Ciência Rural, Santa Maria, v.53:5, e20210832, 2023 http://doi.org/10.1590/0103-8478cr20210832
ISSNe 1678-4596
CROP PRODUCTION

Pantoea ananatis in *Oryza sativa* in Brazil

Laísa Maindra Lima Horn[1*] Ricardo Trezzi Casa[1] Mayra Juline Gonçalves[1] Flávio Chupel Martins[1] Bruno Tabarelli Scheidt[1] Fernando Sartori Pereira[1] Alba Nise Merícia Rocha Santos[1] Valdemir Rossarola[2] Eduardo Silva Gorayeb[1]

[1]Programa de Pós-graduação em Produção Vegetal (PPGPV), Universidade do Estado de Santa Catarina (UDESC), 88520-000, Lages, SC, Brasil. E-mail: maindrac@gmail.com. *Corresponding author.
[2]Agronomia, Universidade do Estado de Santa Catarina (UDESC), Lages, SC, Brasil.

ABSTRACT: *The state of Santa Catarina is the second-largest producer of rice seeds in Brazil. Research on phytopathogenic bacterias in this crop is scarce and the high frequency of panicle diseases leads to the hypothesis that seeds may be infected by bacteria. This research quantified the incidence of bacteria in the seeds, verified the bacteria viability during the storage period and characterized the associated bacteria. Seeds from the 2018/19 and 2019/20 seasons were analyzed. To check the incidence, the seeds were disinfected, plated on a nutrient agar + fungicide culture medium, and incubated for seven days at 27 °C. To assess viability, every 45 days, three cultivars stored in a processing unit were subjected to the same detection methodology. To characterize, prevalent colonies were isolated on semi-selective culture medium Pantoea genus-specific agar (PGSA), where the ones that showed growth were subjected to deoxyribonucleic acid (DNA) extraction and Polymerase Chain Reaction (PCR), DNA sequencing, and sequence comparison on GenBank. The hypersensitivity reaction (HR) in tobacco was performed using a bacterial suspension of each isolate. All seed samples had an average incidence of 83%. During storage, the seeds maintained stable bacterial viability, with an average incidence of 95% at the beginning of storage and 99% at the end of it. All isolates that grew in PGSA culture medium were identified by molecular characterization with 100% identity with two specimens of Pantoea ananatis and one of them induced RH in tobacco.*
Key words: *Oryza sativa, seed health, phytobacteria, identification, Pantoea.*

Pantoea ananatis em *Oryza sativa* no Brasil

RESUMO: *O estado de Santa Catarina é o segundo maior produtor de sementes de arroz do Brasil. As pesquisas com bactérias fitopatogênicas nesta cultura são escassas e a alta frequência de doenças da panícula leva à hipótese de que sementes podem estar infectadas por bactérias. O objetivo desta pesquisa foi quantificar a incidência de bactérias nas sementes, verificar a viabilidade das bactérias durante o período de armazenamento e caracterizar as bactérias associadas. Foram analisadas sementes das safras 2018/19 e 2019/20. Para verificar a incidência, as sementes foram desinfestadas, plaqueadas em meio de cultura ágar nutriente + fungicida e incubadas por sete dias a 27 °C. Para avaliar a viabilidade, a cada 45 dias, três cultivares armazenadas em uma unidade de beneficiamento foram submetidas à mesma metodologia de detecção. Para caracterizar, colônias prevalentes foram isoladas em meio de cultura semisseletivo Pantoea genus-specific ágar (PGSA), onde as que apresentaram crescimento foram submetidas à extração do ácido desoxirribonucléico (DNA) e Reação em Cadeia da Polimerase (PCR), sequenciamento do DNA e comparação de sequências no GenBank. A reação de hipersensibilidade (HR) em tabaco foi realizada utilizando uma suspensão bacteriana de cada isolado. Todas as amostras de sementes apresentaram incidência média de 83%. Durante o armazenamento, as sementes mantiveram viabilidade bacteriana estável, com incidência média de 95% no início do armazenamento e 99% ao fim. Todos os isolados que cresceram no meio de cultura PGSA, foram identificados por caracterização molecular com 100% de identidade com dois espécimes de Pantoea ananatis e um deles induziu HR em tabaco.*
Palavras-chave: *Oryza sativa, sanidade de sementes, fitobactérias, identificação, Pantoea.*

INTRODUCTION

Rice (*Oryza sativa* L.) is the second most cultivated cereal in the world, being the basis of the diet of about three billion people, and in Brazil, is a traditional and highly consumed food. The state of Santa Catarina, located in the south of the country, is the second-largest producer of rice in Brazil, with a cultivated area of approximately 149 thousand hectares and a production of 1.2 million tons,

Received 11.24.21 Approved 05.17.22 Returned by the author 07.09.22
CR-2021-0832.R2
Editors: Leandro Souza da Silva Geri Eduardo Meneghello

predominantly under an irrigated cultivation system (CONAB, 2021).

One of the limiting factors of rice productive potential is the occurrence of diseases caused by several phytopathogens. In the southern region of Brazil, we can cite as examples the rice blast (*Pyricularia oryzae* (Cavara), the brown spot (*Bipolaris oryzae* (Breda de Haan) Shoemaker), the narrow brown spot (*Passalora janseana* (Racib.) U. Braun) and the leaf scald (*Microdochium albescens* (Thüm.) Hern.-Restr. & Crous), which affect the productivity and quality of harvested grains. The most common disease that affects spikelets is glume blotch, which can be caused by the association of various bacteria and fungi (SOSBAI, 2018).

Bacterial infections in rice were reported in Colombia (ZAPATA & VÉLEZ, 2011), Japan (MIZOBUCHI et al., 2018), Russia (EGOROVA et al., 2015) and South Korea (KIM et al., 2010).

According to CARRER FILHO et al. (2018), there are no studies focused on monitoring bacteriosis in panicles. Seeds from a US rice collection received at the Active Germplasm Bank (BAG) through an exchange with the Agricultural Research Service / United States Department of Agriculture (ARS / USDA) did not germinate when an attempt to multiply these accessions for storage in the BAG was performed, and, during the rescue of embryos, bacterial contamination was observed, which was later identified as caused by *Pantoea agglomerans* (CARRER FILHO et al., 2018).

The infected seed, in the epidemiological aspect, is certainly one of the main mechanisms of survival and long-distance dissemination of pathogens and a serious problem related to the transport of bacteria through seeds is the difficulty of execution and/or fragility of the methods used in their detection and quantification (STEILMANN et al., 2019).

The state of Santa Catarina, despite being the second largest national producer, does not have studies regarding the importance of seed-associated phytopathogenic bacteria in rice, concerning the detection and viability of the seed during storage, or characterization studies at the genus or species level. Therefore, the present study aimed to: a) quantify the incidence of bacteria in rice seeds produced in the Santa Catarina state; b) determine the viability of bacteria infecting seeds during the off-season storage period; c) characterize the most prevalent bacteria associated with infected seeds.

MATERIALS AND METHODS

The experiments contained in this study were conducted at the Laboratório de Fitopatologia (LF) of the Universidade do Estado de Santa Catarina (UDESC), in Lages municipality, with lowland rice seed lots, corresponding the cultivars SCS121 CL (42 lots), SCS122 Miura (38 lots), SCS116 Satoru (29 lots), Epagri 109 (21 lots), SCSBRS TioTaka (14 lots), SCS124 Sardo (8 lots), Epagri 108 (7 lots), Primoriso CL (6 lots), BRS Catiana (2 lots), BRS Pampeira (2 lots), SCS118 Marques (2 lots) and SCS123 Pérola (2 lots), from certified seed-producing areas in the municipalities of Agronômica, Rio do Oeste, Taió, Pouso Redondo, Ascurra, Ermo, Forquilinha, Garuva, Guaramirim, Jacinto Machado, Joinville, Laurentino, Massaranduba, Mirim Doce, Rio dos Cedros, Timbé do Sul, Turvo and Praia Grande, all of them belonging to the state of Santa Catarina.

The seeds were produced in the 2018/2019 (29 lots) and 2019/2020 (144 lots) seasons and processed by the Cooperativa Cravil, located in Rio do Sul, SC. They did not receive chemical treatment and were stored in 25 kg polypropylene bags stacked on pallets, inside a conventional masonry warehouse with natural air humidity and ventilation, with partial temperature control. Seed collection and sample homogenization were carried out according to the Ministry of Agriculture, Cattle and Supplying rules for seed analysis (BRASIL, 2009) and later they were sent to the LF, where they were submitted to seed pathology analysis with a specific test for bacteria detection.

To evaluate the incidence, 200 seeds of each sample were disinfected with a 1% sodium hypochlorite solution for one minute, washed with sterile water, and distributed on sterile filter paper to remove water excess. Then, with the aid of tweezers, the seeds were plated in 80 mm diameter Petri dishes containing a nutritive agar culture medium (Kasvi®) with fungicide [agar, 15 g/L, meat extract, 1 g/L, peptone, 5 g/L, sodium chloride, 5 g/L, yeast extract, 2 g/L + distilled water, 1000 mL, 0.1 mL/L Iprodione + 2 mL/L Cycloheximide (0.15 g diluted in 20 ml of 70% alcohol)], in a vertical laminar flow chamber. Ten seeds were distributed in each petri dish and incubated in a bacteriological oven for seven days at 27±2 °C and with no lighting. The experimental design was completely randomized. The incidence of infected seeds was performed by identifying bacterial colonies associated with the seeds using a stereoscopic magnifying glass, considering the presence or absence of colonized seeds. Incidence data were analyzed considering the average incidence of infected seeds in all analyzed samples. The average incidence values of the two crops were represented in a bar graph with error bars using Sigmaplot software.

The evaluation of the viability of bacteria in stored seeds was carried out with seed samples that

remained in the masonry warehouse of Cooperativa Cravil during the off-season. The evaluations were conducted between April (end of seed processing) to December (sowing season) of 2020, with an interval of 45 days between seed collections (April 20, June 04, July 19, September 03, October 18, and December 3). Three seed lots of three cultivars were evaluated: SCS122 Miura (lots 126, 128 and 129), SCS116 Satoru (lots 08, 09 2 11) and SCS121 CL (lots 739, 742 and 737). The detection of bacterial colonies in infected seeds followed the same method used before to quantify the incidence. Data were submitted to an analysis of variance and non-linear quadratic regression analysis, using Sigmaplot software and considering a significance of 5%.

The prevalent bacterial colonies were isolated to obtain pure culture and morphologically characterized using a method adapted from COSTA JÚNIOR et al. (2009). The isolates were sub-cultured in a semi-selective culture medium, *Pantoea* genus-specific agar (PGSA) developed by KINI et al. (2019) for the detection of *Pantoea* spp. Subsequently, four isolates (11f, 12f, 12g, 21b) that showed growth in this culture medium were pre-selected for molecular characterization.

The isolates were grown in microtubes containing 1 mL of Luria Bertani (LB) liquid culture medium (tryptone, 10.0 g/L; Yeast extract, 5.0 g/L; sodium chloride, 5.0 g/L; distilled water 1000 mL) at 28 ºC, for 24 h, under constant agitation (110 rpm) for subsequent DNA extraction. Total DNA was extracted using the Wizard® Genomic DNA Purification kit (Promega) following the manufacturer's recommendations for Gram-negative bacteria DNA extraction, a characteristic that was proven in these isolates through the direct Gram test with 3% KOH (POWERS, 1995).

After extraction, PCR was performed using the pair of oligonucleotides C1 (AGTCGTAACAAGGTAAGCCG) and C2 (CTATTTGCCAAGGCATCCACC), which amplify a 300bp fragment of the conserved region 16S–23S rDNA of bacteria. In each 200 µl tube, 2 µl of DNA, 10 µl of 5x Buffer buffer, 1 µl of dNTP (10mM), 1 µl of each primer, and 0.25 µl of Taq DNA Polymerase (Promega), followed by the addition of a sufficient amount of nuclease-free water, for a final volume of 50 µl per sample. The PCR cycle performed consisted of an initial denaturation for 2 min at 95 °C, followed by 30 cycles of 45 s at 95 °C, 1 min at 50 °C, and 2 min at 72 °C, with a final extension step of 10 min at 72 °C (IQBAL et al., 2014). The amplicons obtained were examined by 1% agarose gel electrophoresis. The gel was stained with GelRed, visualized in a transilluminator device with UV light, and photographed. The amplified fragments were sent for sequencing at ACTGene (Alvorada, Rio Grande do Sul) and the sequences obtained were compared with the GenBank database, using the Blastn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The pathogenicity of the selected isolates was confirmed by the hypersensitivity test (HR) in tobacco (PVH2254), using a bacterial suspension of each isolate (108 CFU.mL-1), inoculated by the mesophyll infiltration method with a hypodermic syringe without needle. Three replications and one control (sterilized distilled water) were performed. The positive result was confirmed by the observation of tissue necrosis and desiccation in the infiltrated region after 24 h.

RESULTS AND DISCUSSION

Regarding bacterial analysis in rice seeds, few studies encompassing the incidence to the correct diagnosis have been carried out so far, justifying the difficulty of discussing the data presented in this first report, with a predominance of studies related to fungi.

The average incidence of bacterial colonies, considering all rice cultivars and the two seasons, resulted in values that ranged from a minimum of 51.1% for cv. Primoriso to a maximum of 97.5% for cv. SCS118 Marques (Figure 1). However, these two cultivars were represented by a small number of samples (six and two, respectively). This trial did not aim to perform a comparative analysis between cultivars since there was no availability of information relevant to the environment, cultivation systems, and cultural practices adopted in each seed production field. Even so, this survey found the prevalence of positive samples in two rice cultivars, SCS121 CL (n-42) and SCS122 Miura (n-38), in which average incidences above 80% of bacterial-infected seeds were obtained (Figure 1).

According to SCHEIDT et al. (2020), rice seed production in Santa Catarina focuses on high physiological quality over sanitary quality, highlighting the need for further studies on this topic mainly focusing on bacteriology, to better understand its symptomatology and epidemiology, as well as provide more accurate diagnoses. To develop an effective control, preventing seeds from being a vehicle for pathogens among Brazilian states.

During the off-season period in which the lots remain stored in the cooperative under partially controlled conditions, the colonies remain viable in

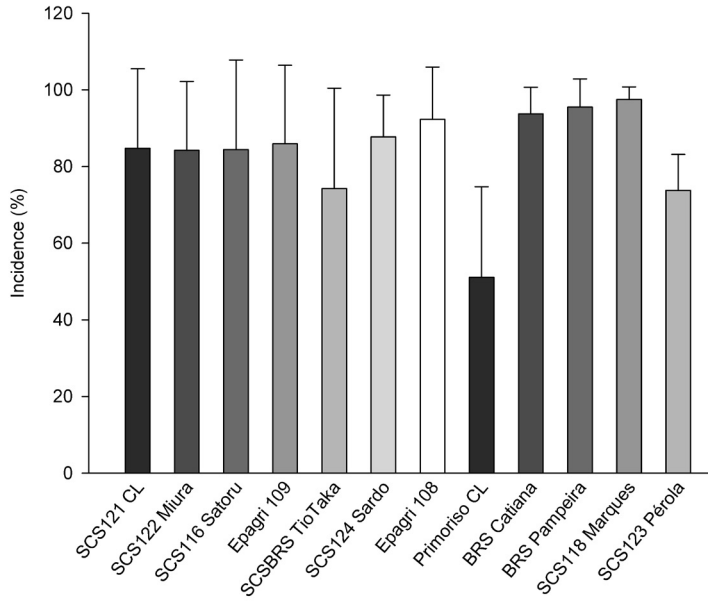

Figure 1 - Average incidence of lowland rice seeds from the state of Santa Catarina infected with bacteria, produced in the 2018/2019 and 2019/2020 seasons.

the seed, confirming that the infected seeds can be a source of primary inoculum and it is possible to transmit the pathogen to the seedling that will produce infected seeds, making pathogen's cycle infinite (Figure 2).

The cv. SCS122 Miura showed an incidence of 95.5% of bacterial colonies in the first evaluation (soon after processing), and at 225 days after processing the incidence was 100%, accounting for one colony per seed, while the cultivar SCS116 Satoru had an initial post-processing incidence of 94.5%, reaching 97.5% at the end of the evaluations, and finally, the cultivar SCS121 CL, which showed an initial incidence of 94% and a final incidence of 99%.

The Brazilian National Company of Supplying (CONAB, 2020), indicated that the agricultural calendar for planting rice in southern Brazil ranges from September to January, and the viability data (Figure 1) obtained from April to December 2020 confirmed that bacterial colonies remain viable in the seed without decreasing throughout this period. This showed that 80% of the crop establishment can be carried out with bacterial-infected seeds.

The prevalent colonies in the analyzed lots were yellow, with circular and elevated shape, and smooth margins, similarly to the results obtained by MONDAL et al. (2011), which reported the occurrence of *P. ananatis* yellow, elevated and with smooth margins colonies, recovered from symptomatic rice leaves in India.

The semi-selective PGSA culture medium, used with the objective of pre-selecting isolates for further molecular characterization, resulted in the growth of four isolates, with highly viscous colonies. As shown by KINI et al. (2019) and AZIZI et al. (2020), *P. ananatis* colonies in PGSA semi-selective medium were also viscous, which can be attributed to the fact that this species has halophilic properties that allow its development in environments with high salt concentration, making this medium a strong candidate for facilitating the diagnosis of *Pantoea* species in laboratory routine, since it uses cheap and readily available reagents, requiring a quick and simple preparation method.

Regarding molecular characterization, fragments of 155 [GenBank accession numbers OM913154 (11f), OM966406 (12f), OM966408 (21b)], and 156 [GenBank accessions OM966407 (12g)] bp were obtained from bidirectional sequencing using the primers C1 and C2, corresponding to the gene that codifies the acetaldehyde-CoA/alcohol dehydrogenase bifunctional protein of the bacteria. Blastn analyses for all isolates resulted in 100% identity with two specimens of *P. ananatis* isolated

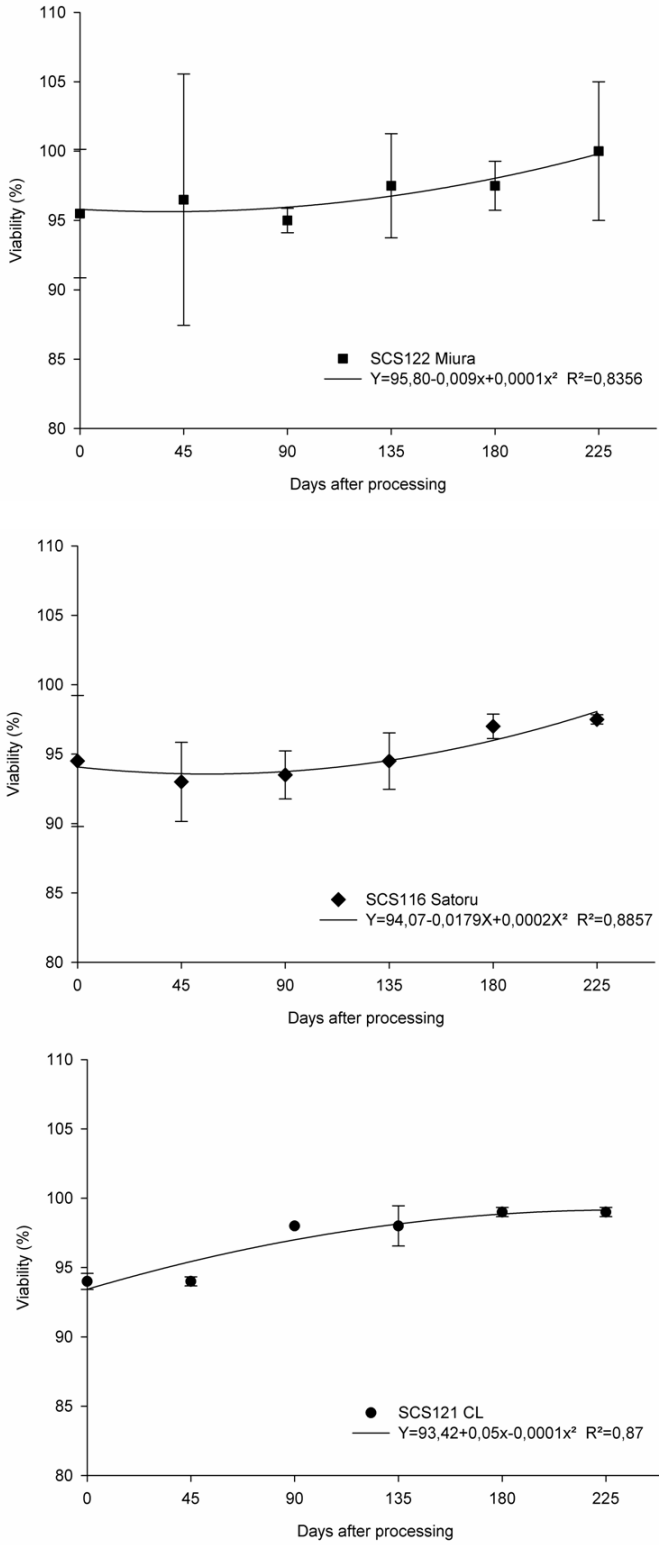

Figure 2 - Viability of bacteria infecting irrigated rice seeds from Santa Catarina produced in the 2019/2020 season, harvested and stored in a Seed Processing Unit during the off-season.

from air samples in Singapore (GenBank accession CP028033) and rice in China (GenBank accessions CP081342). In addition, identities of 97.4% to 98.06% were observed in samples from China, Japan, Vietnam, the United States, and South Africa.

Pantoea ananatis was also isolated from rice plants that showed necrotic spots and discoloration on stems and glumes in South Korea between 2009 and 2010 (CHOI et al., 2012).

In Italy, after observing discoloration in panicles, symptomatic plant samples isolated in a semi-selective medium showed yellow colonies resulting in 40 isolates, with 19 of them being identified by the molecular diagnosis as *P. ananatis* (CORTESI et al., 2007).

When submitting yellow colonies obtained from rice plants from West Africa to molecular diagnosis, KINI et al. (2017) reported for the first time *P. ananatis* in these countries, and later, in 2018, they found the presence of this pathogen also in Burkina Faso, Mali, Nigeria, Niger, Senegal, and Tanzania.

The pathogen identified in this research was reported in Brazil only in maize (*Zea mays*), sorghum (*Sorghum bicolor*), crabgrass (*Digitaria horizontalis*), and onion (*Allium cepa*) isolated from symptomatic plants (PACCOLA-MEIRELLES et al., 2001; COTA et al., 2010; GONÇALVES et al., 2010; RESENDE et al., 2021).

As mentioned in this study, *Pantoea* species occur in rice cultivation worldwide and are responsible for causing several diseases that affect the plant at different vegetative/reproductive stages, and now the presence of *P. ananatis* was also confirmed in some Brazilian lots of seeds, produced and marketed in the state of Santa Catarina. So this is the first report of this pathogen in Brazil affecting rice. More studies to determine the epidemiology, symptomatology, and control strategies for this disease are necessary, seeking to provide consistent diagnostic and control methods, reducing losses in the field.

Hypersensitive response in tobacco plants was observed only for isolate 12f (OM966406), showing that the response can be variable due to the handling of the isolates in terms of culturing and storage during the period of execution of the experiments, leading to losses in virulence.

Mutations in *hrp* genes from phytopathogenic bacteria have been characterized in *Erwinia amylovora,* a species from the same family as *Pantoea*, and can be related to deficiencies in HR elicitations in host species (ALFANO & COLLMER, 1997).

The technical recommendations for cultivating rice do not mention diseases caused by bacteria and not even resistant cultivars or possible chemical control targeting these pathogens, revealing a large gap in rice production in Brazil. Technical assistants and producers need to be aware of the existence of this problem and seek solutions to prevent losses.

The diagnosis of pathogens in rice has been neglected due to a lack of monitoring and correct identification of pathogens or if the causes of symptoms in plants are abiotic. Tests to verify infections by fungi, bacteria, viruses, and nematodes are extremely important to verify the sanitary quality of the seed and must be recommended to the producer.

CONCLUSION

Lowland rice seeds from the state of Santa Catarina produced in the 2018/2019 and 2019/2020 seasons have an incidence of bacteria, regardless of the cultivar or municipality from which they come and the viability of bacteria in seeds during the off-season storage period remains stable.

The four isolates were characterized as *Pantoea ananatis*, being three isolates from two seed lots of the cultivar SCS122 Miura and one isolate from the cultivar SCS116 Satoru, both produced in Santa Catarina, during the 2019/2020 season.

ACKNOWLEDGEMENTS

This research was financed out with the support of the Fundo de Apoio à Manutenção e ao Desenvolvimento da Educação Superior (FUMDES) and in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Finance code 001."

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. Funding sponsors had no role in the study design, collection, analysis, and data interpretation; during the writing of this manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

LMLH and RTC conceived and planned the experiments. LMLH, MJG, FCM, BTS, FSP, ANMRS and VR did the experiments and laboratory analysis. LMLH and ESG performed experimental statistical analysis. LMLH, RTC, MJG and ESG prepared the manuscript. All authors reviewed critically the manuscript and approved the final version of this manuscript.

REFERENCES

ALFANO, J. R.; COLLMER, A. The type III (Hrp) secretion pathway of plant pathogenic bacteria: trafficking hairpins, Avr

proteins, and death. **Journal of bacteriology**, v.179, n.18, p.5655-5662, 1997. Available from: <https://journals.asm.org/doi/pdf/10.1128/jb.179.18.5655-5662.1997>. Accessed: Mar. 20, 2021. doi: 10.1128/jb.179.18.5655-5662.1997.

AZIZI, M. M. F. et al. The emergence of *Pantoea* species as a future threat to global rice production. **Journal of Plant Protection Research**, p.327-335, 2020. Available from: <https://journals.pan.pl/Content/117055/PDF/R_01_JPPR_60_4_767_Azizi.pdf>. Accessed: May, 10, 2021. doi: 10.24425/jppr.2020.133958.

BRASIL. Ministério da agricultura, Pecuária e abastecimento. Secretaria de defesa agropecuária. **Regras para análise de sementes**. Mapa/ACS, 2009.

CARRER FILHO R. et al. Detection of *Pantoea agglomerans* in germplasm rice accessions (*Oryza sativa*) in Brazil. **Plant Disease**, v.102, n.1, p.237, 2018. Available from: <https://apsjournals.apsnet.org/doi/full/10.1094/PDIS-06-17-0905-PDN>. Accessed: Mar. 10, 2021. doi: 10.1094/PDIS-06-17-0905-PDN.

CHOI O. et al. 2012. First report of sheath rot of rice caused by *Pantoea ananatis* in Korea. **The Plant Pathology Journal**, v.28, n.3, p.331-331, 2012. Available from: <https://www.koreascience.or.kr/article/JAKO201228448584501.page>. Accessed: Jan. 05, 2021. doi: 10.5423/PPJ.DR.08.2011.0150.

CONAB. Companhia Nacional de Abastecimento. **Acompanhamento da safra brasileira de grãos** – v.7 – Brasília. Conab, 2018 Safra 2019/20 – N. 12 – setembro, 2020.

CONAB. Companhia Nacional de Abastecimento. **Acompanhamento da safra brasileira de grãos** – Safra 2020/21 e 2021/22 - 1º levantamento, 2021.

CORTESI, P. et al. Palea browning, a new disease of rice in Italy caused by *Pantoea ananatis*. **Journal of Plant Pathology**. v.89, n.Suppl. 3, 2007. Available from: <https://www.cabdirect.org/cabdirect/FullTextPDF/2008/20083005710.pdf>. Accessed: Jan. 12, 2021.

COSTA JÚNIOR, R. C. et al. Isolamento e caracterização morfológica de bactérias em nódulos de leguminosas forrageiras. **Jornada de ensino, pesquisa e extensão da UFRPE**, v.9, 2009. Available from: <http://www.eventosufrpe.com.br/jepeX2009/cd/resumos/R1291-1.pdf>. Accessed: Mar. 10, 2020.

COTA L. V. et al. First report of pathogenicity of *Pantoea ananatis* in sorghum (*Sorghum bicolor*) in Brazil. **Australasian Plant Disease**, v.5, n.1, p.120-122, 2010. Available from: <https://www.publish.csiro.au/dn/Fulltext/DN10044>. Accessed: Mar. 11, 2021. doi: 10.1071/DN10044.

EGOROVA, M. et al. First report of *Pantoea ananatis* causing grain discolouration and leaf blight of rice in Russia. **New Disease Reports**, v.32, n.21. 2015. Available from: <https://bsppjournals.onlinelibrary.wiley.com/doi/10.5197/j.2044-0588.2015.032.021>. Accessed: Mar. 17, 2021. doi: 10.5197/j.2044-0588.2015.032.021.

GONÇALVES, R. M. et al. Capim-colchão: hospedeiro alternativo de *Pantoea ananatis*, agente causal da mancha branca do milho. XXVIII Congresso Nacional de Milho e Sorgo. **Associação Brasileira de Milho e Sorgo**, 4., 2010. Available from: <https://www.alice.cnptia.embrapa.br/bitstream/doc/865479/1/0143.pdf>. Accessed: May, 20, 2021.

IQBAL M. A. et al. Biochemical and molecular identification of *Xanthomonas translucens* pv. *undulosa* causing bacterial leaf streak of wheat in Punjab, Pakistan. **Archives of Phytopathology and Plant Protection**, v.47, n.4, p.417-424, 2014. Available from: <https://www.tandfonline.com/doi/abs/10.1080/03235408.2013.811030>. Accessed: Mar. 17, 2021. doi: 10.1080/03235408.2013.811030.

KIM, J. W. et al. Occurrence of *Burkholderia glumae* on rice and field crops in Korea. **The Plant Pathology Journal**, v.26, n.3, p.271-272, 2010. Available from: <https://www.koreascience.or.kr/article/JAKO201028552427793.page>. Accessed: Jan. 25, 2021. doi: 10.5423/PPJ.2010.26.3.271.

KINI K. et al. 2017. First report of a new bacterial leaf blight of rice caused by *Pantoea ananatis* and *Pantoea stewartii* in Benin. **Plant Disease**, v.101, n.1, p.242-242, 2017. Available from: <https://apsjournals.apsnet.org/doi/full/10.1094/PDIS-06-16-0940-PDN>. Accessed: Jan. 22, 2021. doi: 10.1094/PDIS-06-16-0940-PDN.

KINI K. et al. 2017. First report of a new bacterial leaf blight of rice caused by *Pantoea ananatis* and *Pantoea stewartii* in Togo. **Plant Disease**, v.101, n.1, p.241, 2017. Available from: <https://apsjournals.apsnet.org/doi/full/10.1094/PDIS-06-16-0939-PDN>. Accessed: Jan. 22, 2021. doi: 10.1094/PDIS-06-16-0939-PDN.

KINI K. et al. 2019. A semi-selective medium to isolate and identify bacteria of the genus *Pantoea*. **Journal of General Plant Pathology**, v.85, n.6, p.424-427, 2019. Available from: <https://link.springer.com/article/10.1007/s10327-019-00862-w>. Accessed: Apr. 19, 2020. doi: 10.1007/s10327-019-00862-w.

MIZOBUCHI, R. et al. Evaluation of major Japanese rice cultivars for resistance to bacterial grain rot caused by *Burkholderia glumae* and identification of standard cultivars for resistance. **Breeding science**, p.18018, 2018. Available from: <https://www.jstage.jst.go.jp/article/jsbbs/advpub/0/advpub_18018/_article/-char/ja/>. Accessed: May, 7, 2021. doi: 10.1270/jsbbs.18018.

MONDAL, K. K. et al. A new leaf blight of rice caused by *Pantoea ananatis* in India. **Plant Disease**, v.95, n.12, p.1582-1582, 2011. Available from: <https://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-06-11-0533>. Accessed: May, 12, 2021. doi: 10.1094/PDIS-06-11-0533.

PACCOLA-MEIRELLES L. D. et al. Detection of a bacterium associated with a leaf spot disease of maize in Brazil. **Journal of Phytopathology**, v.149, n.5, p.275-279, 2001. Available from: <https://www.cabdirect.org/cabdirect/abstract/20013081629>. Accessed: May, 13, 2021. doi: 10.1046/j.1439-0434.2001.00614.x.

POWERS, E. M. Efficacy of the Ryu nonstaining KOH technique for rapidly determining gram reactions of food-borne and waterborne bacteria and yeasts. **Applied and environmental microbiology**, v.61, n.10, p.3756-3758, 1995. Available from: <https://journals.asm.org/doi/abs/10.1128/aem.61.10.3756-3758.1995>. Accessed: Apr. 28, 2021. doi: 10.1128/aem.61.10.3756-3758.1995.

RESENDE, R. S. et al. First report of *Pantoea ananatis* causing a foliar and bulb disease on onion in Brazil. **Journal of Plant Pathology**, p.1-2, 2021. Available from: <https://link.springer.com/article/10.1007/s42161-021-01004-y>. Accessed: Mar. 3, 2022.

SCHEIDT, B. T. et al. Viability and location of *Microdochium albescens* in irrigated rice seeds produced in pre-germinated cropping system. **Summa Phytopathologica**, v.47, p.57-59, 2020. Available from: <https://www.scielo.br/j/sp/a/VzFdwxx6mBN9Sv

vDh4pcKLK/?lang=en&format=html>. Accessed: Apr. 28, 2021. doi: 10.1590/0100-5405/237223.

SOSBAI. **Reunião técnica da cultura do arroz irrigado**, Arroz irrigado: recomendações técnicas da pesquisa para o Sul do Brasil. Farroupilha - RS, 32, p.205, 2018.

STEILMANN, P. et al. Detecção, quantificação e identificação de fitobactérias em sementes de trigo. **Nativa**, v.7, n.4, p.349-355, 2019. Available from: <https://periodicoscientificos.ufmt.br/ojs/index.php/nativa/article/view/6710>. Accessed: Jun. 12, 2021. doi: 10.31413/nativa.v7i4.6710.

ZAPATA, N. M. V. F.; VÉLEZ, D. U. Determination of the Infeccion of Burkholderia glumae in Comercial Colombian Rice Varieties. **Revista Facultad Nacional de Agronomía Medellín**, v.64, n.2, 2011. Available from: <https://revistas.unal.edu.co/index.php/refame/article/view/29370>. Accessed: Feb. 10, 2021.