International Scholars Journals

International Journal of Virology and Parasitology, Vol. 13 (1), pp. 001-009, January, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Exploring the interactions between bacterial biocontrol agents and *Xanthomonas axonopodis pv. phaseoli* strains: Implications for bean common blight management

Bianca Obes Corrêa[1], Vanessa Nogueira Soares[2], Mauricio Sangiogo[2], José Rogério de Oliveira[3] and Andréa Bittencourt Moura[2]*

[1]Master in Agroindustrial Production and Management, Anhanguera – Uniderp University, Zip Code 79037-280 Campo Grande, Brazil.
[2]Phytosanitary Department, Agronomy Faculty, Federal University of Pelotas, P. O. Box 354, 96010-970 Pelotas, Brazil.
[3]Phytopathology Department, Federal University of Viçosa, 36571-000 Viçosa, Brazil.

Accepted 07 October, 2023

The bacterial biocontrol agents (BCAs) treatments were used for seed microbiolization; the bacterial biocontrol agents (BCAs) used selected from previous study on the control of *Xanthomonas-axonopodis* pv. *phaseoli* Xap) includes: *Bacillus* (DFs093, DFs348 and DFs769), *Pseudomonas* (DFs513, DFs831 and DFs842), *Rhodococcus* (DFs843 and DFs912), and the combinations C01 (DFs093+DFs769+DFs831), C02 (DFs093+DFs769+DFs842) and C03 (DFs093+DFs769+DFs348). Sixteen *Xap* strains were collected from several Brazilian regions, and were inoculated in cotyledonary leaves. The symptom development was followed for 10 days. The treatments were compared by the area under the disease progress curve for disease incidence, severity, and index. In general, majority of the BCAs reduced, to some degree, the disease caused by different *Xap* strains. The combination C01, and the isolate DFs831 resulted in highest mean disease control. The data from this study suggest a relationship between the region of origin of *Xap* strain and the efficacy of BCA to control the disease caused by it. The use of combinations of these organisms increased the efficacy of the biocontrol of several strains of the same pathogen. A strain-BCA interaction was shown by data collected from this study, which evidence the importance of selecting a BCA or a combination of BCAs with a wider spectrum of action.

Key words: Biological control, co-inoculation, *Bacillus*, *Pseudomonas*, *Rhodococcus,* bean common blight.

INTRODUCTION

The bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*) of common beans (*Phaseolus vulgaris* L.) occurs worldwide and is especially serious in regions of high temperatures and relative humidity. In

*Corresponding author. E-mail: abmoura@ufpel.edu.br.

Brazil, the disease commonly occurs in the states of Rio Grande do Sul, Rio de Janeiro, São Paulo, Minas Gerais, Paraná, Santa Catarina and in the central-west region. The disease is very damaging in rainy season crop (Almeida et al., 2015; Fourie, 2002) and losses of up to 50% have been reported (Vieira and Souza, 2000).

Being widely distributed in Brazil, difference in the virulence *Xap* strains occurring in different regions are expected, which can affect the control strategies, especially development of resistant cultivars (Vieira and Souza, 2000). The association between the origin of a *Xap* strain and its virulence has been reported. The strains from temperate regions were found to be less virulent than those from tropical regions (Rava and Romeiro, 1990; Mutlu, 2008). Frequent failure of disease control through the use of resistant cultivars and chemicals has been attributed to the differences in the virulence of the pathogen (Mutlu, 2008), which warrants developing alternate methods, including biological control.

The biological control using bacterial biocontrol agents (BCA) is an alternative that has been tested and has shown potential for control of foliar pathogens (Singh and Siddiqui, 2015; Akhtar and Siddiqui, 2010). There are also many researches that show good results for bean diseases such as *Macrophomina phaseolina* (Torres et al., 2016), *X. axonopodis* pv. *phaseoli* (Zanatta et al., 2007; Sallan, 2011), *Pseudomonas savastanoi* pv. *phaseolicola* (Garret and Schwartz, 1998), *Colletotrichum lindemuthianum* (Corrêa et al., 2008; Bardas et al., 2009) and *Pseudocercospora griseola* (Corrêa et al., 2014). However, the studies that evaluate the efficacy of BCA in controlling the diseases caused by different strains of the same pathogen are rare. Corrêa et al. (2014) pointed out that the control of bacterial pathogens by bacterial BCA may be strain dependent, leading to variability in control efficacy. *In vitro* evaluation of BCA to control bacterial blight of beans gave varied results when confronted with different strains of *Xap* (Silva et al., 2008), although *in vitro* studies are not sufficient to evaluate the efficacy of a BCA (Köhl et al., 2011). Thus this study was done to evaluate the potential of several BCAs, alone or in combination, to control the disease development induced by different strains of *Xap* and determine the interaction between them.

MATERIALS AND METHODS

Origin of the isolates of bacterial BCAs and of *Xap*

The isolates of bacterial BCAs *Bacillus cereus* DFs93 and DFs769 (isolated from soil and snap bean respectively), *Bacillus* sp. DFs348 (isolated from onion leaf), *Pseudomonas veronii* DFs513 (isolated from onion tunic), *P. fluorescens* DFs831 and DFs842 (respectively isolated from snap and rhizosphere soil of common bean), DFs843 and DFs912 (*Rhodococcus fascians* isolated from bean leaf) used in this study were selected from a previous work on the control of *Xap* (Zanatta et al., 2007) and are maintained in the collection of the Plant Bacteriology Laboratory of the Federal University of Pelotas.

The *Xap* strains were collected from several regions of Brazil (Figure 1), and were tested for virulence before use.

Seed treatment with BCA

Bean seeds were treated with either of the BCAs cultivated for 24 h on medium 523. The bacterial suspensions (20 mL) were prepared in saline solution (NaCl 0.85%) and the concentration of the cell suspension was adjusted to A_{540} = 0.50. Combinations C01 (DFs093+DFs769+DFs831), C02 (DFs093+DFs769+DFs842) and C03 (DFs769+DFs348+DFs831) were prepared by mixing in equal volumes (20 mL) suspension of each component isolate (A_{540} = 0.50). The selection of these combinations was based on an earlier study about control of *Xap* and growth promotion of bean plants (Santos, 2006).

Twenty-five seeds of bean cultivar BRS Valente were immersed in the respective suspensions of BCA (50 mL), for five hours at 10°C under constant orbital agitation. Control seeds were immersed in saline solution only (Zanatta et al., 2007). The treated twenty-five seeds were planted in a commercial substrate Plantmax® in 500 g pots. The pots were randomly distributed on greenhouse benches. Cotyledonary leaves were detached after 12 days for inoculation with the respective strain of the pathogen.

BCAs spectrum to control bacterial blight caused by different strains of Xap

The strains of Xap, belonging to the collections of the Federal Pelotas University and Federal Viçosa University, were cultivated on the medium 523 (Kado and Heskett, 1970) for 48 h at 28°C. The bacterial suspension of each strain was prepared in saline solution and the bacterial cell concentration was adjusted to A_{540} = 0.2. The detached leaves from seedlings originating from seeds treated with the either of the BCA (individually or in combination) or from the control were inoculated with the respective *Xap* strain, with the use of the cutting technique. The two cotyledonary leaves were cut at five locations each with a scissor previously dipped into the inoculum suspension, as described previously by Zanatta et al. (2007). The experiment was conducted in three replications of each treatment.

The symptom development was followed for 10 days with evaluation starting 2-days after inoculation and subsequently at 2-days interval. The disease incidence (DI) was determined by counting the number of cuts with symptoms and the disease severity (DS) estimated on the scale of 0 to 6: 0- no symptoms, 1- discontinuous chlorosis at the cuts, 2- continuous chlorosis at the cuts, 3- chlorosis at the cuts and leaf wilting at leaf border, without crossing the lateral veins, 4- chlorosis and wilt that crossed the lateral veins, 5- chlorosis and wilt at the internal levels of the cut and 6- advanced chlorosis and wilt of the cut area (Rava, 1984).The disease index (IDX) was calculated by multiplying the values of incidence and its respective severity at each day (DIx DS). the general mean disease control was also calculated using [(ID + SD + IDX)/3].

The treatments were compared by the area under the disease progress curve (AUDPC) for disease incidence, severity, and index by the by Scott-Knott test with the use of the R Core Team (2015). The results were expressed as percent of disease control relative to the control plants (without BCA) took as 0% control.

The data of incidence, severity and disease index AUDPCs were subjected to analysis of variance and comparison by Dunnet test (p =5%) using statistical software R (2015). The general means disease control were clustered by Toche method (Cruz, 2006) using the software Statistica® using Euclidean distance.

Strain	Origin
Xap02	RS
Xap06	RS
Xap08	RS
Xap12	RS
Xap13	RS
Xap16	RS
Xap19	RS
Xap20	RS
Xap23	SP
Xap24	PR
Xap25	SP
Xap26	MG
Xap28	RS
Xap29	MS
Xap32	MG
Xap36	DF

Figure 1. Brazilian states where the pathogenic isolates of *X. axonopodis* pv. *phaseoli (Xap)* came from. DF: Distrito Federal; MG: Minas Gerais State; MS Mato Grosso do Sul State; PR: Paraná State; RS: Rio Grande do Sul State; SP: São Paulo State.

RESULTS

The 16 strains of *Xap* showed different aggressiveness, mainly for severity (ranging10 to 33, average = 25) and disease index (varying from 58 to 260, average = 170) (Figure 2). The most aggressive strains were *Xap*12, *Xap*8, *Xap*13, *Xap*2 and *Xap*28 and the less ones were *Xap*24, *Xap*32, *Xap*6, *Xap*26 and *Xap*25 for all the three diseases variables.

Although the aggressiveness was quite different among the strains, in general, the majority of the BCAs reduced, to some degree, the disease caused by different *Xap* strains (Figures 3 to 5); however, the degree of control as indicated by reduction of DI, DS and the disease index differed significantly among the BCA isolates. Some BCAs did not reduce one (all BCAs), two (six BCAs) or none (nine BCAs) of the variables (DI, DS or IDX) used to quantify the disease induced by a particular *Xap* strain.

The combination C01 and the isolate DFs831 alone, resulted in maximum mean disease control of 36 and 27%, respectively, based on general mean percentage calculated by combining all the three disease indicators, in contrast to only 9% by the least effective isolate DFs093 (Figure 5).

The combination C01 was most efficient of all other combinations and the individual BCA isolates, since it reduced the overall disease induced by any of the *Xap* strain, although no decline in the disease incidence caused by Xap 26 or Xap28 (Figure 3), nor disease severity caused by strains Xap13 or Xap16 or Xap 32 (Figure 4) was observed. For all other *Xap*-strains, despite high variation (0 to 68%) the combination C01 reduced, on an average, DI by 35% (range 0 to 81%), DS by 28% (range 0 to 68%) and disease index by 48% (range 0 to 94%).

The seed treatment with the isolate DFs831 alone (Figures 3 to 5) showed widest spectrum against *Xap* strains compared to the other isolates used singly. The mean DI was reduced by 29% (range 0 to 76%), DS by 28% (range 0 to 82%) and the disease index by 41% (range 0 to 93%). Its performance can be considered only slightly lower than that of the combination C01, not only for general mean disease control, but also because it did not reduce the DS caused by five *Xap* strains only (including same two isolates controlled by C01), and the DI and the disease index caused by only two other strains, which shows that the strain spectrum of DFs831 was narrower than that of the combination C01.

The effects of other BCAs or their combinations were less pronounced. The dendrogram (Figure 6A) shows that seed treatment with the C01 or with DFs831 alone, formed a distinct group, suggesting higher efficacy to control disease induced by strains of *Xap*. The small distance between BCAsDFs769, DFs842 and DFs912 and the combination C02 formed an intermediate group, suggesting a similar spectrum for disease control

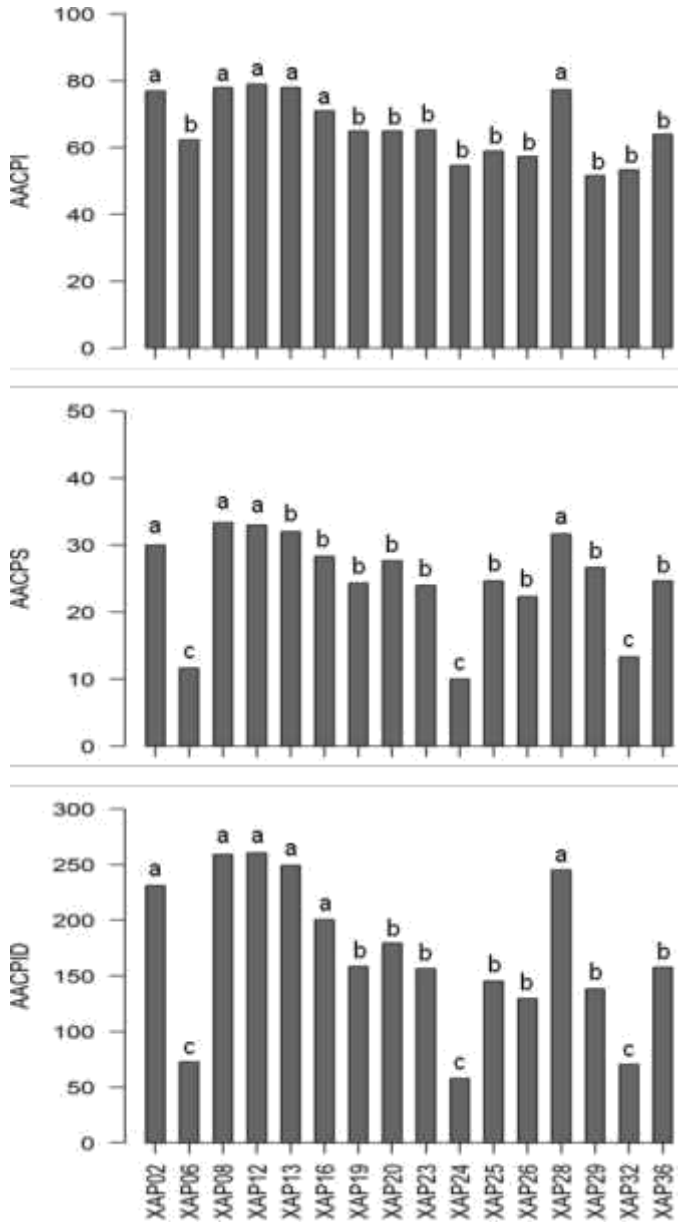

Figure 2. Aggressiveness of different strains of *X. axonopodis* pv. *phaseoli (Xap)* expressed by area under disease progress curve of incidence (AACPI), severity (AACPS) and index (AACPID) on detached cotyledonary leaves of seedlings arising from beans seeds treated with NaCl 0.85% and inoculated by cutting technique. *Means followed by the same letter do not differ by the Scott-Knott test at a significance level of 5%.

(general mean percentage) caused by different strains of *Xap*.

The analysis of the efficacy of all BCAs and their combinations on DI, DS and disease index, showed (Figure 6B) that control of the disease caused by strains *Xap*25 (49.2%) and *Xap*24 (47.8%) formed a separate group, the strain *Xap*12 (38,6%), formed the second group. The BCA shad low disease control efficacy against *Xap*13 and *Xap*8, each of which formed a single separate group. The control of the disease caused by strains *Xap*6, *Xap*19, *Xap*20, *Xap*23, *Xap*26 and *Xap*29, forming another group was intermediate, showing close similarity among them.

DISCUSSION

The data from this study suggest a relationship between the region of origin of *Xap* strain and the efficacy of BCA to control the disease caused by it. Except for the strain *Xap*12, the strains originating from Rio Grande do Sul were less sensitive to BCAs obtained from the same region, which, at least partially, can be attributed to the co-existence of these organisms (Cook and Baker, 1983). On the other hand, the BCAs which is more efficient in controlling these strains (C01 and DFs831) were obtained from rhizosphere or parts of the bean plant, which confirms the importance of locality or the site from where the BCA was obtained. It is well known that in some pathosystems, the most appropriate site for the finding an effective antagonist is the host itself (Jensen et al., 2016; Mercier, 2006).

The varying effect of BCAs against different strains of the pathogen may also be related to the virulence variability among *Xap* strains (Rava and Romeiro, 1990; Vieira and Souza, 2000; Mutlu, 2008). Generally, in this study, the strains that were more effectively controlled (*Xa*p24 and *Xap*25) were less aggressive and in other way, the less controlled (*Xap*8 and *Xap*13) were the more aggressive. However, *Xap*12 was effective controlled (40%) and was one of the most virulent strain (values were same or close to *Xap*8 and slightly bigger than *Xap*13). Additionally, *Xap*6 and *Xap*32 showed low virulence but were in the intermediate group of control (19 and 14% respectively).

The studies showing the disease control ability of BCAs on different strains of the same pathogen are rare, but Naik and Sen (1993) reported considerable variation in the efficacy of a bacterial BCA to control the disease on watermelon caused by nine strains of *Fusarium oxysporum* and *F. solani*, and attributed it to the variability in the virulence of the strains, as also found in this work. Reinforcing results of this study, Corrêa et al. (2014) also observed efficiency variations when the same BCAs of this study to control five isolate of *Curtobacterium flaccumfacien s*pv. *flaccumfaciens* in common bean were used.

It is noteworthy that the most effective BCA treatment on different Xap strains was the combination C01, consisting of BCA isolates known for their efficacy when they were used singly (Zanatta et al., 2007; Corrêa et al., 2008; Silva et al., 2008). The use of combinations of these organisms increased the efficacy of the biocontrol of several strains of the same pathogen, and also of the other pathogens, by exercising different modes of action

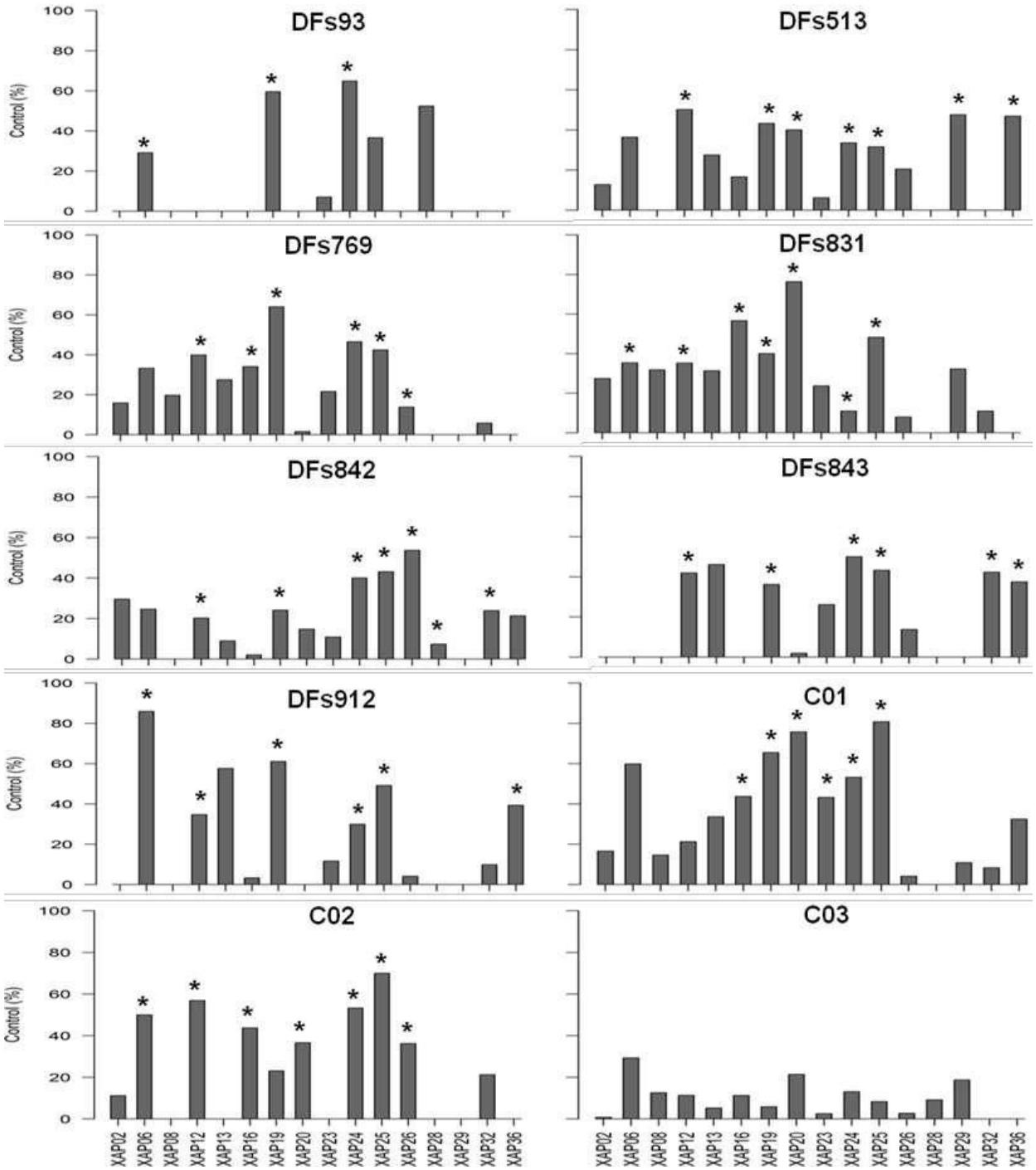

Figure 3. Percent of disease control (incidence) caused by the sixteen strains of *X. axonopodis* pv. *phaseoli* (*Xap*), on detached cotyledonary leaves of seedlings arising from bean seeds treated with biocontrol agents (NaCl control = 0). *Bacillus* (DFs93 and DFs769), *Pseudomonas* (DFs513, DFs831 and DFs842), *Rhodococcus* (DFs843 and DFs912) and the combinations C01 = DFs93+DFs769+DFs842; C02 = DFs93+DFs769+DFs831; C03 = DFs348+DFs769+DFs831. Means followed by * differ by Dunnett test at 5% probability of the control.

(Guetzky et al., 2002; Boer et al., 2003; Wu et al., 2014).

It is also noteworthy that the high spectrum of the isolate DFs831 and its involvement in the combination C01 shows that this isolate has an effective mechanism to control common bacterial blight, and its efficacy increases when combined with the other isolates, probably by synergism. This synergistic effect is generally due to combination of different modes of action of each

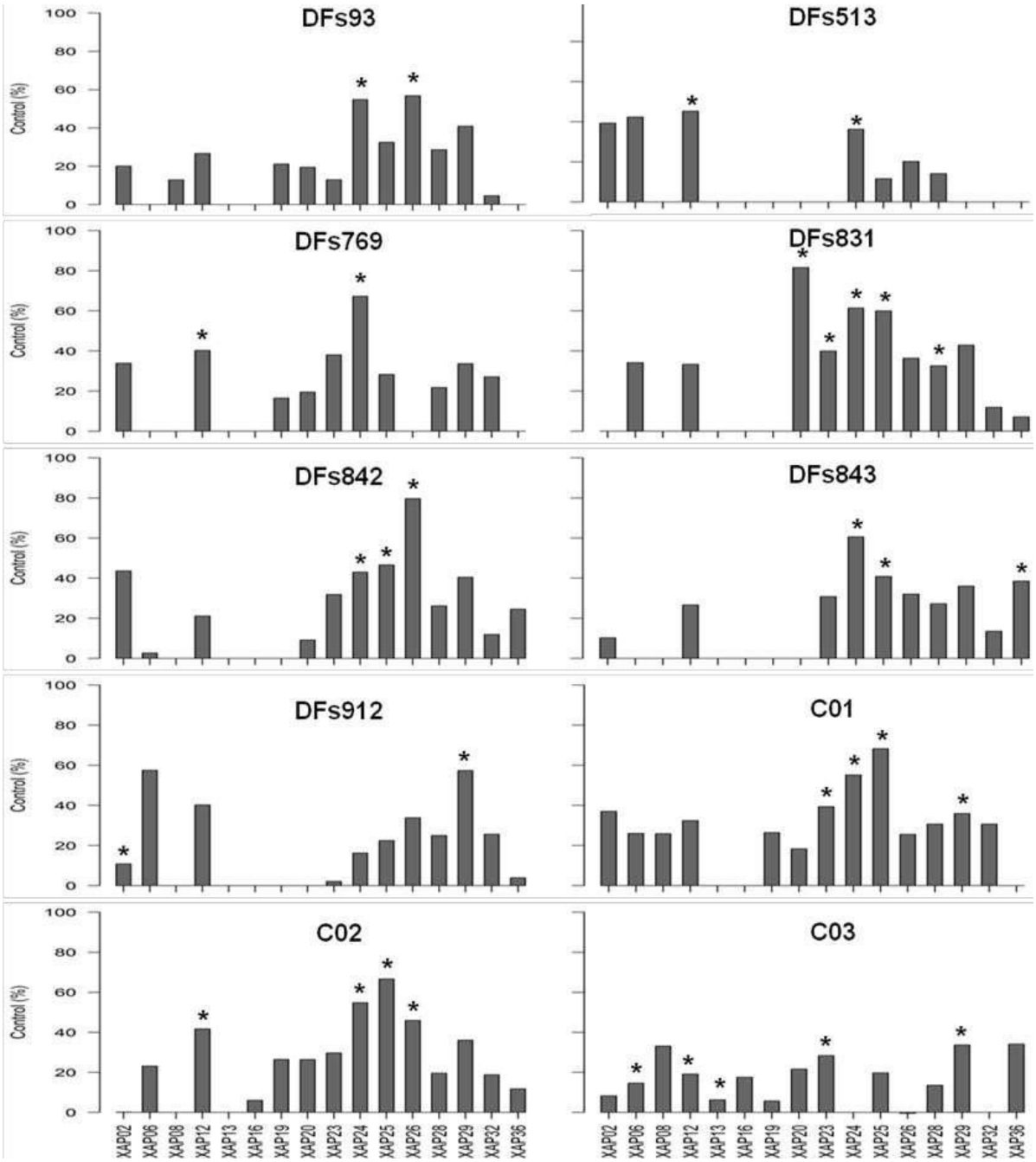

Figure 4. Percent of disease control (severity) caused by the sixteen strains of *X. axonopodis* pv. *phaseoli* (*Xap*), on detached cotyledonary leaves of seedlings arising from bean seeds treated with biocontrol agents (NaCl control = 0). *Bacillus* (DFs93 and DFs769), *Pseudomonas* (DFs513, DFs831 and DFs842), *Rhodococcus* (DFs843 and DFs912) and the combinations C01 = DFs93+DFs769+DFs842; C02 = DFs93+DFs769+DFs831; C03 = DFs348+DFs769+DFs831. Means followed by * differ by Dunnett test at 5% probability of the control.

component BCAs as shown in the combination of *Pichia guilliermondii* and *Bacillus mycoides* to control *Botrytis cinerea* on strawberry leaves, which involves parasitism and production of fungitoxic compounds (Guetzky et al., 2002).

Other mechanisms, however, such as resistance inducement and competition for iron as shown for isolates of *Pseudomonas putida* (RE8 and WCS358, respectively) should not be underestimated, which have been shown to reduce *Fusarium* wilt in radish (Boer et al., 2003). Involvement of several mechanisms can increase the efficacy of BCAs as shown by Mishra and Arora (2012)

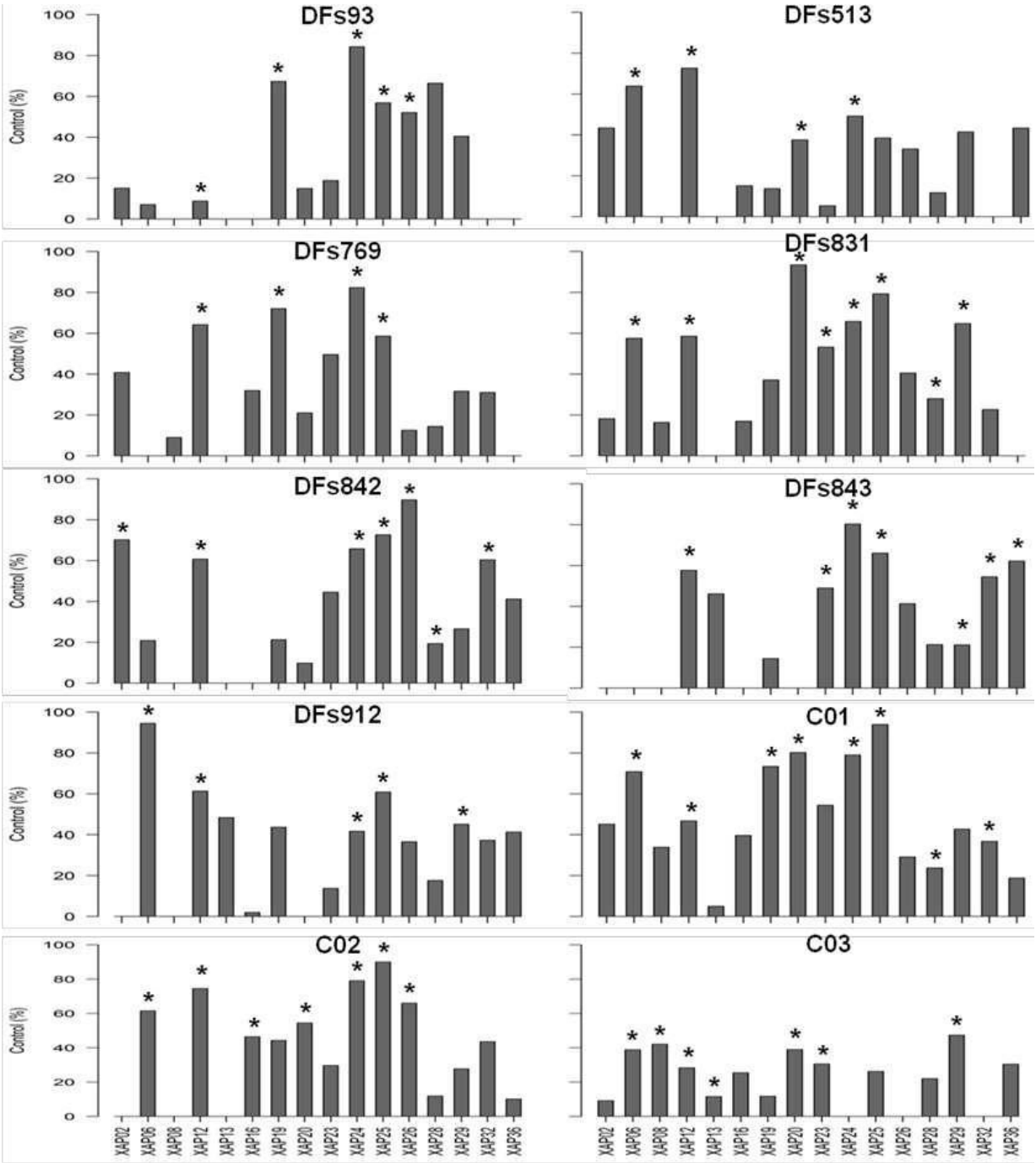

Figure 5. Percent of disease control (index) caused by the sixteen strains of *X. axonopodis* pv. *phaseoli* (*Xap*), on detached cotyledonary leaves of seedlings arising from bean seeds treated with biocontrol agents (NaCl control = 0). *Bacillus* (DFs93 and DFs769), *Pseudomonas* (DFs513, DFs831 and DFs842), *Rhodococcus* (DFs843 and DFs912) and the combinations C01 = DFs93+DFs769+DFs842; C02 = DFs93+DFs769+DFs831; C03 = DFs348+DFs769+DFs831. Means followed by * differ by Dunnett test at 5% probability of the control.

who combined *Pseudomonas* and *Bacillus* to control *Xanthomonas campestris*pv. *campestris,* and found that improved control was due to production of siderphores, autolisines and AHL-lactonases.

The wider spectrum of action and greater control efficacy also can be achieved by combining agents that induce resistance and produce antibiotic as shown for *Bacillus pumilus* (INR7), *Curtobacterium flaccumfaciens* (ME1), *B. subtilis* (GB03) which in different combinations controlled *Pseudomonas syringae*pv. *lachrymans,*

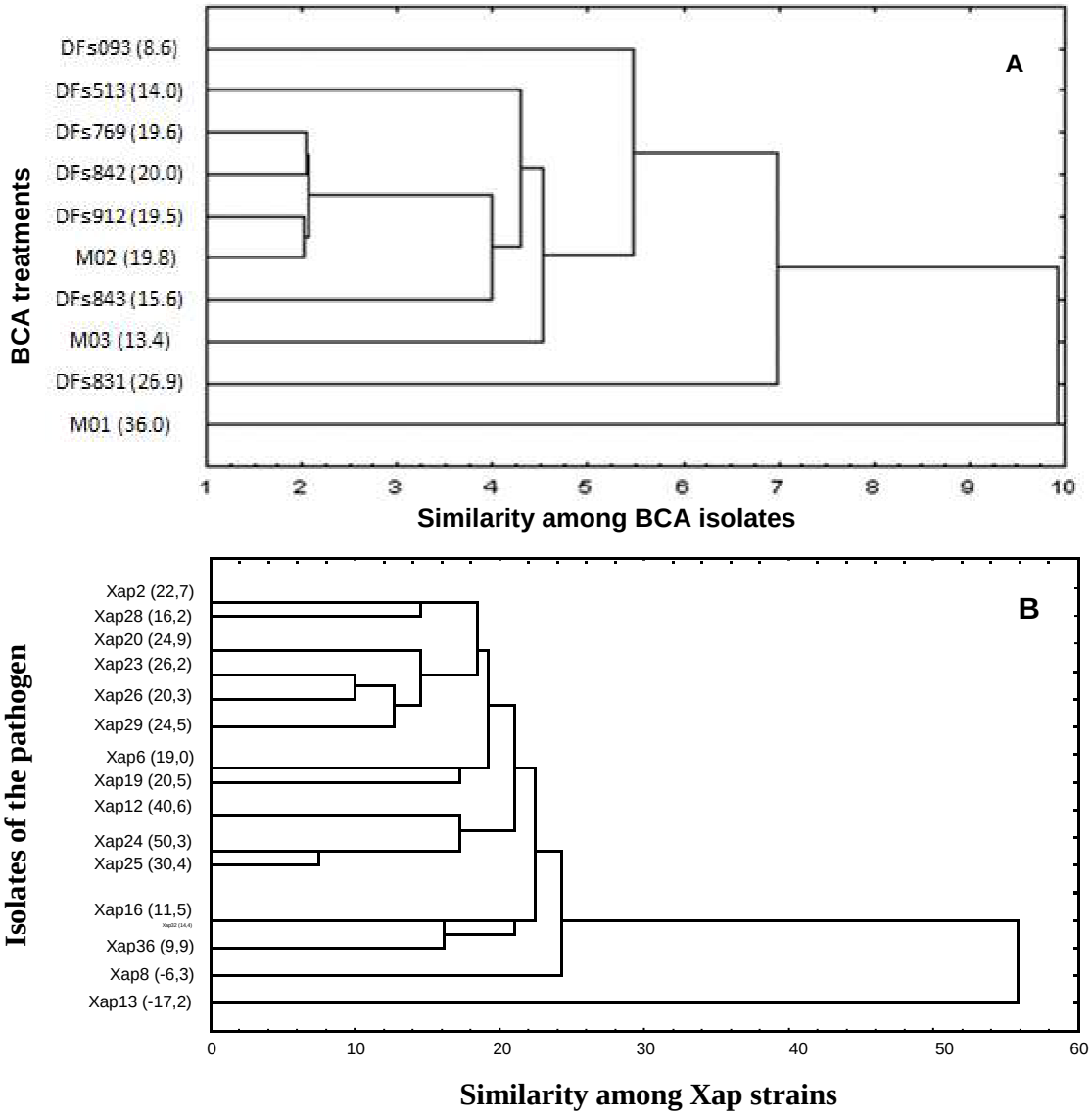

Figure 6. Dendrogram constructed by the software Statistica® relating all the treatments by all biocontrol agents (DFs) considering the general mean percentage of disease control of the sixteen strains of *X. axonopodis* pv. *phaseoli* (A); considering the general mean percentage of control of the sixteen strains of *X. axonopodis* pv. *phaseoli* (*Xap*) (B).

Erwinia tracheiphila and *Colletotrichum orbiculare* (Raupach and Kloepper, 1998) or combining lytic and antibiotic activities with systemic resistance to control bacterial wilt, *Fusarium* wilt, charcoal rot and angular leaf spot of common beans (Corrêa et al., 2014).

The data of this study show that the independent of the virulence of the pathogen´s strain there was strain-BCA interaction, which shows the importance of selecting a BCA or a combination of BCAs with wider spectrum of action, allowing for greater effect under different situations, thus adding to the product stability (Boer et al., 2003; Mercier et al., 2006). It appears that the combination C01 and the isolate DFs831 have the potential for developing into a practical BCA to control bacterial blight of common beans.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Akhtar MS, Siddiqui ZA (2010). Role of plant growth promoting rhizobacteria in biocontrol of plant diseases and sustainable agriculture. In. Maheshwari DK. Plant Growth and health promoting bacteria. Springer. pp.157-195.

Almeida IMG, Rodrigues LMR, Beriam LOS (2015). *Xanthomonas fuscans* subsp. *fuscans* causing wilt symptoms in bean plants (*Phaseolus vulgaris*) in Brazil. Rev. Arq. Inst. Biol. 20:1-3.

Bardas GA, Lagopodi AL, Kadoglidou K, Tzavella-Klonari K (2009). Biological control of three *Colletotrichum lindemuthianum* races using *Pseudomonas chlororaphis* PCL1391 and *Pseudomonas fluorescens* WCS365. Biol. Control 49:139-145.

Boer M, Bom P, Kindt F, Keurentjes JJB, Sluis I,Van Loon LC, Bakker PAHM (2003). Control of *Fusarium* wilt of radish by combining *Pseudomonas putida* strains that have different disease suppression mechanisms. Phytopathology 93:626-632.

Cook RJ, Baker KF (1983). The nature and practice of biological control of plant pathogens. The American Phytopathological Society, 539p.

Corrêa BO, Moura AB, Denardin NA, Soares VN, Schäfer JT, Ludwig J (2008). Influence of bean seed microbiolization on the transmission of *Colletotrichum lindemuthianum* (Saac e Magn.). Rev. Bras. Sementes 30:156-163.

Corrêa BO, Schafer JT, Moura AB (2014). Spectrum of biocontrol bacteria to control leaf, root and vascular diseases of dry bean. Biol. Control 72:71-75.

Cruz CD (2006). Genes Program - Multivariate Analysis and Simulation. Viçosa, MG: Editora UFV.

Fourie D (2002). Distribution and severity of bacterial diseases on dry beans (*Phaseolus vulgaris* L.) in South Africa. J. Phytopathol. 150:220-226.

Garret KA, Schwartz HF (1998). Epiphytic *Pseudomonas syringae* on dry beans treated with copper-based bactericides. Plant Dis. 82:30-35.

Guetzky R, Shtienberg D, Elad Y (2002). Dinoor A. Improving biological control by combining biocontrol agents ach with several mechanisms of disease suppression. Phytopathology 92:976-985.

Jensen DF, Karlsson M, Sarrocco S, Vannacci G. (2016) Biological Control Using Microorganisms as an Alternative to Disease Resistance. In. Colligne DB. Plant Pathogen Resistance Biotechnology. John Wiley & Sons, Inc. pp. 341-363.

Kado CI, Heskett, MG (1970). Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. Phytopathology 60:969-976.

Köhl J, Postma J, Nicot P, Ruocc M, Blum B (2011). Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. Biol. Control 57:1-12.

Mercier J (2006). Dynamics of foliage and thatch populations of introduced *Pseudomonas fluorescens* and *Streptomyces* sp. on a fairway turf. BioControl 51:323-337.

Mishra S, Arora NK (2012). Evaluation of rhizospheric *Pseudomonas* and *Bacillus* as biocontrol tool for *Xanthomonas campestris* pv *campestris*. World J. Microbiol. Biotechnol. 28:693-702.

Mutlu N (2008). Differential pathogenicity of *Xanthomonas campestris* pv. *Phaseoli* and *X*. *fuscans* subs. *fuscans* strains on bean genotypes with common blight resistance. Plant Dis. 92:546-554.

Naik MK, Sen B (1993). Effectiveness of biocontrol agents against a spectrum of *Fusarium* isolates causing wilt of watermelon. Indian J. Plant Prot. 21:19-22.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raupach GS, Kloepper JW (1998). Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88:1158-1164.

Rava CA (1984). Pathogenicity of *Xanthomonas axonopodis* pv. *phaseoli*. Pesqui. Agropecu. Bras. 19:445-448.

Rava CA, Romeiro RS (1990). Variability of isolates of *Xanthomonas campestris* pv. *phaseoli*. Summa Phytopathol. 16:225-232.

Sallan NM (2011). Biological control of common blight of bean (*Phaseolus vulgaris*) caused by *Xanthomonas axonopodis* pv. *phaseoli* by using the bacterium *Rahnellaaquatilis*. Arch. Phytopathol. Plant Prot. 44:1966-1975.

Santos AS (2006). Biocontrol of Xanthomonas axonopodis pv. *phaseoli* and growth promotion of bean plants by the microbiolization of seeds with bacteria. MS thesis, Federal University of Pelotas.

Silva EG, Moura AB, Bacarin MA, Deuner CC, Farias DR (2008). Study of biocontrol mechanisms of common bean blight by bactéria. Ceres 55:377-383.

Singh N, Siddiqui ZA (2015). Effects of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Aspergillus awamori* on the wilt-leaf spot disease complex of tomato. Phytoparasitica 43:61-75.

Torres MJ, Pérez BC, Petroselli G, Erra-Balsells R, Audisio MC (2016). Antagonistic effects of *Bacillus subtilis* subsp. *subtilis* and *B. amyloliquefaciens* against *Macrophomina phaseolina:* SEM study of fungal changes and UV-MALDI-TOF MS analysis of their bioactive compounds. Microbiol. Res. 182:31-39.

Vieira AAH, Souza RM de (2000). Virulence of *Xanthomonas axonopodis* pv. *phaseoli* and its variant *fuscans.* Ciênc. Agrotec. 24:94-102.

Wu Y, Yuan J, Raza W, Shen Q, Huang Q (2014). Biocontrol traits and antagonistic potential of *Bacillus amyloliquefaciens* Strain NJZJSB3 against *Sclerotinia sclerotiorum*, a causal agent of canola stem rot. J. Microbiol. Biotechnol. 4:1327-1336.

Zanatta ZGCN, Moura AB, Maia LC, Santos AS (2007). Bioasay for selection of biocontroller bacteria against bean commnon blight (*Xanthomonas axonopodis* pv. *phaseoli*). Braz. J. Microbiol. 38:511-515.