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Arbuscular Mycorrhizal Fungi: Biocontrol against Fusarium Wilt of Pea

M.K. Yadav* and Sushreeta Naik

Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi - 221 005, India.

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Pea (*Pisum sativum* L.) is an important source of human food that also helps in the management of soil fertility, particularly in dry lands. Fusarium wilt, caused by *Fusarium oxysporum*, is one of the most important limiting factors of Pea production in India. The present study shows the status of Mycorrhization in three test varieties of Pea. As the results revealed irrespective to the crops when Arbuscular Mycorrhizal fungi (AMF) were assessed under the influence of soil quality better results were obtained with unsterile soil in comparison to sterile soil in earthen pots. Interest in biological control has increased recently concerns over the use of chemicals in the environment in general, and the need to find alternatives to the use of chemicals for disease control in particular. Pea variety Pant Matar 2 showed the best result against the Fusarium wilt and Pea variety AZAD P-2 was susceptible against the Fusarium wilt. *Acaulospora spinosa* showed best results with JAWAHAR MATAR 4, *Glomus mosseae* with AZAD P-2 and *Glomus fasciculatum* showed best results with Pant matar 2. Inoculum of *Glomus mosseae* for variety AZAD P 2, *G. fasciculatum* for AZAD P-2 and *Acaulospora spinosa* for variety JAWAHAR MATAR 4 consisted of colonized pieces of root, soil and spores, derived from pot cultures prepared with *Zea mays* grown in 1:9 soil-sand (pH 7.3) or 1:9 soil-sand (pH 5.6) to take into account pH preferences of the fungi . The AMF mix was 1:1 mixture of this inoculum. The lowest percentage mycorrhizal colonization was found on plants with the most severe disease symptoms.

Key words: *Fusarium oxysporum,* Arbuscular Mycorrhizal fungi, Colonization, AZAD P-2 and *Acaulospora spinosa.*

Pea (*Pisum sativum*L.) is an important source of human food that also helps in the management of soil fertility, particularly in dry lands. Fusarium wilt, caused by *Fusarium oxysporum*, is one of the most important limiting factors of Pea production in India. Fusarium wilt epidemics cause important annual losses of Pea yields that may reach 100 % under conditions favourable for disease. *Fusarium oxysporum* is a soil-borne fungus pathogenically specialized on *Pisum sativum* that causes wilt.

* To whom all correspondence should be addressed.
E-mail: manojbhu87@gmail.com

Interest in biological control has increased recently fuelled by public concerns over the use of chemicals in the environment in general, and the need to find alternatives to the use of chemicals for disease control in particular. The key to achieving successful, reproducible biological control is the gradual appreciation that knowledge of the ecological interactions taking place in the soil and root environments is required to predict the conditions under which biocontrol can be achieved. The major feature involves improving plant nutritional status, perhaps water balance and thus plant growth, biocontrol of plant pathogens is generally viewed as a secondary role

MATERIALS AND METHODS

The experiments were conducted a factorial glasshouse experiment using plants of Pea (*Pisum sativum*L.) varieties Pant matar 2, AZAD P-2and JAWAHAR MATAR 4 which were grown as follows: (1) Pea varieties with Foc (2) with AMF species (3) with Foc + AMF species and (4) without Foc or AMF as control. All 4 experiments were done in both soil conditions i.e. sterile (autoclaved at 120° C for 24 h) and unsterile.

Fungal cultures

F. oxysporum was grown on Czapek Dox agar at 25°C in dark. Under sterile conditions, fungal cultures were flooded with sterilized water and the suspension was filtered through three layers Whatman no. 1 of filter paper. The spore-suspension was concentrated by centrifugation at 3,000×g for 10 min and adjusted to 1.5×107 microconidia/ml water using a haemocytometer.

Arbuscular Mycorrhizal Fungi

Inoculum of *Glomus mosseae* for variety AZAD P 2, *G. fasciculatum* forPant Matar 2 and *Acaulospora spinosa* for variety JAWAHAR MATAR 4 consisted of colonized pieces of root, soil and spores, derived from pot cultures prepared with *Zea mays* grown in 1:9 soil-sand (pH 7.3) or 1:9 soil-sand (pH 5.6) to take into account pH preferences of the fungi. The AMF mix was 1:1 mixture of these two inocula.

Planting and growth conditions

Seeds of Pea varieties were surface sterilized in 10% sodium hypochlorite for 10 min, rinsed thoroughly with distilled water and germinated on moist filter paper in dark at 24°C for 2 days. Seedlings of the 3 varieties were transplanted to the pots in the appropriate combinations. The experiment was carried out during July- Oct, 2011–12 in an environmentally controlled glasshouse at the Department of Mycology and Plant Pathology, IAS, BHU Varanasi. Night-day temperature range in the glasshouse was 28-35°C. Pots were watered twice in a week with distilled water.

RESULTS AND DISCUSSION

The three test Pea varieties viz. Pant matar 2, AZAD P-2and JAWAHAR MATAR 4 showed different per cent root colonization when they were treated with selected AMF species. Ten AMF species were evaluated for their root colonization under sterilized and unsterilized soil conditions. In AZAD P-2*Glomus ambisporum* and *G. mosseae* showed greater colonization followed by *G. clarum, G. heterosporum, G. hoi, G. fasciculatum, Acaulospora nicolsonii, G. lacteum, A. spinosa. Gigaspora albida* was found poor colonizer in sterilized soil condition but in unsterilized soil *G. mosseae* showed greater colonization followed by *G. hoi, G. heterosporum, G. lacteum, G. clarum, Gigaspora albida, A. spinosa, G. fasciculatum, A. nicolsonii* and *G. ambisporum*. *Glomus fasciculatum* showed greater colonization in sterile soil condition with Pea varietyPant Matar 2 followed *G. mosseae, G. hoi, Gigaspora albida, G. heterosporum, G. lacteum, A. spinosa, G. ambisporum, G. clarum* and *A. nicolsonii* whereas, *G. mosseae* showed greater per cent root colonization in unsterileize soil condition followed by *G. hoi, G. heterosporum, G. ambisporum, G. fasciculatum, G. calrum, G. lacteum, A. spinosa, A. nicolsonii* and *Gigaspora albida*. In variety JAWAHAR MATAR 4 , *A. spinosa* showed more per cent root colonization followed by *G. lacteum G. mosseae, G. hoi, G. heterosporum, G. calrum, G. fasciculatum, G. ambisporum, A. nicolsonii*, and *Gigaspora albida* under sterilized soil, whereas, in unsterilize soil also *A. spinosa* was found better than *G. lacteum, G. fasciculatum, G. mosseae, G. hoi, G. heterosporum, G. Ambisporum, A. nicolsonii, G. calrum* and *Gigaspora albida*.

The introduction of AM fungi (*G. mosseae, G. fasciculatum* and *A. spinosa* with their respective Pea varieties i.e. AZAD P 2, Pant Matar 2 and JAWAHAR MATAR 4) in the soil suppressed the effect of pathogen in the rhizoplane as well as in the rhizosphere. The data suggest that respective AM fungi played an important role to protect the plant and therefore we found that all the three varieties Pant matar 2, AZAD P-2and JAWAHAR MATAR 4 were well protected by their respective AM fungal inoculants. Thus, no mortality of the plants inoculated with their respective AM fungi was recorded with any case. But inoculation of pathogen alone caused 80.0% of mortality in AZAD P 2, 24.0% in Pant Matar 2 and 26.0% in JAWAHAR MATAR 4. Inoculation of respective AM fungi with pathogen reduced the detrimental effect of pathogen and therefore

we observed remarkable recovery in the mortality of test plants. Therefore, the mortality rate in AZAD P-2was 43.5% in Pant Matar 2 per cent, 12.5% and 15.0% in JAWAHAR MATAR 4. Not much influence on dry weight (g/plant) of nodules g/ plant in all the three varieties of Pea was seen. AM fungi inoculated plants showed greater nodulation in comparison to AM fungi + pathogen and pathogen treated Pea plants.

Data on yield (i.e. dry weight of grains g/ plant) are presented and the results revealed that AM fungi inoculated plants produced greater yield in all the three varieties and maximum yield was obtained in Pea variety JAWAHAR MATAR 4 followed by Pant Matar 2 and minimum in AZAD P 2. As usual effect of pathogen was severe, causing loss in yield in sterile soil condition in each variety. The plants inoculated with AMF and Foc together showed phytoprotection. The susceptible variety AZAD P-2did not show much increment in the yield g/plant in comparison to the resistant varieties of PeaPant Matar 2 and JAWAHAR MATAR 4 .

Experiment with unsterile soil suggested that there was no change in trends. Results revealed that test plants showed protection when inoculated with AMF inoculants. Phytoprotectant role of AMF can be well marked because only 39.0% mortality was recorded with pre-inoculation of by AMF in AZAD P 2, 20.9 % in Pant matar 2, and 22.6% mortality in C11. AM fungi inoculation reduced the pathogenic effect of Foc, resulting reduced mortality rate even in resistant varieties 12.5%Pant Matar 2 and 15.0% in JAWAHAR MATAR 4 .

Arbuscular mycorrhizal fungi influenced nodulation in all the 3 Pea varieties. In case of AZAD P-2andPant Matar 2 it showed 0.05 g nodule wt when inoculated with AM fungi but the same varieties inoculated with Foc resulted into a significant reduction in nodules wt (0.02 g). However, when AM fungi inoculated with pathogen Foc, recovery in nodules wt was 0.04 g. In case of JAWAHAR MATAR 4 when plants were inoculated only with AM fungi the nodule wt was recorded 0.03 g, but when Foc alone was introduced, nodule wt was reduced to 0.01 g and when AMF + Foc was inoculated a recovery in nodules wt was 0.02 g.

The results are clearly indicating response of individual AM fungi with individual

Table 1. Effect of AM Fungi and Foc inoculated alone or in combination on mortality, nodulation and yield under sterilized soils in pea varieties

Treatment	Pea varieties								
	AZAD P 2			PANT MATAR 2			Jawahar matar 4		
	Total Mortality (%)	DW of nodules (g/plant)	Yield DW of grains (g/plant)	Total Mortality (%)	DW of nodules (g/plant)	Yield DW of grains (g/plant)	Total Mortality (%)	DW of nodules (g/plant)	Yield DW of grains (g/plant)
Control	0.0	0.02	1.75	0.0	0.03	1.75	0.0	0.02	1.95
AMF*	0.0	0.03	1.95	0.0	0.03	2.17	0.0	0.04	2.15
Foc	81.20	0.02	0.66	25.0	0.01	1.60	26.0	0.01	1.65
AMF + Foc	42.5	0.03	1.72	13.0	0.02	2.05	15.0	0.01	2.13
CD (P= 0.05)	3.22	0.08	0.25	2.05	0.01	0.34	4.00	0.01	0.50

Pea variety. *G. ambisporum* and *G. mosseae* colonized 90% in AZAD P-2under sterile condition, but in unsterile condition *G. ambisporum* was failed to show its potential but *G. mosseae* maintained its compatibility. This result also suggested that under unsterile condition *G. mosseae* has ability to maintain its dominance and suppressiveness against any other AM fungal species or other microbe's competition for the carbon source. Poor performance of *G. ambisporum* probably might be due to its poor compatibility or grazing by other microbes.

In the resistant Pea varietyPant Matar 2 best result was found with *G. fasciculatum* wherein 80.0% root colonization under sterile condition was recorded, followed by 70.0% root colonization of *G. mosseae* but in unsterile soil condition *G. mosseae* was better candidate and could colonizes 89.0% of the root. It was deduced that host preference or root exudation influenced variable response of the AM fungi with different test varieties. JAWAHAR MATAR 4 exhibited totally different responses. Here, we found that *A. spinosa* and *G. lactuem* were better which colonized 78.0 and 70.0% of the root under sterile condition, 75.0% root colonization recorded with *A. spinosa* and followed by *G. lacteum* (70.0%) under unsterile condition. These results allowed us to draw an inference that AM fungal association was regulated by host and their leachets. AM fungi are also strong candidate for providing biocontrol through competition for space by virtue of their ecological obligate association with roots. Therefore, we may attribute that our results are in good accord with earlier reports made by some workers on role of AM fungi in bioprotection.

Our results with AZAD P-2clearly revealed that presence of native AM fungal species has good potential to protect the plant from Foc and not only they protected the host plant but also they influenced their developing nodules and percent recovery of yield loss. Mycorrhizal and nodule symbiosis often act synergistically on infection rate, mineral nutrition and plant growth. Caron observed reduction in *Fusarium* population in the soil surrounding mycorrhizal tomato roots and suggested that there was a potential role of AM fungi for biocontrol of soilborne diseases. Priming seems to be the main mechanism operating in MIR (mycorrhizal induced resistance). The biocontrol role of AM fungi studied here under sterile and unsterile conditions provide almost similar results. This confirms that AM fungi consortia used in this study have potential role as a biocontrol agent under any conditions they are used. It will be illuminating to extend our approach to investigate the outcomes of competition between a host plants that is positively responsive to AMF colonization. A further challenge will be to scale up the findings obtained with our pot based approach to elucidate mechanisms underlying the direct and indirect effects in fields situations which involve many AMF and hence an enormous range of interactions and responses.

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