

Changes in cellular metabolism of *Brassica nigra* due to infection by certain seed borne fungi

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Present study has been carried out to observe changes in metabolism of *Brassica nigra* due to infection caused by certain seed borne fungi viz., *Fusarium oxysporum*, *Alternaria brassicae* and *Pythium aphanidermatum*.

Key words : *Brassica nigra*, Seed borne fungi, Metabolic studies

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INTRODUCTION

Seeds and seedlings often suffer from the structural and physiological disorders following the infection of the pathogens. Physiological disorder or structural abnormality is harmful to the plant or to any of its parts or products that reduces its field. Young seedlings are particularly susceptible to a number of diseases because of their tender tissues and often have difficulty in establishing themselves. Vigour of seedling, further growth and yield are to a considerable extent dependent on the quality of seeds. Since seeds are primary of planting stock and improved seeds are expensive, it is necessary to investigate the seed and seedling, pathogens and if necessary some treatment should be given either before showing the seeds or at the seedling stage.

Since seeds are major source of propagation in higher plants it should be free of any pathogen in order to have healthy plant and fair field. Several fungi have been found associated with seeds of angiosperms and gymnosperms. As a result of infection several physiological processes such as membrane permeability, photosynthesis, respiration and nitrogen metabolism are affected. Disease symptoms appear in the host cell due to these physiological disturbances. The fungi associated with seeds may cause infection and can reduce yield by altering the physiological processes.

Loss of production due to fungal infection is great loss of the agriculturists and to the normal economy of the country. Thus, study of physiological aspects of the infected plants will give an idea of actual way of losses incurred and

thus, a suggestion can be made for effective control measure of seed borne disease. In view of the above facts the present works has been proposed for investigation.

RESEARCH METHODOLOGY

Metabolic studies of diseased as well as healthy mustard plants were performed by measuring various metabolists such as total chlorophyll, photosynthesis, total water soluble sugars, starch, total nitrogen, organic nitrogen, protein, phosphorus and nucleic acids. The structure of chloroplast was studied and the number of chloroplast (per cell) was determined in hand cut sections of fresh mustard leaf infected with three different pathogenic fungi viz., *Pythium aphanidermatum*, *Alternaria brassicae* and *Fusarium oxysporum*. Sections were stained mounted in DPX and observed under high power of microscope.

Total chlorophylls was estimated as per method suggested by Arnon(1949). Photosynthesis(Net assimilation rate) was determined by applying the formula given below:-

$$NAR = \frac{(W_2 - W_1) 2.303 (\log_{10} A_2 - \log_{10} A_1)}{(t_2 - t_1) (A_2 - A_1)}$$

W_1 and W_2 = Dry weight at times t_1 and t_2 , respectively

A_1 and A_2 = leaf area at times t_1 and t_2

It is commonly reported as $\text{gdm}^{-2} \text{week}^{-1}$.

Total water soluble sugar was estimated by applying the method of Dubios (1951). Starch was estimated by the method of Clegg (1956). Total nitrogen was estimated calorimetrically using Nessler's reagent. Fifty milligrams

of dried material was digested by Doneen's method (1932) for nitrogen estimation.

Total protein was estimated as per method of Snell and Snell (1949). Fifty milligrams of dried leaf was homogenised with 10 ml solution of 10 per cent trichloroacetic acid. Total phosphorus was estimated by the colorimetric method of Allen (1940). 10 milligrams of dried sample after adding 0.4 ml perchloric acid and a drop of 30 per cent hydrogen peroxide (H₂O₂). Nucleic acids were extracted and estimated by the modified procedure of Smillie and Krotkov (1960).

RESEARCH FINDINGS AND ANALYSIS

The results obtained are listed in the Tables 1 to 4. The salient features of the findings areas under. Apparently the size and shape of chloroplast in diseased plants were similar to control plants and their number were also equal in both the diseased and healthy plants. Total chlorophyll content was measured as per method of Arnon (1949) in green leaves of *Brassica nigra*. The chlorophyll content was found low in comparison of control plant. The chlorophyll content of all the infected plants was almost equal. A decrease in the sugar content of infected plants was recorded against sugar content

of control plants. Maximum decrease was found in the leaves infected with *Fusarium oxysporum* and minimum in those infected with *Alternaria brassicae*. However, difference of sugar content amongst infected and healthy plants was quite narrow. Starch content was measured in the leaves and a decrease was recorded in the infected plants. Maximum decrease in the starch content was found in the leaves of host plant inoculated with *Alternaria brassicae* and minimum in those infected with *Fusarium oxysporum*. However, there was a narrow difference of amount between the healthy and diseased plants.

Net assimilation rate was found quite low in the infected plants. Maximum decrease was found in the plants infected with *Pythium aphanidermatum* and those infected with *Alternaria brassicae* and *Fusarium oxysporum* had slightly higher net assimilation rate. Total nitrogen was estimated by Doneen (1932) method. There was overall decrease in total nitrogen in all the infected plants. Maximum decrease in nitrogen content was found in the leaves infected with *Pythium aphanidermatum* followed by *Alternaria brassicae* and minimum in *Fusarium oxysporum*.

Organic nitrogen was also found low in the infected

Table 1: Per cent total nitrogen, organic nitrogen and protein contents of *Brassica nigra* (cv. MAHI GOLD) infected with different fungal species (after 120 days).

Sr.No.	Days after inoculation	Fungal species			
		<i>A. brassicae</i>	<i>F. oxysporum</i>	<i>P. aphanidermatum</i>	Control
1.	2	0.5	1.0	0.5	1.5
2.	4	2.0	1.5	1.0	2.6
3.	6	3.0	2.0	1.5	3.5

Table 2: Total water soluble sugar (%) starch (%) and total chlorophyll (mgs/g) contents of the *Brassica nigra* (cv. MAHI GOLD) raised from the seeds inoculated with different fungal species (after 120 days).

Sr.No.	Content	Fungal species			
		<i>A. brassicae</i>	<i>F. oxysporum</i>	<i>P. aphanidermatum</i>	Control
1.	Total water soluble sugar	3.70	3.36	3.63	3.92
2.	Starch	2.10	12.30	2.19	2.99
3.	Phosphorus	1.29	1.97	1.08	2.70
4.	Chlorophyll	0.70	0.97	0.80	1.12

Table 3: Net assimilation rate' (gdm-2week-1) in leaves of *Brassica nigra* (cv. MAHI GOLD) raised from the seeds inoculated with different fungal species at certain time intervals

Sr.No.	Days after inoculation	Fungal species			
		<i>A. brassicae</i>	<i>F. oxysporum</i>	<i>P. aphanidermatum</i>	Control
1.	60	2.30	2.45	2.22	2.60
2.	120	2.45	2.60	2.30	3.80

Table 4: ug/mg of DNA and total RNA in dry leaves of *Brassica nigra* (cv. MAHI GOLD) infected with different pathogenic fungi (after 120 days of inoculation)

Sr.No.	Nucleic acids	Fungal species			
		<i>A. brassicae</i>	<i>F. oxysporum</i>	<i>P. aphanidermatum</i>	Control
1.	DNA	0.55	0.68	0.80	0.48
2.	RNA	2.50	1.90	2.02	1.80

leaves. Highest decrease was in the plants infected with *Pythium aphanidermatum*. The total protein content decreased in the infected plants. Maximum decrease was found in the leaves infected with *Pythium aphanidermatum* followed by *Alternaria brassicae* and minimum in *Fusarium oxysporum*.

Total phosphorus content was found decreased in the infected plants. Maximum decrease was found in the plants which infected with *Pythium aphanidermatum* followed by *Alternaria brassicae* and minimum in those infected with *Fusarium oxysporum*.

Unlike other cellular content nucleic acids DNA and RNA, recorded an increase in infected plants against healthy plants. DNA was found more in plants infected with *Pythium aphanidermatum* than *Fusarium oxysporum* and *Alternaria brassicae* followed by *Pythium aphanidermatum* and minimum in *Fusarium oxysporum*.

Total chlorophyll content was found less in all the infected plants in comparison to healthy plants, similar results were found by other investigators. Khare *et al.* (1996) observed and concluded that the metabolites produced by the pathogen of purple blotch, *Alternaria porri* induced chlorosis in onion might be due to inhibition of chlorophyll synthesis. Pounds *et al.* (1951) reported the production of a toxic material by *Alternaria solani* in tomato which caused yellowish spots on the leaves due to the reduction of chlorophyll synthesis. However, difference of sugar content between infected and healthy plants was quite narrow. Similar results were found by other investigators. Pesis *et al.* (1997) studied the compositional changes in kiwi fruit infected with *Botrytis cinerea* and found a decrease in reducing sugar. A decrease in starch content was found and maximum decrease was in leaves infected with *Alternaria brassicae*. Similar results were found by Reddy *et al.* (2008) in rice plant infected with *Aspergillus*, *Fusarium* and *Penicillium* resulted a decrease in starch content. The rice plant infected by *Alternaria alternata*, the seed borne fungi caused more a decrease in starch content.

Net assimilation rate was measured by the author in the present investigation which was lower in the infected host plants than in control plants. Similar observations were recorded by some other investigators. Suri *et al.* (2008) studied the photosynthesis in mustard leaf infected by *Alternaria brassicicola* and found loss of photosynthesis due to infection and pathogenesis. Singhal *et al.* (2002) found loss of photosynthesis in the host plant (wheat) infected by *Alternaria* species and *Ustilago nuda*. More reduction in phosphorus content was found in the plants infected with *Pythium aphanidermatum*. Unlike other metabolites nucleic acids (DNA and RNA) recorded an increase in infected plants over control which might be possibly due to increase in the amount of nitrogenous bases and nucleotides. Similar observations have been by other investigators. Konstant *et al.* (2002) reported an increase in DNA content due to development of specific primer in carrot infected by *Alternaria radicina*. Goswami *et al.* (2006) performed the genomic analysis of host-pathogen interaction between *Alternaria* and *Fusarium* and found an increase in DNA content.

Loss of phosphorus content in rice plant infected by *Fusarium Moniliforme* was reported by Paszkowski *et al.* (2002). Raymond *et al.* (2003) reported low phosphorus content in corn and wheat grains naturally contaminated with *Fusarium moniliforme*.

In the present investigation the author observed overall decrease in total nitrogen, organic nitrogen and protein content of the host plant infected by the fungal species. It is assumed that some phytotoxins might be responsible for the decrease in nitrogen and protein content. Similar results were found by other investigators. Srivastava *et al.* (1970) found a decrease in nitrogen content of rice plant infected by *Fusarium moniliforme* while studying the role of sulphanilamide in the control of rice disease. Hussaini *et al.* (2007) reported decrease in protein content in rice plant infected by *Alternaria alternata* due to fungus and mycotoxin contamination.

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