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Response of some important Iranian wheat cultivars to *Fusarium culmorum* under genetic diversity of indigenous bio-control agent fluorescent *Pseudomonas* spp.

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Abstract

Wheat root and crown rot caused by *Fusarium culmorum* is known as one of the most common diseases among wheat diseases in Iran. Recently, application of new resistant cultivars and biological control by antagonistic bacteria, such as *phlD*+ (a key biocontrol gene) fluorescent *Pseudomonas* spp. have received much attention. An important scientific gap in this strategy is the interaction between disease resistant level of cultivar and composition of beneficial soil rhizosphere bacteria. Therefore, the resistance levels of some Iranian wheat cultivars to *Fusarium culmorum* were assessed under diversity of naturally occurring fluorescent *Pseudomonas* spp. The resistance level of seven wheat cultivars and lines (Falat, Niknejad, Marvdasht, Chamran, N83, Durum and line X) to *F. culmorum* were tested in greenhouse conditions. Then, the rhizosphere and endorhizosphere population of bacterial isolates were evaluated. Then diversity of *phlD*+ isolates were assessed using restriction fragment length polymorphism (RFLP). Finally, root colonization by four *phlD*+ isolates in the line X (resistant) and Falat (the most susceptible) cultivars at greenhouse level were analyzed. Based on resistance analysis, the cultivars were classified in to three groups: resistant, tolerant, and susceptible. The results showed that the indigenous rhizosphere population size of fluorescent *Pseudomonas* spp. was significantly different among the cultivars and correlated to the level of plant resistance. The results of RFLP analysis of the *phlD* gene among the isolated bacteria indicated that the composition of the genotypes enriched by the individual cultivars was differed. Four distinct *phlD* alleles, including A, B, D and F were detected. The highest genotype diversity was observed among *phlD*+ strains, obtained from line X rhizosphere (resistant cultivar). Comparison of the root colonization by the inoculated strains showed that *phlD*+ isolates were consistently recovered from the rhizosphere of the resistant cultivar (line X), and were commonly present at populations higher than those recovered from susceptible cultivar (Falat). Based on the results, it can be concluded that amazingly, there is a positive correlation between the wheat resistance level and the population size and diversity of fluorescent *Pseudomonas* spp. in their rhizosphere.

Keywords: DAPG (2, 4-diacetylphloroglucinol); fluorescent *Pseudomonas*; *Fusarium culmorum;* PCR-RFLP; Wheat cultivars.
Abbreviation: DAPG_2,4-Diacetylphloroglucinol, *Fps*_fluorescent *Pseudomonads*, KB^{+++} _King's medium B amended with ampicillin (100 µg mL^{-1}), chloramphenicol (13 µgmL^{-1}) and cycloheximide (75 µg mL^{-1}), Phl_2, 4-diacetlyphloroglucinol.

Introduction

Beneficial soil bacteria, such as 2,4-Diacetylphloroglucinol (DAPG)-producing *Pseudomonas spp*. have received considerable attention in promoting plant growth and biological control of various soil-borne plant pathogens (Lutz et al., 2004). DAPG-producing pseudomonads have widespread occurrence and broad capacity in root disease suppression in various cropping systems (Iavicoli et al., 2003). *PhlD*, one of the main genes responsible for the biosynthesis of DAPG in *Pseudomonas* spp., has been commonly used as a genetic marker to study DAPG-producing fluorescent *Pseudomonas* (*Fps*) (Bangera and Thomashow, 1999). In previous studies, root colonization by populations of certain *Fps* genotypes correlated with root disease control (Landa et al., 2002; De Souza et al., 2003; Ramette et al., 2003). However, because of inconsistent performance in various environmental conditions, only a few strains have been commercially used for bio-control of plant diseases (Bergsma-Vlami et al., 2005; Garbeva et al., 2008; McSpadden-Gardener et al., 2005; Notz et al., 2001). The types of host plant species/cultivars and their exudates have significant effect on the root colonization and bio-control activity of antagonistic bacteria in diverse environment conditions. The root exudates and root morphology differ among varying host species/cultivars, and can influence the abundance and variety of indigenous *Fps* (De La Fuente et al., 2006). This has been revealed for DAPG-producing *Pseudomonas* spp., in particular (Meyer et al., 2010). A wide range of secondary plant compounds released from plant roots are important signals in complex communication processes in the rhizosphere. Therefore, identifying host genotypes with superior populations of beneficial soil bacteria, such as *Fps* would help to improve disease control (Mazzola and Gu, 2002; Mazzola et al., 2004; Meyer et al., 2010). Soil-borne disease is known as an important wheat disease in Iran and the wheat root and crown rot caused by *Fusarium culmorum* is one of the most common diseases among them. Unfortunately, because of soil-borne properties of these diseases, none of the conventional chemical control methods have been effective yet (Kazemi, 2008; Mansori and Pazhomand, 2005).

A new strategy, which recently received much attention, is the application of resistant cultivars. One of the unclear subjects in the interactions between the cultivar type and beneficial soil bacteria in the rhizosphere is the relationship of cultivar resistance to the plant pathogens and the population and diversity of those bacteria. So, the objective of the present study was (i) to evaluate the resistance level of some important Iranian wheat cultivars to *F. culmorum* (ii) characterization of rhizosphere population of indigenous *Fps* (iii) the genotypic diversity of indigenous *phlD*$^+$ Pseudomonas isolates and (iv) the population densities of introduced 2, 4- DAPG-producing pseudomonads.

Results

Cultivars reaction to F. culmorum

The resistance level of cultivars to *F. culmorum* was evaluated. The average quantity and intensity of crown and sub-crown internode rot, seed shrinkage and white heads were significantly different among the cultivars. Cultivars were grouped as follows: susceptible (Chamran, Falat, Durum, N-83 and Niknejad), tolerant (Marvdasht), and resistant (Line x) (Table1). Among the susceptible cultivars, Falat and N-83 showed the maximum (90%) and minimum (27.5%) crown and sub-crown internode rot rate, respectively (Fig. 1).

Wheat seedling cycling and determination of indigenous Pseudomonas spp. population

The cultivars were grown in the same soil and *Fps* were isolated to evaluate population size and diversity of *Pseudomonas* spp. in the rhizospheres. The results indicated that the population sizes were significantly different ($P \leq 0.001$) among different cultivars (Fig. 2). Totally, based on analysis of mean population densities at the end of all three cycles, rhizosphere of Line X (4.3×10^8 CFU/g fresh root) and Chamran, Falat and Dorum ($3\text{-}4\times10^5$CFU/g fresh root) contained the maximum and minimum population size of *Fps*, respectively (Fig. 2). The maximum density of *Fps* for all cultivars was observed at the end of the first growth cycle, whereas after the second cycle, population density on N83, Durum, Chamran and Falat declined gradually. The cultivars Falat and Line X showed the most variation, with both a decline and an increase of bacterial population size during growth cycles (Fig. 3). Evaluation of *Pseudomonads* populations prior to the endorhizosphere population enumeration on the samples showed that 99% of the *Pseudomonads* on the root surface were removed during the sterilization process. No significant differences were found among the cultivars for *Pseudomonads* populations recovered from endorhizosphere sample.

Genotype diversity of Pseudomonas spp. isolates from different wheat cultivars

To evaluate the effect of wheat resistance on diversity of *Fps*, the presence and diversity of *phlD* gene (a key gene involving in DAPG production) was studied in *Fps* isolated from rhizosphere of the cultivars. PCR results showed that *phlD*$^+$ *Fp* isolates were present in the rhizosphere of all the studied cultivars except for Durum. Totally, 24 isolates out of 350 isolates contained the *phlD* gene. The maximum and minimum presence of *phlD*$^+$ isolates was observed for line X (52.9%) and Durum (0%), respectively. *phlD*$^+$ isolates in the rhizosphere of Falat, Chamran, Niknejad, Marvdasht and N83 comprised 9.58%, 4.16%, 12%, 5% and 20.83%, respectively. None of the isolates obtained from endorhizosphere contained the *phlD* gene. To evaluate diversity and dominance of phlD$^+$ alleles for isolates obtained from each cultivar, restriction fragment length polymorphism (RFLP) analysis was performed. The results showed that the genetic composition of the *phlD*+ populations varied among cultivars. Four distinct *phlD* alleles, including A, B, D and F, were detected among the 24 indigenous *phlD*$^+$ *Pseudomonas* isolates as following: (i) three alleles were found in the line X (A, B and D alleles); (ii) two alleles were present in Marvdasht (A and D alleles); (iii) only one allele was detected in the in Falat (allele B), Chamran (allele D) and Niknejad (allele B) and N-83 (allele F).

Comparison of colonization of wheat cultivars by four phlD+ isolates

The efficiency of four *Fps* isolates (E6, G30, B4 and C25) in the colonization of the roots of two resistant and susceptible cultivars was measured to evaluate the effect of wheat resistance to *F. culmorum* on the colonization ability of the strains. To inoculate seeds before planting, the initial populations of the selected isolates were the same and estimated about 1.3×10^7cells seed^{-1}. As it is shown in Table 2, the quantity of colonization of the *Fps* isolates for two studied cultivars is significantly different (α= 0.01), and population of each isolate is different on two cultivars. The isolates E6, G30 and C25 did not show persistence in the rhizosphere of Falat, and their populations gradually decreased during a 30-day period, as they were not detected in 20 days after inoculation. The isolate B4 showed a different manner and colonized the rhizosphere of cultivar Falat in all cycles in the same extent (Table 2). Experiments on line X showed that the persistence of the inoculated isolates in the rhizosphere over a 30-days period is significantly different (α= 0.05), also the persistence rate is different for each isolate. Populations of E6 and C25 exhibited continuous increase rate, whereas those of G30 and B4 declined over a 30-day period. The maximum and minimum population size at the end of 30 days after planting was observed in E6 and G30, respectively (Table 2).

Discussion

The exploitation of host response to beneficial microorganisms carries great potential, which allow breeders to select traits that cause positive effect on plant-beneficial *Pseudomonads* interaction (Wissuwa et al., 2009). Different factors, such as bio-control agent genotype, environmental aspects (e.g. temperature, soil texture and moisture), plant genotype, and metabolites have a significant effect on the bio-control ability of bacterial antagonists. Host cultivar effect is one of the most important factors on bio-control characteristics of *Fps* (De Souza et al., 2003; Meyer et al., 2010; Weller, 2007). The population and genetic diversity are the key regulatory mechanisms in the production of antimicrobial compounds in *Fps* (De Werra et al., 2008). The current study represents the first work presented regarding the influence of wheat cultivars resistance level to *F. culmorum* on accumulation and genetic diversity of indigenous *Fps* in the rhizosphre. The results of this study, which determined the resistance level of the studied wheat cultivars to crown and root rot caused by *F. culmorum*, confirms the previous studies on the susceptible cultivars:

Table 1. Reaction of wheat cultivars to *F. culmorum* the cause of crown and root rot of wheat.

Cultivars/lines	Disease index	Seed shrinkage	White head	Reaction
Chamran	2	+	+	S
Falat	2	+	+	S
Dorom	2	+	+	S
N-83	2	+	+	S
Niknejad	2	+	+	S
Marvdasht	1	-	-	T
Line X	0	-	-	R

0=No symptoms; 1= Mild discoloration at crown and sub-crown internode; 2=Typical symptoms of acute foot rot, with brown discoloration of crown and sub-crown internode. T= Tolerant, S= Suseptible, R= Resistant.

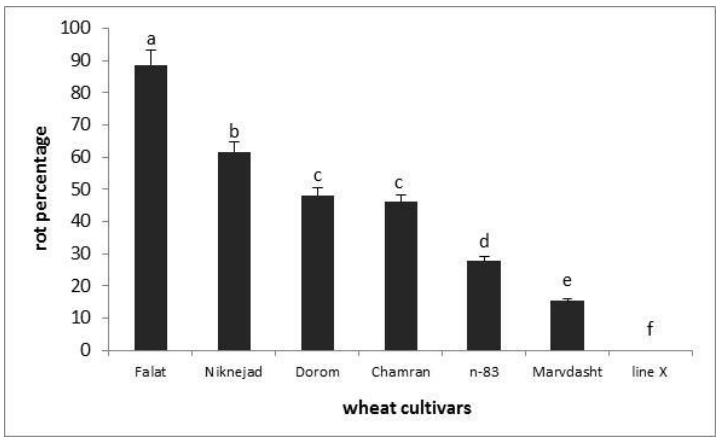

Fig 1. Mean comparison percentage of crown and sub-crown internode rot in wheat cultivars inoculated with *F. culmorum* 49 days after planting. Each column is mean of three replications. For each wheat cultivar, data are shown as means ±SE (n = 7) and significant differences (P <0.01; 0.05) are indicated by letters (a,b,c,d,e,f). Means in the same column followed by the same letter are not significantly (P > 0.05) different according to Fisher's protected least significant.

Falat and Niknejad (Mansori and Pazhoumand, 2005), Durum (Statler and Darlington, 1972) and Marvdash as a tolerant cultivar (Mansori and Pazhoumand, 2005). However, the results on susceptibility of Chamran cultivar contradicted with the results of Mansori and Pazhoumand (2005), who described this cultivar as tolerant. This may have occurred because of a difference in the aggressiveness of the pathogen strains used in both studies. Line X was analyzed for the first time in this study and was determined as resistant to *F. culmorum*. In the study of the indigenous population, *Fps* isolates were detected in the rhizosphere of all seven cultivars. However, population sizes were significantly different among various cultivars. Line X contained the maximum population of *Fps* isolates, whereas Falat and Chamran contained the minimum population of isolates. This shows that the resistant cultivar promotes inhabitation of indigenous *Fps* in its rhizosphere rather than susceptible cultivars. The evaluation of *Pseudomonads* populations on endorhizosphere showed no significant differences between the cultivars. In accordance with the previous studies (Mazzola et al., 2004; Meyer et al., 2010), the results of this study showed that the quantity of colonization by bacterial strains depend upon various plant genotypes. One remarkable find from this study is the discovery that *Fps* populations enhance in different degrees according to the cultivar employed and according to the level of resistant to the pathogen in each cultivar. The most susceptible cultivar (Falat) and resistant cultivar (line X) showed the most variation with a decline and increase of bacterial population size during growth cycles. Other cultivars followed this pattern with mediocrity effect. This shows that according to cultivar susceptibility and resistance, cultivars have negative or positive effects on *Fps* population over the time. Line X showed positive effects on *Fps* population enhancement. With this characteristic it could be used as a qualified cultivar for future breeding programs. The ability of a cultivar to promote or support beneficial interactions is important at low-input agriculture (Mital and Johri, 2008). The genetic composition of the *Fp*s population is a major factor in their bio-control activity and plant growth promotion. A prerequisite in many naturally disease-suppressive soils is the enrichment of *phlD+ Pseudomonads* (DAPG producer *Fp*s) that only occurs on specific crops (De Souza et al., 2003; Meyer et al., 2010). Also, there is a direct relation between genotypic group *phlD+ Fp*s strains and their rhizosphere competence and disease suppression (Landa et al., 2006; Ramette et al., 2006). Isolates containing allele D outweighed on roots of wheat field, exhibiting take-all decline (Frapolli et al., 2010). In this study, we observed that wheat cultivars possess a differential capacity to demonstrate resident presence of *phlD+ Pseudomonads*. The maximum and minimum presence of *phlD* gene was observed in the *Fp*s isolated from resistant (line X) and susceptible (Durum, Falat and chamran) cultivars, respectively. Surprisingly, none of the isolates obtained from endorhizosphere contained *phlD* gene. The result of the genotype diversity experiment was also related to resistant level in cultivars. Based on PCR-RFLP of *phlD*, strains detected on rhizosphere of resistant cultivar (line X) showed more genetic diversity and contained three different alleles of *phlD* gene, including A, B and D, whereas susceptible cultivars (Chamran, Durum, Falat, Niknjad, N-83) contained only one allele. Tolerant cultivar (Marvdasht) had two alleles. Additionally, genotype D was the most prominent in line X, as genotype D isolates have superior colonization abilities. It can provide a level of take-all control (Mazzola et al., 2004). The high presence and genetic diversity of *phlD+* strains in line X imply that this line has a positive interaction and adaptation with *phlD+*

Table 2. Population density (cfu/g root) of four studied *phlD+* fluorescent *Pseudomonas* strains during three successive growth cycle on Falat and Line X cultivars (10 days for each cycle).

Isolates	Initial population (cfu/g fresh root)	10 days		20 days		30 days	
		Falat	Line X	Falat	Line X	Falat	Line X
E6	1.5×10^7	ND*	6.3×10^7	ND	8.1×10^7	ND	2.4×10^8
G30	1.3×10^7	5.1×10^5	2.3×10^5	ND	1.1×10^5	ND	3.1×10^4
C25	1.4×10^7	2.1×10^5	4.1×10^6	ND	8.8×10^6	ND	1.7×10^7
B4	1.3×10^7	2.3×10^6	1.1×10^6	1.1×10^6	4.9×10^6	7.4×10^7	7.5×10^5

Each value is a mean consisting of four replicates. ND*: Not detected.

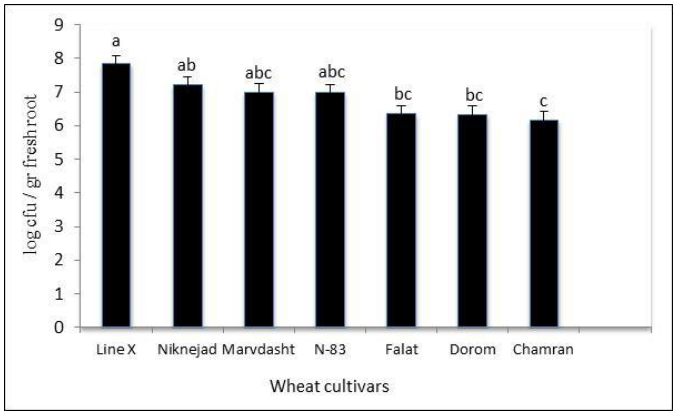

Fig 2. Population density of indigenous *Fp*s in the rhizosphere of the studied cultivars. Number of each bar is average of population density from three cycle and data were expressed as log of the mean CFU g–1 of root (fresh weight after washing). For each wheat cultivar, data are shown as means ±SE (n = 7) and significant differences (P <0.1; 0.05) are indicated by letters (a,b,c,d,e,f). Means in the same column followed by the same letter are not significantly (P > 0.05) different according to Fisher's protected least significant.

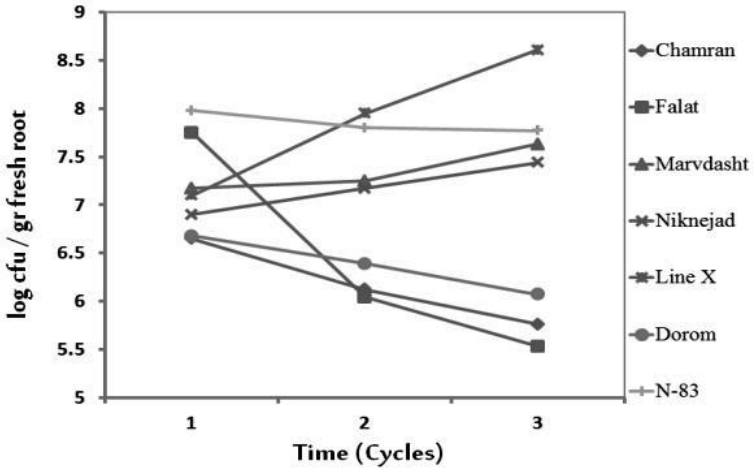

Fig 3. Colonization rate of indigenous fluorescent *Pseudomonas* spp. in the rhizospheres of the seven different wheat cultivars. The cultivars were grown in three successive 28-day growth cycles in the same soil in order to isolate fluorescent *Pseudomonas* spp. from the rhizosphere. After each cycle the population of isolated fluorescent *Pseudomonas* spp. evaluated for each cultivar individually. Data were expressed as log of the mean CFU g–1 of root (fresh weight after washing).

Pseudomonads. Successful root colonization and the maintenance of threshold population by introduced bio-control rhizo-bacteria is an essential factor in determining effective biological disease control in certain systems (Lugtenberg et al., 2001). Raaijmakers et al. (1999) showed that a threshold of 10^5 cfu/g root of *phlD*$^+$ *Pseudomonads* required a successful suppression for the control of take-all of the wheat. Mazzola et al. (2004) showed that this quality completely depends on the particular host plant genotype. In the current study, the cultivars showed variable responses to colonization by *phlD+ Fp*s. Okubara et al. (2002, 2008) had a similar finding, where the definite *Fp* strains were maintained in the rhizosphere of some wheat cultivars. Interestingly, line X and Falat cultivar as resistant and susceptible cultivars, showed maximum and minimum support of the introduced isolates, respectively. Additionally, the strains E6, C25 increased in the rhizosphere of line X during three successive wheat cropping cycle, while in the rhizosphere of Falat, the population of all inoculated strains gradually decreased and were undetected during the final cropping cycle. The results obtained from this experiment add a new idea on previous works (Mazzola et al., 2004), which showed that definite strains has particular population changing pattern in different cultivars. This trait may also

have a direct correlation with resistant level in cultivars. Another interesting result was that the Falat cultivar could only maintain the strain B4 at a low level (genotype D). Meanwhile, this strain was isolated from the rhizosphere of Falat, indicating that it only has adaptation to its own indigenous populations, as compared to the other strains. Therefore, cultivars such as Falat that can only maintain specific bio-control genotype are not recommended for breeding program in sustainable agriculture systems. Persistence of all of the inoculated isolates with different *phlD* genotype in rhizosphere of line X suggests that bio-control strains could quickly adapt with this cultivar. Based on these findings, selection and continuous wheat cropping systems, which employ the appropriate wheat cultivar, such as line X, could substantially reduce the soil borne disease.

Material and Methods

In total, seven winter varieties and lines, including Falat, Niknejad, Marvdasht, Chamran, N83, Durum and line X (X is the name of line), were selected based on their importance in the breeding programs as potential parents. Seeds and all data of the selected cultivars and lines were kindly provided by the Seed and Plant Improvement Institute (SPII), Karaj, Iran.

Pathogenicity test and cultivars reaction

A native strain of *Fusarium culmorum* was kindly provided by the Plant Protection Research Institute (PPRI), Tehran, Iran. Isolates were stored on silica beads at 4ºC and autoclaved barley seeds were stored at –80ºC. Inoculum was prepared by growing individual isolates on 0.2-strength potato-dextrose agar (PDA) medium (Merck, Darmstadt, Germany) and was then transferred by colonized agar blocks into twice-autoclaved millet seed. Colonized millet was air dried, sieved to eliminate clumps, and stored at room temperature (approximately 24ºC) in paper bags. The pathogenicity test for *F. culmorum* was performed according to the previous studies (Cook, 1980; Tinline, 1997; Smiley and Yan, 2009), and it was measured based on the quantity and intensity crown and sub-crown internode rot. During 49 and 109 days after planting, severity of infection for each of the seven cultivars per fungal isolate was assessed using 0-to-2 rating scale of Cook (1980). Additionally, percentages of crown and sub-crown internode rot infection were measured similar to Tinline (1997).

Evaluation of wheat genotypes effect on Pseudomonas spp. populations

Soil samples were taken from the upper 30 cm of the soil profile of the wheat field, located at the Tehran University fields in Karaj, Iran. Soil (1kg) was added to pots (15 by 10 by 6.5 cm) and each pot was used for planting one of seven cultivars. For each cultivar, 10 seeds were sown per pot, and in total, four pots were prepared for each cultivar. Prior to sowing, seeds were surface disinfected according to the protocol described by Keijer et al. (1997). Pots were arranged in a completely randomized design on a greenhouse bench at 24 ± 1°C, with a 12-h photoperiod. The soil in each pot was cropped in three successive cycles (each cycle lasting 28 days) with the same cultivar. Pots were watered every 3-days, and no fertilizer was used during the course of plant growth. After each growth cycle, the *Pseudomonas* populations in two different locations, including the rhizosphere and endorhizosphere were evaluated.

Isolation of fluorescent *Pseudomonas* spp. from the rhizosphere, and enumeration of population were performed by King's medium B (KB^{+++}) according to Mazzola et al. (2004). After enumeration of Pseudomonas populations, fifteen colonies with different morphology for each cultivar and growth cycle (n=45 for tree cycles) were sub-cultured and stored at -80 °C for each subsequent test. To isolate fluorescent *pseudomonas* spp. strains from endorhizosphere, the root section from prior test washed with 0.9% NaCl solution, and afterward isolation was performed according to Meyer et al. (2010). Subsequently, root surfaces were sterilized with ethanol, and, without maceration, were shaken again in 0.9 % sterile NaCl solution for 15 min at 500 xg. The number of pseudomonads was determined as described for the rhizosphere. Because of low morphology differences between colonies isolated from endorhizosphere, only 5 colonies were selected, subcultured and stored at -80º C for subsequent tests.

Data analysis

First population data were converted to log CFU per gram of root fresh weight. A standard ANOVA determined the population densities among treatments. The mean comparisons tests among treatments were performed using Fisher's protected least significant difference (LSD) test at $P \leq 0.05$ using the SPSS software (SPSS version 15).

Evaluation of the effect of wheat genotypes on diversity of phlD gene in Fps

Pseudomonas isolates containing the *phlD* gene were identified as previously described by Bangera and Thomashow (1999). PCR amplification of *phlD* was conducted using gene-specific primers Phl2A and Phl2B (Mazzola and Gu, 2002). Total genomic DNA from 350 individual bacterial isolates (n = 50 per wheat cultivar; N = 350 total), was extracted as previously described by Mazzola and Gu (2002). PCR reactions were 20 µl volumes, including 1 µl of the heat-lysed cell suspension, 1 µl PCR buffer, 200 lM, of each dNTP, 1.5 mM MgCl2, each primer at 0.1 lM, and 2.5 U AmpliTaq Gold (Applied Biosystems). Samples were amplified in conditions which consisted of a hot start cycle at 94°C for 4 min, then 35 cycles at 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, followed by an extension cycle at 72°C for 5 min. In each PCR, DNA templates extracted from CHAO strain served as positive control. PCR products were verified by electrophoreses on 1% agarose gels, containing of GELRED solution for treatment.

PCR/RFLP analysis

To identify the diversity in $phlD^+$ alleles in *Pseudomonas* isolates, PCR products in 24 $phlD^+$ isolates were analyzed. Restriction analysis was conducted with 5 µl of amplified product and 1.5 U of *Hae*III and *Msp*I (Fermentas, Vilnius, Lithuania). Reactions were incubated 3 h at 37 °C (*Hae*III and *Msp*I), and then stored at -20 °C. Restriction fragments were separated by PAGE. A 100-bp ladder (Fermentas, Vilnius, Lithuania) was used as a size marker. For each sample, this test was conducted twice, each time resulting in the same results. The isolates were classified based on RFLP profiles of reference strains for *phlD* (McSpadden-Gardener et al., 2001, 2005).

Evaluation of the effect of wheat genotypes on the root colonization by DAPG-producing Fps strains

Soil sample collected from wheat field (Tehran University fields, Karaj, Iran) was used to test the capacity of the Line X and Falat to support 4 selected DAPG producing *Fp*s isolates (E6, G30, C25 and B4) when introduced as seed inoculants. Line X and Falat were the two cultivars that showed completely different responses during the previous experiments as the resistant and the most susceptible cultivars, respectively. Bacterial strains were selected based on their *phlD* genotypes. Spontaneous rifampicin-resistant mutants for all strains were prepared according to the methods developed by Mazzola et al. (2004). All seeds were inoculated with the same concentration (10^8 cfu/ ml) of each isolate. Seeds in control plant treatment only were inoculated with a sterile 0.5% (w/v) methylcellulose solution. Plants were grown at 24°C with a 16-h photoperiod for 30 days, and 5 seedlings were harvested at 10, 20 and 30 days after planting. The population density for each inoculated isolate in the rhizosphere was determined as previously described (Mazzola et al., 2004). Pots were watered every 3-days and no fertilizer was used during the course of plant growth. Three replications were used for each pot, and the experiment was set in a completely randomized design.

Conclusion

It can be concluded that cultivar resistance levels have a different pronounced effect on indigenous population, diversity and activity of inoculated *Fp*s strains in the rhizosphere. We also concluded that there is a positive correlation between the wheat resistance level to certain pathogens (*F. culmorum*) with population size and diversity of bio-control agents (*Fp*s). Understanding how the resistance levels have positive or negative effects on maintaining rhizosphere populations and diversity of one strain, as well as further exploration into which plant gene controls these traits will provide insights to the breeding of cultivars, which are specifically adapted to support beneficial microbes.

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