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Integrated Biological and Chemical Control of Powdery Mildew of Barley Caused by *Blumeria graminis* F.sp. *Hordei* Using Rhizobacteria and Triadimenol

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Abstract: In this study, ability of triadimenol and rhizobacteria to control the powdery mildew of barley caused by *Blumeria graminis* f.sp. *hordei* were investigated in field conditions on eight barley cultivars with different resistance genes. The pathogen fungus was isolated from the diseased plants in fields. Rhizobacteria were isolated from the healthy barley plants via dilution method and applied to seeds and leaf surface when plants were in the first or second leaf stage. Application of rhizobacteria alone gave the best control in Bornova-92, Yercil and Yildirim cultivars by 26.96, 13.92 and 17.65%, respectively. Triadimenol and rhizobacteria combination controlled the disease by 60.31-72.89% while triadimenol alone prevented the disease by 58.25-65.34%. Protection in both treatments showed variation in tested cultivars ($p<0.05$). Disease index was found lower in barley cultivars with more than one resistance genes.

Key words: Powdery mildew, barley, integrated control, triadimenol

INTRODUCTION

The disease powdery mildew of barley (*Hordeum vulgare* L.) caused by *Blumeria graminis* f.sp. *hordei* is one of the most important diseases of barley on a worldwide basis[1]. The disease has been caused losses in barley production in western Turkey and many part of world. The two main strategies to control this fungus are the application of fungicides and the use of resistance cultivars. Chemical control has been preferred as control measure when compared with other ones for years. Fungicides such as triazol group were applied as seed dressing to control the disease. Intensive use of triazole fungicides has resulted in practical resistance in both wheat and barley mildew in some areas of Europe. In the course of time, fungicide resistance pathogen populations have appeared in many barley growing areas[2,3]. Consequently, chemical control alone is not satisfactory for controlling the disease. To overcome these sorts of problems, chemical control must be evaluated with different control management such as biological control.

During the past 30 years, a great diversity of soil microorganisms have been described, characterized and tested as biocontrol agents of diseases caused by soil borne plant pathogens. Several strategies of control have been developed based on the introduction of single or mixtures of biocontrol agents. Unfortunately, this approach to disease control has not been widely adopted for a variety of reasons. Some of the introduced microorganisms could control only one of several important diseases of a particular crop although others provided only partial control of the disease[4]. Although certain diseases can be controlled either completely or partially by the use of biological agents, the best method to control diseases is the integrated pest management, where a biological control component would be significant. In the past, biocontrol agents have only been used in the biological control of plant pathogens. Their integrated use with other management practices has been inadequately studied and practiced. The integrated biological control of plant pathogens has been studied by several workers[5-8].

Pest management practices used jointly with biocontrol agents include the use of pesticides and resistant varieties as well as other cultural methods including solarization. In this study, powdery mildew disease was tried to control by an integrated approach using jointly rhizobacteria and fungicide, triadimenol in barley plants with different resistance genes. However, additional aim of this study was to find out that whether there is relationship between biological effectiveness of biocontrol agents and resistance genes or not.

MATERIALS AND METHODS

Mildew source and barley cultivars used in experiment: Barley mildew isolates were collected on diseased plants as cleistothecia from experiment sites in 2003 and stored at 4°C. Pathogen isolates used in these tests were

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Table 1: Barley cultivars used in all experiments and their resistance genes

Barley cultivars	Resistance genes
Bornova 92	Mla1
Bülbül	-
Gem 8889	Mlra
Orza	Mlra
Tokak 157/3	Mlg
Yesilköy	Mla8
Yercil 147	Mlra, Mlg
Yildirim	Mlh, Mla8

inoculated as mix because of there was no knowledge on the composition of barley mildew populations. To obtain mildew source for experiment, barley cultivars were planted as mixture in greenhouse, where the temperature was maintained at 15-22°C. When seedlings are in the first leaf stage, diseased leafs with cleistothecia were placed on soil among tested plants. And then, every morning these diseased leafs were sprayed with sterile tap water to induce ascospores formation. Eight selected barley cultivars (Table 1) were used as test plants in all experiments.

Isolation and application of rhizoplane bacteria: Rhizoplane bacteria were isolated from healthy barley roots. Therefore, eighty different barley plants were collected from the experiment site in Dalaman, Mugla province. Each sample was put in a sterile plastic bag in the field. Bacterial isolation was carried out according to the method of Rouatt and Katznelson[9]. The roots were first freed from the adhering coarse soil particles. Exactly 55 g root was washed three times with 0.5 L sterile demineralized water so that all residual soil particles were removed. This mixed root sample was placed in a 250 mL flask with 90 mL of 0.1% Proteose-peptone (PP) solution and shaken vigorously for 10 min on a shaker. Then, this bacterial suspension was adjusted 10^{-4} cfu mL^{-1} concentration. Bacterial populations were determined by surface plating 0.1 mL from suspension, on the nutrient agar (NA:Oxoid) and King's -B culture mediums respectively. Four replicate plates were used for surface plating. After incubation during two days at 28°C, all bacterial colonies growing on nutrient agar and King's Broth (KB) petri media, were scraped and suspended in 100 mL of 0.1 M sodium phosphate buffer (pH 7.0). The concentration was adjusted to 10^{9} cfu mL^{-1} for seed treatment. Eight hundred gram of barley seeds for each barley cultivar were surface disinfected in 1% NaOCI for 10 min, washed in sterile water and air dried. The bacterial mixture, which are prepared above, was applied to surface disinfected seeds as 2 mL of bacterial suspension, at 10^{9} cfu/mL/200 g of barley seeds in a 250 mL flask. In other flasks, 200 g of disinfected barley seed were wetted with 2 mL of sterilized water and treated with triadimenol at recommended dose (150 g 100^{-1} kg seed). To assess the combined effects of triadimenol and bacteria, five minutes following the bacterial application to seeds, triadimenol (Baytan DS. 7.5) was applied to 200 g of barley seed from each selected barley cultivar at recommended dose and they were planted in experiment plots. Control seeds (200 g/plot) were treated with only disinfected tap water. These processes were done for each cultivar separately.

Field trial: Seeds of each barley cultivar (200 g/plot), which were treated with rhizobacteria, triadimenol and triadimenol plus rhizobacteria, were planted at plots (5x1.20 m) in March 2003. When seedlings arrived the first or second leaf stage, bacterial mixture was sprayed on barley seedling at 10^{9} cfu L^{-1} except for control plants. After two days, the all barley seedling were inoculated artificially by shaking heavily infected plants grown in greenhouse over the seedlings so that the conidiospores were uniformly distributed on the leaves. When disease development stopped, 50 plants per each plot were chosen randomly. Disease severity (0 to 100) for each barley cultivar was evaluated using a scale of 0-4 developed by Welz[10] and calculated by equation of McQuilken *et al.*[11]

RESULTS AND DISCUSSION

The indexes for each treatment including controls showed difference among both barley cultivars. Average disease index in plots with treatment triadimenol alone and triadimenol and rhizobacteria combination were not different statically in six barley cultivars except for Yercil and Yildirim. The lowest diseases index were determinated in all treatments for Bornova and Tokat cultivars. Also, rhizobacteria were found to be effective in controlling the disease in barley cultivars with more than one resistance genes. Bornova cultivar showed the lowest disease index. This cultivar have resistance gene Mla1. It was though that Mla1 and unknown resistance gene(s) were the reason of the resistance against the pathogen population in bornova cultivar. Diseases indexes in plots with triadimenol showed difference among cultivars. The most important reason of the situation is that cultivars used in experiment have different resistant genes. One of the evidence supporting this opinion is the fact that the highest disease index and the lowest protection appeared in Bülbül cultivar in control plot.

The most important aim of the study was to find out the combined effect of non pathogen rhizobacteria and chemical control. When taken into consideration data in both Table 2 and 3, it was obtained satisfactory results, especially from biological control of powdery mildew with rhizobacteria in certain cultivar. Application of rhizobacteria alone gave the best protection (26.96, 13.92 and 17.65%, respectively) against disease in

Table 2: Disease index obtained from test plants treated with each treatment

	Barley cultivars							
Treatments	Bornova	Bülbül	Gen 8889	Orza	Tokak 157/3	Yeşilköy	Yercil 147	Yildirim
Control	26.56aD	96.25aA	86.25aB	86.56aB	45aC	89.69aAB	81.55aB	87.19aAB
Rhizobacteria (Seed and phylloplane)	19.4bD	93.20bA	83.2bB	83.5bB	40.2bD	85.12bB	70.2bC	71.8bC
Triadimenol	10cD	40.2cA	32.4cAB	30cB	20cC	35cAB	30.2cB	33.2cAB
Triadimenol+ Rhizobacteria (Seed and phylloplane)	7.2dD	38.2dA	28.2dAB	27.2 dB	16.4dC	30.1dAB	14.2dCD	15.4dCD

Values within a column followed by the same letter are not significantly ($p>0.05$) different according to Duncan Test
Values within a line followed by the same capital letter are not significantly ($p>0.05$) different according to Duncan Test
Results are means of four replicate for each treatment

Table 3: Effect of the treatments on the disease incidence compared to control (%)

	Barley cultivars							
Treatments	Bornova	Bülbül	Gen 8889	Orza	Tokak 157/3	Yeşilköy	Yercil 147	Yildirim
Rhizobacteria	26.96	3.16	3.54	3.54	10.67	5.10	13.92	17.65
Triadimenol	62.35	58.23	62.43	65.34	55.56	60.98	62.97	61.92
Triadimenol+ Rhizobacteria	72.89	60.31	67.30	68.58	63.56	66.44	82.59	82.34

Bornova, Yercil and Yildirim cultivars. Triadimenol plus rhizobacteria gave good protection for controlling the disease compared with individual application of both chemical and rhizobacteria. These results are indicating the importance of combinations of biocontrol agents with other plant protection practices, such as chemical control and biological activities on both rhizosphere and phyllosphere.

Disease control by rhizobacteria, applied to both seed and phyllosphere, is associated with induced resistance and microbial antagonism between pathogen and antagonistic bacteria on the leaf. These bacteria are present in large numbers on the root surface, where nutrients are provided by plant exudates and lysates[12,13]. Certain rhizobacteria suppress the plant diseases by means of antagonistic activity and Induced Systemic Resistance (ISR), While Certain strains of rhizosphere bacteria are referred to as Plant Growth-promoting Rhizobacteria (PGPR), because their application can stimulate growth and improve plant stand under stressful conditions[14,15]. For instance, Flourescent *Pseudomonas* spp. are among the most effective rhizosphere bacteria in reducing soil-borne diseases in disease-suppressive soils[16]. Certain strains of fluorescent pseudomonads are important biological components of agricultural soils that are suppressive to diseases caused by pathogenic fungi on crop plants. The biocontrol abilities of such strains depend essentially on aggressive root colonization, induction of systemic resistance in the plant and the production of diffusible or volatile antifungal antibiotics[17].

All plants possess active defense mechanisms against pathogen attack. If defense mechanisms are triggered by a stimulus prior to infection by a plant pathogen, disease can be reduced. It is possible that a rhizobacterial strain can induce resistance by different mechanisms, depending on the local conditions in the rhizosphere. For instance, a strain could induce ISR through a constitutive mechanism, while starting to produce SA and consequently trigger further resistance through SA production when iron-limiting conditions are encountered. The other bacterial determinants of induced systemic resistance are lipopolysaccharide, salicylic acid (SA), siderophore. For instance, in the systemic protection of carnation against Fusarium wilt by *Pseudomonas fluorescens* WCS417, heat-killed bacteria or the purified bacterial outer membrane lipopolysaccharide (LPS) were found as effective in inducing resistance[18]. This observation indicated that the bacterial LPS acts as a determinant of resistance induction by WCS417 in carnation. In this study, it was estimated that disease control by rhizobacteria treatment alone or with fungicide was due to these sort of rhizobacteria-mediated active defense mechanisms. More recently, several microorganisms were identified in suppressive composts capable of inducing systemic resistance in radish and cucumber[19]. *T. hamatum* 382 and *Pantoea agglomerans* 278A applied to roots of radish seedlings induced resistance to foliar bacterial spot caused by *Xanthomonas campestris* pv. *armoraceae*. Several types of rhizosphere microorganisms can induce this effect in plants[20]. In addition, it was first reported that composts induce systemic resistance to powdery mildew of wheat[21]. Although little is known about how composts induce systemic resistance in plants it could be suggested that several types of microorganisms will be likely reason of resistance.

Due to powdery mildew pathogen, *E. graminis* f.sp. *hordei*, is air-borne and obligate pathogen, disease control by rhizobacteria are attributed to induced systemic resistance and antagonistic activity on phyllosphere. Therefore, it must be focused on this matter briefly.

Induced resistance is characterized by an accumulation salicylic acid and pathogenesis-related proteins[22-26]. Both pathogen and salicylic acid-induced resistance are associated with the induction of several families of pathogenesis-related proteins (PRs)[22,25,26]. Some of these PRs are β-1,3-glucanases and chitinases and, capable of hydrolyzing fungal cell walls. Other PRs have more poorly characterized antimicrobial activities or unknown functions. At least, eight of the ten major PRs induced in tobacco in response to pathogens causing hypersensitive necrosis, were found in the intercellular washing fluid of leaves of plants grown in autoclaved soil in the presence of *P. fluorescens* CHAO, which is likely to produce SA in the rhizosphere[27].

In addition, certain strains of bacteria could cause structural alterations in plant. These structural alterations are remarkable impediment to pathogens. Such impediment to fungal ingress might involve cellular alterations in the epidermal and cortical cells that inhibit further colonization. Evidence for such PGPR-induced structural modifications was described recently for pea root tissue[28]. Pea roots pre-inoculated with the endophytic biocontrol strain *Bacillus pumilis* SE34 were protected against the root-rot fungus *Fusarium* f.sp. *pisi* (Fop). Upon challenge inoculation, however, pathogen growth was restricted to the epidermis and the outer cortex. The wall of these cells were strengthened at sites of attempted fungal penetration by appositions containing large amounts of callose and phenolic materials, effectively preventing fungal ingress. Phenolic materials were also seen in intercellular spaces as well as at the surface or inside the invading hyphae of the pathogens. Thus, protection afforded by SE34 involved host cell wall signification upon challenge inoculation with Fob[28]. Similar wall appositions and papillae were seen in peat roots treated with the either Fob or *Pythium ultimum*[29,30], indicating a general induction of defensive physical barriers to pathogen ingress. *P. fluorescens* WCS417 also has been reported to induce a thickening of cortical cell walls in tomato roots, if epidermal or hypodermal cell were colonized densely by the bacterium[31].

Consequently, to reduce the dependence on chemical crop protectants for disease control in agriculture, biological agents must be received increasing attention. Resistance-inducing rhizobacteria offer an attractive alternative in providing a natural, safe, effective, persistent and durable type of protection. Protection based on biological agents is not always reliable and is seldom as affective as chemical treatments. However, different treatments may be combined and combinations of biocontrol agents that suppress diseases by complementary mechanisms may further reduce disease losses. Rhizobacteria-mediated ISR will likewise be a valuable addition to the options available for environmentally friendly plant disease control.

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