

Effect of the Endomycorrhizal Fungus *Glomus mosseae* on Soybean Mosaic Virus in Soybean

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(Accepted date for publication: June 15, 1985)

摘 要

Green, S. K., 鄧汀欽 1985。內生菌根 *Glomus mosseae* 對大豆嵌紋病毒感染大豆之影響。植保會刊 27 : 353~358 (亞洲蔬菜研究發展中心, 臺灣省農業試驗所植物病理系)

在大豆 (*Glycine max*) 植株上測定 *Glomus mosseae* 感染對大豆嵌紋病毒的影響, 發現有菌根的大豆植株, 其第七節及第八節葉片所含病毒的濃度均比無菌根的植株有顯著性增高。在株高、葉面積、每株莢數及種子數、地上部及根部乾重、每株產量等比較, 同樣接種大豆嵌紋病毒的情形, 有菌根的植株均顯著高於無菌根的對照組, 但是在百粒重方面無明顯的差異。由於 *G. mosseae* 對產量的影響極為顯著, 因此植株遭病毒感染但有菌根的情況和無菌根且無病毒的健全株比較, 在產量方面無顯著差異。

ABSTRACT

The effects of colonization with *Glomus mosseae* were examined in soybean (*Glycine max*) infected with soybean mosaic virus (SMV). The virus concentration of the seventh and eighth trifoliolate leaves was found to be significantly higher in mycorrhizal plants than in nonmycorrhizal plants. Plant height, leaf size, pod and seed number per plant, shoot and root dry weight, and yield per plant were all significantly higher in the SMV inoculated mycorrhizal plants than in SMV inoculated non-mycorrhizal control plants. One hundred seed weight, however, was unaffected by mycorrhizae. The effect of *G. mosseae* was so pronounced that the yield of SMV-infected mycorrhizal plants was not significantly different from that of healthy non-mycorrhizal plants.

INTRODUCTION

Plants colonized with vesicular arbuscular mycorrhizae (VAM) are known

to show a different response to plant pathogens than do nonmycorrhizal plants (5,14,18). Studies conducted with soil-borne fungi and nematodes have shown that

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VAM decrease disease severity and reduce infection by these pathogens^(5,7,8,12,13,14). Other reports, however, indicate that mycorrhizal plants may be more susceptible to rust, mildew, and leaf spot fungi⁽¹⁴⁾. In one instance, VAM have also been implicated as the cause of disease⁽⁶⁾. The effect of VAM on infection with viruses has been examined for tobacco mosaic virus, tomato aucuba mosaic virus, potato virus X and arabis mosaic virus^(4,15), and in each case virus multiplication was higher in mycorrhizal than in non-mycorrhizal plants.

The objectives of the present study were to measure the effects of colonization with *Glomus mosseae* on soybean (*Glycine max*) infected with soybean mosaic virus (SMV).

MATERIALS AND METHODS

Seeds of soybean cultivar TN-4 were planted in 17 cm plastic pots containing autoclaved soil (120°C/30 min.) from an AVRDC field known to be low in soluble phosphorus. The crop succession pattern of this field prior to this experiment was rice, fallow, corn, sweet potato, fallow, and sweet potato. After autoclaving the available phosphorus in the soil was measured and found to be 9 ppm. Before seeding each seedhole was inoculated with 1 ml of a solution containing 7.5×10^7 *Rhizobium* bacteria/ml, prepared from surface sterilized (10 min. in 0.1% HgCl₂, followed by 3 rinses with distilled water) crushed soybean nodules. The nodules had been collected from field-grown soybean plants. Seedholes to be treated with mycorrhizae received an additional 10 g soil inoculum, containing 200 spores of *Glomus mosseae*. This species was one of

the three most common endomycorrhizal fungi reported by Tschanz and Wong⁽¹⁷⁾ on soybean roots in the soybean-rice cropping system in Taiwan. Mycorrhizal chlamydospores used in all tests were produced in a soybean pot culture. After 10 days, seedlings were thinned to three per pot. Inoculation with SMV (strain G1, isolated from soybean in Taiwan) was at the primary leaf stage, using a homogenate of 1 g fresh SMV infected *Glycine max* cv. 'TN-4' leaves in 3 ml 0.1 M phosphate buffer, pH 6.5. The treatments, each consisting of 3 plants/pot, were replicated 10 times and arranged randomly on a greenhouse bench. Plants were watered daily with tap water.

Plant height, shoot and root dry weight, nodule number, and pod and seed number were determined at harvest. Successful mycorrhizal infection was assessed visually by staining randomly selected root segments of each treatment in acid fuchsin⁽¹⁰⁾. The leaf area of the sixth trifoliolate leaf was determined when the leaf was fully expanded. Each of the measured variables was subjected to an analysis of variance. Orthogonal contrasts of treatment means were used to estimate the main effects (virus and mycorrhizae) and their interactions. The significance of each contrast was determined from its "t" value (value of the contrast divided by its standard error). The relative virus content of SMV infected leaves was measured in the fully expanded leaves of the fifth, seventh, and eighth trifoliolate leaves. Five leaf discs of 27.75 mm² each were cut with a corkborer at the same position of each leaf. Three discs were cut from the tip of the middle leaflet and one was cut from each of the

two side leaflets. The five leaf pieces were then pooled and triturated in 1 ml of 0.01 M phosphate buffer, pH 6.5. Twenty μ l of this homogenate was placed on two half-leaves of a fully developed unifoliolate leaf of *Phaseolus vulgaris* cv. Topcrop and was spread evenly with a glass rod, exercising gentle pressure. The number of local lesions produced was used as a measurement of the relative virus concentration in the infected plant.

RESULTS

All root systems of mycorrhiza inoculated treatments were successfully colonized by *G. mosseae* at harvest and considerable growth enhancement was observed in mycorrhizal plants (Fig. 1) irrespective of the presence or absence of virus.

Plant height, leaf size, the number of pods and seeds per plant, shoot and root

dry weight and yield per plant were all higher in the SMV inoculated mycorrhizal plants than in SMV-inoculated non-mycorrhizal plants (Table 1). The main effects, increase due to *G. mosseae* and decrease due to virus, were significant for all growth parameters, except for the 100 seed weight, which was the same in mycorrhizal and non-mycorrhizal plants.

Enhancement of the leaf area was most marked. The leaf size of SMV infected mycorrhizal plants showed an increase of more than 70% over the non-mycorrhizal healthy controls. Significant interactions between virus and mycorrhizae were observed only for leaf size and nodule number. The effect of mycorrhizae on both parameters was higher in the absence of virus than in its presence. Yield reduction due to SMV in mycorrhizal plants was only 39%, compared with 65% in non-mycorrhizal plants.

Mycorrhizal infection significantly

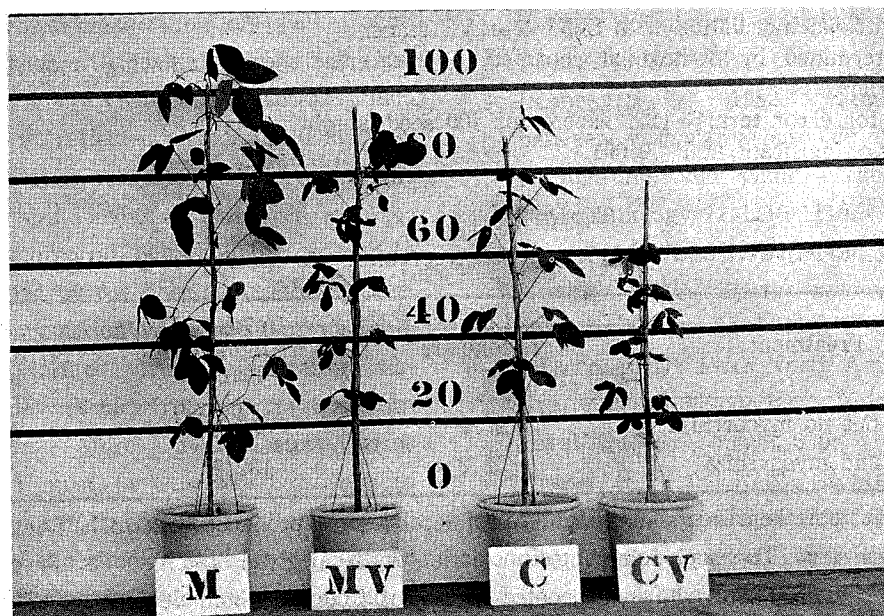


Fig. 1. Growth response of soybean inoculated with *Glomus mosseae* (M), soybean mosaic virus (CV), *G. mosseae* and SMV (MV), and noninoculated (C).

increased soybean mosaic virus concentration in the seventh and eighth trifoliolate leaves (Table 2). In the fifth trifoliolate leaf, SMV concentration was also higher in mycorrhizal than in non-mycorrhizal plants; but not significantly.

Table 1. Plant response to infection with *G. mosseae* and soybean mosaic virus (SMV)

Treatment	Plant height (cm)	Leaf ¹ size (cm ²)	Pod No./ plant	Seed No./ plant	Shoot dry weight (gm)	Root dry weight (gm)	Nodule No./ plant	Yield/ plant (gm)	100 seed weight (gm)
Control	86.20	59.63	5.93	9.77	1.37	0.82	13.32	2.01	20.57
Virus ²	62.79	39.72	2.97	4.07	0.64	0.43	7.90	0.71	17.50
<i>G. mosseae</i>	105.57	150.30	9.00	15.90	1.97	0.97	30.45	3.04	19.12
<i>G. mosseae</i> + virus ²	81.80	103.31	6.97	10.73	1.31	0.82	8.64	1.87	17.40
..... Total value and significance of main effects and interactions ^{3,4,5}									
Interaction	-0.18	13.53***	0.47	0.27	0.03	0.12	8.9***	0.06	0.67
Increase due to <i>G. mosseae</i>	19.19***	77.12***	3.50***	6.40***	0.64***	0.27***	13.6***	1.09***	-0.77
Decrease due to presence of virus	23.59***	33.46***	2.50***	5.43***	0.70***	0.27***	8.2***	1.23***	2.39***
Standard error of contrasts	2.57	2.29	0.37	0.58	0.08	0.07	1.42	0.11	0.52

1) Area of trifoliolate leaf at node No. 6.

2) Artificial inoculation with SMV-1 at VC stage.

3) Determined by orthogonal contrasts of treatment means following analysis of variance.

4) df for error terms=115, except for 100 seed weight, df=10

5) ***=significant at $P \leq 0.001$.

Table 2. Relative virus titer in leaves of mycorrhizal and nonmycorrhizal soybean plants

Treatment	5th trifoliolate leaf	7th trifoliolate leaf	8th trifoliolate leaf
Virus but no mycorrhiza	27.9 ¹	87.8	33.4
Virus and mycorrhiza	31.2	132.9*	79.0**

1) Each value represents the mean No. of local lesions produced on two half leaves of *P. vulgaris* 'Topcrop' following inoculation with a given volume (20 μ l) of leaf homogenate prepared from a pooled sample of five 27.75 mm² leaf pieces. The five pieces came from one trifoliolate leaf each of 10 test plants.

* and ** indicate significant difference from control (no mycorrhiza) at 5% and 1% levels, respectively.

DISCUSSION

The general growth enhancement of soybean plants by mycorrhizae in pot culture, as observed in this study, has been demonstrated before^(1,2,3,11,16). The increase of virus concentration in mycorrhizal plants has also been reported^(4,15). The higher virus concentration in mycorrhizal plants has been attributed to the more vigorous plant growth⁽⁵⁾ and to an increased phosphorus uptake⁽⁹⁾ making more phosphorus available for the replication of viral nucleic acid. In this study it was found that even though the virus concentration in mycorrhizal plants increased significantly in the seventh and eighth trifoliolate leaves, the total yield of virus-infected mycorrhizal plants was still much higher than that of virus-infected non-mycorrhizal plants and was not significantly different from healthy non-mycorrhizal plants. The effect of mycorrhizal colonization on plant growth thus appears to be more important than its effect on virus multiplication. Such effect is reflected in a 2.5-fold increase in the photosynthetic leaf area of the mycorrhizal plants as compared with the non-mycorrhizal plants. The benefits to be gained by the host plant from mycorrhizal inoculation would seem to outweigh the accelerated virus multiplication that may also result from mycorrhizal inoculation. More VAM/hostplant/virus systems must be studied, however, to determine if this is universally true and further experiments will be required to verify these results under field conditions.

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