The onion (Allium cepa) is cultivated on almost all continents (Kunz et al., 2009), and is the third largest value-added vegetable in the world, together with potatoes and tomatoes (El Balla et al., 2013). In Brazil, the onion has high socioeconomic importance, and stands out as the most produced crop within the genus Allium, generating income directly and indirectly (El Balla et al., 2013). According to the latest regional update, in 2017 the Santa Catarina State localized in the South region of Brazil was the main onion producer with 431,759 tons. In turn, Northeastern region of Brazil was responsible for the production of 296,455 tons, and the states of Bahia and Pernambuco stood out with a production of 265,465 tons and 28,152 tons, respectively, which accounted for 99.04% of the regional production (IBGE, 2019).

Several diseases may occur in onion bulbs, standing out slippery skin, which can affect onions from cultivation to the commercialization stage of the bulbs (Romeiro, 2000; Wordell Filho et al., 2006). Slippery skin is caused by the bacteria Burkholderia gladioli pv. alliicola (Burkholder, 1942),
which is present in the main onion production areas of the semi-arid region of Brazilian Northeast (Oliveira et al., 2019). Additionally, the disease is called slippery skin because it occurs in the inner scales or in the centre of the bulb, which soften and produce a sulphurous odour, and the center of the infected bulb can slip out of the top of the onion bulb when squeezed (Silva et al., 2018).

The use of resistant cultivars is the most efficient, economical, and environmentally safe disease control method because it reduces the use of agrochemicals and is compatible with other plant diseases management practices (Camargo, 2011). As such, genetic improvement of the onion has been carried out until the present day, in both red and yellow onions, with the aim of developing cultivars containing high productive potential and resistance to the phytosanitary problems that occur in the region of the São Francisco Valley. Methods have also been developed to improve postharvest conservation, and to produce onions with moderate pungency and good adaptation to the environmental conditions of this region (Souza et al., 2008). However, there are still no onion cultivars that have been proven to be resistant to slippery skin, which could considerably reduce losses caused by this disease (Gava & Tavares, 2016).

Considering the potential threat of this disease, research aimed at selection of sources of tolerance to slippery skin which will greatly contribute to increase productivity of the onion crop in the Northeastern semi-arid region of Brazil. Therefore, this study aimed to evaluate the reaction of onion genotypes to slippery skin caused by B. gladioli pv. alliicola, and to analyze the tolerance stability of the most promising genotypes to different strains of the bacteria.

MATERIAL AND METHODS

Cultivation of accessions

We evaluated 58 genotypes from the onion germplasm collection maintained at Embrapa Semiárido located in Petrolina-PE, Brazil (Table 1). The genotypes belong to different agronomic types: Baia Periforme, Péra, Crioula and Grano (Santos et al., 2010).

Seedlings of different genotypes were produced in nurseries in the experimental Station of Embrapa Semiárido using styrofoam trays with 200 cells filled with substrate Bioplat and afterwards, manually transplanted to the field when plants reached 20 cm height, with two to three true leaves, around 35 days after sowing. The management practices for bulb production were those recommended for the crop in that region (Souza et al., 2008), and plants were irrigated by drip irrigation, three times per week. Plants were sprayed with fungicides to control mainly Alternaria porri fungus, and with insecticides to control Thrips spp. and Liriomyza spp.

Strains, cultivation conditions, and preparation of bacterial suspensions

Nine strains of B. gladioli pv. alliicola (CCRMBG07, CCRMBG38, CCRMBG39, CCRMBG47, CCRMBG89, CCRMBG165, CCRMBG172, CCRMBG175, and CCRMBG212) obtained from the Rosa Mariano Culture Collection of Phytophacteriology Laboratory (LAFIBAC) of the Federal Rural University of Pernambuco, Brazil, were used in the present study. The bacterial strains were obtained from onion bulbs showing slippery skin symptoms in the region of the São Francisco Valley, and were identified by sequencing and phylogenetic analysis of the 16S rRNA region in a previous study (Oliveira et al., 2019). Although several bacteria may cause rotting in onions scales similarly to B. gladioli pv. alliicola (Silva et al., 2018), we decided to use only this bacterium to evaluate the reaction of onion genotypes because it has been identified as one of the bacterial species that predominate in the region of the São Francisco Valley and has shown high aggressiveness to onion bulbs (Oliveira et al., 2019).

The strains were grown in nutrient yeast dextrose agar (NYDA, 10 g dextrose, 5 g yeast extract, 3 g meat extract, 5 g peptone and 20 g agar, supplemented with up to 1,000 mL distilled water) (Mariano et al., 2016), at 27±2°C for 36-48 h at biochemical oxygen demand (BOD). The bacterial suspensions were prepared in sterile distilled water (SDW) and the concentration was adjusted in a spectrophotometer (Analyzer 500 M, Brazil), for A₅₇₀ = 0.54, which corresponds to 10⁶ colony forming units (CFU) mL⁻¹. All the experiments were performed in duplicate.

Analysis of the aggressiveness of B. gladioli pv. alliicola

Onion bulbs cv. Baia Periforme, characterized as “class 2” (35-50 mm diameter), purchased at the wholesale market [(CEASA Centro Estadual de Abastecimento) State Supply Center] of Pernambuco, were injured at a depth of approximately 2.5 mm with the aid of an entomological pin. Individually, 10 μL of bacterial suspensions of the strains CCRMBG07, CCRMBG38, CCRMBG39, CCRMBG47, CCRMBG89, CCRMBG165, CCRMBG172, CCRMBG175, and CCRMBG212 were deposited in the wounds. The bulbs were then placed on Petri dishes and maintained in plastic trays containing four sheets of absorbent paper soaked in 20 mL SDW. Trays were covered with transparent plastic bags to create a moist chamber environment and incubated at 27±2°C for 48 h in the BOD. Bulbs treated similarly with SDW were used as the negative control. The experimental design was completely randomized, with four replicates per treatment (bacterial strains), and each repetition consisted of one bulb. We choose to optimize the experimental resources and to standardize all the experiments using only one bulb per repetition due to the low availability of onion bulbs of the genotypes used in the experiments. Disease severity was evaluated by measuring the diameter of the lesion in two opposite directions 48 h after inoculation and computing the mean lesion size. Data were analyzed by analysis of variance (ANOVA), after checking that assumptions were met using the Shapiro-Wilk and Levene’s tests using the Software Statistix (v. 9.0, Tallahassee, Florida, USA). The severity of the lesions caused by the strains were compared using the least significant difference (LSD) test at the
were used as the negative control. The experiments were carried out in a completely randomized experimental design in a factorial arrangement, represented by three strains and 16 genotypes, with four replications, each consisting of a bulb containing one inoculation point. We evaluated disease severity measuring the diameter of the lesion in two opposite directions and computing the mean lesion size. The severity induced by the strains was submitted to ANOVA, and means were compared using Scott-Knott test at 5% probability level using Sisvar statistical software v. 5.6 (Ferreira, 2011).

RESULTS AND DISCUSSION

Analysis of the aggressiveness of B. gladioli pv. alliicola

The strains CCRMBG39, CCRMBG212, and CCRMBG172 showed the highest levels of aggressiveness and were not significantly different (p>0.05) from each other (Table 2). However, strain CCRMBG172 also did not differ from strains CCRMBG38, CCRMBG175, CCRMBG89, CCRMBG7, and CCRMBG47, which showed moderate levels of aggressiveness. In contrast, strain CCRMBG165 was found to be less aggressive. The difference in severity between the most aggressive strain (CCRMBG39) and the less aggressive one (CCRMBG165) was 4.5 mm. Therefore, strains CCRMBG39, CCRMBG172, and CCRMBG212 were selected to be used in the following experiments, in the present study. Similarly, Oliveira et al. (2019) also observed the existence of different levels of disease severity caused by different strains of B. gladioli pv. alliicola.

Several bacteria such as some B. cepacia complex (BCC) species, Pseudomonas aeruginosa (Wordell Filho et al., 2006), and Serratia marcescens (Malavolta Júnior et al., 2008) may cause rotting in onions scales similarly to B. gladioli pv. alliicola (Silva et al., 2018; Oliveira et al., 2019). However, according to Oliveira et al. (2019), B. gladioli pv. alliicola seems to predominate in the region of the São Francisco Valley. In addition, based on pathological characterization of strains of these bacteria, the authors found that strains of BCC and B. gladioli pv. alliicola resulted in higher values of severity, disease index, and area under the disease progress curve and had a shorter incubation period than P. aeruginosa strains. Nevertheless, so that tolerant genotypes may be used successfully in breeding programs or for the management of the rotting caused by these bacteria is also essential to evaluate the tolerance of the onion genotypes to species present in the BCC.

In the present study, only the severity of slippery skin (measured as lesion size) was used to evaluate the reaction of the onion genotypes. This variable was selected because of the high aggressiveness and the low incubation period of this pathogen, which varies from 7 to 16 h (Oliveira et al., 2019), which demonstrates that the pathogen colonizes and begins maceration of the bulb tissues very quickly. In addition, Silva (2016) observed positive correlations between severity and area below the disease progression curve, as well as negative correlations between these variables and the incubation period, indicating that any of these variables can be used in the research on slippery skin.

Screening of onion genotypes to B. gladioli pv. alliicola

No significant differences were observed (p>0.05) for the variances of the replicates of the experiments, and thus the data were evaluated as repetitions over time. Significantly different levels of tolerance to slippery skin (p≤0.05) were observed among the 58 genotypes, 48 h after inoculation (Table 1). The genotypes were clustered in three groups of severity, a, b, and c, which were classified in this study as tolerant, moderately tolerant, and susceptible, respectively. Thirty-four genotypes were considered tolerant, with severity ranging from 9.79 to 13.42 mm; 21 genotypes were considered moderately tolerant, with severity ranging from 13.89 to 16.88 mm; and three genotypes were considered susceptible, with severity ranging from 18.39 to 19.86 mm. None of the 58 genotypes tested showed immunity (absence of disease)
to strain CCRMBG39 (Table 1).

Pereira et al. (2016) analyzed the resistance of 64 onion genotypes to purple blotch (*Alternaria porri*), and were able to classify the evaluated genotypes in four distinct groups (resistant, moderately resistant, susceptible, and highly susceptible), with proportions of 16.41%, 47.76%, 26.86%, and 4.47%, respectively. The genotypes Crioula Mercosul, Bola Precoce, Juporanga L2, Juporanga L7, and Roxa do Barreiro were considered resistant to purple blotch by these authors and were also classified as tolerant to strain CCRMBG39 of *B. gladioli pv. alliicola* in the present study.

Souza et al. (2008) evaluated the productive performance of onion genotypes in the Northeastern semi-arid region of Brazil and demonstrated that the ‘Régia’ genotype was one of the most promising in the region of the São Francisco Valley. In addition, when evaluating onion genotypes to purple blotch, Pereira et al. (2016) verified that this genotype showed moderate tolerance to this disease. However, in the present study, the ‘Régia’ genotype was found to be susceptible to *B. gladioli pv. alliicola*.

**Evaluation of tolerance stability of onion genotypes to *B. gladioli pv. alliicola***

Among the 34 genotypes considered to be tolerant in the present study, 15 genotypes that presented the lowest average lesions [Cascuda T7, EHCEB 20142028, Cascuda T5, EHCEB 20111036, EHCEB 20122003, EHCEB 20141038, Juporanga, Cascuda T6, Alfa SF C-XI, F2 (EHCEB 20151030 x EHCEB 20133015), Crioula Mercosul, EHCEB 201124, EHCEB 201423, IPA 12 and IPA 11] and the most susceptible genotype (Optima PF) were studied for tolerance stability.

No significant differences were observed (p>0.05) for the variances of the replicates of the experiments, and thus the data were evaluated as repetitions over time. No significant interactions were observed between genotypes and strains at 5% probability. Therefore, data regarding the genotypes

| Genotype1 | AT2 | Severity3 | Tolerance class4 |
|-----------|-----|-----------|-----------------|
| Alfa SF ‘A’ | Ba  | 14.30 b1 | MT |
| Alfa SF ‘B’ | Ba  | 16.88 b | MT |
| Alfa SF C-XI | Ba  | 11.42 a | T |
| Alvorada | NI  | 15.15 b | MT |
| Bola Precoce | Ba  | 13.10 a | T |
| BRS 367 | NI  | 13.22 a | T |
| Cascuda T5 | NI  | 10.54 a | T |
| Cascuda T6 | NI  | 11.14 a | T |
| Cascuda T7 | NI  | 9.79 a | T |
| Cascuda T8 | NI  | 12.60 a | T |
| Conquista | Ba  | 12.71 a | T |
| Crioula Mercosul | Cr  | 11.51 a | T |
| EHCEB 20101003 | NI  | 15.68 b | MT |
| EHCEB 20101017 | NI  | 12.39 a | T |
| EHCEB 20101019 | NI  | 13.11 a | T |
| EHCEB 20102017 | NI  | 12.64 a | T |
| EHCEB 20102019 | NI  | 14.24 b | MT |
| EHCEB 20111006 | NI  | 16.27 b | MT |
| EHCEB 20111036 | NI  | 10.63 a | T |
| EHCEB 20112006 | NI  | 14.53 b | MT |
| EHCEB 20112036 | NI  | 12.16 a | T |
| EHCEB 201124 | NI  | 11.52 a | T |
| EHCEB 20122003 | NI  | 10.95 a | T |
| EHCEB 20141008 | NI  | 15.95 b | MT |
| EHCEB 20141027 | NI  | 13.00 a | T |
| EHCEB 20141028 | NI  | 13.37 a | T |
| EHCEB 20141038 | NI  | 10.98 a | T |
| EHCEB 20141040 | NI  | 15.49 b | MT |
| EHCEB 201412 | NI  | 12.29 a | T |
| EHCEB 20142008 | NI  | 19.86 c | S |
| EHCEB 20142027 | NI  | 14.96 b | MT |
| EHCEB 20142028 | NI  | 10.08 a | T |
| EHCEB 20142038 | NI  | 12.89 a | T |
| EHCEB 20142040 | NI  | 15.60 b | MT |
| EHCEB 201423 | NI  | 11.69 a | T |
| EHCEB 201426 | NI  | 15.58 b | MT |
| EHCEB 201427 | NI  | 13.19 a | T |
| EHCEB 201446 | NI  | 14.51 b | MT |
| EHCEB 201513 | NI  | 12.88 a | T |
| EHCEB 201515 | NI  | 15.67 b | MT |
The absence of significant interactions between genotypes and strains could be assigned to the uniformity of reaction of the genotypes in relation to the strains of *B. gladioli* pv. *alliicola*. In turn, this must be regarded as a good signal because indicate that genotypes could be equally tolerant to the strains of this bacterium.

Regarding the reaction to the CCRMBG39 strain, all genotypes were found to be tolerant and were significantly different (p≤0.05) from Optima PF, which was susceptible (Table 3). This result was expected and confirmed the reaction of these genotypes to strain CCRMBG39, which was observed in the experiment for screening onion genotypes. According to the reaction of onions to the strain CCRMBG172, the genotypes Cascuda T5, Cascuda T6, Cascuda T7, Crioula Mercosul, EHCEB 20111036, EHCEB 20122003, EHCEB 20141038, EHCEB 20142028, EHCEB 201423, F2 (EHCEB 20151030 x EHCEB 20133015), IPA 11 and Juporanga behaved as tolerant, while the other genotypes were considered as susceptible. In turn, when the strain CCRMBG212 was inoculated, we verified that the genotypes Cascuda T5, Cascuda T6, Crioula Mercosul, EHCEB 20111036, EHCEB 20141038, EHCEB 20142028, EHCEB 201423, F2 (EHCEB 20151030 x EHCEB 20133015), IPA 11 and Juporanga were tolerant, while the other genotypes were susceptible. Unfortunately, there are no studies about the genetic diversity of strains of *B. gladioli* pv. *alliicola* that may help explain the reasons for these variations. However, such variations are likely due to genetic variations between the strains, which may reflect in the different aggressiveness levels observed. The genotype Optima PF showed to be susceptible while the genotypes F2 (EHCEB 20151030 x EHCEB 20133015), Cascuda T5, Crioula Mercosul, Juporanga, EHCEB 20111036, Cascuda T6, and EHCEB 20142028 remained stable about their tolerance to slippery skin considering the three strains of *B. gladioli* pv. *alliicola* tested (CCRMBG39, CCRMBG212, and CCRMBG172). Therefore, we concluded these genotypes were found

### Table 1. Disease severity caused by nine strains of *Burkholderia gladioli* pv. *alliicola* artificially inoculated in onion bulbs, cv. Baia Periforme class 2 (35-50 mm diameter). Recife, UFRPE, 2016.

| Genotype1 | AT2 | Severity3 | Tolerance class4 |
|-----------|-----|-----------|------------------|
| Express   | NI  | 14.33 b   | MT               |
| F2 (EHCEB 20131006 x EHCEB 20133014) | NI | 12.42 a | T |
| F2 (EHCEB 20151030 x EHCEB 20133015) | NI | 11.44 a | T |
| Imperatriz | NI  | 12.71 a   | T                |
| IPA 10    | NI  | 13.89 b   | MT               |
| IPA 11    | Ba  | 11.76 a   | T                |
| IPA 12    | Ba  | 11.74 a   | T                |
| Juporanga | Ba  | 11.00 a   | T                |
| Luminosa do Enza | NI | 12.26 a | T |
| Optima F1 | NI  | 14.79 b   | MT               |
| Optima Pf | Gl  | 18.39 c   | S                |
| Primavera | Ba  | 14.48 b   | MT               |
| Rainha    | NI  | 13.42 a   | T                |
| Regia     | Gl  | 18.91 a   | S                |
| Roxa do Barreiro | NI | 12.37 a | T |
| São Paulo | Gr  | 14.48 b   | MT               |
| Serrana   | Ba  | 16.62 b   | MT               |
| Sirius F1 | NI  | 14.70 b   | MT               |

CV (%) = 26.136

1Genotypes from the onion germplasm bank of Embrapa Semiárido, Petrolina-PE, Brazil. 2AT = agronomic types; NI = no information available; Ba = Baia periforme; Cr = Crioula; Gl = Gladalan; Gr = Grano; 3Disease severity estimated by measuring the diameter of the lesion. 4Tolerance class: T = tolerant, MT = moderately tolerant, and S = susceptible; determined according to severity groups observed in this study. 5Mean of five replicates. Mean scores in the column followed by the same letter do not differ significantly (p>0.05) from each other by Scott-Knott test. 6Variation coefficient.

### Table 2.

| *Burkholderia gladioli* pv. *alliicola* strain |Severity1 |
|---------------------------------------------|----------|
| CCRMBG7                                    | 11.94 bc2|
| CCRMBG38                                   | 13.29 bc |
| CCRMBG39                                   | 16.01 a  |
| CCRMBG47                                   | 11.88 bc |
| CCRMBG89                                   | 12.94 bc |
| CCRMBG165                                  | 11.50 c  |
| CCRMBG172                                  | 13.89 ab |
| CCRMBG175                                  | 12.95 bc |
| CCRMBG212                                  | 15.59 a  |

CV (%) = 20.133

1Severity estimated by measuring the diameter of the lesion. 2Mean of four replicates. Mean values in the column followed by the same letter did not differ significantly (p>0.05) from each other by the LSD test. 3Variation coefficient.
Table 3. Reaction of 16 onion genotypes to slippery skin when artificially inoculated with strains CCRMBG39, CCRMBG172, and CCRMBG212 of Burkholderia gladioli pv. alliicola. Petrolina, Embrapa Semiárido, 2016.

| Onion genotype | Severity1 | CCRMBG39 | CCRMBG172 | CCRMBG212 |
|----------------|-----------|----------|-----------|-----------|
| Alfa SF C-XI   | 12.50 a   | 14.54 b  | 15.10 b   |
| Cascuda T5     | 11.57 a   | 9.94 a   | 11.53 a   |
| Cascuda T6     | 10.39 a   | 10.23 a  | 13.01 a   |
| Cascuda T7     | 9.99 a    | 10.46 a  | 13.86 b   |
| Crioula Mercosul | 10.41 a | 9.13 a   | 12.08 a   |
| EHCEB 20111036 | 14.19 a   | 11.64 a  | 12.95 a   |
| EHCEB 201124   | 12.02 a   | 13.21 b  | 15.99 b   |
| EHCEB 20122003 | 13.34 a   | 11.47 a  | 14.37 b   |
| EHCEB 20141038 | 11.65 a   | 12.91 b  | 12.19 a   |
| EHCEB 20142028 | 10.75 a   | 10.64 a  | 13.14 a   |
| EHCEB 2014243  | 10.30 a   | 12.40 b  | 11.84 a   |
| Optima PF      | 20.30 b   | 21.57 b  | 16.67 b   |
| F2 (EHCEB 20151030 x EHCEB 20133015) | 10.88 a | 10.31 a | 10.40 a |
| IPA 11         | 13.87 a   | 11.20 a  | 15.32 b   |
| IPA 12         | 11.13 a   | 14.23 b  | 12.25 a   |
| Juporanga      | 12.25 a   | 10.05 a  | 12.52 a   |
| CV (%)1        | 17.57     | 16.01    | 18.07     |

1Disease severity estimated by measuring the diameter of the lesion on onion bulbs caused upon inoculation of three strains of Burkholderia gladioli pv. alliicola. 2Mean of four replicates. Mean scores in the column followed by the same letter do not differ significantly (p<0.05) from each other by the Scott-Knott test. 3Variation coefficient.

to be promising sources of tolerance to slippery skin and could be better explored in breeding programs.

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