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Potential of Endo-mycorrhizae and Bacterial Antagonist on the Growth Performance of Tomato Cultivars against Bacterial Wilt

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ABSTRACT

The application of *Glomus mosseae* (Gm1, Gm2 and Gm3), *Pseudomonas fluorescens* and *Bacillus cereus* individually and in combination reduced the wilt disease significantly as compared to control in cv. Solan gola and BWR-5. Interestingly, the antagonist *Pseudomanas fluorescens* with isolates of vesicular arbuscular mycorrhizae showed synergistic effect against wilt incidence, while as no such effect was found with *Bacillus cereus*. The concomittent application of *Pseudomanas fluorescens* with all the three isolates of *Glomus. mosseae* and that of *B. cereus* with VAM isolates Gm1 and Gm2 synergistically reduced wilt, whereas *Bacillus cereus* in combination with Gm3 did not influence wilt incidence. The combined application of vesicular arbuscular mycorrhizae and the antagonist had much more pronounced effect on all. The plant height, root length and number of fruit per plant were also significantly increased.

Key words: *Glomus mosseae*, *Pseudomonas fluorescens*, *Bacillus cereus*, *Ralstonia solanacearum,* tomato cv. Solan gola and BWR-5

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* is the most important disease in tropical and sub-tropical regions of the world. Recently great emphasis on stable sources of resistance (Wang *et al*., 1996) and other alternative control measures such as biological and cultural control have been laid (Trigalet *et al*., 1994). Studies conducted in several countries have shown the potential of antagonistic rhizobacteria, soil

solarization and VAM (Vesicular Arbuscular Mycorrhizae) to inhibit or displace soil borne pathogens at root soil interface and there by protect the crop plants (Hayward, 1991). Vesicular Arbuscular Mycorrhizae (VAM) fungi have also been inflicted in the biological control of a few soil borne fungal (Jalali *et al.,* 1990) and bacterial pathogens (Halos and Zorilla, 1979). The use of VAM alone and in combination with bacterial antagonists also needs to be explored for the control of soil borne diseases like bacterial wilt of tomato. The present work has been initiated to integrate bio-agents with VAM and observe its effect on the disease development of bacterial wilt of tomato.

MATERIAL AND METHODS

VA-mycorrhizal Isolates

Three isolates of vesicular arbuscular mycorrhizal *Glomus mosseae* designated as Gm_1 Gm_2 and Gm_3 were used in the present study. Isolate Gm_1 was obtained from DR. D.J. Bhagyaraj; G.K.V.K, Campus University of Banglore, the isolate Gm2 was procured from Punjab Agricultural University Ludhiana, whereas the third isolate Gm_3 was isolated from soil of Palampur in the Department of Plant Pathology.

Multiplication and Maintenance of VA-mycorrhizal Isolates

VA-mycorrhizae isolates were multiplied and maintained separately on the roots of tomato plants (cv. Roma and Solan Gola) in plastic pots in the glass house. The roots of 30-45 day old plants of the respective isolates were checked for the colonization under the microscope. The entire root system was subsequently chopped of along with the adhering soil and inbreed with fresh soil obtained from the field for further multiplication in a similar fashion. The process was repeated several times for the production of VAM enriched soil for experimentation and maintenance of isolates.

Evaluation of VA-mycorrhiza and Antagonist against Wilt

The effect of soil enriched with the three VAM isolates individually and with the combination of the antagonists, *Pseudomonas fluorescens* and *Bacillus cereus* against wilt was studied by conducting a pot experiment in glass house during two years using tomato cultivars Solan Gola and BWR-5 (HR). The experiments were laid out in a completely randomized block design with three replications. Each replication consisted

of three pots, each pot containing three plants. Soil enriched with each of three VAM isolates was taken into the pots for determining the effect of respective isolates. In order to study the effect of individual antagonist, soil bacterization was done with the respective antagonist. For studying combined effect of the VAM isolates was further bacterized. The sterilized soil + FYM (1:1) taken in pots was used for control treatment. Twenty five to twenty eight day old seedlings of Solan Gola and BWR-5 (HR) raised separately in iron trays containing sterilized mixture of soil + FYM (1:1) were transplanted in the month of May during both the years and then inoculated with 20 ml cell suspension of virulent *Ralstonia solanacearum.* The inoculated plants along with control were observed regularly for 25 days for the appearance of wilt symptoms. The data on plant height root length and number of fruits per plant were also recorded and analyzed statistically.

RESULTS AND DISCUSSION

The enrichment of soil with all the three isolates of *Glomus mosseae* (Gm1, Gm2 and Gm3) and application of antagonist *P. fluorescens* and *B. cereus* individually and in combination reduced the wilt significantly as compared to control in cultivar Solan Gola and BWR-5 (Table 1 & 2). Table 1 revealed that lowest per cent of wilt incidence of 0% and 11.11% was observed on BWR-5 and Solan Gola cvs. When Gm_1 was used in combination with P. *fluorescens* compared to control were 37.33% and 95.66% wilt incidence per cent was observed during the year 1996. Similar trend was also observed in the year 1997 with wilt incidence of 0% and 19.99% compared to control where disease was 28.33% and 91.66%. Interestingly the application of antagonist *P. fluorescens* with all the three isolates of VAM showed synergistic effect on R. *solanacearum* resulting in reduction of wilt incidence, whereas no synergistic reduction in wilt was not observed, when the antagonist *B. cereus* was applied in VAM enriched soil. The combination of VAM with *B. cereus* reduced less wilt in Solan Gola as compared with BWR-5.

Variable results were obtained with comparatively less susceptible cultivar BWR-5 (HR). In this case only the VAM isolate Gm1 resulted in significant reduction in wilt but the isolate Gm2 and Gm3 had no effect in the reduction of wilt. Similarly the antagonist *B. cereus* did not influence wilt incidence. The combined application of *P. fluorescens*

with all the three isolates of *G. mosseae* and that of *B. cereus* with VAM isolates Gm1 and Gm2 synergistically reduced wilt, whereas *B. cereus* in combination with Gm3 did not influence the wilt incidence.

Table 1. Effect of VA-mycorrhizae and antagonists on the performance of tomato cultivars against bacterial wilt during 1996.

Treatment	Terminal wilt incidence (%)	
	Solan Gola	**BWR-5 (HR)**
Gm_1	66.66 (54.70)	25.00 (29.98)
Gm_2	61.10 (51.46)	33.33 (35.24)
Gm_3	65.00 (53.70)	40.55 (39.46)
P. fluorescens	50.00 (44.98)	24.33 (29.53)
B. cereus	55.55 (48.22)	31.11 (33.88)
Gm_1 + *P. fluorescens*	11.11 (19.46)	0.00 (0.00)
Gm_2 + *P. fluorescens*	25.00 (29.98)	15.00 (22.77)
Gm_3 *P. fluorescens*	33.33 (35.24)	20.00 (26.55)
Gm_1 + *B. cereus*	50.00 (44.98)	0.00 (0.00)
Gm_2 + *B. cereus*	55.55 (48.22)	19.33 (25.95)
Gm_3 *B. cereus*	55.55 (48.22)	33.33 (35.24)
Control	95.66 (82.92)	37.33 (37.64
CD (5%)	**11.51**	**3.77**

*Average of three replications
Figures in parentheses are angular transformed values
Gm: *Glomus mosseae*

Almost similar trend was obtained during second year also. The combined application of VAM isolates Gm1, Gm2 and Gm3 and of the antagonist *B. cereus* produced synergistic effect on the reduction of wilt incidence. The extent of terminal wilt incidence in general was low during second year than first year.

Table 2. Effect of VA-mycorrhizae and antagonists on the performance of tomato cultivars against bacterial wilt during 1997.

Treatment	Terminal wilt incidence (%)	
	Solan Gola	**BWR-5 (HR)**
Gm_1	38.88 (38.39)	20.66 (27.00)
Gm_2	30.55 (33.49)	20.33 (26.78)
Gm_3	33.33 (35.24)	23.33 (28.80)
P. fluorescens	29.44 (32.81)	17.33 (24.58)
B. cereus	44.44 (41.73)	20.66 (26.99)
Gm_1 + *P. fluorescens*	19.99 (26.49)	0.00 (0.00)
Gm_2 + *P. fluorescens*	25.00 (29.98)	8.77 (17.21)
Gm_3 *P. fluorescens*	33.33 (35.24)	13.66 (21.66)
Gm_1 + *B. cereus*	20.00 (26.57)	0.00 (0.00)
Gm_2 + *B. cereus*	25.00 (29.98)	10.66 (19.01)
Gm_3 *B. cereus*	33.33 (33.24)	24.44 (28.44)
Control	91.66 (79.96)	28.33 (31.12)
CD (5%)	**9.68**	**3.74**

*Average of three replications
Figures in parentheses are angular transformed values
Gm: *Glomus mosseae*

The enrichment of soil with VAM isolates and the alone application of the antagonist significantly increased plant height, root length and number of fruits per plant during both years in tomato cultivars viz. Solan Gola (Table 3) and BWR-5 (Table 4). Maximum plant hight of 84.55 cm, root length of 17.44 cm and 5.12 fruits/plant were observed in the year 1996 when Gm_1 was used in combination with P. *fluorescens.* Similar trend with 87.44 cm, 19.44 cm and 5.66 fruits/plant were observed during the 1997 as is revealed by the Table 4.

The combined application of VAM and the antagonists had much more pronounced effect on all the three crop parameters (plant height, root length and number of fruit per plant) as compared to their individual effect.

Table 3. Effect of VA-mycorrhizae and antagonists on plant height, root length and fruit number of tomato cv. Solan Gola during 1996.

Treatment	Terminal wilt incidence (%)		
	Plant height (cm	Root length (cm)	No. of fruits/plant
Gm_1	48.21	11.98	3.33
Gm_2	47.71	12.07	3.00
Gm_3	41.66	9.91	3.21
P. fluorescens	51.55	12.10	4.77
B. cereus	52.44	11.10	4.10
Gm_1 + *P. fluorescens*	84.55	17.44	5.10
Gm_2 + *P. fluorescens*	67.10	14.03	4.22
Gm_3 *P. fluorescens*	62.55	12.92	4.32
Gm_1 + *B. cereus*	82.22	16.38	4.44
Gm_2 + *B. cereus*	62.66	14.92	4.48
Gm_3 *B. cereus*	59.33	13.47	4.47
Control	26.33	5.00	1.00
CD (5%)	**8.67**	**1.42**	**1.05**

*Average of three replications
Gm: *Glomus mosseae*

The previous studies conducted by Parashar (1995) have shown the efficacy of VA-mycorrhizal fungi (*Glomus* spp.) and antagonistic rhizobacteria (*P. fluorescens* and *Bacillus* sp.) individually against bacterial wilt using tomato cv. Solan Gola. However, in the present study, it was endeavoured to determine the combined effect of VAM and antagonists on the performance of tomato cvs. Solan Gola and BWR-5(HR) with varying degree of terminal wilt incidence. During first year, the combined use of VAM and with antagonists reduced wilt incidence in both cultivars synergistically as compared to their individual effect, the VAM isolate Gm1 alongwith the antagonist *P. fluorescens* reduced wilt by six times in the highly susceptible cv. Solan Gola and 25 times less in moderately susceptible cv. BWR-5 (HR) as compared to Gm1 alone. Like wise the VAM isolate Gm1 alongwith the antagonist *B. cereus* reduced wilt by 25 times as compared to Gm1 alone

in BWR-5 (HR) but the combination had no significant effect in case of Solan Gola. Similar trend was also obtained during second year except combined use of VAM (Gm1) with *B. cereus* reduced by 50 per cent as compared to alone. The yearly variation could be explained due to the reason that both the VAM and the antagonist could get more time for colonization of the roots of tomato during second year. The response of *B. cereus* on the performance of tomato cvs. could be done to its relative degree of colonization of the roots of two tomato cvs. The combined use of at least VAM (Gm1) and antagonist (*P. fluorescens*) not only reduced wilt synergistically but also resulted in significant increase in plant height, root length and the number of fruits per plant in both the cultivars. Thus the combined use of VAM and antagonists can prove one of the potential parameters in integrated management for bacterial wilt.

Table 4. Effect of VA-mycorrhizae and antagonists on plant height, root length and fruit number of tomato cv. BWR (HR) during 1997

Treatment	Terminal wilt incidence (%)		
	Plant height (cm	Root length (cm)	No. of fruits/plant
Gm_1	58.10	14.99	3.21
Gm_2	54.28	14.81	4.00
Gm_3	57.33	12.84	3.88
P. fluorescens	52.33	15.32	4.77
B. cereus	56.55	14.32	4.11
Gm_1 + *P.Fluorescens*	87.44	19.44	5.66
Gm_2 + *P .fluorescens*	67.77	16.03	4.77
Gm_3 *P. fluorescens*	66.10	14.92	4.44
Gm_1 + *B. cereus*	86.66	19.21	4.44
Gm_2 + *B. cereus*	73.10	17.62	4.55
Gm_3 *B. cereus*	67.88	15.84	4.77
Control	34.99	7.75	2.77
CD (5%)	**7.26**	**1.37**	**0.93**

*Average of three replications
Gm: *Glomus mosseae*

The role of VAM fungi for the control of soil borne diseases has been well documented (Caron *et al*., 1986; Jalali and Chand, 1988; Jalali *et al*., 1990 and Chandra, 1992), but relatively little work has been done on bacterial pathogens. The present study corroborates with the findings of Halos and Zarilla (1979) who maintained that prior infection with mycorrhiza reduced wilt incidence in sterilized and unsterlized soil when inoculated with *R. solanacearum*. Garcia – Garrido and Ocampoo (1989). In another study found significant interaction between *G. mosseae* and *Erwinia carotovora* and envisaged its effect on the growth of tomato plants. Dochow and Abov-Shaar (1990) also found that combination of *Glomus* sp. and *B. subtillis* strain 799 resulted in impeding disease of tomato and increasing growth and yield. Suresh and Rai (1991) shall further observed that extracts from mycorrhizal tomato roots infested with *G. fasiculatus* reduced the population of *R. solanacearum*.

The possible reasons for wilt reduction and significant increase in growth parameters of tomato plants could be that mycorrhizal colonization induces strengthening of cell walls by enhanced production of lignins. Lignins, acting as induced resistance substances, are believed to provide effective barriers to pathogen attacks. Increased plant growth was achieved because mycorrhizae had got developed on non-mycorrhizal plants, like tomato which ensured the plants with increased availability of water and nutrients. Further, stronger vascular system in VAM colonized tomato plants imparts greater mechanical strength. The synergistic effect of VAM and antagonists was presumably because both are strong root colonizers of tomato plants.

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