First signs of late blight resistance in traditional native potatoes of Pasco—Peru, a preliminary assay

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Abstract
Background: The native Andean potatoes, despite their low yield, have a large diversity that is conserved by subsistence farmers in Peru, due to their culinary characteristics and other qualities. However, this diversity is threatened by the impacts of climate change, which would directly affect the food security of these people, and eventually ours. Among its qualities of resistance to pests and diseases, there could be a genetic source of resistance to late blight, one of the most damaging diseases of the potato crop in the world. In this assay, 103 native landraces collected from local farmers in the Pasco region of Peru were subjected to natural infection conditions with Phytophthora infestans to identify potential resistant landraces within them.

Results: The 103 landraces assessed showed a broad variety of responses and were classified as “resistant” (22%), “moderately resistant” (57%), and “susceptible landraces” (21%). A relative effect of the disease in the yield is also shown, which is already low for commercial intentions.

Conclusion: Within this representative sample of the native potato diversity of the Pasco region, at least 23 local varieties grown by subsistence farmers have resistance qualities against eventual late blight disease.

Keywords: Potato landraces, Andean highlands, Subsistence farmers, Climate change, Food security

Introduction
Andean native potato landraces own a high diversity that contributes as an important source of resistance against pests and diseases, and possess valuable qualities such as chipping quality, high solids content, resistance to frost, and medicinal uses [1]. Despite having a very low average yield (7 t/ha) [2], farmers keep them for cultural heritage and food safety, because of their good taste and softness after cooking [3]. Notwithstanding, the difficult and time-consuming labor, the cultivation of native potatoes is a source of pride for older community members in particular [4].

Over the past few decades, this diversity is decreasing due to lack of access to water for irrigation, lack of recognition of indigenous people to own their land, changes in agricultural practices, migrations of inhabitants to urban areas, the impossibility of obtaining clean potato seeds of their local landraces, and the restricted access to markets [1, 3]. In addition, forecasted scenarios state that climate change will increase temperatures in cold highlands that could promote the proliferation of pests and diseases in these areas [5].

One of the most detrimental diseases of the potato crop in the world is late blight (LB), and so is in Peru [2]. The LB disease is caused by the pathogen Phytophthora infestans (Mont.) de Bary, and is sensitive to weather...
conditions, thus a warmer and more humid environment—due to climate change—will be a great challenge for farmers [6]. Studies have found that a 20% increase in LB severity could cause a 1 t/ha reduction in yield [7].

In the Peruvian Andes, subsistence agriculture is rainfed, which forces farmers to plant potatoes during rainy season—the season for major P. infestans attack—[7]. There is the case of the peasant community Challabamba located at 4100 m.a.s.l. (meters above the sea level) in Cusco—Peru, where climate change has caused the loss of 90% of its native potato diversity due to LB [2]. The disease affects farmers in other aspects as well: when the fungicides damage the leaves, the photosynthetic capacity is reduced and consequently, crop yield; it is also risky for their health, and represents an income lost [8]. Traditionally, farmers have used highlands as late blight-free areas, planting more susceptible potato varieties because of low temperatures inhibiting the disease [5]. However, climate change effects could force them to go into more fragile ecosystems, such as Paramo and Puna lands in the Andes, with direct impact on soil organic carbon reservoirs [9].

Over the years, there have been many studies and attempts to combat the disease without complete success. Among the reasons for this, could be the ability of the pathogen to evolve over time, as well as the variable level of resistance of a genotype that depends on the place where it lives, which together would cause the resistance genotype less effective [8]. In fact, P. infestans is known to be a highly variable pathogen which easily adapts to factors restricting its development, such as systemic fungicides [10]. Furthermore, considering the strong influence of climate, the actual resistance of varieties may not be known in all locations [8].

In the Pasco region, farmers produce potato landraces under an indigenous farming system called “Chaqru” in the Quechua language, and like in the Huanacavilca region—center of high intraspecific diversity—they plant a mixture of native bitter and floury cultivars that correspond to various species with different ploidy levels [11]. The most common Solanum species found in the Central Andes are S. stenotomum (2×), S. goniocalyx (2×), S. chaucha (3×) and S. tuberosum subsp. andigenum (4×) that contains the majority of landraces in a mixed potato field [12]. Referring to the farmers culinary preferences, they have three primary “use-categories” in which the mentioned species are located: “boiling potato” (S. stenotomum, S. goniocalyx, S. × chaucha and S. tuberosum subsp. andigena), “soup potato” (S. tuberosum subsp. tuberosum), and “freeze-drying potato” (S. curtilobum and S. × juzepczukii) [13]. Yet, little is known about the incidence and severity of pests and diseases on traditional landraces of the region [14].

To support farmers adapt to climate change impacts is necessary to contribute to a better description and understanding of those impacts on potato crop in its center of origin [5]. According to Byarugaba et al. [15] and Gabriel et al. [16], the most economical and environmentally feasible solution to control LB is to sow a host resistance, which is why many research programs are still looking for genetic resistance in native or wild species for contributing to extreme climatic conditions adaptation [17]. This study was carried out to identify native potato landraces traditionally cultivated in the Pasco region with potential field resistance to Phytophthora infestans.

Materials and methods
Location
An on-farm field trial was carried out at the Yanay peasant community, in Paucartambo, Pasco region, Peru (10° 45′ 56.3″ S 75° 46′ 22.7″ W, 2745 m.a.s.l.) from December 4th, 2020 to February 23rd, 2021 during the rainy season. The site’s weather is characterized by an annual rainfall of 1010.1 mm, and average relative humidity of 60.7 ± 1.4%, in addition to average values of maximum, medium and minimum temperature of 19.0 ± 0.2, 11.8 ± 0.2 and 7.0 ± 2.1 °C, respectively [18]. The place is known for having LB presence on crops (E. Zevallos, personal comment). During the evaluation period (59 days), the accumulated rainfall and average values of relative humidity, maximum and minimum temperature were 228.6 mm, 83.4 ± 0.6%, 16.3 ± 0.2 °C, and 8.7 ± 0.1 °C, respectively (Fig. 1). Due to technical issues, these data were obtained as reference from NASA POWER access data [19].

Plant material and management
The genotypes assessed in this trial are part of a collection of 358 traditional potato landraces from subsistence farmer’s fields in the Pasco region, made by the UNDAC Native Potatoes Project. The collection is maintained at natural highlands conditions (10° 45′ 06.8″ S 75° 47′ 53.4″ W, 3555 m.a.s.l.), where native varieties are normally cultivated by locals, and the same genotypes were grown in a greenhouse (10° 46′ 14.1″ S 75° 48′ 53.4″ W, 2949 m.a.s.l.), since August 20th, 2020. At the greenhouse (12 h photoperiod; 15–20 °C) each individual came from a tuber sprout planted in a pot filled with 6 kg mixture of sphagnum moss, perlite, vermiculite, and arable land that was irrigated at field capacity (750 ml). On December 4th, 2020 (106 days after planting—DAP), a selection of those with better market qualities and available replications was taken to the field at a height where improved varieties are cultivated, for assessing LB resistance under natural infection conditions in the open field, and irrigated by rainfall. This type of study is used to select large populations of potato genotypes [20]. The next day, Greenzit
pH (Neoagrum, Peru), Cyperklin 25 (TQC, Peru), and Antracol (Bayer, Germany) were applied to protect plants until evaluations. The harvest was made on February 23rd, 2021 (187 DAP); where a preliminary fresh tuber yield (FTY) was calculated from the fresh weight of the total number of tubers collected per plant.

Experimental design
In the field, the pots (experimental unit) were distributed in a randomized block design, with three replications (composed by a single pot) per landrace. Based on local knowledge, the accession UNDAC_153 representative of the landrace “Cajacino” was considered as a susceptible control. In addition, four known susceptible varieties were included within each block as dispersal genotypes (“Yungay”, “Canchán”, “Negra andina”, and “Huayro”). The separation between pots was 0.35 m long and 0.9 m wide, simulating plant density used by local farmers. The total area occupied by the experimental units was 102 m².

Evaluation of late blight resistance
A visual inspection was carried out to estimate the percentage of leaf area affected by *P. infestans* every 5 days, starting at 114 DAP. These data were used to calculate the area under the disease progress curve (AUDPC), a single measure that quantifies the amount of disease in an epidemic, widely used in the literature. However, it is only applicable within a single experiment, for which the relative AUDPC (rAUDPC) has been proposed as a standardized and more stable measure, but still with limited use to quantify susceptibility [21]. The scale of susceptibility (SS) applied in this study is an adaptation to the conditions of the tropical highlands agroecosystems made by Yuen and Forbes [21], because the common references come from European cultivars that are not well suited to these conditions.

For the inspection, three composite leaves were selected at each experimental unit (one from the lower, middle and upper third), and a standardized scale was used to determine the percentage of leaves affected by LB, following [22]. The calculus of AUDPC responds to Eq. 1, where “t” is the time of every measurement in DAP, “y” the percentage of foliage affected in each inspection and “n”, the number of inspections [20]:

$$
\text{AUDPC} = \sum_{i=1}^{n-1} \left( \frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)
$$

The rAUDPC is calculated by dividing the AUDPC by the “maximum potential AUDPC”, the value that a genotype would have with 100% of infection at every measurement, and is obtained from multiplying the total number of days between the first ($I_1$) and last ($I_n$) inspection by 100 [20]:
The SS is the value of the standard genotype coefficient (the most susceptible can receive a measure of \( \approx 9 \)) multiplied by the observed rAUDPC of the candidate genotype (\( r_{\text{AUDPC}_c} \)) and divided by the rAUDPC of the standard genotype (\( r_{\text{AUDPC}_s} \)), which is chosen carefully [21]:

\[
SS = 9 \times \frac{r_{\text{AUDPC}_c}}{r_{\text{AUDPC}_s}}
\]

**Statistical analysis**

For determining significant differences between the accessions, it was applied an analysis of variance (ANOVA) and a Tukey test from the “Agricolae” package [23]. In addition, a correlation between the AUDPC values and tuber yield was made for assessing the effects of LB on potato yield. All the analyses were run using R Studio software [24].

**Results**

The first evidence of LB affection on the assessed accessions of Pasco region’s traditional potato landraces was registered in the second evaluation (December 17th, 2020). From this date, the disease incidence spread quickly until the sixth evaluation (January 6th, 2021), considering that the pathogen can complete the cycle from infection to sporulation in 4 days (Fig. 1), considering that the pathogen can complete the cycle from infection to sporulation in 4 days (Fig. 1), considering that the pathogen can complete the cycle from infection to sporulation in 4 days [25]. Nevertheless, the assay showed a broad variability of responses to the disease, which were grouped according to susceptibility scale (SS) ranges and those from Fig. 3A (SS = 6–9) could be considered as “susceptible landraces”, Fig. 3B (SS = 4–5) as “moderately resistant”, and Fig. 3C (SS = 2–3) as “resistant” [20]. The lowest AUDPC values—part of Fig. 3C—were due to a late onset and a slow development of the infection, which is how a resistant cultivar responds [16].

Within the complete list of landraces, there were some accessions with the same folk name. According to
| Code      | Folk name                  | rAUDPC  | SS | Code      | Folk name                  | rAUDPC  | SS |
|-----------|----------------------------|---------|----|-----------|----------------------------|---------|----|
| UNDAC_4   | Coleto                     | 0.29±0.07 | 5.7 | UNDAC_133 | Milagro                    | 0.26±0.04 | 5.1 |
| UNDAC_8   | Amarilla legítima           | 0.24±0.04 | 4.7 | UNDAC_134 | Pillush                    | 0.28±0.04 | 5.6 |
| UNDAC_14  | Azul siku                  | 0.29±0.05 | 5.9 | UNDAC_139 | Yurac maco                 | 0.28±0.07 | 5.6 |
| UNDAC_18  | Queqorani I                | 0.35±0.01 | 7  | UNDAC_141 | Leona                      | 0.23±0.03 | 4.7 |
| UNDAC_23  | Muru huayro                | 0.25±0.04 | 5  | UNDAC_142 | Queqorani II               | 0.19±0.04 | 3.8 |
| UNDAC_24  | Baraza                     | 0.28±0.03 | 5.6 | UNDAC_146 | Sunccu suwacc              | 0.14±0.01 | 2.7 |
| UNDAC_26  | Huayro moro                | 0.27±0.05 | 5.5 | UNDAC_147 | Negra pestaña blanca       | 0.22±0.04 | 4.3 |
| UNDAC_29  | Chaulina                   | 0.17±0.02 | 3.4 | UNDAC_151 | Vitelete                   | 0.25±0.03 | 5   |
| UNDAC_32  | Pico de oro                | 0.28±0.06 | 5.6 | UNDAC_153 | Cajacino                   | 0.36±0.05 | 7.3 |
| UNDAC_33  | Muru allupu nuy            | 0.21±0.05 | 4.2 | UNDAC_157 | Chaucha curvians           | 0.23±0.03 | 4.7 |
| UNDAC_42  | Ishco puro arnillero       | 0.15±0.04 | 3  | UNDAC_160 | Huanuqueñeto               | 0.24±0.04 | 5.7 |
| UNDAC_43  | Ichis puro                 | 0.11±0.02 | 2.3 | UNDAC_163 | Jara callu                 | 0.31±0.02 | 6.1 |
| UNDAC_48  | Yurac mata                 | 0.16±0.01 | 3.3 | UNDAC_164 | Huanuco suytu              | 0.27±0.08 | 5.3 |
| UNDAC_49  | Comun ojo negro            | 0.33±0.04 | 6.6 | UNDAC_168 | Higo suytu                 | 0.2±0.03  | 4   |
| UNDAC_51  | Escuela nueva              | 0.13±0.03 | 2.6 | UNDAC_169 | Callhuain                  | 0.29±0.01 | 5.7 |
| UNDAC_52  | Queso suytu               | 0.11±0.01 | 2.3 | UNDAC_170 | Pikura                     | 0.27±0.03 | 5.4 |
| UNDAC_53  | Coletu suytu              | 0.23±0.03 | 4.6 | UNDAC_171 | Negro callash              | 0.09±0.01 | 1.8 |
| UNDAC_55  | Arnachi                   | 0.22±0.01 | 4.4 | UNDAC_172 | Cocho negro                | 0.25±0.05 | 5   |
| UNDAC_57  | Sonia canteña              | 0.21±0.03 | 4.2 | UNDAC_178 | Valicha                    | 0.16±0.03 | 3.3 |
| UNDAC_58  | Seda galeta               | 0.2±0.03  | 3.9 | UNDAC_182 | Yurac allua                | 0.17±0.01 | 3.3 |
| UNDAC_59  | Tumbay I                  | 0.39±0.04 | 7.8 | UNDAC_184 | Camotillo                  | 0.23±0.06 | 4.6 |
| UNDAC_60  | Pampiña                    | 0.11±0.02 | 2.2 | UNDAC_188 | Garhua suytu               | 0.24±0.05 | 4.8 |
| UNDAC_61  | Shiruco                   | 0.12±0.04 | 2.4 | UNDAC_190 | Puca clavelina             | 0.14±0.02 | 2.9 |
| UNDAC_65  | Capay blanco              | 0.22±0.05 | 4.5 | UNDAC_191 | Huacapa gallum             | 0.24±0.01 | 4.7 |
| UNDAC_72  | Tatash                    | 0.17±0.01 | 3.4 | UNDAC_193 | Yuquis suytu               | 0.14±0.01 | 2.8 |
| UNDAC_74  | Galleta roja              | 0.13±0.01 | 2.5 | UNDAC_195 | Chiaquil rosado            | 0.23±0.04 | 4.5 |
| UNDAC_76  | Puca rosa                 | 0.1±0.01  | 2.1 | UNDAC_196 | Yurac pña                   | 0.26±0.05 | 5.1 |
| UNDAC_77  | Huayro moro rojo          | 0.16±0.01 | 3.1 | UNDAC_198 | Manzana II                 | 0.41±0.01 | 8.1 |
| UNDAC_79  | Rosada                    | 0.3±0.09  | 6  | UNDAC_200 | Shashai warmi I            | 0.28±0.02 | 5.5 |
| UNDAC_84  | Wichic wichic             | 0.17±0.04 | 3.4 | UNDAC_201 | Negra milagro              | 0.28±0.04 | 5.6 |
| UNDAC_85  | Tarmenía                  | 0.25±0.03 | 5  | UNDAC_202 | Rojiza acaciuli pecho      | 0.25±0.02 | 5   |
| UNDAC_92  | Huamali                   | 0.21±0.02 | 4.2 | UNDAC_203 | Yana caurina               | 0.26±0.07 | 5.1 |
| UNDAC_95  | Conchucano                | 0.31±0.02 | 6.2 | UNDAC_206 | Santo domingo              | 0.23±0.06 | 4.7 |
| UNDAC_97  | Clavel suytu              | 0.22±0.02 | 4.4 | UNDAC_208 | Morales rojo               | 0.19±0.05 | 3.8 |
| UNDAC_100 | Yawar suytu               | 0.22±0.07 | 4.3 | UNDAC_212 | Muru caurina               | 0.2±0.05  | 4   |
| UNDAC_101 | Suytu rojo                | 0.27±0.02 | 5.4 | UNDAC_214 | Huayro moro liso           | 0.26±0.05 | 5.2 |
| UNDAC_105 | Tumbay II                 | 0.22±0.03 | 4.3 | UNDAC_215 | Shashai warmi II           | 0.19±0.02 | 3.8 |
| UNDAC_106 | Chiaquil                  | 0.18±0.02 | 3.5 | UNDAC_218 | Gaiwa suytu                | 0.18±0.02 | 3.7 |
| UNDAC_113 | Orgo runtush              | 0.29±0.02 | 5.8 | UNDAC_222 | Ganto suytu                | 0.24±0.04 | 4.8 |
| UNDAC_114 | Huayra chuco              | 0.31±0.03 | 6.2 | UNDAC_224 | Mosqueña                   | 0.09±0.02 | 1.7 |
| UNDAC_117 | Mama lucha                | 0.27±0.05 | 5.4 | UNDAC_226 | Yurac negro                 | 0.2±0.04  | 4   |
| UNDAC_118 | Juyu huayro               | 0.2±0.02  | 4.1 | UNDAC_230 | Paña tahuina               | 0.26±0.07 | 5.2 |
| UNDAC_120 | Corta                     | 0.23±0.01 | 4.7 | UNDAC_232 | Yana tulo                  | 0.19±0.03 | 3.9 |
| UNDAC_121 | Jilguero                  | 0.21±0.03 | 4.3 | UNDAC_239 | Pampa machay               | 0.19±0.02 | 3.8 |
| UNDAC_122 | Huamantanga               | 0.19±0.02 | 3.7 | UNDAC_249 | Jerga suytu                | 0.21±0.06 | 4.2 |
| UNDAC_123 | Pallanchacrina            | 0.21±0.03 | 4.1 | UNDAC_250 | Conchuna                   | 0.19±0.01 | 3.9 |
| UNDAC_125 | Manzana I                 | 0.19±0.02 | 3.8 | UNDAC_251 | Niña papa                  | 0.2±0.03  | 4   |
Table 1 (continued)

| Code       | Folk name             | rAUDPC  | SS  | Code       | Folk name             | rAUDPC  | SS  |
|------------|-----------------------|---------|-----|------------|-----------------------|---------|-----|
| UNDAC_126  | Colorada              | 0.21 ± 0.02 | 4.3 | UNDAC_252  | Higos                 | 0.26 ± 0.04 | 5.1 |
| UNDAC_127  | Huayllino rojo        | 0.26 ± 0.03 | 5.3 | UNDAC_255  | Yana juyto            | 0.28 ± 0.08 | 5.7 |
| UNDAC_129  | Rojo ayacuchana       | 0.25 ± 0.01 | 5   | UNDAC_256  | Chucas                | 0.15 ± 0.01 | 3.1 |
| UNDAC_130  | Alga huayro           | 0.21 ± 0.04 | 4.3 | UNDAC_259  | Muru chingus          | 0.19 ± 0.03 | 3.9 |
| UNDAC_131  | Huancaína             | 0.16 ± 0.05 | 3.3 |            |                       |         |     |

rAUDPC: Relative area under the disease progress curve, SS: susceptibility scale based on Forbes et al. [20].
* Most of folk names are in Quechua language.

Clausen et al. [1], this could be due to coming from a different locality or farmer and sharing morphological similarities, or the result of the exchange of varieties between farmers. In fact, time ago Zimmerer [12] stated that the spatial coalescence of endemic distributions conforms most closely to agricultural areas articulated through networks of seed exchange, so-called cultivar regions. Whereby, two accessions with the same folk name, e.g., “Corta”, “Huamali”, “Leona” and “Pampa machay” that came from different farmers or localities, and had similar AUDPC curve behavior with SS = 5, were assumed to be the same landrace and just one of them was conserved for the analysis. Only those with different AUDPC.

Table 2 Analysis of variance between the assessed accessions

|               | Degree of freedom | Sum square | Mean square | F value | Pr (> F)  |
|---------------|-------------------|------------|-------------|---------|-----------|
| Accessions    | 102               | 24,001,557 | 235,309     | 3.526   | 9.49e−15***|
| Blocks        | 1                 | 4,439,849  | 4,439,849   | 66.532  | 3.41e−14***|
| Residuals     | 205               | 13,680,147 | 66,732      |         |           |

Significance codes: 0 ***

Fig. 2 Disease incidence across the 103 accessions assessed in the evaluation period (106–164 days after planting—DAP)
Fig. 3  AUDPC values (area under the disease progress curve) of the 103 landraces assessed, subdivided according to susceptibility scale ranges: 6–9 (A), 4–5 (B), and 2–3 (C) based on [20]. Different letters mean significant differences according to a Tukey test.
curve behavior were considered different and kept with a Roman numeral added to the name. It is not possible to assume that those with different behaviors were genetically different without molecular analysis. If many accessions were representatives of the same genotype with different behavior against *P. infestans*, it could be an expression of wide resistance related to horizontal resistance [10].

Regarding the crop yield, the influence of LB could not be assured because of the low correlation ($r = -0.4$) between them, but when considering only “resistant landraces” the negative correlation slightly increases ($r = -0.6$). Almost 70% of the landraces had a FTY greater than 110 g/plant, with the maximum values over 300 g/plant. Although it does not exist statistics on the annual production of native potatoes in Peru [26], a recent study with native potatoes from Peru showed FTY values (considering 20% dry matter) between 500 and 3000 g/plant under optimal conditions [27]. Another publication, a catalog of native potato varieties from Apurimac—an agrobiodiverse region of Peru—presented FTY of 500–1450 g/plant [28]. When comparing to these, it is evident that the LB disease affected yield. It is not feasible to establish the proportion of the impact due to preliminary yield data. These landraces are typically harvested after 8 months of cultivation, but this study was carried out at 6 months. Still, preliminary yield allows predictions of its final values, which strongly influence the selection, e.g., for breeding programs [29]. Given that a resistant genotype maintains an acceptable production during the rainy season [30], it is proposed that those within the group (Fig. 3C) and with a yield greater than 200 g/plant, such as “Puca clavelina”, “Negro callash” and “Mosqueña” could withstand a high pressure of the disease, or in times of low pressure, they would respond better than the rest.

The reasons for maintaining these types of landraces despite their low yield rely in farmer’s culinary preferences, based in cultivar mixtures for home consumption, the co-existence with other crops, and the fact that the marketable varieties subsidize the maintenance of non-commercial potato landraces [31]. Therefore, they maintain an ecologically dynamic agro-ecosystem [1].

Finally, the statistical difference between blocks according to ANOVA may have been biased by the small sample size, since it was only one plant representing an accession at each block. Given the large number of landraces evaluated, it was difficult to assess more repetitions for each one. However, it should also be considered that the AUDPC trait is sensitive to a lack of homogeneity of the disease in open field conditions [20]. The rAUDPC could be useful to compare with future trials within the UNDAC Native Potatoes Project and other studies.

**Conclusion**

This preliminary assay suggests that at least 23 native potatoes landraces cultivated by subsistence farmers from the Pasco region present a potential resistance to late blight that could contribute as genetic resources for food security. It is necessary to continue with this type of study in other areas and seasons to be aware of what is happening with native landraces in the Andean localities.
highlands, which is the ideal scenario to evaluate the impacts of climate change.

Abbreviations
UNDAC: National University Daniel Alcides Carrion; LB: Late blight; m.a.s.l.: Meters above sea level; DAP: Days after planting; FTY: Fresh tuber yield; AUDPC: Area under the disease progress curve; rAUDPC: Relative AUDPC; SS: Susceptibility scale; ANOVA: Analysis of variance.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s40066-021-00330-9.

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Authors’ contributions
EZ, JL FA and KM participated in the conception and design of the study; GR and IV in the project administration; EZ, GR and RP in the data collection; EZ, JL, FA, KM, RP IV, DB, GR and CSD in the data analysis; CSD in the manuscript writing; and all the authors made a final critical revision. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article [and its additional information files].

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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