

## Regular Article

## Mycorrhizal Application as a Biocontrol Agent against Common Root Rot of Barley

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This study was conducted to assess the biocontrol efficacy of vesicular arbuscular mycorrhizae (VAM) against barley common root rot caused by *Cochliobolus sativus*. Mycorrhization of barley was achieved by growing the plants in expanded clay mixed with 10% (v/v) VAM fungus inoculum in pots experiments. Large differences in disease reactions were observed among genotypes and among treatments. VAM treatments significantly reduced the percentage of disease severity in infected barley plants and increased significantly root biomass, which could be attributed to enhanced nutrients uptake, via an increase in the absorbing surface area. It can be concluded that the application of VAM as a biocontrol agent played an important role in plant resistance and exhibit greater potential to protect barley plants against *C. sativus*.

**Key words:** biocontrol, mycorrhizae, barley, root-rot

Common root rot (CRR), caused by the fungus *Cochliobolus sativus* (Ito & Kurib.) Drechl. ex Dast. (anamorph: *Bipolaris sorokiniana*) (sacc. In Sarok.) Shoem), is one of the most widespread diseases of barley and other small-grain cereals worldwide (Mathre, 1990; Meldrum *et al.*, 2000; Arabi and Jawhar, 2002). CRR infections are especially noticeable on the sub-crown internode and coleoptile but are also found on crowns and roots, which affects yield through a reduction in tiller number (Kokko *et al.*, 1995).

The development of resistant genotypes, application of fungicides and biocontrol agents are considered to be the most common ways for controlling this disease (Kumar *et al.*, 2002). However, avoiding the build-up of soil-borne inoculum by exploitation and stimulation of microbial regulatory mechanisms in the field is probably one of the key strategies in sustainable barley production.

Vesicular arbuscular mycorrhizae fungi (VAM) constitute an important component of the microbial soil community and there is ample evidence that these symbioses are of significant benefit for plants (Yinsuo *et al.*, 2004; Sukhada *et al.*, 2010). However, the obligate symbiotic nature of VAM fungi and limited inoculum supplies continue to impede research aimed at managing these beneficial fungi. The application of selected VAM fungi will not only benefit plant growth and development, but it offers the possibility of increasing the resistance to soil-borne plant pathogens as well (Upadhyaya *et al.*, 2000; Ziedan *et al.*, 2011). On the other hand, under field conditions, the amount of inoculum, time of infection, genotype and environmental interactions are factors that make conducting an error free estimation for *C. sativus*-mycorrhizae interaction practically impossible.

The objective of the present study was to evaluate the mycorrhizal activity as biocontrol agent against common root-rot disease of barley under greenhouse conditions.

## Materials and Methods

**Barley genotypes:** Three different genotypes (Arabi Abiad, Igri and WI2291,) of barley (*Hordeum vulgare* L.) were used in the study. They were chosen for their differential reactions (Arabi and Jawhar, 1999; 2002). WI2291 (susceptible) originated from the Waite Institute, Glen Osmond, Australia, Igri (resistant) is a German genotype and Arabi Abiad (moderately susceptible) is a local genotype (heterogeneous landrace).

**C. sativus inocula:** Nine isolates of *C. sativus* selected on the basis of cultural morphology and virulence (Arabi and Jawhar, 2002), were used in our experiments. These isolates were obtained from subcrown internodes of barley showing CRR symptoms. Each isolate was grown on potato-dextrose agar (PDA, DIFCO, Detroit, MI. USA) for 10 days at 22 ±1°C in the dark. After 10-12 days, conidia were collected by flooding the plate with 10 mL of sterile distilled water and then scraping the agar surface with a glass slide to dislodge the conidia. Equal volumes of conidial suspension from each isolate were combined and filtered through a double layer of cheeseclose. The conidial suspension was adjusted to 5 X 10<sup>5</sup> conidia/mL.

Disinfected seeds were inoculated by mixing thoroughly with peat-gum conidia inoculum (5 x 10<sup>5</sup> conidia/mL) and planted in plastic pots (8 Litre) filled with sterilized soil. To increase the severity of disease, seeds were planted on 6 cm depth (Kokko et al., 1995). The genotypes were arranged in a randomized complete block design with three replicates.

**AFM inocula:** Four different isolates of mycorrhizal inoculum were used; M4 (205

is a Syrian isolate, whereas, the three isolates (M1; *Glomus intraradice*, M2; *G. constrictum* and M3; *G. claroideum*) were received from Spain (Deparment de Microbiologia, Zaidin Research Center). The isolates were supplied as propagules in expanded clay.

**Greenhouse experiment:** Mycorrhization of the barley was achieved by growing the plants in expanded clay mixed with 10% (v/v) arbuscular mycorrhizal fungus inoculum. The inoculum was prepared by homogenizing and mixing surrounding soil with the *Mycorrhizae* sp. root systems. The soil characteristics were a deep vertisol, with low levels of organic matter (1.8%), soluble and exchangeable potassium 0.026 g/ 100 m.e.q. and 0.13 g/ 100 m.e.q, phosphorus 0.039 g/ 100 m.e.q, with a soil pH between 6.5 and 7. Non-mycorrhizal treatments received inoculum that had been autoclaved at 121 °C for 20 min.

The plants were placed under growth room conditions (18-22°C). Light supplementation was not necessary due to the high light intensity in the spring. The pots were watered every second day to field capacity (same weight) to maintain a saturated soil moisture condition.

**CRR assessment:** Sub-crown internodes (SCIs) were examined after 8 weeks post inoculation and rated for root rot damage by measuring the percentages of SCIs surface showing CRR symptoms (Kokko et al. 1995).

Roots were washed by gently rubbing under running tap water and the roots were cleared for CRR evaluation, and then the root biomass was measured in each treatment after drying it at 40 °C for 3 days. The experiment was repeated twice. Mycorrhizal tips were observed under a dissecting microscope. They were identified by the presence of a mantle (usually a different colour and texture than the root), external hyphae, the absence of root hairs, a slightly swollen apex, and in more mature tips, branching.

**AFM colonization:** A subsample of fresh root material of each genotype was taken randomly after the whole root sample had been cut into about 1 cm long segments. This subsample was used for determination of AM colonization. AFM fungal structures in roots were stained with 0.05% trypan blue at 60°C for 5 min in a water bath (Phillips and Hayman, 1970) after heating in 2.5% KOH at 60°C for 12 min, rinsing then in a few changes of water, and acidifying the roots in 1% hydrochloric acid at room temperature for 1 h. The stained root segments were stored in distilled water at 4°C until they were used for slide preparation. Root segments (about 1 cm long) were mounted on three slides in a polyvinyl alcohol-lactic acid-glycerol solution (Koske and Tessier, 1983) and examined at 1000x magnification under an Olympus YS100 light microscope.

**Statistical analysis:** Statistical analyses were carried out using the STAT-ITCF

program (Anonymous, 1988). Analysis of variance (Newman-Keuls test) was performed to estimate the barley infection level in the different treatments, and to investigate whether the mycorrhizae had an effect on the CRR severity.

## Results and Discussion

None of the seedlings of the three genotypes showed any obvious disease symptoms in their shoots (i.e., stunting, chlorosis,); however, upon closer examination of the root systems of the diseased plants, evidence of VAM and CRR, including general root decay, missing cortical tissue, and fine roots, was observed (Fig 1). The disease symptoms (discoloration and necrosis of the SCIs) were always more severe in the susceptible genotype WI2291 after 8 weeks post inoculation. SCI discoloration was typically observed in infected plants. Infection responses of barley genotypes to *C. sativus* are summarized in (Table 1).

**Table 1. Common root rot disease severity (%) of three barley genotypes co-inoculated with four isolates of mycorrhizae.**

Genotype	Treatment	Disease severity <sup>A</sup>				
		Without Mycorrhizae	With Mycorrhizae isolates			
			M1	M2	M3	M4
Arabi Abiad	Healthy plants	0.0	0.0	0.0	0.0	0.0
	Diseased plants	87.27a	52.95c	49.27c	70.69b	53.68c
	% of root rot reduction		39.33	43.54	18.99	38.49
WI 2291	Healthy plants	0.0	0.0	0.0	0.0	0.0
	Diseased plants	90.00a	59.65c	45.08d	72.00b	28.00e
	% of root rot reduction		33.72	49.91	20.00	68.88
Igri	Healthy plants	0.0	0.0	0.0	0.0	0.0
	Diseased plants	49.00a	13.05	38.59	10.23	11.53
	% of root rot reduction		73.33	21.24	79.12	76.47
Mean			48.79%	38.24%	39.37%	61.28%

<sup>A</sup>Measured as % of damaged SCI area (Kokko et al., 1995)

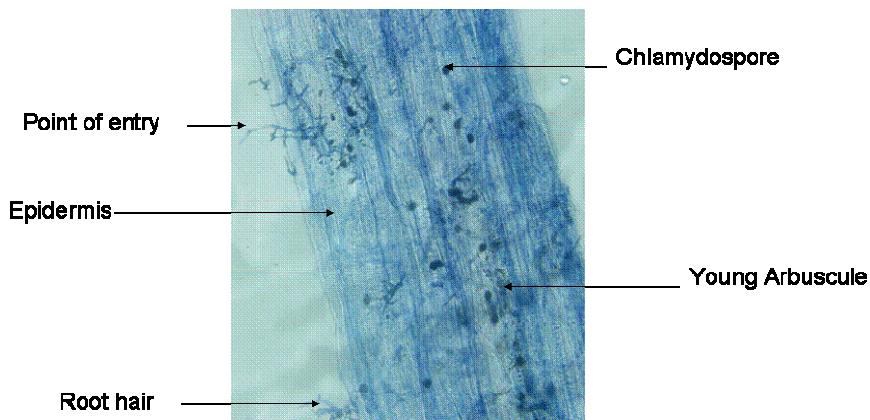
Means followed by different letters are significantly different ( $P<0.05$ ).

The results show highly significant differences ( $P<0.05$ ) between mycorrhizal treatments and CRR severity on the three barley genotypes (Table 1). Compared with

the control, the level of infection across the mycorrhizal treatments was reduced by 43.54 % (M2) and 39.33 % (M4) in Arabi Abiad, and 68.88.19% (M4) and 49.91% (M2)

in WI 2291, and by 79.12% and 76.4% in Igri for isolates M3 and M4 respectively. Mycorrhizal treatment caused high reductions in disease severity on Igri genotype. In general, the mycorrhizal treatments had a positive effect in reduction

of disease severity as compared with the control which had a high infection level of CRR (Table 1). The Syrian Micorrhizal isolate M4 was the best among other isolates with 61.28% reduction of CRR severity (Table 1).



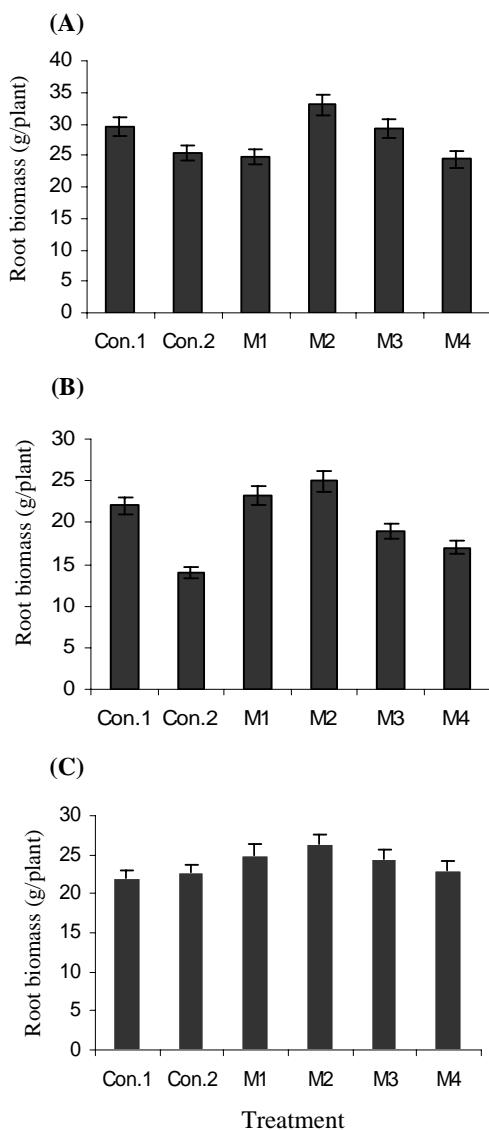
**Fig. 1. Light microscopy of barley mycorrhizal roots cv. Arabi Abiad.**

As Figure 2 shows, inoculating barely with VAM significantly increased the root biomass production. This is in good agreement with results obtained by Castillo *et al.* (2012) who found that treatment of wheat, barley and oats with VAM significantly increased their root biomass. VAM infection has been reported to increase both the uptake of nutrients by the roots and the concentration of nutrients in the plant tissues (Garg and Chandel, 2010.) However, even though the root biomasses differed between the genotypes, the genotypes differ in the intensity of root infection (Table 1; Fig2). This is interesting as it could mean that genetical differences between the genotypes influenced the mycorrhizal infection process. The subject of susceptibility and compatibility of VAM fungi with plant genotypes was and currently still is a matter of great scientific interest (Castillo *et al.* 2012).

Clearly, the study shows that infection with *C. sativus* decreased after mycorrhizal treatments with different isolates. Similar results were obtained by Kjoller and Rosendahl (1996), who found a positive effect of VAM treatments on

decreasing root rot disease caused by pathogen *Aphanomyces euteiches* in pea. Recently, El-Mohamedy (2012) found also that biological control could be used for controlling Pythium root rot of broccoli plants under greenhouse conditions. However, the increased resistance in mycorrhizal plants found in this study should be further examined, to understand the function of disease resistance of mycorrhiza correctly.

The mechanisms of pathogen suppression by VAM are poorly understood. In the present experiment, there was an indication of an induced resistance factor as suggested by Bodker *et al.* (1998). The mode of interaction between VAM and *C. sativus* is more likely to be competition for nutrients and for physical space, because different degrees of discoloration of SCI and reduction in root biomass were observed. This hypothesis is supported by the results of Kjoller and Rosendahl (1996) who found that the *G. mosseae*-pea symbiosis had an effect on disease severity caused by *A. euteiches* and reduced the biomass and the level of energy reserves of the pathogen.



**Fig. 2.** Root biomass of barley cv. Arabi Abiad (A), cv. WI2291 (B) and cv. Igi (C) after 7 weeks of inoculation with *C. sativus*. Con.1: non-infected plant with pathogen and without mycorrhiza treatment. Con.2: infected plants with the pathogen only.

On the other hand, the establishment of VAM in barley roots was shown to be accompanied by the accumulation of putrescine and agmatine amides of 4-coumarate and ferulate, respectively (Peipp *et al.*, 1997) and leads to marked increases of Jasmonate levels (Hause *et al.*, 2002). This might suggest a possible role of these compounds in increasing barley resistance to CRR.

The results from the present study question the beneficial effect of VAM on the suppression of CRR disease in barley under controlled conditions. However, the rate of increase in pathogen infection potential will likely be lower in barley with active isolates of VAM. The existence of certain specificity among VAM isolates in their ability to suppress disease caused by *C. sativus* in barley should be investigated by more field trials by testing a number of different fungal species. VAM treated plants also increased significantly root biomass formation comparing with the controls, which could be attributed to that VAM enhanced nutrients uptake, via an increase in the absorbing surface area. However, information obtained from this work should be further examined, because it may help the plant breeder to understand the function of disease resistance of mycorrhiza correctly, and to use mycorrhizae as a new biocontrol method to control soil-borne diseases in eco-agriculture in future.

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