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Effect of ultraviolet-B (UV-B) radiation on bacterial community in the soybean phyllosphere

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The impact of ultraviolet-B (UV-B) radiation on the culturable and unculturable bacterial communities were studied in field experiments using soybean plants grown under increased UV-B (UV-B+), negative control (UV-B-) and solar UV-B. Sampling of leaves exposed to UV-B was performed in different developmental stages of the plant. The data obtained demonstrate that UV-B radiation did not alter the culturable and unculturable bacterial community. In contrast, culture independent analysis revealed major differences between cultivars for the stage of development (cultivation time) of the plants. Regarding the bacterial communities of the phyllosphere, it is possible to observe a similar behavior of the analyzed groups (Bacteria, β-Proteobacteria, and *Pseudomonas*) on both cultivars (BRS-262 and IAC-100) according to the analyzed environmental factors (increased UV-B, negative control and solar UV-B). These results indicate that leaf surfaces are composed of epiphytic bacterial communities that have survival mechanisms of different environmental conditions, such as repair of DNA damage, pigmentation and production of exopolysaccharides (EPS).

Key words: Ultraviolet-B (UV-B), phyllosphere, pigmented bacteria, survival.

INTRODUCTION

The surface of plant leaves, called phyllosphere represents a niche with large agricultural and environmental significance. It is considered to be exposed to rapid changes in temperature and humidity, limited nutrients and ultraviolet radiation harsh environment, however, successful phyllosphere inhabitants can be expected to multiply and occupy newly formed niches while the leaves are expanding (Vorholt, 2012).

There is emerging evidence that increased solar ultraviolet-B (UV-B) radiation is reaching the earth's atmosphere, due to stratospheric ozone depletion (Madronich et al., 1998; McKenzie et al., 2011). Individual climate change factors, such as UV-B are known to have direct biological effects on cultivated and native plants

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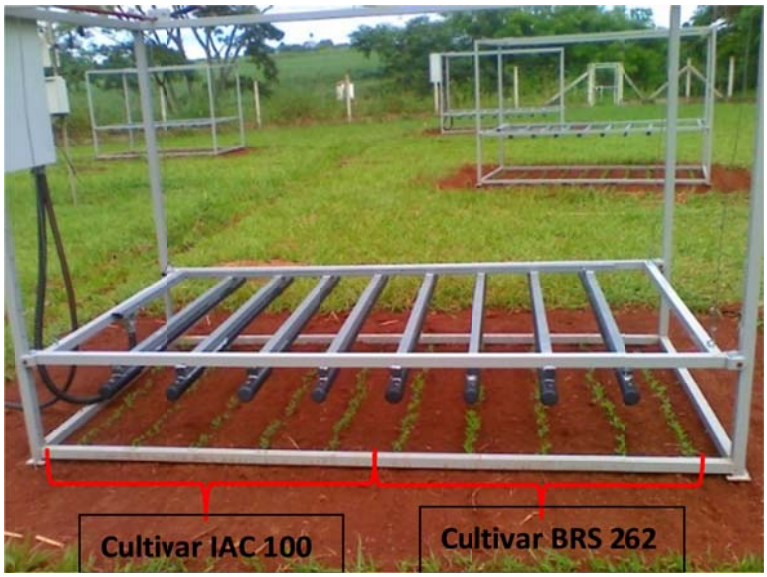

Figure 1. One parcel of the experiment showing the assembled structure bounded by steel structures. Each plot is sown with both cultivar soybean seeds as displayed.

(Ballare et al., 2011) and can indirectly affect the incidence and severity of plant diseases (Chakraborty et al., 2000).

The daily influx of solar UV radiation includes UV-A and UV-B wavelengths. High energy UV-B wavelengths (280 to 320 nm) are particularly inhibitory to organisms and cause direct DNA damage due to the strong absorption at wavelengths below 320 nm among others by the DNA molecule (Lucas et al., 2006). However, few studies (Jacobs and Sundin, 2001; Sundin and Jacobs, 1999) have reported the impact of UV-B radiation on both culturable and unculturable microbial communities inhabiting the phyllosphere. UV-B radiation may directly and indirectly affect the incidence of associated microorganisms, many of them, beneficial microbiota that may result in significant alterations in the ecological balance in the phyllosphere. The ecological success of bacteria exposed to UV radiation is conferred by the ability of organisms to effectively repair DNA damage, produce pigments or even to avoid damage by the reduced bacterial growth and colonization of sites protected from UV radiation. These niches are protected interior locations of plant leaves, or external shady places physically, at the base of the trichomes (Wilson et al., 1999).

According to Paul et al. (1997), UV-B radiation has negative impacts on individual microbial species and complex microbial communities encompassing a number of trophic levels. We know little about increased UV-B effects on bacteria. Most attention has focused on potential interactions with fungi.

We have investigated the effects of UV-B on the phyllosphere bacterial community on field-grown soybean, where the main goal was to determine the effects of UV-B radiation on culturable and unculturable bacterial communities from the phyllosphere of two soybean cultivars.

MATERIALS AND METHODS

Experimental plots

The field experiments with soybean were performed at the Brazilian Enterprise for Agricultural Research (EMBRAPA), in Jaguariúna (Brazil). Nine parcels were assembled and bounded by galvanized steel structures (1.2 m wide, 2.3 m long and 1.8 m high). In each plot, seeds of two soybean cultivars, IAC 100 and BRS 262, were sown (Figure 1). The experimental design was randomized in blocks with three treatments in triplicates as follows: [1] solar UV-B, [2] negative control (UV-B-) and [3] increased UV-B (UV-B+). The cultivars were chosen because they are of great importance in Brazil, pest resistance and good grain production (Lourencao et al., 2002).

In UV-B+ treatment, UV-B radiation was for 4 h per day, using 8 units of fluorescent lamps (UVB EL-313, Q-Lab, USA), wrapped with a 0.1 mm thick film of cellulose acetate (Crystal) to filter the spectrum of UV-C. The lamps were positioned approximately 40 cm above the top of the plant canopy. The film of cellulose acetate was changed every five days to prevent the passage of radiation during the experiment. For UV-B-treatment, the plots were covered with clear polyester film (DuPont) of 0.152 mm, which absorbs the UV-B radiation. The radiance of each treatment was measured by a spectrometer (USB2000+RAD, OceanOptics, USA) in order to prove the efficacy of the employed treatments. Throughout the growth of the soybean, the height of the lamp and the filter were adjusted to approximately 40 cm above the canopy, according to the growth of the plant.

During the experiment, the UV-B radiation was monitored two or three times per week using the spectrometer. The spectrometer was placed at approximately at the same height as the canopy, solar

solar UV readings were integrated over six hours, producing a quantitative output in $kJ.m^{-2}$. This unit was calibrated using the equation proposed by Quaite et al. (1992). The average from the measurements throughout the experiment was calculated and the amount of solar UV-B radiation received by plants was 6.82 $kJ.m^{-2}$ per day.

The plots with UV-B+ were also monitored and the readings were integrated over 4 h per day and the average for the whole experiment was 11.28 $kJ.m^{-2}$ per day. Therefore, UV-B+ treatment received (in average) 4.46 $kJ.m^{-2}$ per day above the solar UV-B radiation. The plots with UV-B- treatment were also measured and showed no UV-B incidence (0 $kJ.m^{-2}$).

Plant sampling

The first sampling was performed 20 days after sowing (stage of development - V3), followed by two other samplings, one after fifty days (stage of development - V6) and the last one after ninety days (stage of development - R1). Samples consisted of five individual leaves taken from each replicate (fifteen leaves per treatment). The leaves of the same size were randomly chosen from the top of the plant and in the center of the structure. Each leaf was placed in a sterile plastic bag and transported to the laboratory for immediate processing.

Isolation of epiphytic bacteria

For the isolation of epiphytic bacteria, 10 leaf discs (1.76 cm^2 each) were placed in 50 ml of PBS buffer. The flasks were subjected to ultrasound (25 kHz) for 30 s and then agitated (100 rpm) at 25°C for 2 h. Serial dilutions of cell suspensions were performed and were plated in culture medium containing Tryptone Soya Broth-Agar (TSBA). The plates were incubated at 25°C for 10 days. The count of colony forming unit (CFU) per square centimeter of plant tissue fresh weight ($CFU.cm^{-2}.fw^{-1}$) was performed for the estimation of bacterial abundance. Bacterial colonies were purified and kept at -80°C (Araújo et al., 2002).

Resistance of bacterial isolates from soybean phyllosphere to UV-B

The resistance of individual bacterial isolates to UV-B (peak at 310 nm) was assessed by determining the minimum inhibitory dose (MID) needed to inhibit cell growth on TSBA medium as compared to cells grown in non-irradiated plates and also for inhibition of *Escherichia coli* cells, sensitive to UV radiation (Kuhlman et al., 2005).

Cell densities were adjusted to an optical density of 0.1 (550 nm) which corresponded to a population of about 10^8 $CFU.mL^{-1}$. The cells were serial diluted in NaCl (0.85%) until the concentration of 10^4 cells was reached and 25 µL were plated onto TSBA (10%).

Lamps (UVB EL-313, Q-Lab, USA) were covered with a 0.1 mm thick film of cellulose acetate (Crystal) in order to filter a portion of the spectrum of UVC (280-290 nm). Irradiance was measured with a spectrometer. Under the conditions used in this study, the weighted irradiance value was approximately 0.52 $J.m^{-2}.s^{-1}$.

The lamp was turned on fifteen minutes before the use to allow radiation stabilization. Bacterial cultures grown on TSBA (10%) were exposed for 0, 30, 60, 90, 120 and 150 min. Then, they were incubated for 72 h at 25°C in the dark to minimize photoreactivation, and CFU was determined.

DNA extraction from phyllosphere samples

The bacterial wash solution was centrifuged at 3,000 x*g* for 10 min and the cells re-suspended in 500 µL volumes of TE (10mM Tris-HCl, pH 8.0). The cells were added to 0.2 g of beads and shaken in a homogenizer (Mine-Beadbeater TM, Biospec Products) for 30 s at 350 rpm. Subsequently, extraction was performed according to Araújo et al. (2002).

Effects of UV-B radiation on unculturable bacteria from phyllosphere by denaturing gradient gel electrophoresis (DGGE) analysis

First, a specific PCR reaction was performed containing 1 µL of DNA in thirty-five cycles of amplification with primers selective for 16S rRNA gene of Bacteria, primers R1378 (5´-CGGTGTGTACAAGGCCCGGGAACG-3´) and 27F (5'-AGAGTTTGATC(A/C)TGGCTCAG-3) (Heuer et al., 1997) and Class β-Proteobacteria, primers 1492R (5′-TACGG(C/T)TACCTTGTTACGACTT-3) and F948β (5'-CGCACAAGCGGTGGATGA-3') (Gomes et al., 2001). β-Proteobacteria was chosen because there are some evidence that members of this class are representative in plant surfaces (Andrews and Harris, 2000) and *Pseudomonas* spp. because they show tolerance to UV radiation (Sundin et al., 1996; Kim and Sundin, 2000), probably due to a set of *uvr* genes that could enhance resistance to ultraviolet radiation (Molina et al., 2011). The selective primers for *Pseudomonas* spp. were used as described by Garbeva et al. (2004).

The amplification product of each specific group reaction was used in a second PCR amplification with primers for DGGE. These reactions were performed in a volume of 50 µL containing approximately 20 ng of template DNA and 400 nM of each universal primer U968-GC and R1378 using thirty-five amplification cycles with annealing temperature of 55°C. The PCR products were evaluated by electrophoresis on agarose gel (1% w/v) in 1X Tris-acetate-EDTA (TAE buffer), stained with ethidium bromide (1 $mg.L^{-1}$) and viewed under ultraviolet light. The DGGE was performed according to Heuer et al. (1997).

Statistical analysis

The DGGE profiles were analyzed and compared using the software BioNumerics version 6.01 (Applied Maths, Belgium), according to Andreote et al. (2009).

Multivariate analysis was held in the software Canoco (Canoco 4.5, Biometry, Wageningen, The Netherlands). The bands were considered as species and their relative intensities considered as frequency of occurrence. The environmental variables used in this analysis were the plant genotype (two cultivars), the stage of plant development (three samplings) and the UV radiation (3 dosages).

RESULTS AND DISCUSSION

The knowledge on the structure and composition of species that make up bacterial communities associated with plants is fundamental to understanding how biological processes can be influenced by environmental factors. The interaction between plants and bacteria can occur in several ways, leading to disease or benefits in plant development (Ballare et al., 2011).

Epiphytic culturable bacteria in soybean leaves

The count of the culturable density of epiphytic bacterial

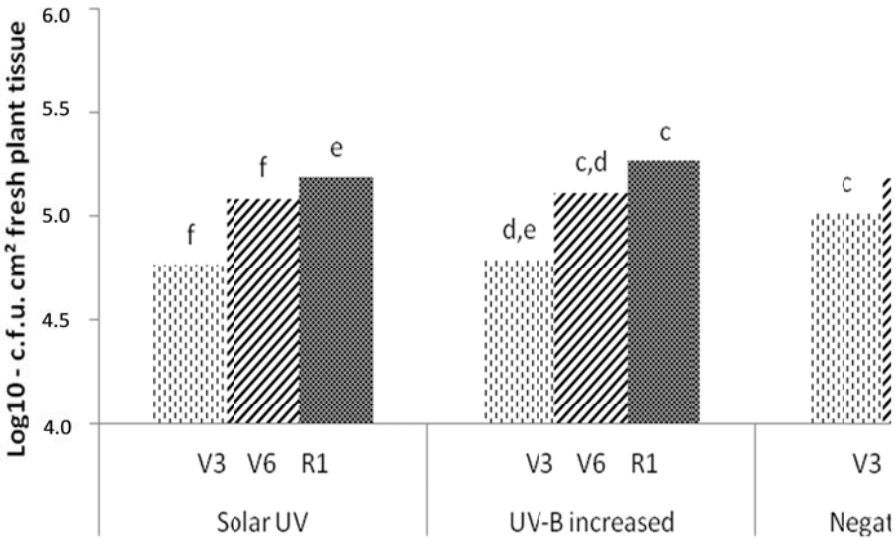

Figure 2. Total population of epiphytic bacterial community of soybean during stage of plant development: V3, V6 and R1, under solar UV radiation, UV-B increased and negative control (UV-B-). Means with the same letter are not significantly different by Tukey test (P< 0.01).

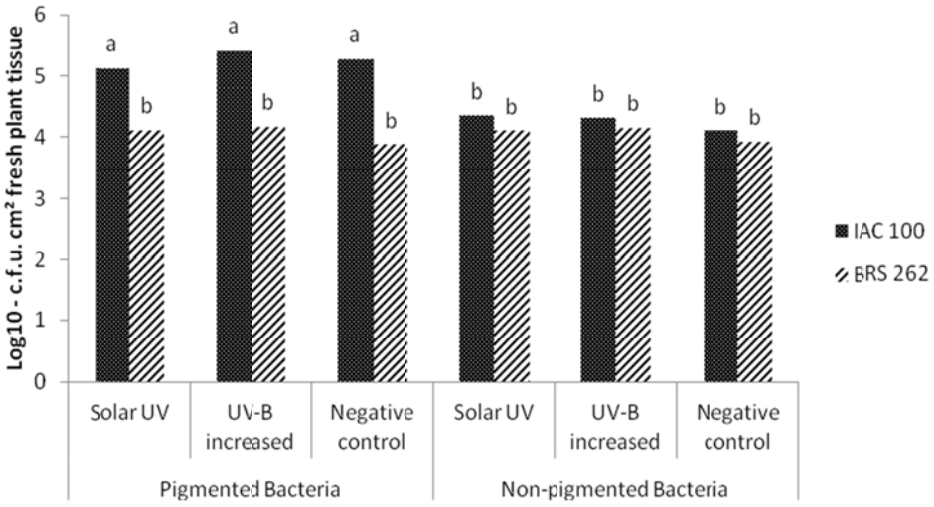

Figure 3. Total population density of epiphytic bacterial community pigmented and non-pigmented from IAC 100 and BRS 262 soybean, under solar UV radiation, UV-B increased and negative control. Means with the same letter are not significantly different by Tukey test (P< 0.01).

community from both cultivars, ranged from 10^3 to 10^4 CFU.cm^{-2}. The epiphytic bacterial density was significantly different for the three treatments and developmental stages, where host R1 stage (flowering) showed a higher density of epiphytic bacterial populations in both cultivars, when compared with the other two phases (V3 and V6) (Figure 2).

According to Figure 2, a statistically ($p < 0.01$) higher number of pigmented colonies in variety IAC 100 when compared to variety BRS 262 was observed. This difference was not observed for non-pigmented colonies. An additional observation was made for pigmented bacteria. The number of colored colonies showed differences according to cultivars, with greater values for IAC 100, whereas there was no difference for BRS 262 (Figure 3).

UV-B+ and solar UV-B had no significant influence on the epiphytic bacterial population density. However, the developmental stages of the host had an influence, suggesting that the physiological stage of the host is

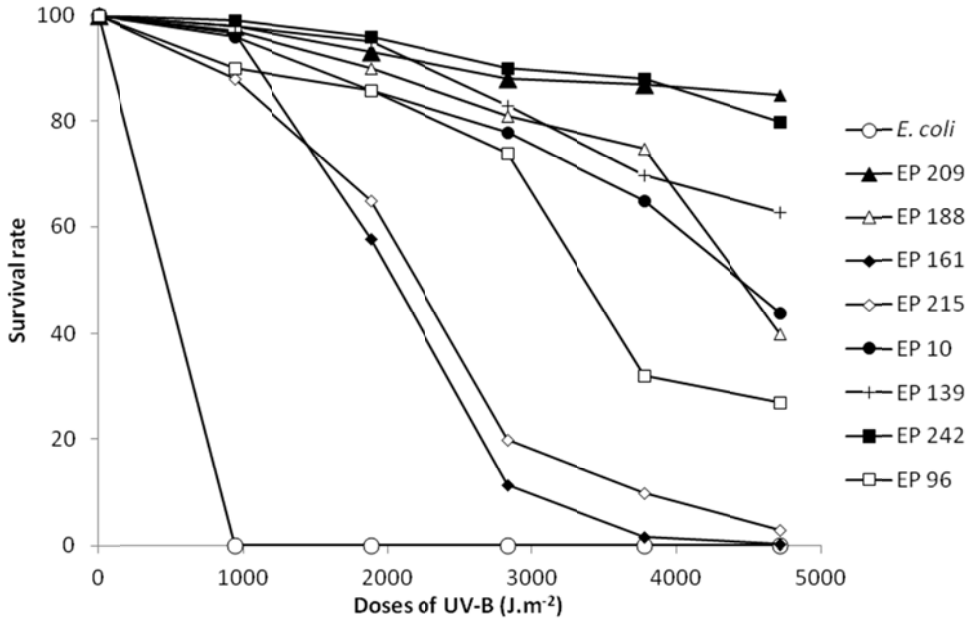

Figure 4. Survival of non-pigmented EP 209 (x), EP 188 (△), EP 161 (□), EP 215 (◇) and pigmented strains EP 10 (●), EP 139 (○), EP 242 (▲), EP 96 (■) isolated from soybean phyllosphere and *E. coli* (◆) as negative control after irradiation with UV-B. Each point represents the mean of triplicates.

connected with the capacity for colonization and establishment of bacterial groups (Kuklinsky-Sobral et al., 2004).

On the other hand, UV-B- affected the population size of culturable bacteria on both cultivars. Cell counts showed that numbers of bacterial isolates were higher than those previously reported (Jacobs and Sundin, 2001), suggesting that the protection promoted by the plastic filters may have affected the canopy microclimate, as compared to the treatment without the filter. Also, the plant cover by the plastic film can act as a barrier for possible leaf colonizers, like those bacteria transported by insects, water and wind.

Kuklinsky-Sobral et al. (2004) observed that the density of bacteria in soybean leaves is influenced by factors such as plant genotype, seasonal variations, cultural methods, developmental stage and plant tissue. Physiological stages under different environmental conditions have significantly affected bacterial population density.

The high incidence of UV radiation is one of the most prominent features of the leaf surface environment in which, presumably, the epiphytic organisms had to adapt. Currently, the vast majority of epiphytic bacteria are believed to be pigmented, which possibly provides protection against radiation (Lindow and Brandl, 2003), corroborating the data obtained in this work, where a large part of epiphytic bacteria isolated from soybeans produced pigments (52.61%) with little differentiation among the studied cultivars.

UV-B resistance of bacteria from soybean phyllosphere

Eight bacterial isolates, four pigmented and four non-pigmented, randomly chosen, were exposed to UV-B, at a wavelength of 310 nm for 150 min (4,720 $J.m^{-2}$). All bacterial isolates exhibited significant resistance to UV-B (Figure 4), when compared with *E. coli* that was readily killed within the first 30 min of exposure (940 $J.m^{-2}$).

An orange-pigmented isolate (EP 242), a pink-pigmented (EP 139) and a non-pigmented isolate (EP 209) exhibited high resistance to the exposure. One of the mechanisms by which bacteria resist to UV-B radiation is conferred by the ability of effective repair of DNA damage. As it would be expected, bacteria from the phyllosphere exposed to UV-B for long periods, showed a high degree of UV resistance. As shown by these data, the pigments produced by these bacteria probably contribute to their UV resistance as well as other mechanisms, such as exopolysaccharides production and repair of DNA damage, that should be further investigated.

The epiphytic isolates tested for UV-B resistance that displayed radiation protection mechanisms, such as pigment, appeared to be more tolerant to high levels of radiation. These results suggest that the pigment production might be an important adaptive mechanism in the phyllosphere. These data are in accordance with that of Sundin and Jacobs (1999), where a greater percentage of pink or orange isolates tolerant to UV-B in the

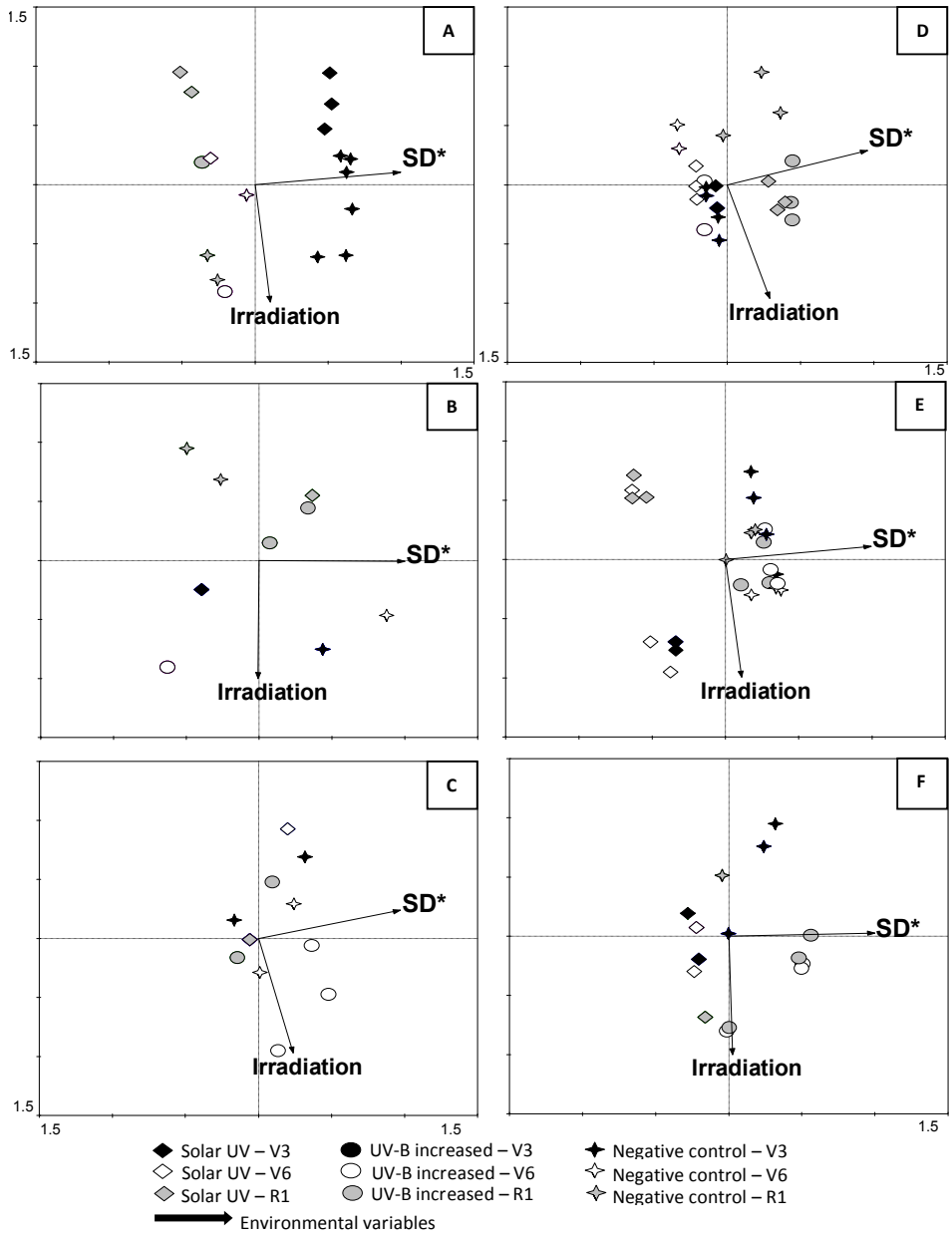

Figure 5. Redundancy analysis (RDA) between the profiles of DGGE bands, samples obtained from the leaves of soybean cultivar IAC 100 (A, B and C) and BRS 262 (D, E and F), with universal primers for Bacteria (A and D); β-proteobacteria-specific (B and E) and for the genus *Pseudomonas* (C and F). The environmental factor marked with * is significant for determining the composition of bacterial communities ($p < 0,05$) according to the permutation test of Monte Carlo. SD - Stage of development.

phyllosphere of peanut leaf was observed.

DGGE analysis of bacterial community epiphytic soybean

Redundancy analysis (RDA) showed a significant correlation between species distribution (bands in DGGE profiles) and environmental factors (Figure 5). The main factor influencing the epiphytic bacterial community composition of soybeans was the developmental stage. The lambda-1 values led to the separation of samples on the first axis according to their stage of development, and in the second axis according to UV-B treatments. However, significant values of Monte Carlo permutation test were obtained only for the effect of plant stages of

Table 1. Variance explained by each of the environmental variables (solar UV-B, increased UV-B and negative control) and stage of plant development, in phyllosphere bacterial communities (Bacteria, β-Proteobacteria and Pseudomonas) from IAC 100 and BRS 262 soybean. Values were obtained by RDA correlating DGGE patterns with environmental variables on the basis of Monte Carlo permutation test.

Environmental variable	IAC 100		BRS 262		Group
	Lambda-1	*p* value	Lambda-1	*p* value	
Stage of development	0.26	0.002	0.22	0.002	Bacteria
	0.29	0.002	0.17	0.002	β-Proteobacteria
	0.12	0.008	0.2	0.004	*Pseudomonas*
Irradiation	0.05	0.142	0.06	0.062	Bacteria
	0.05	0.102	0.04	0.368	β-Proteobacteria
	0.02	0.688	0.04	0.312	*Pseudomonas*

development ($p < 0.05$) (Table 1).

In general, there was a significant difference in epiphytic bacterial community of soybean cultivars according to the phenological stage of the plant as compared to the increase of irradiation received. This becomes apparent when we look at the group of Bacteria (Figure 4A and D), unlike the group of β-Proteobacteria and *Pseudomonas*, which has, a unique feature in the composition of its community, having no interaction with the environmental factors studied, that is, irradiation nor stage of development.

Also, it is possible to observe that samples obtained for Bacteria (Figure 5A and D) correlated with the stage of development V3 for IAC 10 and R1 for BRS 262. In contrast, communities of β-Proteobacteria and *Pseudomonas* showed a more disperse grouping and were not significantly influenced by the studied environmental factor (stage of development and irradiation).

It was possible to observe that stage of development R1 (flowering) in group Bacteria was more clustered than the remaining samples in both analyzed genotypes, confirming the data from bacterial isolation, in which there was a greater wealth of physiological bacterial groups at this stage of the host. This may suggest that during this phase, bacterial populations are well established and under favorable conditions, enabling the microbial population to grow faster (Kuklinsky-Sobral et al., 2004; Beattie and Lindow, 2009).

In general, the differences observed on epiphytic bacterial community of soybean due to the phenological stage were more pronounced as compared to the effect of received radiation. This occurs when we look at β-Proteobacteria and Bacteria, however, *Pseudomonas* showed no significant correlation to any of the evaluated parameters. The genus *Pseudomonas* is widely studied in association with plants and it has been described as highly competitive in natural environments and known to produce antibiotics (De Souza and Raaijmakers, 2003), siderophores (Zawadzka et al., 2006; Bakker et al., 2007) and others compounds, which may explain this feature of high competitiveness and ability to colonize niches present in the plant.

Several studies have shown that different cultivars of the same plant species have different microbial populations in the phyllosphere, for example, between cultivars of tomato and pepper (Correa et al., 2007; Rasche et al., 2006) and among varieties of potato (Sessitsch et al., 2002). Nevertheless, microbial communities selected for different genotypes may show similar responses to environmental variables (Whipps et al., 2008).

Although the plant genotype appears to be an important factor in determining the structure of microbial communities of the phyllosphere, the control mechanisms of these interactions need to be elucidated. Different from what has been observed in this study, other reports indicate that the tolerance to UV radiation is probably important in the selection for survival and growth in the phyllosphere using culture independent techniques (Kadivar and Stapleton, 2003; Stapleton and Simmons, 2006).

Despite the progress made in elucidating the structure and distribution of microbial communities in the phyllosphere, little is known about the functions of its community to adapt to different genotypes of plants. In this study, cultivation-independent analysis showed that the difference in the structure of bacterial communities from phyllosphere of soybean, especially for Bacteria and β-Proteobacteria, is related to the stage of plant growth and does not show a significant influence of different field treatments (solar UV-B, UV-B+ and negative control).

In general, the exploitation of the microbial diversity associated with the phyllosphere and their functional roles in the ecosystem are essential not only to the understanding of the ecology of microorganisms, but also for the comprehension of climate changes of global importance. This is due to the several environmental changes that are being imposed on terrestrial ecosystems,

including the increase in UV-B radiation. With this, it is possible to understand the response of microbial communities towards this problem (Caldwell et al., 2003). Moreover, the knowledge of such biodiversity may result in new biotechnology products for medical and environmental importance, such as pigments that could be further applied in cosmetic products and sunscreen.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Andreote FD, Azevedo JL, Araujo WL (2009). Assessing the diversity of bacterial communities associated with plants. Braz. J. Microbiol. 40:417-432.

Andrews JH, Harris RF (2000). The ecology and biogeography of microorganisms on plant surfaces. Annu. Ver. Phytopathol. 38:145-80.

Araujo WL, Lima AOS, Azevedo JL, Marcon J, Kuklinsky-Sobral J, Lacava PT (2002). Manual de isolamento de microorganismos endofíticos. ESALQ, Departamento de Genetica, Piracicaba.

Bakker P, Pieters CMJ, Van Loon LC (2007). Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytophathology 97:239-243.

Ballare CL, Caldwell MM, Flint SD, Robinson SA, Bornman JF (2011). Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. Photochem. Photobiol. Sci. 10:226-241.

Beattie GS, Lindow SE (1999). Bacterial colonization of leaves: a spectrum of strategies. Phytopathology 89:353-359.

Caldwell MM, Bornman JF, Ballaré CL, Flint SD, Kulandaivelu G (2003). Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climatic change factors. Photochem. Photobiol. 2:29-38.

Chakraborty S, Tiedemann AV, Teng PS (2000). Climate change: potential impact on plant diseases. Environ. Pollut. 108:317-326.

Correa OS, Romero AM, Montecchia MS, Sorria MA (2007). Tomato genotype and *Azospirillum* inoculation modulate the changes in bacterial communities associated with roots and leaves. J. Appl. Microbiol. 102:781-786.

De Souza JT, Raaijmakers JM (2003). Polymorphisms within the *prnD* and *pltC* genes from pyrrolnitrin and pyoleteorin-producing *Pseudomonas* and *Burkholderia* spp. FEMS Microbiol. Ecol. 43:21-34.

Garbeva P, VanVeen JA, VanElsas JD (2004). Microbial diversity in soil: selection of the microbial populations by plant and soil type and implementations for disease suppressive. Annu. Rev. Phytopathol. 42:243-270.

Gomes NCM, Heuer H, Schönfeld J, Costa R, Mendonça-Hagler L, Smalla K (2001). Bacterial diversity of the rizosphere of maize (Zea mays) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant and Soil, The Hague. 232:167-180.

Heuer M, Krsek P, Baker K, Smalla K, Wellington MHE (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gelelectrophoretic separation in denaturating gradients. Appl. Environ. Microbiol. 63:3233-3241.

Jacobs JL, Sundin GW (2001). Effect of solar UV-B radiation on a phyllosphere bacterial community. Appl. Environ. Microbiol. 67:5488-5496.

Kadivar H, Stapleton AE (2003). Ultraviolet radiation alters maize phyllosphere bacterial diversity. Microb. Ecol. 45:353-361.

Kim JJ, Sundin GW (2000). Regulation of the *rulAB* mutagenic DNA repair operon of *Pseudomonas syringae* by UV-B (290 to 320 nanometers) radiation and analysis of *rulAB*mediated mutability *in vitro* and in plant. J. Bacteriol. 182:6137-6144.

Kuhlman KR, Allenbach LB, Ball CL, Fusco WG, La Duc MT, Kuhlman GM, Anderson RC, Stuecker T, Erickson IK, Benardini J, Crawford RL (2005). Enumeration, isolation, and characterization of ultraviolet (UV-C) resistant bacteria from rock varnish in the Whipple Mountains. California. Icarus 74:585-595.

Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004). Isolations and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ. Microbiol. 6:1244-1251.

Lindow SE, Brandl MT (2003). Microbiology of the phyllosphere. Appl. Environ. Microbiol. 69:1875-1883.

Lourencao AL, Braga NR, Miranda MAC, Valle GE, Pereira JCVNA, Reco PC (2002). Avaliação de danos de percevejos e de desfolhadores em genótipos de soja de ciclos precoce, semiprecoce e médio. Neotrop. Entomol. 31:623-630.

Lucas R, McMichael T, Smith W, Armstrong B (2006). Solar ultraviolet radiation global burden of disease from solar ultraviolet radiation. Environmental Burden of Disease Series 13, Geneva; World Health Organization 250p.

Madronich S, McKenzie RL, Biorn LO, Caldwell MM (1998). Changes in biologically active ultraviolet radiation reaching the Earth's surface. J. Photochem. Photobiol. B Biol. 46:5-19.

McKenzie RL, Aucamp PJ, Bais AF, Bjorn LO, Ilyas M, Madronich S (2011). Ozone depletion and climate change: impacts on UV radiation. Photochem. Photobiol. Sci. 10:182-198.

Molina L, Duque E, Gomez MJ, Krell T, Lacal J, Garcia-Puente A, Garcia V, Matilla MA, Ramos J-L, Segura A (2011). The pGRT1 plasmid of *Pseudomonas putida* DOT-T1E encodes functions relevant for survival under harsh conditions in the environment. Environ. Microbiol. 13:2315-2327.

Paul ND, Rasanayagam S, Moody SA, Hatcher PE, Ayres PG (1997). The role of interactions between trophic vels in determining the effects of UV-B on terrestrial ecosystems. Plant Ecol. 128:296-308.

Quaite FE, Sutherland BM, Sutherland JC (1992). Action spectrum for DNA damage in alfafa lowers predicted impact of ozone depletion. Nature 358:576-578.

Rasche F, Trondl R, Naglreiter C, Reichenauer TG, Sessitsch A (2006). Chilling and cultivar type affect the diversity of bacterial endophytes colonizing sweet pepper (*Capsicum anuum*L.). Can. J. Microbiol. 52:1036-1045.

Sessitsch A, Reiter B, Pfeifer U, Wilhelm E (2002). Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and *Actinomycetes*specific PCR of 16S rRNA genes. FEMS Microb. Ecol. 39:23-32.

Stapleton AE, Simmons SJ (2006). Plant control of phyllosphere diversity: genotype interactions with ultraviolet-B radiation. In: Bailey MJ, Lilley AK, Timms-Wilson PTN, Spencer- Philips PTN (Ed.). Microbial ecology of the aerial plant surface. pp. 223-238.

Sundin GW, Jacobs JL (1999). Ultraviolet radiation (UVR) sensitivity analysis and UVR survival strategies of a bacterial community from the phyllosphere of field-grown peanut (*Arachishypogeae* L.). Microb. Ecol. 38:27-38.

Sundin GW, Kidambi SP, Ullrich M, Bender CL (1996). Resistance to ultraviolet light in *Pseudomonas syringae*: sequence and functional analysis of the plasmid-encoded *rulAB* genes. Gene 177:77-81.

Vorholt JA (2012) Microbial life in the phyllosphere. Nature 10:828-840.

Whipps JM, Hand P, Pink D, Bending GD (2008). Phyllosphere microbiology withspecial reference to diversity and plant genotype. J. Appl. Microbiol. 5:1744-1755.

Wilson M, Hirano SS, Lindow SE (1999). Location and survival of leaf-associated bacteria in relation to pathogenicity and potential for growth within the leaf. Appl. Environ. Microbiol. 65:1435-1443.

Zawadzka AM, Vandecasteele FPJ, Crawford RL, Paszczynski AJ (2006). Identification of siderophores of *Pseudomonas stutzeri*. Can. J. Microbiol. 521:164-1176.