

THE INFLUENCE INTERACTION BETWEEN VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI, *Rhizobium leguminosarum* AND BEAN YELLOW MOSAIC VIRUS ON FABA BEAN PLANTS

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(Received, Mar., 12, 2003)

ABSTRACT: *The interaction between VA mycorrhizal fungi, *Rhizobium leguminosarum* and bean yellow mosaic virus (BYMV) on plants dry weight, chlorophyll content, virus infectivity, nodulation status and N₂-ase activity, nitrogen and phosphorus contents, density of mycorrhizal spores and mycorrhizal root infection of faba bean plants was studied in a pot experiment under sterilized condition. Plants were sampled at 30, 45 and 60 days of sowing.*

Results indicate that inoculation of faba bean with VA mycorrhizal fungi and virus (BYMV) caused a increased significant the virus concentration in plant leaves as compared with infected plants with the virus alone and caused decreased significantly the number of mycorrhizal spores and rate of root infection of faba bean plants. The dry weight of plants and contents of nitrogen, phosphorus and chlorophyll were markedly increased enhanced in the double inoculation (VAM+virus) in presence or absence of rhizobia when compared with the treatment of virus only at the end of experiment. On the other hand, the inoculation with VAM and rhizobia clearly increased significantly in all parameters, while the inoculation of rhizobia and virus which lead to a significant decrease in nodulation status and N₂-ase activity. Generally, the inoculation with VAM and/ or rhizobia overcome the deleterious effect of BYMV on the plants.

Key words: *vesicular arbuscular mycorrhizal fungi-Rhizobium leguminosarum, Bean yellow mosaic virus – Faba bean plants.*

INTRODUCTION

Faba bean (*Vicia faba*) is the most important leguminous food crop in Egypt, due to its high protein value and cheap price. It can be considered as the most popular crop for both humans and animal. Faba bean crop is subjected to infection with many viruses (Allam and El-Kady, 1966, and Makkouk et al., 1988). The faba bean virus infections cause great economic losses and considered to be the major limiting factor affecting faba bean production in Egypt

Many previous studies have shown that effect of vesicular arbuscular mycorrhiza fungi on crop growth are well established. Moreover, it was found that double infection of plants with virus and VA-mycorrhizal fungi and/or *Rhizobium-leguminosarum* caused synergistic damage effects (Sahab et al., 1978; Dehne, 1982; Mikhail, et al., 1988; Ishac et al., 1989 and Abd El-Mageed and Zaghloul, 1997). Reduction in phosphorus, nitrogen and potassium content of the infected plants with virus as compared to healthy ones was also emphasized (Tu et al., 1970; Daft and Okusanyo, 1973; Wongkaew and Peterson, 1986; Fawzy and Abd El-Mageed, 1990 and El-DougDoug and Othman, 1991).

The present investigation aimed at studing the effect of inoculation with VAM fungi and/or rhizobia on the infection with bean yellow mosaic virus on the growth of faba bean as well as their nutrient contents.

MATERIAL AND METHODS

Seed and soil

Faba bean seeds (*Vicia faba* c.v Giza 402) were obtained from Field Crops Res. Inst., Agriculture Research center (ARC), Giza, Egypt. The soil was of clay - loam texture with pH 7.5, organic matter 1.15%, total nitrogen 0.11%, total phosphorus 0.12%, calcium carbonate 2.53% and available P ppm. The soil was obtained from ARC Experimental field, Giza, Egypt.

Mycorrhizal inoculants

Mycorrhizal spores *Glomus mosseae*, *G. fasciculatum*, *Gigaspora* sp.were originally extracted from rhizosphere of onion plants (*Allium cepa*) using the methods of wet- sieving and decantation (Gerdeman and Nicolson, 1963). The isolated spores suspension containing about 90-100 spores ml⁻¹ was used as standard inoculum.

Bacteria inoculum

Symbiotic N₂ - fixes, (*Rhizobium leguminosarum* ARC 441) was used as seed inoculant. This strain was kindly provided by Agric. Microbiol. Dept., Soil, Water and Environment Res. Inst., Agric. Res. Center, Giza, Egypt. The strain was grown on yeast extract mannitol medium (Vincent, 1982) for 7 days at 28°C.

Virus inoculum

The virus was isolated from naturally infected broad bean plants and identified as follows:

Isolation

The infected young leaves were crushed in (0.1M) phosphate buffer pH 7.2 and indexed on *Chenopodium amaranticolor* and *C. quinoa* (El-DougDoug,1982), as well as of faba bean seeds were inoculated using mechanical inoculation method . Inoculated plants were placed in an wire proof insect greenhouse and symptoms expression was

observed and recorded after 4 weeks and then used as source of inocula in the subsequent experiments.

Properties of virus

Determination in vitro properties of the virus was made with fresh crude infection. Sap dilution was made with distilled water (10^{-1} up to 10^{-6}) to determine dilution end point (DEP). Undiluted infection sap was poured into sterilized test tubes and heated for 10 minutes in water bath at (40, 50, up to 80°C) to determine the thermal inactivation point (TIP). The treated virus with both (DEP) and (TIP) was assayed biologically using *Ch.amaranticolor* a local lesion host. Thereafter the inclusion bodies were examined in epidermal strips from the lower surface of healthy and infected leaves according to the method of McWhorter (1941). Partially purified virus was obtained using procedures described by (Makkouk, et al., 1988).

Fertilizers

Rock phosphate (26% P_2O_5) at the rate of 0.4 g kg^{-1} soil as unavailable phosphate was added before sowing for mycorrhizal inoculation, while superphosphate (15.5% P_2O_5) at the rate of 0.7 g kg^{-1} soil was added to for all treatment as available phosphate source. Ammonium nitrate (33.5% N) was added to soil at a rate of (0.1 g kg^{-1} soil) after sowing at the early stage of broad bean growth.

Experimental design

Sterilized pottery pots (5 kg capacity) were packed with steam sterilized soil (at 121°C for 1 h for 3 consecutive days). Sterilized soil was either amended with superphosphate or rock phosphate. Half of the pots was inoculated before planting with mycorrhizal fungi by layering (2×10^2 spores pot^{-1}) 2-3 cm below the planting holes in each pot. Spores washing was given to the uninoculated control pots. Five seeds of (*Vicia faba* c.v G 402) were sown in each pot^{-1} and inoculated with *R. leguminosorum* ARC 441 grown in yeast extract monnitrol broth by adding 5 ml of the medium contained 10^8 cell ml^{-1} (Vincent, 1982). The control treatment received YEM broth without inoculation for comparison. The plants were treated by the virus by using mechanical inoculation method (El-DougDoug and Othman, 1991) 20-30 days post sowing and the symptoms appear after 15-20 days from artificial infection. The experiment includes eight treatments in four replicates and arranged in a complete randomized design as follows:-

- 1- control.
- 2- Inoculation with *Rhizobium* (R).
- 3- Inoculation with mycorrhizal fungi (M).
- 4- Inoculation with virus (V).
- 5- Inoculation with M + R.

- 6- Inoculation with V + R.
- 7- Inoculation with V + M.
- 8- Inoculation with V + M + R.

The plants were left to grow for 60 days from sowing date under the greenhouse conditions with a natural day length of 8 hr. and an averages temperature of 18°C. During this period the plants were watered when necessary to keep the soil on 60% of its water holding capacity (WHC) .

Plant samples were taken at intervals of 30, 45 and 60 days of planting to determine plant height, dry weight at 80°C for 48 hrs., chlorophyll "a" and "b", (according to Arnon 1949), nodulation status (number and dry weight of nodules plant⁻¹), nitrogenase activity (μ mole C₂H₄ plant⁻¹ h⁻¹) in nodules was determined by the method of acetylene reduction (Hardy et al., 1973), as well as nitrogen and phosphorus content (mg plant⁻¹) were determined according to Jackson, (1973). The percentage of mycorrhizal infection in root tissue was also recorded by using the method of Phillip and Hayman, (1970), also the density of mycorrhiza spores was recorded according to (Gerdeman and Nicolson , 1963). The virus investivity area in the leaves was recorded after 60 days (Makkouk, et al., 1988), and then the virus concentration in leaves were assayed spectrophotometrically (Nordam, 1973). All data were statistically analyzed by Fisher's L.S.D. test (P< 0.050) according to the method described by McIntosh (1983).

RESULTS AND DISCUSSION

Assay of bean yellow mosaic virus in plant leaves

Data in Table (1) revealed that the viral infected plants the infectivity of BYMV increased as a result of previous infection with VA mycorrhizal fungi at rate 31.6%. This increase may due to the effect of VAM fungi on stimulating nutrient uptake and thus increasing growth and development of the host plant leading to excess virus multiplication. The increased exchange of nutrients between the fungus and the host plant cells can be characterized by high rate of phosphate metabolism as well as high contents of nucleic acids and proteins (Daft and Okasanyo, 1973, Ishac et al., 1989 and Abd El-Mageed and Zaghloul, 1997). It is clear from the obtained results that rhizobia inoculation of faba bean plants obviously reduced the infection caused by virus after 60 days of planting being 86.4%. This result may attributed to bacterial nodulation causing that enhance the chlorophyll content in such plants that decreases the host suscepility especially during the later stage of infection. El-Dougdoug (1982) and Fugro and Mishra (1995).

On the other hand, the double inoculation (VAM + rhizobia) reduced the concentration of virus in leaves of plants by a rate of 32.0% (Table 1).

The Influence Interaction Between Vesicular Arbuscular Mycorrhizal Fungi....

Table (1): Effect of inoculation with VA-mycorrhizal Fungi and *R. leguminosarum* on assay BYMV

Inoculum on Faba Bean	No. of L.L. on <i>Ch.amarnticolor</i>
R	-
V	125
M	-
R+M	-
R+V	17
V+M	183
R+V+M	85

R	=	<i>Rhizobium</i>
V	=	Virus
M	=	Mycorrhiza
R+M	=	<i>Rhizobium</i> + Mycorrhiza
R+V	=	<i>Rhizobium</i> + Virus
V+M	=	Virus + Mycorrhiza
R+V+M	=	<i>Rhizobium</i> + Virus + Mycorrhizal

Plant growth

Data of (Table 2) indicate that plants inoculated with VAM spores and rhizobia had greatly recorded the highest height and dry weight of faba bean plants after 60 days period being 121.2 cm and 6.48 g plant⁻¹. The high value were significantly differed positively from the other treatment. Significant increases of plant height and dry weight were noticed also in plants treated (VAM+V) being 58.5 cm and 0.67 g plant⁻¹ in comparison with virus infected ones. It was concluded that virus was a significant acts as repressing factor, through its effect on plant growth decreasing (Orellania et al.; 1978, El-DougDoug, 1982 and Mikhail et al., 1988). The plants inoculated with VAM+ rhizobia + virus developed less severe symptoms than those infected with the virus only, this may due to the effectiveness of the inoculated *Rhizobium* strain that negatively affect the severity symptomes of the virus infected plants (El-DougDoug, 1982 and Mikhail et al.,1988).

Chlorophyll content

Mycorrhizal plants Table (2) gave increases in chlorophyll a&b contents as compared with non-mycorrhizal plants. The double inoculation with (VAM + *Rhizobium*) generally gave the highest significant content of chlorophyll a and b (4.49 and 2.435 mg/g fresh leaves, respectively) at 60 days period in

Table (2): Effect of inoculation with mycorrhizal fungi, *R. leguminosarum* and bean yellow mosaic virus on height (cm), dry weight (g) and chlorophyll a and b of faba bean plants.

Treatments	Height of Plants (cm)			DW of Plants (g plant ⁻¹)			Chlorophyll "a" (mg g fresh leaves)			Chlorophyll "b" (mg g fresh leaves)		
	30 Days*	45 Days*	60 Days*	30 Days*	45 Days*	60 Days*	30 Days*	45 Days*	60 Days*	30 Days*	45 Days*	60 Days*
Control	66.5 d	70.7 c	100.2 e	0.82 c	1.12 b	1.28 c	2.620 d	2.229 d	1.650 c	1.395 c	0.986 c	0.824 d
<i>Rhizobium</i> (R)	76.4 f	90.5 f	116.0 f	2.27 f	3.62 l	3.21 f	3.500 f	3.554 g	2.327 f	1.975 e	1.851 e	1.128 e
VAM (M)	72.5 e	86.9 f	111.9 f	1.22 e	2.38 d	2.88 e	3.513 f	3.319 f	2.126 e	1.879 d	1.942 f	1.968 f
Virus (V)	44.5 a	51.6 a	50.0 a	0.29 a	0.39 a	0.46 a	0.723 a	0.350 a	0.477 a	0.136 a	0.120 a	0.105 a
R + M	112.3 g	116.5 g	121.2 g	3.34 g	5.07 f	6.48 g	3.621 g	3.670 h	4.490 g	2.112 f	2.168 g	2.435 g
R + V	60.3 c	78.5 d	93.0 c	1.03 d	1.13 b	1.44 d	2.853 e	2.484 e	1.731 d	1.315 c	1.215 d	0.899 d
M + V	49.0 b	55.6 b	58.5 b	0.32 a	0.42 a	0.67 b	0.950 b	0.701 b	0.782 b	0.157 a	0.155 a	0.191 c
R + M + V	62.2 c	71.5 c	85.1 d	0.77 b	1.22 c	1.24 c	2.370 c	1.733 c	1.603 c	1.229 b	0.851 b	0.791 c

In a column, means followed by a common letter are not significantly at 5% level by (DMRT).

* From planting.

comparison with all treatments at all other periods. However healthy nodulated faba bean plants showed considerable increase in photosynthetic activity over other treatments. Such increase could explain the highest content of carbohydrates found in faba bean shoots of nodulated plants (El-DougDoug, 1982 and Fugro and Mishra, 1995). The inoculated plants with VAM + virus increase the content of chlorophyll a and b in plants (0.782 and 0.191 mg/g fresh leaves, respectively) compared to the plants inoculated with virus only. These result could confirmed with (Hayman, 1983) who noted that the precence of VA mycorrhiz had encouraged the root exudation and rhizosphere population.

Nodulation status and nitrogenase activity

Results recorded in Table (3) shown that the increases in number, size and dry weight of nodules at the different tested growth periods (30,45, 60 days from planting) were recorded for plants inoculated with VAM and rhizobia compared to rhizobial inoculation only. These data are in harmony with results obtained by Ishac et al. (1989) and Badr El-Din et al., (2001). On the other respect, the nodulation status was decreased significantly in the plants inoculated with virus as compared with those inoculated with rhizobia only. These results recorded the corresponding number of nodules reduction were 44.9,52.9 and 54.9% after 30,45 and 60 days respectively. However, the virus infection caused the size of nodules and the dry weight of nodules to be decreased with increasing the growth period (Table 3). The effect of BYMV infection on nodulation status was related to the fact that virus multiplication causes physiological change including photosynthesis reduction or respiration increase and enzymes changes which were directly or indirectly affected the plant rhizobia symbiotic relationship. (Mikhail et al., 1988). These findings may be also lead to an insufficient tissue differentiation that caused a suppression of leghemoglobin synthesis and symbiotic N_2 – fixation activity (Orellana, et al., 1978).

Significant differences in N_2 – ase activity were observed between the inoculated treatments. Data in Table (3) shown that the nodules of the plants inoculated with *Rhizobium* + VAM gave a significantly increase in N_2 – ase activity values in comparison to those formed from plants inoculated whith rhizobia only. In case of nodules formed on virus infected plants a significant reduction in N_2 – ase activity in comparison with those inoculated rhizobia only. The reduction percentage were 27.2, 19.9 and 22.5%, respectively for 30, 45 and 60 days from planting. This trend may due to the effects of virus infection on the root nutrients uptake capacity and their transportation to other plants parts (Matthwes, 1981 and Ishac et al., 1983). On the other hand, the multi – inoculation (rhizobia + VAM + virus) had increased N_2 – ase activity compared with the treatment Rhizobia + virus by the 63.2% at 60 days from planting. These data are in harmony with the results obtained by Ishac et al., (1989) and El-DougDoug and Othman, (1991).

Table (3): Effect of inoculation with mycorrhizal fungi, *R. leguminosarum* and bean yellow mosaic virus on nodulation status and N_2 - ase activity.

Treatment	No. of Nodules Plant ⁻¹			Size of Nodules Plant ⁻¹			DW of Nodules mg Plant ⁻¹			N ₂ -ase Activity μ mole C ₂ H ₄ plant ⁻¹ h ⁻¹		
	30 Days*	45 Days*	60 Days*	30 Days*	45 Days*	60 Days*	30 Days*	45 Days*	60 Days*	30 Days*	45 Days*	60 Days*
R	24.50 b	35.00 c	37.75 b	0.595 c	0.89 c	1.223 c	0.409 b	0.550 b	0.612 c	396.30 c	417.06 c	318.03 c
R + V	13.50 a	16.50 b	17.00 a	0.162 b	0.785 b	0.485 b	0.023 a	0.054 a	0.065 b	200.43 a	304.21 b	106.3 a
R + M	33.31 c	42.12 d	46.32 c	1.011 d	1.354 d	1.501 d	1.33d	2.125 c	2.097 d	509.22 d	585.40 d	428.11 d
R + V + M	10.11 a	31.21 a	41.01 a	0.100 a	0.135 a	0.155 a	0.019 a	0.0375 a	0.0140 a	231.09 b	323.33 a	173.53 b

In a column, means followed by a common letter are not significantly at 5% level by (DMRT).

* From Planting

Nitrogen and phosphorus contents

Data in (Table 4) obviously revealed that mycorrhizal fungi addition to plants inoculated with rhizobia recorded the highest nitrogen and phosphorus contents 272.0 mg N and 28.16 mg P plant⁻¹ after 45 days from planting than single inoculation (Ishac et al., 1986 and Badr El-Din et al., 2001). On the other hand, mycorrhizal plants which treated with virus gave the highest values of N and P contents 53.32 mg N and 2.24 mg P plant⁻¹ after 60 days from planting compared with plants infected with virus. Such increase is of great importance since VAM fungi can overcome the deleterious effect of virus (Ishac et al., 1989). This behavior may be due to the chemical content of the virus. (Abd El-Mageed and Zaghloul, 1997).

The rate of mycorrhizal root infection and density of mycorrhizal spores:-

The relation between mycorrhizal root infection of faba bean plants and inoculation with rhizobia and / or bean yellow mosaic virus was evaluated and results are recorded in Table (4). Data indicated that inoculation with mycorrhizal fungi and rhizobia gave the maximal mycorrhizal root infection being 66.3% at 60 days period, while the inoculation with VAM + virus gave lower percentage of mycorrhizal infection being 30.3% at same period.

On the other hand, the inoculated plants with VAM + rhizobia produces maximal spores density (885.2 spores/kg soil). The lowest yield of mycorrhizal spores was collected from around the roots of plants infected with VAM + virus 201.2 spores/kg soil after 60 days. The virus infection led to reduce the number of VAM spores and percentage of mycorrhizal infection in roots. This finding may be due to the increased virus concentration in mycorrhizal plant roots, which was mostly diminish the cells containing contain arbuscular stage of the endophyte. (Dehne, 1982, El-DougDoug and Othman, 1991 and Fugroand Mishra, 1995).

Table (4) : Effect of inoculation with mycorrhizal fungi, *R. leguminosarum* and bean yellow mosaic virus on N and P contents, rate of infection and number of spores of mycorrhizal fungi of faba bean plants

Treatment	N Content (mg Plant ⁻¹)			P Content (mg Plant ⁻¹)			Rate of Infection With VAM (%)			Number of Spores (kg ⁻¹ soil)		
	30 Days*	45 Days*	60 Days*	30 Days*	45 Days*	60 Days*	30 Days*	45 Days*	60 Days*	30 Days*	45 Days*	60 Days*
Control	22.85 b	58.12 b	59.30 c	2.08 b	5.60 b	6.55 b	-	-	-	-	-	-
<i>Rhizobium</i> (R)	70.33 f	128.31e	180.40 g	5.65 d	8.19 c	9.36 c	-	-	-	-	-	-
VAM (M)	47.32 e	117.90d	119.01 f	9.15 e	17.10 d	18.75 d	41.2 c	47.3 b	56.5 c	309.3 c	321.b	824.3 d
Virus (V)	16.30 a	37.71 a	35.11 a	1.30 a	1.55 a	1.04 a	-	-	-	-	-	-
R + M	105.91g	272.00g	269.13 h	13.88 f	28.16 e	24.62 e	48.4 d	54.3 c	66.3 d	332.4 d	411.3 d	885.2 c
R + V	40.22 d	90.72 c	92.80 e	3.95 c	7.61 c	6.44 b	-	-	-	-	-	-
M + V	21.51 b	55.01 b	53.32 b	1.26 a	2.92 a	2.24 a	20.5 a	26.4 a	30.3 a	262.4 b	350.4 c	201.2 a
R + M + V	35.72 c	87.21 c	85.51 d	2.05 b	5.78 b	6.29 b	25.3 b	30.2 a	42.2 b	210.2 a	250.2 a	232.1 b

In a column, means followed by a common letter are not significantly at 5% level by (DMRT).

* From planting.

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أثر التفاعل بين فطريات الميكوريزا والريزوبيا وفيروس موزايك الفاصوليا الأصفر على نبات الفول البلدي

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الملخص العربي :

في هذا البحث تم دراسة التفاعل بين فطريات الميكوريزا والريزوبيا وفيروس موزايك
الفاصوليا الأصفر على نباتات الفول البلدي وتأثير ذلك على الوزن الجاف للنبات ومحتوى
الكلوروفيل ونشاط الفيروس وعدد العقد الجذرية. ونشاط انزيم النيتروجينز ومحتوى النبات من
النيتروجين والفوسفور وعدد جراثيم الميكوريزا بالتربة ونسبة إصابة جذور نباتات الفول
البلدي وذلك في تجربة أصص وتحت ظروف التعقيم وقد أوضحت النتائج ما يلي:-

- أدى التلقيح بفطريات الميكوريزا وفيروس موزايك الفاصوليا الاصفر الى زيادة معنوية في
تركيز الفيروس في أوراق نباتات الفول البلدي مقارنة بالنباتات المصابة بالفيروس فقط كما
أدت الإصابة بالفيروس الى انخفاض معنوي في اعداد جراثيم الميكوريزا في منطقة
ريزوسفير نباتات الفول البلدي وكذلك انخفاض معدل إصابة جذور نباتات الفول البلدي
بالميكوريزا.

- كما أدى التلقيح السابق في وجود أو عدم وجود الريزوبيا الى زياده في الوزن الجاف للنبات
ومحتوى الكلوروفيل والمحتوى النباتي من النيتروجين والفوسفور مقارنة بالنباتات الملحقة
بالفيروس فقط وذلك في نهاية عمر النبات.

- أدى التلقيح المزدوج بين الميكوريزا والريزوبيا الى زياده معنوية في جميع القياسات السابقة ما عدا التلقيح بالريزوبيا والفيروس الذي أدى إلى انخفاض معنوى في تكوين العقد الجذرية ونشاط إنزيم النيتروجينيز.
- أدى التلقيح بالميكوريزا في وجود او عدم وجود الريزوبيا الى عدم تدهور نمو نباتات الفول البلدي المصابة بفيروس موزايك الفاصوليا الاصفر.