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Expansion of the biocontrol spectrum of foliar diseases in rice with combinations of rhizobacteria[1]

Ampliação do espectro do biocontrole de doenças foliares em arroz com combinações de rizobactérias

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ABSTRACT - The cultivation of irrigated rice is subject to the occurrence of various diseases, where damage causes losses in productivity. Alternatives are currently being sought to control these diseases, with biocontrol a viable possibility. With the aim of broadening the spectrum of action of biocontrol, this study evaluated the effect of combinations of biocontrol bacteria on rice blast (*Pyricularia grisea*), brown spot (*Bipolaris oryzae*) and leaf scald (*Gerlachia oryzae*) in rice plants. The effect of isolate combinations was assessed by microbiolization of seeds from the rice cultivar El Paso L144 with suspensions of the bacteria DFs185 (*Pseudomonas synxantha*), DFs223 (*P. fluorescens*), DFs306 (unidentified), DFs416 and DFs418 (*Bacillus* sp.), used individually or in a combination of two, three or four compatible isolates. Seeds treated with Carboxin Thiram fungicide (T+F) at a final dosage corresponding to 3 ml kg^{-1} were used as control. Considering the leaf diseases together, eight treatments significantly controlled all three diseases, and were superior to the treatment with fungicide. Among these treatments, seven were combinations. Only the DFs306 bacteria were able to control the three diseases when used individually. It can therefore be presumed that combinations of bacteria, when microbiolized in rice seeds, can broaden the spectrum of action for the control of rice blast, brown spot and leaf scald.

Key words: *Oryza sativa*. *Pyricularia grisea*. *Bipolaris oryzae*. *Gerlachia oryzae*. Seed microbiolization.

RESUMO - A cultura do arroz irrigado está sujeita à ocorrência de várias doenças cujos danos provocam perdas na produtividade. Atualmente buscam-se alternativas para o controle destas doenças, sendo o biocontrole uma possibilidade viável. No sentido de ampliar o espectro de ação do biocontrole, o objetivo deste trabalho foi avaliar o efeito de combinações de bactérias biocontroladoras sobre a brusone (*Pyricularia grisea*), mancha parda (*Bipolaris oryzae*) e escaldadura (*Gerlachia oryzae*) em plantas de arroz. O efeito das combinações de isolados foi avaliado por microbiolização de sementes de arroz, cultivar El Paso L144, com suspensões das bactérias DFs185 (*Pseudomonas synxantha*), DFs223 (*P. fluorescens*), DFs306 (não identificada), DFs416 e DFs418 (*Bacillus* sp.) usadas individualmente ou combinadas em arranjos de dois, três e quatro isolados compatíveis. Como controle foram utilizadas sementes tratadas com o fungicida Carboxin+Thiran (T+F) na dosagem final correspondente a 3 mL kg^{-1}. Considerando as doenças foliares em conjunto, oito tratamentos controlaram significativamente as três doenças, sendo superiores ao tratamento com fungicida. Dentre estes tratamentos, sete foram combinações. Somente a bactéria DFs306 foi capaz de controlar as três doenças quando usada individualmente. Assim, acredita-se que combinações de bactérias quando microbiolizadas em sementes de arroz ampliam o espectro de ação para o controle das doenças, brusone, mancha parda e escaldadura.

Palavras-chave: *Oryza sativa*. *Pyricularia grisea*. *Bipolaris oryzae*. *Gerlachia oryzae*. Microbiolização de sementes.

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INTRODUCTION

The productivity of rice crop (*Oryza sativa* L.) may become reduced as a result of several factors, such as viruses, nematodes, as well as insect pests (OU, 1985). Among the most important diseases are rice blast, brown spot, and an emerging disease known as leaf scald. It has been estimated that these diseases are responsible for losses of up to 50% in rice crops cultivated in Rio Grande do Sul (NUNES; RIBERO; TERRES, 2004).

Rice blast, caused by the fungus *Pyricularia grisea* (Cooke) Sacc. (Teleomorph: *Magnaporthe grisea* Barr.), is considered the most important disease of rice worldwide, as a result of the high destructive potential of the pathogen and the susceptibility of the cultivars in use (NUNES; RIBEIRO; TERRES, 2004). Losses can reach 100%, when conditions are favorable for the occurrence of this disease (OU, 1985).

Bipolaris oryzae (Breda de Haan) Shoemaker [(teleomorph: *Cochliobolus miyabeanus* (Ito & Kuribayashi)], the causal agent of brown spot, causes greater damage in rice fields in Rio Grande do Sul during the germination of seeds, thereby resulting in death of the seedlings (BEDENDO; PRABHU, 2005). The occurrence of brown spot has been on the increase, due to the use of susceptible cultivars, and has become the main crop disease in some regions (NUNES; RIBEIRO; TERRES, 2004). The disease can also increase the sterility of flowers (OU, 1985), reduce the number of grains per panicle, and cause smaller and stained grains (NUNES; RIBEIRO; TERRES, 2004).

Rice leaf scald caused by *Gerlachia oryzae* (Hashioka & Yokogi) W. Gams [(teleomorph: *Monographella albescens* (Thumen) Parkinson, Sivanesan & C. Booth)] has been occurring at considerably high levels in the rice regions of Brazil, and is associated with susceptible cultivars and excessive nitrogen fertilization (NUNES; RIBEIRO; TERRES, 2004). There are still no estimates regarding the damage caused by this disease, which provokes radicle and coleoptile rot, photosynthetic area reduction and flowers sterility.

In order to control these diseases, it is necessary, in most cases, to use resistant cultivars and apply protective or systemic fungicides (BEDENDO; PRABHU, 2005). However, due to the pathogens variability and the favorable environmental conditions, the resistance of the cultivars (NUNES; RIBEIRO; TERRES, 2004) and the emergence of pathogen populations resistant to fungicides, can reduce the efficiency of the control (PRABHU; FILIPPI, 1997). In addition, the use of fungicides implies an increase in production costs and an increased risk of contamination, both of the environment and workers. Associated with this, there is still consumer pressure for pesticide-free food.

The biological control, carried out by the application of antagonistic microorganisms, presents as a viable alternative in several cultures, including rice crop (CHANDLER *et al*., 2015; LUDWIG; MOURA, 2007; VIDHYSEKARAN *et al*., 2001). In this sense, satisfactory results have been obtained with bacteria selected for the control of *B. oryzae* and *G. oryzae* (LUDWIG *et al*., 2009); however, in each year, a bacterium stood out as the most efficient. To overcome this behavioral instability, as well as intensify the effect, or even extend the biocontrol spectrum, the use of combinations of biocontrollers is suggested (MAKETON; APISITSANTIKUL; SIRIRAWEEKUL, 2008; RAUPACH; KLOEPPER, 1998; SOUZA JÚNIOR *et al*., 2010).

Thus, the aim of this work was to evaluate the potential of different combinations of bacteria, comparing them with their use individually, and aiming at the control of three rice foliar diseases: rice blast, brown spot and leaf scald.

MATERIALS AND METHODS

Rhizobacteria isolates - The bacteria used in the trials were individually selected for the *in vivo* control of brown spot and scald (LUDWIG *et al*., 2009). In this study were used the bacterial isolates, DFs185 (*Pseudomonas synxantha* (Ehrenberg) Holland), DFs223 (*P. fluorescens* Migula), DFs416 and DFs418 (*Bacillus* sp. Cohn), identified by 16S rDNA gene sequencing (unpublished data), DFs306 (unidentified) and the following compatible combinations: DFs185/306, DFs185/416, DFs185/418, DFs306/416, DFs306/418, DFs416/418, DFs185/306/416, DFs185/306/418, DFs185/416/418, DFs306/416/418, DFs185/306/416/418 (SOUZA JÚNIOR et al., 2010).

Microbiolization of seeds and seeding - Bacteria were cultured in Kado 523 solid medium for 24 h at 28 °C. Afterwards, the bacteria were suspended in saline (0.85% NaCl) and the cell concentration was adjusted to an absorbance of A_{540}=0.5. The combinations of isolates were prepared by mixing equal volumes of the individual bacterial suspensions. Rice seeds (cv. El Paso L144) were microbiolized by immersing and agitating for 30 min at 10°C in the cell suspension. The controls were composed of seeds immersed in saline solution and fungicide solution containing Carboxin+Thiran (C+F), at the final dosage corresponding to 3 mL.kg^{-1}. Each trial was conducted in a completely randomized experimental design with four replicates, with 1.0 L pots containing two plants, grown on non-sterile soil and kept in a greenhouse.

Plant inoculation and evaluation of control - Isolates of *P. grisea* and *B. oryzae* were obtained from rice plants exhibiting symptoms typical of rice blast or brown spot, respectively. The *G. oryzae* isolate was obtained from naturally infested rice seeds. Spore suspensions were obtained from pure colonies of fungi after 15 to 20 days at concentrations of $1.2x10^5$ (*P. grisea*), $1.0x10^5$ (*B. oryzae*) and $1.0x10^4$ (*G. oryzae*) conidia.mL^{-1}, respectively.

The plants were inoculated in the V4 stage by spore spraying and kept in a humid chamber, 24 h before and 48 h after inoculation. In addition, those inoculated with *P. grisea* were kept until the end of evaluations at night in the humid chamber, at 22 ± 2 °C under greenhouse conditions.

The evaluations were performed at 7, 14 and 21 days after pathogen inoculation, according to the severity of the disease in the second leaf. For rice blast, the scale recommended by the IRRI (1996) was used, and grades were assigned from 1 to 9, according to the intensity of symptoms and percentage of area attacked. For brown spot, a scale was used to evaluate seedlings and plants until the beginning of tillering, considering the affected leaf area (IRRI, 1975). For leaf scald, the scale developed by IRRI (1996) was used, and the grades were given according to the affected leaf area.

From the severity notes, the area under the disease progress curve (AUDPC) was calculated using the Gwbasic® program (MAFFIA, 1986). The data were submitted for analysis of variance and the means were compared by the Scott-Knott test (α = 0.05), using the SASM-Agri program (CANTERI *et al*., 2001). The percentage of control was calculated from the AUDPC (control = 0%).

To establish the participation of each bacterium used in the combinations, the collaboration established by the sum of the control percentage of all the bacterial treatments in which each bacterium participated, divided by the total number of treatments, was calculated.

For the treatments that significantly controlled the three diseases, was calculated the mean performance (sum of the control of each treatment divided by 3) and the weighted performance [multiplying the control value of each disease by its economic importance (rice blast = 3; Brown spot = 2; leaf scald = 1), dividing by the sum of the weights].

Antagonism by antibiosis - Antibiosis was evaluated qualitatively, by pairing cultures of fungi *P. oryzae*, *B. oryzae* and *G. oryzae* with the bacterial colonies and the formation or absence of an inhibition halo of fungus mycelium was observed. For the bacterial isolates that were able to inhibit the three fungi, a quantitative evaluation was performed, where the metabolic liquid of each bacterium was used by gel diffusion (ROMEIRO, 2007). When the control reached the edges of the plates, the evaluation was performed using a digital caliper. The experiment has a completely randomized design, with four replications.

Production of siderophores - The isolates were evaluated using the Schwyn and Neilands universal method, by using a solution of chrome azurol S (CAS) as described by Romeiro (2007), and checking for a change in coloration to reddish yellow, which is indicative of siderophores production. The experiment has a completely randomized design, with four replicates, and one isolate known as siderophore was used as the positive control.

Root colonization capacity - The rice cultivar (El Paso L144), whose seeds were submitted for superficial disinfestation, was used in this study. The rhizobacteria were suspended in saline solution (NaCl 0.85%) individually and their concentrations adjusted by the Mc Farland scale (tube 5). Subsequently, the seeds were immersed in the suspension of each bacterium, and the control in saline solution (NaCl 0.85%). The seeds were shaken for 4 h at 4 °C, deposited in agar-water (0.7%), one seed per tube and incubated in a growth chamber under a photoperiod of 12 h at 28 °C for seven days. The experimental design was performed in completely randomized blocks, with six replicates. The tubes were observed with the naked eye and the turbidity of the medium around the root system was verified to be due to colonization by bacteria. The incidence (percentage of plants with colonized roots) and intensity of colonization were evaluated using the Habe and Uesugi (2000).

RESULTS AND DISCUSSION

Regarding the severity of the rice blast, a significant increase from the first to the second evaluation was observed, but not from the third evaluation. The number of treatments that differed from the control varied throughout the evaluations. The progress of rice blast (AUDPC) was reduced by 11 treatments (Table 1). The control of rice blast as provided by the bacterial treatments, ranged from 35 to 57% (mean of 45%), with the DFs306/416 combination (57% of control). In general, there was a tendency for the bacterial combinations to provide greater rice blast control than each individual rhizobacterium. Among the bacterial combinations, it was only DFs306/416/418 that did not differ significantly from the control (C). The only individually effective bacterium was DFs306, which provided a 55% reduction in severity of disease. On the other hand, when double or triple treatments with DFs185, DFs416 and DFs418 were used, higher control averages were observed as compared to these alone, demonstrating the synergistic effect of the combinations (Table 1).

Table 1 - Severity of rice blast at 7, 14 and 21 days after inoculation of *P. grisea* in rice plants which originated from seeds microbiolized with bacteria, showing the respective area under the disease progress curve (AUDPC) and percentage of control (PC)

Treatments	Days after inoculation			AUDPC	PC
	7 d	14 d	21 d		
DFs185/306	1,38 b	2,00 a	2,00 b	25,81 b	40
DFs185/416	1,00 b	2,13 a	2,13 b	25,81 b	40
DFs185/418	0,75 b	2,25 a	2,50 a	27,00 b	38
DFs306/416	1,25 b	1,38 a	1,38 b	18,81 b	57
DFs306/418	1,00 b	2,00 a	2,00 b	24,50 b	43
DFs416/418	0,88 b	1,75 a	1,75 b	21,44 b	51
DFs185/306/416	1,50 b	2,13 a	2,25 b	28,00 b	35
DFs185/306/418	0,00 b	2,00 a	2,00 b	21,00 b	52
DFs185/416/418	0,88 b	2,13 a	2,13 b	25,31 b	42
DFs306/416/418	1,88 a	2,25 a	2,25 b	30,19 a	30
DFs185/306/416/418	1,00 b	1,50 a	1,50 b	19,25 b	56
DFs185	1,38 b	2,38 a	2,38 a	29,75 a	31
DFs223	0,88 b	2,63 a	2,63 a	30,63 a	29
DFs306	0,88 b	1,50 a	1,50 b	19,56 b	55
DFs416	1,38 b	2,38 a	2,38 a	29,75 a	31
DFs418	1,75 a	2,88 a	2,88 a	36,31 a	16
Control	2,50 a	3,13 a	3,63 a	43,31 a	0
C + F	2,25 a	2,50 a	2,50 a	34,13 a	21
Mean	1,25 B	2,16 A	2,21 A		
CV%	61,1	30,5	28,74	29,92	

* Values followed by the same lowercase letter in the column and upper case in the row do not differ by the Scott-Knott test, at the 5% level of significance. C = saline solution; C + F = saline solution plus the fungicides (Carboxin + Thiran)

In the evaluation of the control of the brown spot by the bacterial treatments, it was observed that there was a significant increase in the severity of the disease throughout the 21 days. The number of treatments that differed from the control increased between the first and second evaluations. Plants that originated from microbiolized seeds with treatments DFs185/306, DFs306/416, DFs185/306/418, DFs223 and fungicide treated plants did not provide a decrease in the severity of the disease. On average, the other bacterial treatments provided 41% of the control (reducing AUDPC between 29 and 58%). In general, it was observed that there was no tendency of the combinations to provide greater control (Table 2).

The severity of the leaf scald increased significantly over the assessed period. The number of treatments that differed from the control increased between the first and third evaluations. When analyzing the accumulated effect over time (AUDPC), all treatments, including the chemical, differed from the control. It was possible to observe the formation of two groups of efficient treatments: the intermediates, varying between 21 and 39% of control, and the superiors whose control varied between 47 and 56%. The first group consisted of nine treatments, eight of which are combinations. The second group consisted of biological treatments, four of which utilized bacteria individually. In general, no additional effect was observed to be provided by the use of combinations for leaf scald control (Table 3).

Although the trials were conducted under different environmental conditions, when the control was considered for the three foliar diseases evaluated, it was observed that the bacteria in different combinations provided close collaboration values. However, when each disease was observed separately, it was verified that different bacteria were responsible for the control of different pathogens. On the other hand, it was observed that DFs223, which did not participate in any combination, produced lower values for the control of rice blast, brown spot and higher values for leaf scald (Figure 1A).

Table 2 - Severity of the brown spot at 7, 14 and 21 days after inoculation of *B. oryzae* in rice plants which originated from seeds microbiolized with bacteria, showing respective area under the disease progress curve (AUDPC) and percentage of control (PC)

Treatments	Days after inoculation			AUDPC	PC
	7 d	14 d	21 d		
DFs185/306	1,25 a	1,50 a	2,75 a	24,50 a	21
DFs185/416	0,88 a	1,00 b	3,25 a	21,44 b	31
DFs185/418	0,88 a	1,25 b	2,75 a	21,44 b	31
DFs306/416	1,50 a	1,75 a	2,50 a	26,25 a	15
DFs306/418	0,88 a	1,25 b	1,75 a	18,06 b	42
DFs416/418	0,38 b	1,13 b	3,00 a	19,69 b	36
DFs185/306/416	0,75 a	1,00 b	2,50 a	18,88 b	39
DFs185/306/418	1,13 a	1,88 a	2,75 a	26,69 a	14
DFs185/416/418	0,13 b	0,25 b	3,25 a	13,56 b	56
DFs306/416/418	0,25 b	0,38 b	3,50 a	15,75 b	49
DFs185/306/416/418	0,88 a	1,00 b	2,75 a	19,69 b	36
DFs185	0,63 b	0,88 b	2,25 a	16,19 b	48
DFs223	1,00 a	1,25 b	3,00 a	22,75 a	27
DFs306	0,75 a	1,13 b	3,25 a	21,88 b	29
DFs416	0,25 b	0,63 b	2,25 a	13,13 b	58
DFs418	0,50 b	1,13 b	2,50 a	18,38 b	41
Control	1,25 a	2,00 a	3,50 a	30,63 a	0
C+F	1,00 a	1,75 a	3,25 a	28,88 a	6
Mean	0,79 C	1,17 B	2,82 A		
CV%	63,16	50,29	27,83	30,99	

* Values followed by the same lowercase letter in the column and upper case letter in the row, do not differ by the Scott-Knott test, at the 5% level of significance. T = saline solution; C+F = saline solution plus the fungicides (Carboxin + Thiran)

Table 3 - Severity of leaf scald at 7, 14 and 21 days after inoculation of *G. oryzae* in rice plants which originated from seeds microbiolized with bacteria, showing the respective area under the disease progress curve (AUDPC) and percentage of control (PC)

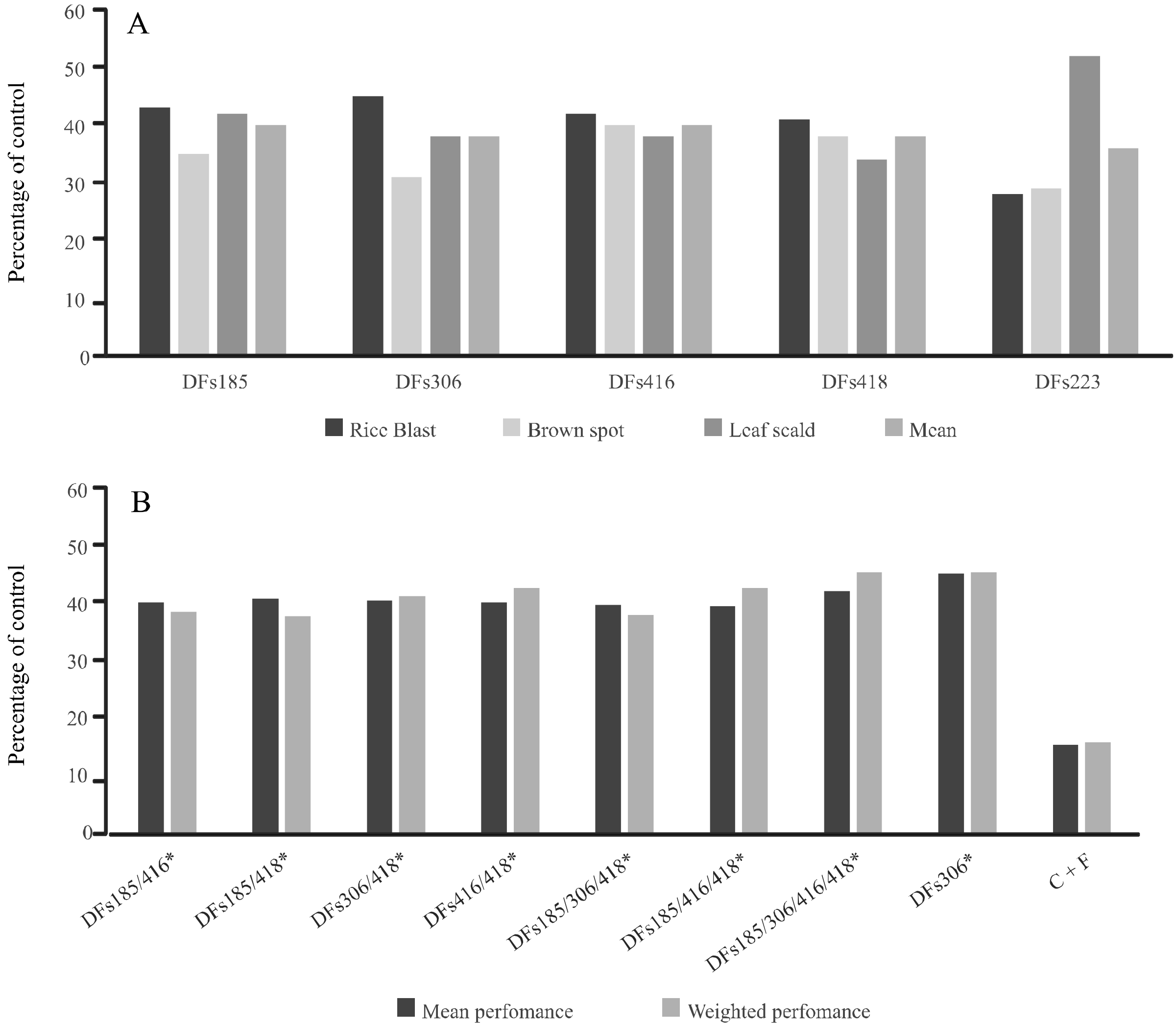
Treatments	Days after inoculation			AUDPC	PC
	7 d	14 d	21 d		
DFs185/306	1,25 b	3,25 a	3,75 c	40,25 b	39
DFs185/416	1,00 b	2,25 b	3,75 c	32,38 c	51
DFs185/418	1,25 b	1,75 b	3,75 c	29,75 c	55
DFs306/416	1,50 b	3,75 a	3,75 c	44,63 b	32
DFs306/418	2,00 a	2,75 b	4,25 c	41,13 b	37
DFs416/418	1,00 b	3,00 a	5,25 b	42,88 b	35
DFs185/306/416	1,50 b	2,50 b	3,50 c	35,00 c	47
DFs185/306/418	1,50 b	3,25 a	4,75 b	44,63 b	32
DFs185/416/418	1,75 a	4,25 a	5,00 b	51,63 b	21
DFs306/416/418	2,00 a	3,50 a	5,00 b	49,00 b	25
DFs185/306/416/418	1,50 b	3,25 a	4,00 c	42,00 b	36
DFs185	1,25 b	2,00 b	3,50 c	30,63 c	53

Continued Table 3

DFs223	1,00 b	1,75 b	3,75 c	28,88 c	56
DFs306	1,00 b	2,50 b	3,00 c	31,50 c	52
DFs416	1,25 b	1,75 b	3,75 c	29,75 c	55
DFs418	2,00 a	3,25 a	4,00 c	43,75 b	33
Control	2,75 a	4,75 a	6,50 a	65,63 a	0
C+F	2,25 a	4,00 a	4,75 b	52,50 b	20
Mean	1,54 C	1,17 B	2,82 A		
CV%	47,74	30,37	17,21	21,97	

* Values followed by the same lowercase letter in the column and upper case in the row do not differ by the Scott-Knott test, at the 5% level of significance. Control = saline solution; C+F = saline solution plus the fungicides (Carboxin + Thiran)

Figure 1 - A. Collaboration of each bacterium on the biocontrol of rice blast, brown spot, and leaf scald in rice plants, which originated from microbiolized seeds and individually inoculated with *P. grisea*, *B. oryzae* and *G. oryzae*, respectively. B. Average and weighted performance control according to the importance of rice blast, brown spot and leaf scald in rice plants whose seeds were microbiolized with rhizobacteria and treated with fungicide. *Treatments whose percentage of control was statistically superior to the control. C + F = saline solution plus the fungicide Carboxin + Thiran



Considering the importance of each leaf disease and the eight treatments that presented significant control for the three diseases, it was observed that the average performance was superior to the treatment with fungicide (Figure 1B). It was also found that among the best treatments, seven are combinations. Only the bacterium DFs306 was able to control the three diseases when used alone.

Although the trials were conducted under different environmental conditions, when the control was considered for the three foliar diseases evaluated, it was observed that bacteria in different combinations provided close collaboration values. However, when each disease was observed separately, it was verified that different bacteria were responsible for the control of different pathogens. On the other hand, it was observed that DFs223, which did not participate in any combination, produced lower values for the control of rice blast, brown spot and higher values for leaf scald (Figure 1A).

Considering the importance of each leaf disease and the eight treatments that presented significant control for the three diseases, it was observed that the average performance was superior to the treatment with fungicide (Figure 1B). It was also found that among the best treatments, seven are combinations. Only the bacterium DFs306 was able to control the three diseases, when used alone.

By observing the control of treatments applied in each disease individually, it was verified that the bacterial combinations showed levels similar to those obtained with the use of a single isolate for brown spot and leaf scald. For rice blast, additive or synergistic effects were observed. In addition, it was found that there was an increase in the spectrum of action against different pathogens, when bacterial combinations were used.

Studies have been conducted with several pathosystems and combinations of different groups of biocontrollers. A few years ago, Pierson and Weller (1994) observed that combinations of Q2-87/Q1c-80/Q8d-80/Q65c-80 and Q1c-80/Q8d-80/Q65c-80 compounds with different *Pseudomonas* isolates had lower rates of wheat foot disease caused by the fungus *Gaeumannomyces graminis* var. *graminis* than when it was used alone. Subsequently, Karthikeyan and Gnanamanickam (2008) demonstrated that isolates of *P. fluorescens* when used individually, showed little control of rice blast in *Setaria italica*, in the field. However, when this isolate was combined with *Bacillus polymyxa*, the suppression of the disease increased to 88%.

Recent studies have reinforced the idea that combinations of biocontrollers can intensify the effect on disease control. The combination of *Bacillus atrophaues* (S2BC-2) +*Burkholderia cepacea* (TEPF-Sungal) and *B. subtilis* (S2BC-1) + *B. subtillis* (GIBC-Jamong) showed antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* and reduction of vascular wilt in tomato plants, besides promoting plant growth (SHANMUGAM; KONOUJIA, 2011). The combination of *P. fluorescens* (Pf 9A-14), *B. subtilis* (BS 8B-1) and *Pseudomonas* sp. (Psp 80-45) provided an additive effect on the control of *Phytophthora capsici* and *Rhizoctonia solani* on cucumber plants (KHABBAZ *et al*., 2015).

The use of antagonists from different kingdoms has also been successful in biocontrol. According to Chaves *et al*. (2009), utilizing a combination of bacteria and endophytic fungi, resulted in 88% reduction of the nematode *Radopholus similis* in banana, a result similar to the nematicide used.

Another successful strategy is the combined use of bacterial and plant secondary metabolites. Dimkic *et al*. (2015) demonstrated that the use of lipopeptides from the isolate of *B. amytoliquefaciens* SS-12.6 together with the essential oil of thyme (0.55 mg/mL) and savory (0.32 mg/mL), showed antifungal activity and synergistic effect in the control of different species of *Fusarium*.

In contrast, Pivic *et al*. (2015) showed that the control of wheat powdery mildew caused by *Blumeria graminis* f. sp. *tritici* was not intensified when a combination of the bacterial antagonists *Bacillus* sp. - Q3 and *P. chlororaphis*-Q16 was used as compared to treatment with the Q16 isolate used individually. A similar behavior was observed by Sneh *et al*. (1984), where combinations between isolates of *Pseudomonas* sp. and other lytic bacteria did not increase the control of fusarium wilt on cucumber.

On the other hand, the broadening of the spectrum of action by the control of different diseases is more frequent. A similar behavior was observed when the bacterium *B. subtilis* and the fungus *Trichoderma harzianum* were used (MAKETON; APISITSANTIKUL; SIRIRAWEEKUL, 2008), as well as when different bacteria were used (RAUPACH; KLOEPPER, 1998). In general, increasing the spectrum makes it possible to control diseases which occur in different parts or stages of plant development or even when caused by different groups of pathogens. This case was reported by MaketonApisitsantikul and Siriraweekul (2008) who obtained control of damping-off (*Pythium aphanidermatum*), bacterial wilt (*Ralstonia solanacearum*) and cercospora leaf spot (*Cercospora nicotianae*) in tobacco plants. In this sense, Corrêa, Schafer and Moura (2014) observed that the microbiolization of commom bean seeds by using a combination of *B. cereus* and *P. fluorescens*, presented a broad spectrum of action, reducing the severity of bacterial wilt, fusarium wilt, charcoal rot and angular leaf spot.

In the qualitative evaluation of the antibiosis, it was observed that the isolate DFs185 inhibited the fungi *B. oryzae* and *G. oryzae* and the isolate DFs306, was unable to inhibit the mycelial growth of any of the fungi *in vitro*.

In the qualitative evaluation of the antibiosis, it was observed that the isolate DFs185 inhibited the fungi *B. oryzae* and *G. oryzae* and that the isolate DFs306, was unable to inhibit the *in vitro* mycelial growth of any of the fungi.

However, the isolates DFs223, DFs416 and DFs418 inhibited the growth of the three leaf pathogens studied. In the quantitative evaluation, it was observed that the isolate DFs223, presented the highest percentages of inhibition for the three pathogens evaluated (Table 4).

All bacterial isolates were able to colonize the rice roots of El Paso L144 cultivar, although they differed in incidence percentage and intensity. The bacteria that colonized 100% of the evaluated plots (DFs223 and DFs416) had a lower colonization intensity (mean of 1.08), while those that colonized more intensely (mean of 1.52) reached only 72% of the plots evaluated (DFs185, DFs306 and DFs418) (Table 4).

The isolates DFs185, DFs223 and DFs418 produced some type of siderophore. This is an important characteristic during the competition for space with other microorganisms, assisting to promote the growth of plants (AHMED; HOLMSTRÖM, 2014). The isolate *P. fluorescens*-2P24, a biocontrol agent of wheat root disease, is capable of producing antifungal compounds, hydrogen cyanide and siderophores. Since the latter is regulated by the quorum sensing signaling system, a deletion of the genes involved in the quorum sensing showed a significant effect on the biocontrol of the disease (WEI; ZHANG, 2006). The isolate *B. subtilis*-CAS15, a siderophore producer, promoted biocontrol in up to 57% of fusarium wilt, and promoted plant growth by up to 55%, resulting in an increase in yield of sweet pepper fruits by 50% (YU *et al*., 2011).

The isolates DFs185, DFs223 and DFs418 produced some type of siderophore. This is an important characteristic during the competition for space with other microorganisms, and therefore promotes plant growth (AHMED; HOLMSTRÖM, 2014). The isolate of *P. fluorescens*-2P24, a biocontrol agent of wheat root disease is capable of producing antifungal compounds, hydrogen cyanide and siderophores. However, since the latter is regulated by a *quorum sensing* signaling system, a deletion of the genes involved in the quorum sensing showed a significant effect on the biocontrol of the disease (WEI; ZHANG, 2006). The *B. subtilis*-CAS15 siderophore producer promoted biocontrol in up to 57% of fusarium wilt, and promoted plant growth by up to 55%, resulting in an increase in the yield of sweet pepper fruits by 50% (YU *et al*., 2011).

Among the bacteria selected in this study, two are of the *Pseudomonas* species (DFs185 and DFs223) while the other two are *Bacillus* species (DFs416 and DFs418). Several species of these genera are able to produce antibiotics, siderophores and lytic enzymes involved in the biocontrol (SIVASAKTHI; USHANARAN; SARANRAJ, 2014) and induction of systemic resistance (CHOUDHARY; JOHRI, 2009), and have been reported as efficient biocontrollers in several pathosystems. The results presented here corroborate this tendency, since the treatments provided protection against the three main leaf diseases of rice.

One of the hypotheses for the difficulty of achieving an intensified effect with the use of combinations may be because of the competition between the bacteria, mainly by the occupation of colonization sites or by competition for nutrients (PAULITZ; AHMAD; BAKER, 1990). This is because all the bacteria used in the present study are able to colonize the rice root system, thus providing evidence of the competitive potential of the isolates.

Table 4 - Qualitative and quantitative antibiosis of bacterial isolates confronted with the fungi *B. oryzae*, *G. oryzae* and *P. grisea*; Colonization of rice roots cv El Paso L144 and siderophores production

Isolates	*B. oryzae*	*G. oryzae*	*P. grisea*	Colonization		Siderophore
				Incidence	Intensity	
DFs185	+*	+	-	83	1,67	Yes
DFs223	+ (61)	+ (51)	+ (46)	100	1	Yes
DFs306	-	-	-	67	1,5	No
DFs416	+ (20)	+ (16)	+ (45)	100	1,16	No
DFs418	+ (35)	+ (50)	+ (51)	67	1,4	Yes

* (+) Inhibition; (-) without inhibition. Values between parentheses represent the percentage of mycelial inhibition in plate, calculated by measuring mycelial growth in two orthogonal axes (mean of the two diametrically opposed measurements

On the other hand, spectrum amplification usually occurs when biocontrollers with different mechanisms are combined, as investigated by Corrêa, Schafer and Moura, 2014 (antibiosis and resistance induction). It is possible to affirm that in the present study, the combinations of a broad spectrum of action utilized different mechanisms, when challenged by different pathogens, since the bacteria used are individually capable of producing antibiotics, siderophores and root colonization.

According to the results, the use of the DFs306 isolate in an individualized way for the control of leaf diseases showed a similar effect to the use of bacterial combinations. This fact can be explained by the capacity of the bacterium to act by resistance induction (LUDWIG; MOURA, 2007). Vidhysekaran *et al*. (2001) have also reported success with the use of a single isolate, for the control of bacterial blight in rice leaf. Chandler *et al*. (1985), in the control sheath blight of rice caused by *Rhizoctonia solani*, the isolate of *Bacillus subtilis*-BBG111 (producer of cyclic lipopeptides as fengicin and surfactin), induced resistance response in plants.

Another factor to consider in using combinations of biocontrollers is the stability of the control. According to some authors, the use of combinations allows increase in stability under different culture conditions and environment (RAUPACH; KLOEPPER, 1998). Therefore, it is possible that the use of broad-spectrum combinations reduces the variations in relation to the biocontrol intensity observed when the bacteria used in this study were evaluated in different years and challenged by the pathogens *B. oryzae*, *G. oryzae* (LUDWIG; MOURA, 2007) and *R. solani* (LUDWIG *et al*., 2009). In addition, Souza Júnior *et al*. (2010) demonstrated that there was an advantage in the use of DFs185/306, DFs185/416, DFs306/416 and DFs185/306/416/418, DFs185/418, DFs306/416, DFs416/418 and DFs185/ 306/418, which provided gains in the growth of rice plants and promoted the biocontrol of *M. graminicola* and *R. solani*.

Thus, this study demonstrated that the use of compatible bacteria combinations is desirable, since it broadens the spectrum, controls different diseases, acts by different mechanisms of action, and amplifies the suppression of pathogens. In addition, the use of biocontroller combinations has a higher probability of at least one species of bacteria surviving in the soil, allowing the stability of the control.

CONCLUSION

The bacterial combinations DFs185/416, DFs185/418, DFs306/418, DFs416/418, DFs185/306/416, DFs185/416/418, DFs185/306/416/418 and the DFs306 isolate, through the rice seeds microbiolization, increases the spectrum of action and control rice foliar fungal diseases, such as, rice blight, brown spot and leaf scald.

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