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NODULATION AND NITROGEN FIXATION PATTERN IN VIRUS INFECTED *VICIA FABA*

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ABSTRACT

Vicia faba is a common leguminous plant grown in many tropical countries. In addition of being a rich source of protein, this plant also fixes considerable amount of nitrogen in the soil. The setback with this plant is that it is susceptible to the infection of Bean Mosaic Virus (BMV). In light of the above, the present study was designed with the aim to study the morphology of the root nodule of BMV infected plant in light of a healthy plant. The growth trend of the nodule was found to be similar, but the magnitude was much lower as compared to a normal healthy plant. In addition, it was also recorded that the nitrogen fixing ability in the BMV infected plant reduces as compared to the normal plants of Vicia faba. Most of the values were found significant either at 5% or at 1% level of significance, through F-test.

***Key words: Root nodules, Vicia faba, nitrogen fixation, Bean Mosaic Virus*.**

Introduction:

Vicia faba is an important protein rich leguminous plant which is widely cultivated in most of the tropical parts in the developing countries. It is used as a feed for poultry, horses, pigs and many countries, at the same time it is used as vegetable or as pulses also by human. Because of high protein contain in addition to minerals, this plant is one of the most preferred plant to farmers and the market as well.

Most of the legumes have nodules on their roots containing bacteria, which have the quality of fixing atmospheric nitrogen. This ability to draw on atmospheric nitrogen is dependent on the presence of particular group of soil-inhabiting bacteria the *Rhizobium.* It is within the root nodules that atmospheric nitrogen is converted into some available form, for neither plant nor microorganism on its own is capable of carrying out this process. Rajagopalan and Raju (1972) studied the influence of infection by *Dolichos* enation mosaic virus on nodulation and nitrogen fixation by field bean. Tu et. al. (1970) studied the factors affecting nodulation and nodular efficiency on soybeans infected by soybean mosaic virus. Parallel study in light of nitrogen fixation by virus infected leguminous plants was studied by Singh and Mall (1974). Although the effect of few viruses, on nodulation of a number of legumes have been studied but information regarding the influence of virus on the nodular physiology and their subsequent effect on bacterial population and nitrogen fixation in *Vicia faba*, one of the important commercial crop is still needed. Present investigation, was, therefore undertaken to study the effect of viral infection on nodulation, bacteria population and nitrogen fixation by *Vicia faba*.

Material and Methods:

During detailed studies, throughout experimental period the effects of virus infection on different physiological aspects of Bean mosaic nodulation, at different periods of infection, seven days old *Vicia faba* seedlings where taken in two groups each consisting of 250 seedlings.

Seedlings of the first group were inoculated with neutral phosphate buffer to serve as the healthy control, while those of the second group were inoculated with Bean mosaic virus. Plants of each group were harvested at 20^{th}, 40^{th}, 60^{th}, 80^{th}, and 100^{th} day of inoculation at the rate of 50 plants per harvest. The harvested samples were used for studying nodulation. Different aspects of the experimental

plants were studied at each harvest by the methods given below:

The soil, compost mixture, in which seeding was done, was tested for the presence of *Rhizobia*. The seeds were not artificially inoculated with the *Rhizobia* because the soil mixture was already infested with the same.

Plants were uprooted carefully to avoid damaging of root system. Nodules from each plant were collected by removing the soil in running water through 40 mesh screen and collecting the nodules from the screen. Those still adhering to the roots were picked up individually with the help of forceps. Nodules were surface dried with filter paper, sealed in small polythene bags, and stored at 4^0C. Nodules from each group of plants were counted and weighed. For dry weight, nodules were kept in oven maintained at 80$\pm$ 5^o C for 24 hours.

Total nitrogen was calculated by the Nessler's colorimetric method as modified by Snell and Snell (1949). For the ease of convenience, mean values of the obtained data is expressed in the respective tables. The obtained values were subjected to F-test of statistical analysis, with respect to days and with respect to the condition to test the significance of the obtained values.

Results and Discussions:

The number of root nodules in infected as well as in the diseased plants during the interval of the selected period is presented in table 1. The number of root nodules increases gradually in both the cases up to 80 days, this is followed by a gradual decrease after 100 days of inoculation. But the difference in the number of root nodules in the case of healthy plants is much higher as compared to the infected samples. Statistical analysis shows that the values for days in the case of number of root nodules are significant at 5% level. Estimation of fresh weight of the root nodules is also expressed in table 1. Trend for fresh weight of the root nodules was found more or less parallel to that of the number of root nodules. Statistical analysis shows that the values for days in the case of fresh weight of root nodules are significant at 5% level; however in the case condition the values are significant at 1% level.

Percent dry matter of the nodules is presented in table 2. The value of Percent dry matter of the nodules is comparatively higher in the case of diseased plants as compared to the normal plants. The highest value of Percent dry matter of nodules is after 80 days of infection in both the cases, mean value in normal plant is 20.604, while in the diseased plants it is 22.126. Statistical analysis shows that the values are significant at 5% level, both for days as well as for the conditions.

Bacterial population in the nodules was expressed as colony content per gram of root nodules and the bacterial population in the rhizosphere was also estimated and it is expressed in the terms of colony content per gram of soil; the values are expressed in table 3. A perusal of the Table 3 indicate that the virus infection increased the bacteria population of the diseased nodules as compared to the healthy nodules that increased up to 80 days of inoculation and after that it showed a decrease. Colony content per gram of nodules shows the values for days significant for 5%. On the other hand colony content per gram of soil shows the values for days significant at 1% and for the condition significant at 5%.

Nitrogen fixed in the soil, during different interval of time was estimated in the terms of nitrogen content in µg/100 mg soil. The findings are expressed in table 4. Continuously increasing trend was recorded for nitrogen fixed, throughout the study period in both the cases; however, the magnitude was higher in the case of normal plants as compared to the diseased plants. Statistical analysis shows the valves significant at 1% level of significance. Singh and Mall (1975) studied the nodulation and nitrogen fixation by virus infected leguminous plants; their study concentrated around the effect of arhar mosaic virus infection on nodulation of pigeon pea. Their findings are parallel to our findings, which confirm our results, barring few exceptions regarding root nodule morphology. This variation may be due to confounding variables.

A perusal of the Tables indicate that Bean mosaic virus infection influenced the population ability of the *Vicia faba* plats nodule from the infected plans were less in number, size and fresh weight, but have increased dry weight. The number, size and fresh weight of nodules increased up to 80 days of inoculation and after that it decreases. The maximum dry matter accumulation was noticed infected nodule number and its size are signification.

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Table 1: Number and fresh weight of root nodules of bean at different of period of BMV infection.

Days after inoculation	Number of nodules		Fresh weight of nodules (mg)	
	Healthy	Diseased	Healthy	Diseased
20	30.32	25.60	0.104	0.064
40	41.30	31.40	0.135	0.084
60	46.36	39.41	0.202	0.086
80	71.05	42.28	0.136	0.118
100	22.45	13.66	0.018	0.112

Table 2: Percent dry matter of nodules of bean at different period of BMV infection.

Days after inoculation	Healthy	Diseased
20	18.850	18.900
40	20.000	20.678
60	20.186	21.336
80	20.604	22.126
100	18.506	20.166

Table 3: Bacterial population at different period of BMV infection.

Days after inoculation	Bacterial population (colony contents/g)			
	In Root Nodules		In Rhizosphere	
	Healthy	Diseased	Healthy	Diseased
20	5390	6620	2920	2810
40	11040	13320	3580	3230
60	23610	24841	3960	3590
80	54840	67320	4468	3848
100	37920	41370	4790	4160

Table 4: Nitrogen (µg/100 mg soil) level of pot soil holding bean plants having different BMV infection period.

Days after inoculation	Healthy	Diseased
20	0.664	0.642
40	0.692	0.672
60	0.741	0.719
80	0.752	0.731
100	0.783	0.753