslim University, Aligarh, India, for award of the degree of Doctor of Philosophy in Botany, in the year 2013.

This thesis is chaptered into five sections. In chapter 1, the problems of aphid herbivores on crop plants, direct and indirect plant defenses, emphasizing the role of jasmonic acid (JA) as simulator of natural herbivory to elicit the plant defense volatiles well before aphid attack has been introduced. The relevant literature available on the topic has been reviewed comprehensively in the Chapter 2. The details of the biological materials used, design of the experimental set up, preparation of chemical reagents, methodology of experiments and analysis has been elaborated in Chapter 3. Chapter 4 covered the graphical representation of recorded data with brief description of findings. The analyzed data tables are annexed at the end of the thesis. The last section (Chapter 5) deals with the explanations of the results extracted from the data and discussed with the findings of relevant work of other researchers in the field. A brief account of experimentation is summarized below.

Five pot experiments were conducted in winter season (October to March) in the years 2009-2011 at Aligarh Muslim University, Aligarh. The objective was to elucidate the simulatory priming effect of JA and aphid (Lipaphis erysimi; Kalt) individually and as follow up treatment after screening of the five cultivars of Brassica juncea viz. Alankar, Pusa Jai Kisan, Varuna, Sakha and Rohini, against selected population of aphid. The effects of predator beetles (Coccinella septempunctata) often also referred as lady beetle on aphid attacks and resulting changes in plant growth. A brief account of the findings of the five experiments is given below:
Experiment 1

This experiment was laid to analyze the relative susceptibility of the five locally grown cultivars of *Brassica juncea* namely, cvs. Alankar, Pusa Jai Kisan, Varuna, Sakha and Rohini. The experiment was set up in the ambient conditions of winter season of the year 2009 in the specially designed net-houses at Aligarh Muslim University, Aligarh. These mustard cultivars were exposed to aphid infestation at the rate of 40 aphids (*Lipaphis erysimi*) per plant at 45 days after sowing (DAS). The seeds of all the selected cultivars were sown in earthen pots. The selected cultivars were infested with selected and identified aphids (*Lipaphis erysimi*) at the rate of 40 adult aphids per plant and a control of each cultivar (0 aphid) was also maintained with suitable replication. Each set of five cultivars were arranged in completely randomized block design in separate net houses of size 185x100x125cm supported with iron rods and each with a zipped entrance. All the five cultivars were sampled at 60 and 75 DAS to analyze the population growth of aphid on each cultivar, the plant growth responses (plant height, length of shoot, root, total plant, leaf number and area and plant fresh and dry mass), photosynthetic pigment concentration (chlorophyll a, b, total chlorophyll and carotenoids), total protein and proline contents. All the growth attributes and biochemical parameters significantly reduced at the onset of aphid herbivory at stages (60 and 75 DAS) except proline which increased on aphid infestation. The damage of all the cultivars increased as the population of aphid multiplied with plant age. On the basis of increasing sensitivity (in terms of per cent loss), the selected cultivars can be arranged from least to most sensitive one in the order as Alankar < Pusa Jai Kisan < Varuna < Sakha < Rohini. Alankar, therefore, was relatively least sensitive to aphid and supported lowest number of aphid population and least resultant damage. Whereas, cultivar Rohini was screened as most susceptible cultivar with highest increase in aphid population and resultant damage.

Experiment 2

From the screening Experiment 1, the two cultivars were selected viz. cvs. Alankar, the least sensitive and cv. Rohini as most susceptible. This experiment was set
up in the growth season of the successive year (2010) keeping all the agricultural practices and statistical design same as in Experiment 1. The selected cultivar (Alankar and Rohini) were independently infested with 0 (control), 50, 100 or 150 aphids per plant at 45 DAS. The comparative response of growth, physiological, biochemical and other parameters were recorded at 60 and 75 DAS and analyzed in detail. The yield was recorded finally at harvest (120 DAS). Besides, reduction in growth parameters (length of shoot, root, total plant, leaf number and area and plant fresh and dry mass), aphids also induced adverse changes in the structure of stomata and their dynamics (relative stomatal closure index, frequency of stomata) and hence affected the gaseous exchange, net photosynthetic rate, photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids), protein content and nutritional quality (NPK level) of the two selected cultivars. The increase of proline content reflected the protective response of cultivars against aphid induced water stress in plants. The decline in all these parameters eventually reduced yield attributes (pod length, pods per plant, seeds per pod, 1000 seed weight and seed yield) including seed oil content. This decrease was more pronounced in cv. Rohini as compared to cv. Alankar. Higher level of proline in cv. Alankar suggested requirement of a better protective mechanisms in this cultivar as compared to Rohini. One set was kept in open environment to naturally invite the beetle on treated plants and beetles number was counted. The cultivar Alankar attracted more beetles than Rohini.

Experiment 3

This experiment was conducted simultaneously along with experiment 2 in the growth season of 2010. Two selected cultivars of mustard were infested with 0, 50, 100, 150 aphids per plant at 45 day growth stage and 5 days later (50 DAS), two beetles (Ladybirds; *Coccinella septempunctata*) per plant were introduced. The experimental design was same as in Experiment 1. The plant samples were collected at 60 and 75 DAS to record growth, physiological, and biochemical characteristics of the two cultivars of Alankar and Rohini and finally harvested at maturity (120 DAS) for yield parameters. The aphid herbivory with 50 and 100 aphids per plant together with two predatory beetles per plant, reduced plant growth (length, fresh and dry mass of shoot and root, area and number of leaves per plant), altered adversely the stomatal dynamics (relative stomatal...
closure index, number of stomata), photosynthetic performance (net photosynthetic rate, stomatal gaseous exchange, pigment concentration), nutritional quality (NPK level) of plants and yield attributes (pod length, oil content, pods per plant, seeds per pod, 1000 seed weight and seed yield). The impact of aphids was not as prominent as in Experiment 2 (with beetles). This improvement was higher at late growth stage (75 DAS) as the aphid population decreased considerably at this stage. The results were much better in cv. Alankar as compared to most susceptible one; Rohini. The improved defense mechanism was further supported with the higher levels of proline in cv. Alankar, as compared to cv. Rohini.

**Experiment 4**

This experiment was conducted to study the different concentrations of JA on the two selected cultivars of *Brassica juncea* cvs. Alankar and Rohini. This experiment was performed in the growth season of year 2010. The cultivation practices and experimental design were same as in Experiment 1. Jasmonic acid (0, 0.5, 1.0 or 1.5 mM) was sprayed on the foliage of Alankar and Rohini at 45 DAS. Plants were sampled at 60 and 75 DAS. No significant change in growth parameters (length, fresh and dry mass of shoot and root, area and number of leaves per plant) and nutritional quality (NPK level) was observed on either of the cultivar treated with 0.5 or 1.5 mM JA. Only treatment with 1.0 mM JA enhanced the growth and yield characteristics (seed yield and oil content) of two the cultivars, significantly. This response was more prominent in Alankar than that of Rohini at late growth stage (75 DAS). Jasmonic acid application strongly stimulated the level of photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids content) and proline level. But, the level of proline enhanced was lower as compared to that of aphid infested plants (Experiment 2). Jasmonic acid induced stomatal closure in dose dependent manner in both the varieties. This concentration of JA (0.1 mM) also increased the plant fresh and dry mass more effectively in Alankar as compared to Rohini. Larger number of beetles was attracted in JA treated cv. Alankar as compared to JA treated Rohini at the 60 DAS.
Experiment 5

This experiment was conducted with the objective to elucidate the effect of foliar spray of JA prior to aphid infestation on two selected cultivars of mustard (Alankar and Rohini). This experiment was set up in the growth season of mustard in the year 2011, keeping all the cultivation practices same as in Experiment 1. Both the cultivars of Brassica juncea; Alankar and Rohini, were sprayed with 0.1 mM of JA solution at 45 DAS followed (5 days later) by aphid infestation (0, 50, 100 or 150 aphids per plant). The population of aphids significantly reduced with the progressing age of the plant (60 to 75 DAS). The JA treatment improved growth (length, fresh and dry mass of shoot and root, area and number of leaves per plant), structure and stomatal dynamics (relative stomatal closure index, number of stomata), photosynthetic performance (net photosynthetic rate, stomatal gaseous exchange, pigments content), nutritional quality (NPK) and yield attributes (pod length, oil content, pods per plant, seeds per pod, 1000 seed weight and seed yield) of plants. Cultivar Alankar excelled in its growth response and resistance to aphid than Rohini. The increase in proline level in JA treated plants confirmed its defense role and protective nature in aphid induced water stress in plants.

It emerged from the data of experiment 5, that JA treatment induced only marginal increase in plant growth. But, findings elucidates that JA treatment enhanced defensive and protective abilities of the cultivars. To elucidate the role of volatile chemicals in plant defense including beetle attraction and chemical deterrence to aphid, GC-MS analysis was carried out. Analysis revealed that volatile organic compounds (VOCs) viz. allyl isothiocyanate and 3-Hexan-1-ol increased in cultivar Alankar treated with JA (0.1 mM) and aphids as compared to plants infested alone with aphids. In addition to these, morphological defense features, leaf trichome density, hypertrophied pods, induction of extra floral nectarines, stomata leaf surface features indicated that aphid induced damage and reciprocal plant defense responses and signaling extended up to third trophic level in mustard-aphid-beetle food chain.