Possibility of Controlling Bean Yellow Mosaic Virus on Faba Bean (*Vicia faba* L.) by *Rhizobium* Inoculation*

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Abstract : Bean Yellow Mosaic Virus (BYMV) inoculum was applied after two, three and half or five weeks from sowing to faba bean cv. Agabat which was either uninoculated or inoculated with *Rhizobium leguminosarum* biovar. *viceae* strain TAL1397 in a pot experiment. Inoculation with the virus after two and three and half weeks significantly decreased shoot and root dry weight, nodule number, nodule dry weight, number of flowers and pods per plant, total plant nitrogen and nitrogen fixation, whereas inoculation with *Rhizobium* has significantly increased these parameters. The reduction in plant dry weight and nodule number due to the viral treatments were correlated with the time of virus inoculation in a statistical model. The results indicate that faba bean plants suffer greatly from the virus infection when virus inocula are added early in the season. The results also indicate that *Rhizobium* strain TAL1397 is effective in fixing nitrogen in normal and in virus infected faba bean plants.

INTRODUCTION

Faba bean (*Vicia faba*) is one of the most important leguminous crops in the Sudan-being an important cash crop and is also rich in protein. It is usually grown in the Northern State. A significant increase in nodulation, yield and nitrogen fixation in response to inoculation was reported by Mahdi (1992; 1993), and it was also reported that the crop

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suffers from Bean Yellow Mosaic Virus and Broad Bean Mottle Virus infections (Ahmed, 1986; Hussein, 1979). The magnitude of losses in nodulation and bean yield due to viral diseases varies according to variety and the time of virus infection (Hago, 1991). The aim of this work was to study the effect of nitrogen fixing bacteria (*Rhizobium leguminosarum* biovar. *viceae*) in reducing the harmful effect of viral infection on faba bean.

MATERIALS AND METHODS

Rhizobium leguminosarum biovar *viceae* strain TAL1397 and Bean Yellow Mosaic Virus (BYMV) were kindly provided by NifTAL Project (USA), and the Department of Crop Protection, University of Khartoum (Sudan), respectively. The bacterial strain was maintained at 4°C on yeast extract mannitol (YEM) agar slopes incorporating 3.0 gl^{-1} $CaCO_3$, and the virus was maintained at 4°C on leaves of systematically infected faba bean. These bacterial and viral strains were used in this study to inoculate a local cultivar of faba bean, namely Agabat which was kindly provided by the Agricultural Research Corporation (Sudan).

Five surface sterilized seeds of Agabat cultivar were sown in pots (5.0 kg soil pot^{-1}). The soil used was a "Gerf"[1] soil containing 0.11 total nitrogen, 1.44 dSm^{-1} ECe, pH value of 7.63, and 30%, 30% and 40% silt, sand and clay, respectively. The plants were thinned to two plants pot^{-1} after two weeks from sowing. The group of plants which were randomly selected for the viral infection were inoculated with BYMV after two, three and half or five weeks from sowing. A factorial experiment with four replicates was designed to give all possible interactions. Treatments were as follows:

(a) Faba bean plants infected with BYMV after 2 weeks from sowing.

(b) Faba bean plants infected with BYMV after 3.5 weeks from sowing.

[1] Gerf soil : Very fertile loamy soil in the direct vicinity of both sides of the River Nile and its tributaries.

Faba bean plants infected with BYMV after 5 weeks from sowing.
Non-infected faba bean plants (control).

Each of these treatments was either uninoculated or inoculated after two weeks from sowing with *Rhizobium leguminosarum* biovar *viceae* strain TAL1397. The experiment was conducted in the glass house of the Faculty of Agriculture, Shambat, during the winter season of 1991/1992.

Plants were harvested after six and eight weeks from sowing. Shoot and root dry weight, nodule number, nodule dry weight, number of flowers and pods per plant, nitrogen fixation and total plant nitrogen were determined. Nitrogen fixation was determined by the Nitrogen Difference Method (I - U) where I is the total nitrogen content in plants inoculated with *Rhizobium* and U is the total nitrogen in uninoculated plants.

RESULTS

Effect of Treatments on Dry Weight

All faba bean plants treated with BYMV produced less fresh (data not shown) and dry weights of shoot and root compared with the control (no virus) treatments (Table 1). The first treatment (the virus inoculation after two weeks from sowing) significantly reduced the dry weight of shoots and roots of all treatments when sampled after six and eight weeks from sowing. The third treatment (the virus inoculation after five weeks from sowing) slightly reduced the dry weight of shoots and roots of all treatments six weeks after viral inoculation, but significantly reduced them after eight weeks. The reduction of shoot dry weight due to the first, second and third treatment, after eight weeks was about 29%, 26% and 14% for plants with no *Rhizobium* treatment; whereas the reduction in *Rhizobium*-treated plants was 43%, 40% and 19%, respectively. Similar reductions were observed for root dry weight.

Inoculation with *Rhizobium leguminosarum* biovar *viceae* strain TAL1397 significantly increased the dry weight of shoots and roots of plants of all treatments after six and eight weeks, as compared with their homologous uninoculated plants. Inoculation with strain TAL1397 increased the shoot dry weight after eight weeks by about 48%, 18%, 19% and 38% for the uninoculated control, the first, second and third viral treatments, respectively, as compared with their homologous treatments with no *Rhizobium* inoculation, whereas the increase in the root dry weight due to strain TAL1397 after eight weeks was about 37%, 14%, 15% and 30% for the same treatments, respectively.

Effect of Treatments on Flowers and Pods

The first, second and the third viral treatments significantly reduced the number of flowers per plant by about 32%, 23% and 21%, respectively, after eight weeks (Table 2). On the other hand, inoculation with *Rhizobium leguminosarum* biovar *viceae* TAL1397 significantly increased the number of flowers per plant by about 56%, 27%, 19% and 25% for the uninoculated control, the first, second and third viral treatments, compared with their homologous treatments with no *Rhizobium* inoculation.

The virus infection in the first, second and the third treatment resulted in a significant reduction in the number and dry weight of pods (Table 2). The reduction in number of pods per plant due to the viral infection at the first, second and the third treatment after eight weeks was about 48%, 39%, 27%, respectively, compared with the control plant. Similar reduction was observed for the dry weight of pods. Inoculation with *Rhizobium leguminosarum* biovar *viceae* strain TAL1397 significantly increased the mean number of pods per plant by 50%, 17%, 21% and 23% for the uninoculated control, the first, second and third viral treatment, respectively, compared with the uninoculated control treatment. Similar increments were found for pod dry weight.

Table 1. The effect of time (weeks) of Bean Yellow Mosaic Virus (BYMV) infection on shoot and root dry weight (g plant^{-1}) of faba bean cultivar Agabat, either uninoculated control or inoculated with *Rhizobium* strain TAL1397.

Treatment	Shoots		Roots	
	6 weeks SE = ± 0.12	8 weeks SE = ± 0.17	6 weeks SE = ± 0.04	8 weeks SE = ± 0.05
No inoculation				
BYMV (2 weeks)	1.20	1.28	0.33	0.43
BYMV (3.5 weeks)	1.31	1.34	0.35	0.47
BYMV (5 weeks)	1.46	1.56	0.48	0.53
Control (no virus)	1.51	1.81	0.50	0.62
***Rhizobium* TAL1397**				
BYMV (2 weeks)	1.47	1.51	0.44	0.49
BYMV (3.5 weeks)	1.50	1.54	0.47	0.54
BYMV (5 weeks)	2.06	2.15	0.66	0.69
Control (no virus)	2.15	2.67	0.69	0.85

Table 2. The effect of time (8 weeks) of Bean Yellow Mosaic Virus (BYMV) infection on number of flowers (plant^{-1}), number of pods (plant^{-1}) and pods dry weight (plant^{-1}) of faba bean cultivar Agabat, either uninoculated control or inoculated with *Rhizobium* strain TAL1397.

Treatment	Number of flowers/plant SE = ± 0.25	Number of pods/plant SE = ± 0.16	Dry weight of pods SE = ± 19.5
No inoculation			
BYMV (2 weeks)	3.22	1.85	449
BYMV (3.5 weeks)	3.67	2.17	522
BYMV (5 weeks)	3.75	2.58	614
Control (no virus)	4.75	3.54	855
***Rhizobium* TAL1397**			
BYMV (2 weeks)	4.10	2.17	534
BYMV (3.5 weeks)	4.40	2.63	659
BYMV (5 weeks)	4.67	3.18	806
Control (no virus)	7.42	5.33	1346

Effect of Treatments on Nodulation and N Fixation

The presence of nodules on the roots of uninoculated faba bean plants indicated the presence of indigenous Rhizobia (Fig. 1). Inoculation with *Rhizobium leguminosarum* biovar *viceae* strain TAL1397 increased nodulation in all treated plants. The increment in nodulation due to this strain was 60.3% and 64.6%, at six and eight weeks, respectively, compared with uninoculated control. *Rhizobium* strain TAL1397 increased the nodulation by more than 50% in all viral treatments after six and eight weeks, which indicate the infectivity of the strain in normal and in virus-infected plants. The first and the second viral treatment significantly reduced nodulation in inoculated and uninoculated plants of all treatments at six and eight weeks. The third viral treatment showed the least reduction as compared with the other viral treatments. The significant effect of the different treatments on nodule dry weight per plant showed a similar pattern to that of nodulation (Fig. 2).

Inoculation with the studied *Rhizobium* strain significantly increas-ed the total nitrogen of faba bean plants at six and eight weeks, compared with their homologous uninoculated plants (Table 3). Although the application of the viral inoculum after five weeks had decreased the total plant nitrogen of inoculated plants compared with *Rhizobium*-inoculated and virus-free plants, this nitrogen content value (59.24) is more than the value (40.24) of the control (no *Rhizobium* and no virus treatments) which indicates the efficiency of this strain.

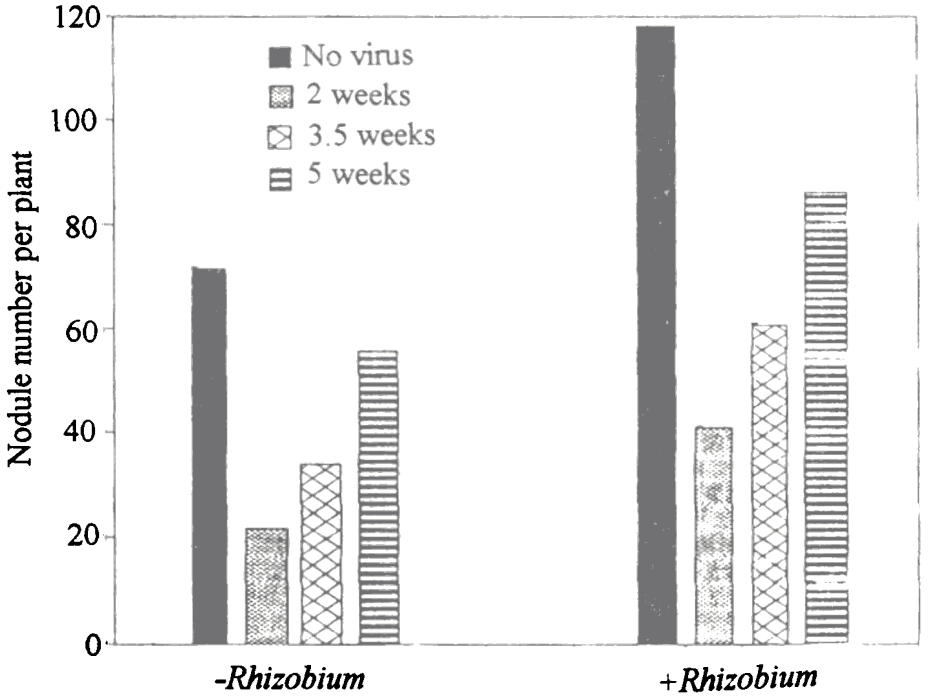

Fig.1 The effect of time (8 weeks) of Bean Yellow Mosaic Virus (BYMV) infection on total number of nodules (plant^{-1}) of faba bean cultivar Agabat, either uninoculated control or inoculated with *Rhizobium* strain TAL1397.

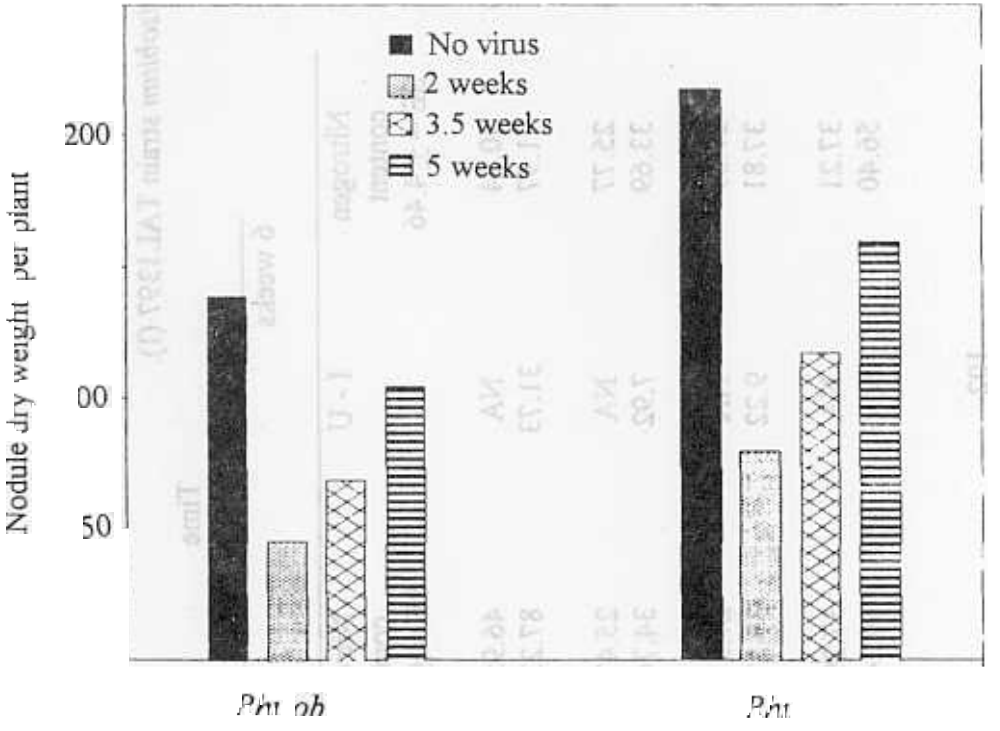

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Table 3. The effect of time (weeks) of Bean Yellow Mosaic Virus (BYMV) infection on total nitrogen (mg plant^{-1}) and nitrogen fixation (I - U) of faba bean cultivar Agabat, either uninoculated control (U) or inoculated with *Rhizobium* strain TAL1397 (I).

Treatment	Time			
	6 weeks		8 weeks	
	Nitrogen content SE = ± 4.46	I - U	Nitrogen content SE = ± 4.77	I - U
No virus infection				
Control (no *Rhizobium*)	40.24	NA	46.95	NA
Rhizobium TAL1397	71.97	31.73	87.23	40.28
BYMV (2 weeks)				
Control (no *Rhizobium*)	25.77	NA	25.47	NA
Rhizobium TAL1397	33.69	7.92	34.76	9.29
BYMV (3.5 weeks)				
Control (no *Rhizobium*)	27.59	NA	27.51	NA
Rhizobium TAL1397	37.81	9.22	39.27	11.76
BYMV (5 weeks)				
Control (no *Rhizobium*)	37.21	NA	37.29	NA
Rhizobium TAL1397	56.40	19.19	59.24	21.95

NA = not applicable.

DISCUSSION

In this study, the presence of nodules in the roots of uninoculated faba bean plants indicates the presence of indigenous rhizobia in the "Gerf" soil (Fig.1). The presence of indigenous *Rhizobium leguminosarum* biovar. *viceae* in the Sudanese soils was reported to vary with different soils depending upon soil physicochemical properties, cropping and moisture content (Mahdi, 1993; Mukhtar and Naib, 1988). Inoculation of Agabat cultivar with *Rhizobium leguminosarum* biovar *viceae* strain TAL1397 significantly increased shoot and root dry weight, nodule number, nodule dry weight, number of flowers and pods per plant, in addition to total plant nitrogen and nitrogen fixation. The present positive response of faba bean to inoculation suggests that indigenous faba bean rhizobia in this region are inefficient and not highly competitive to introduced rhizobia; similar results were reported by Mahdi (1992).

Inoculation with Bean Yellow Mosaic Virus significantly decreased dry matter yield, nodulation and nitrogen fixation of all treatments. The highest reduction in these parameters was observed when the plants were infected with BYMV after two and three and half weeks. Virus infection was reported to reduce faba bean nodulation by 14 to 50% depending on the variety, growth stage and time of virus infection (Ahmed, 1986); in other reports, reduction in nodulation may exceed 90% depending on the variety (Hago, 1991). The inhibitory effect of viral infection on nodulation and nitrogen fixation reported in this study supports the findings of the research carried out on other legumes such as haricot bean (*Phaseolus vulgaris*) (Ahmed and Abdelbagi, 1993), cowpea (*Vigna unguiculata*) (Mayoral *et al.*, 1989) and alfalfa (*Medicago sativa*) (Ohki *et al.*, 1986). The reduction in nodulation of viral infected plants could be attributed to the reduction in leghaemoglobin and leaf chlorophyll (Mayoral *et al.*, 1989) or to an imbalance in auxins and enzymes (Hago, 1991).

The relative reduction in the total plant (shoot and root) dry weight and total nodule number per plant were correlated with the time of the virus inoculum application (Fig.3). The following two regression equations were obtained :

(a) For plants without *Rhizobium* inoculation

$$Y = 1.41 - 0.205 X_1 + 0.006 X_2 R^2 = 0.99 \qquad (1)$$

(b) For plants with *Rhizobium* inoculation

$$Y = 7.99 + 891 X_1 + 0.151 X_2 \quad R^2 = 0.99 \qquad (2)$$

where

Y = the reduction in total plant weight of virus-infected plants from the control (*Rhizobium* and virus- free) (g)

X_1 = the time of inoculum application (weeks)

X_2 = the reduction in total nodule number per plant of virus-infected plants from the control (*Rhizobium* and virus-free)

The time (weeks) after which virus infection could not be effective can be estimated by both equations; the estimate of equation (1) is seven weeks and for equation (2) is 4.2 weeks (Fig. 3). This means that the *Rhizobium* can alleviate completely the harmful effect of the virus if faba bean plants are subjected to virus infection after 4.2 weeks (all comparisons based on *Rhizobium* and virus-free control). This clearly indicates the enhancing effect of the *Rhizobium* inoculation in alleviating the harmful effect of the virus infection.

The overall results indicate that virus infection has significantly decreased dry matter yield, nodulation and nitrogen fixation of faba bean. The results also indicate that *Rhizobium* strain TAL1397 is infective and can effectively fix nitrogen in normal virus-infected faba bean plants, and it should be tested fully under the field conditions before being finally recommended to farmers. The results also indicate that nitrogen fixation can be used as a tool to alleviate the reduction caused by viral diseases coupled with breeding for viral resistance.

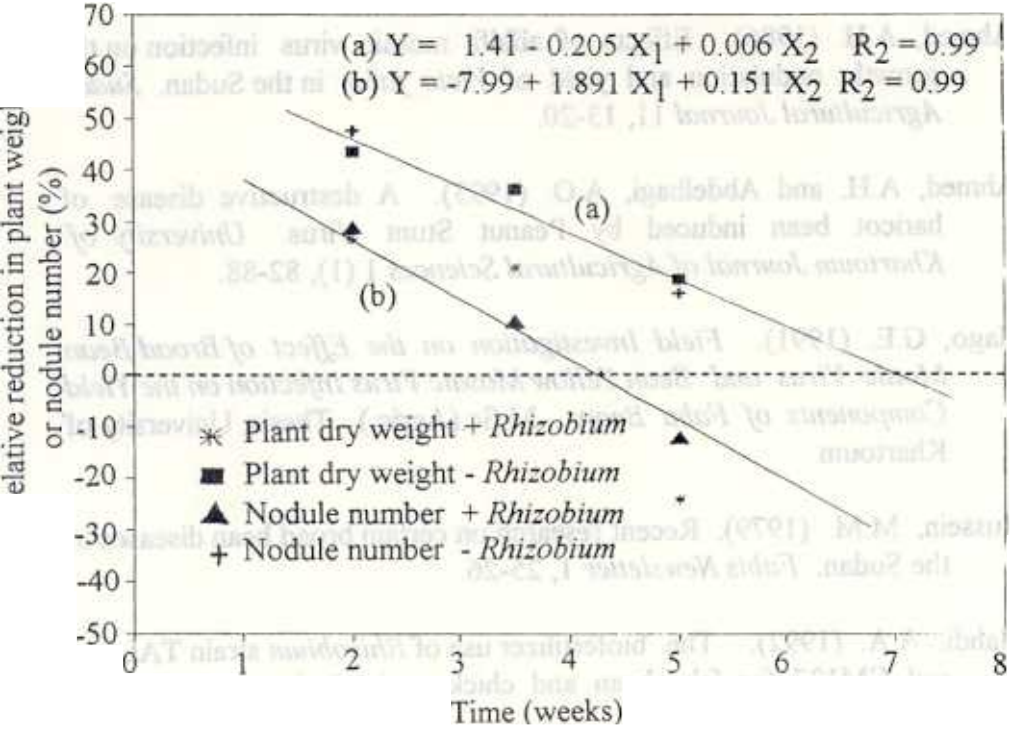

Fig.3. Regression analysis of the relationship between the time (weeks) of virus inoculum application and the relative reduction in plant dry weight(s) and nodule number (plant^{-1}) ; (a) for plants without *Rhizobium* inocu ation; (b) for plants with *Rhizobium* inoculation.

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امكانية استخدام بكتريا العقد الجذرية فى تقليل خطر الإصابة بفيروس موزايك الفاصوليا الأصفر (BYMV) على الفول المصرى

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موجز البحث : أضيف فيروس موزايك الفاصوليا الأصفر (BYMV) بعد اسبوعين أو ثلاثة أسابيع ونصف أو خمسة أسابيع من الزراعة لصنف الفول المصرى " عقبات " والذى تم أو لم يتم تلقيحه ببكتريا العقد الجذرية سلالة TAL 1397 . أدت اضافة الفيروس بعد اسبوعين أو ثلاثة أسابيع ونصف الى نقص معنوى فى الوزن الجاف للمجموعين الخضرى والجذرى وعدد العقد الجذرية ووزنها الجاف وعدد الأزهار والثمار. كما أدت الى نقص فى كمية النتروجين الكلى وفى عملية تثبيت النتروجين الجوى ، بينما أدت البكتريا العقدية الى زيادة معنوية فى هذه الصفات . تم تحليل المعلومات احصائيا ومن ثم وضع انموذج احصائى للعلاقة بين النقص فى عدد العقد الجذرية والوزن الجاف للنبات مع وقت اضافة الفيروس .

دلت النتائج على أن الاصابة بالفيروس فى بداية الموسم تضر نباتات الفول بدرجة كبيرة ، كما دلت النتائج أيضا على أن سلالة TAL 1397 لها القدرة على تثبيت النتروجين الجوى فى نباتات الفول المصرى السليمة والمصابة بالفيروس .