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Precise assessment of angular leaf spot severity using the Leaf Doctor app for common beans

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Angular leaf spot (ALS) is one of the most severe diseases of common bean. However, there is as yet no standardization of the phenological stage, inoculum concentration, diagrammatic scale (DS), and day of assessment for screening ALS resistance in bean lines. The present study aimed to evaluate the potential for assessment of ALS severity in common beans using the Leaf Doctor app and inoculation at the V2 phenological stage under two inoculation concentrations. The experiment was carried out in a completely randomized design in a 3 × 2 × 2 factorial arrangement with three cultivars (AND 277, IAC-Milênio, and IAC-Carioca), two monosporic isolates (31-31 and 10-59), and two inoculum concentrations (2×10^4 and 4×10^4 conidia/mL). Disease severity was evaluated from 11 to 15 days after inoculation (d.a.i) using a diagrammatic scale and the Leaf Doctor. The results showed that 15 d.a.i., the concentration of the inoculum was no longer significant and that the isolates showed no significant difference in terms of pathogenicity. Moreover, in 15 d.a.i. it was possible to separate the cultivars into three phenotypic classes: resistant (AND-277), moderately susceptible (IAC-Milênio) and susceptible (IAC-Carioca). Considering the diagrammatic scale of notes, the cultivar AND 277 had a score of 2.3, while for the image evaluation, it was observed that only 0.65% of the total leaf area was affected by the disease. For this reason, 15 d.a.i. was recommended as the better day for evaluation, and the concentration of 2×10^4 conidia/mL may be adopted in order to reduce the amount of inoculum needed for the experiment. In addition, the evaluation with the Leaf Doctor app increases accuracy and improves discrimination between resistant and susceptible cultivars.

Key words: *Pseudocercospora griseola, Phaseolus vulgaris*, digital analysis, disease resistance.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the grains most consumed by humans throughout the world (Assefa et al., 2019). Regarding the consumption of beans, Brazil stands out as the largest consumer of this

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grain (FAOSTAT, 2021), with an average of 18 kg year^{-1} per capita (Carneiro et al., 2015). The nutritional value of this grain and the potential health benefits explain the relevance that this legume has in the human diet, being a source of carbohydrates, fibers, vitamins and minerals (Pires et al., 2006), as well as a source of polyphenolic compounds with antioxidant properties (Polya, 2003). In addition to being the largest consumer, Brazil is the third largest producer of beans in the world (FAOSTAT, 2021). The estimated production of the last bean crop in Brazil was about 3.1 million tons, with an average yield of 1.074 kg/ha (CONAB, 2021). Bean yield is affected by several factors, and in Brazil, disease is one of the main problems. Angular leaf spot, caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous and U. Braun, is one of the most serious diseases in common beans; it may cause yield losses of up to 80% (Nay et al., 2019a; Librelon et al., 2020).

In bean breeding programs, evaluation of the level of disease resistance is based on disease severity, which is defined as the area of plant tissue affected by the disease-causing organisms and expressed as a percentage of the total amount of plant tissue (Schoonhove and Pastor-Corrales, 1987; Forrest et al., 1991). In evaluation of ALS severity, breeders routinely adopt the V3 (first expanded trifoliate leaf) phenological stage (Pastor Corrales and Jara, 1995; Pereira et al., 2015; Bassi et al., 2017; Vidigal Filho et al., 2020; Almeida et al., 2020). However, when assessing the disease at the initial stages of plant development, Pereira et al. (2011) proposed the use of the V2 phenological stage (expanded primary leaves), making it possible to reduce the amount of inoculum used and the amount of time to perform the analyses.

All the diagrammatic scales (DS) developed to assess ALS resistance were proposed for the V3 stage (Moreno, 1977; Schoonhove and Pastor-Corrales, 1987; Inglis, 1988; Godoy et al., 1997), and they are not appropriate for the V2 stage. Librelon et al. (2015) proposed a specific DS for the V2 stage, which has been successfully used for evaluation and selection of ALS resistant bean lines (Pereira et al. 2019a, b, Librelon et al., 2020; Almeida et al., 2021a). DSs are contingent on the subjectivity of each evaluator, and Librelon et al. (2015) reported that the majority of 14 evaluators tested underestimated ALS severity using a DS.

An alternative to the DS for assessment of disease severity is quantification of infected tissue by digital image analysis, with advantages such as high precision, accuracy, reliability, and the possibility of non-destructive sampling, allowing data to be collected from the same individual at the experimental site over a period of time (Bock et al., 2008; Arnal Barbedo, 2013; Mutka and Bart, 2015). Several programs have been developed to quantify plant diseases, such as “Quant”, “Assess 2.0”, “ImageJ”, “Scion Image”, and “CompuEye” (Vale et al., 2001; Lobet et al., 2013; Mutka and Bart, 2015; Mahlein, 2016). However, some of these programs do not have friendly interfaces and are rather expensive, and require a Windows operating system interface, meaning that image processing must occur on a desktop and/or laptop only after capturing the images by another device (that is, photo camera or scanner).

With the advance of technology, an app for smartphones, named Leaf Doctor (https://www.quantitative-plant.org/software/leaf-doctor), was developed to perform image quantification on a portable mobile device in a fast and simpler way (Pethybridge and Nelson, 2015). The app has an extremely friendly interface, and it only requires the user to take photos with the device in the app itself, indicating the color of healthy and infected leaf tissues. After a few seconds of processing, the app shows the percentage of leaf area affected by the pathogen (Pethybridge and Nelson, 2015). This is advantageous in bean breeding programs, considering that generally many plants need to be evaluated with high accuracy in a short period of time. The purpose of the present study was to test the efficiency of using the Leaf Doctor app to perform assessment of the severity of ALS in common bean at the V2 phenological stage and compare with the evaluation carried out by a DS proposed for the V2 stage. In addition, two different inoculum concentrations were adopted (2×10^4 and 4×10^4 conidia/mL) in order to identify possible interaction and recommendation of better one.

MATERIALS AND METHODS

Three common bean cultivars (that is, AND 277, IAC-Milênio, and IAC-Carioca) with different levels of resistance to ALS were selected from the Germplasm Bank of the Instituto Agronômico - IAC (Campinas, São Paulo, Brazil). The AND 277 cultivar (Andean) is highly resistant and widely used as a source of resistance to angular leaf spot (Gonçalves-Vidigal et al., 2011; Oblessuc et al., 2012; Bassi et al., 2017; Almeida et al., 2020). The IAC-Carioca and IAC-Milênio cultivars (Mesoamerican) are susceptible, though they have different levels of susceptibility; the IAC-Carioca has been used as a check cultivar for susceptibility in some studies (Oblessuc et al., 2012; Bassi et al., 2017). The experiment was carried out in a completely randomized design in a 3 × 2 × 2 factorial arrangement, consisting of three cultivars, two monosporic isolates of *P. griseola* characterized as physiological races 31-31 and 10-59, and two inoculum concentrations (that is, 2×10^4 and 4×10^4 conidia/mL), with three replicates. A plot consisted of a pot with one plant.

Seeds of each cultivar were pre-germinated, aiming at selection of seedlings with the same size and vigor. Plants were inoculated according to the method proposed by Pereira et al., (2011). Plants were kept in a greenhouse for 10 days. When they reached the V2 stage, they were placed in an inoculation chamber, where they remained under controlled temperature (24°C) and moisture (95-100%) conditions in the absence of light for 24 h. The inoculum suspensions of both isolates were prepared as described by Almeida et al. (2020), and the inoculum concentrations were adjusted to those described earlier. Inoculation was performed on the adaxial and abaxial surfaces of the primary leaves using an air compressor (*De Vilbiss*). Twenty-four hours after inoculation, the

Table 1. Analysis of variance and the Tukey mean test for angular leaf spot severity for the following factors: isolate, inoculum concentration, and cultivar and the interactions between the factors, estimated by the diagrammatic scale (1-9) and the Leaf Doctor app at different days of evaluation (from 11 to 15 d.a.i.).

Factor	Number of days after inoculation					Leaf Doctor	AUDPC
	11	12	13	14	15		
Isolate							
31-31	2.05[a]	2.44[a]	2.88[a]	3.44[a]	4.11[a]	9.24[a]	24.38[a]
10-59	2.16[a]	2.33[a]	2.77[a]	3.72[a]	4.22[a]	7.53[a]	25.38[a]
Concentration							
2×10^4 conidia mL^{-1}	1.88[b]	2.27[b]	2.55[b]	3.38[b]	4.00[a]	7.26[a]	24.84[a]
4×10^4 conidia mL^{-1}	2.33[a]	2.50[a]	3.11[a]	3.77[a]	4.33[a]	9.51[a]	24.92[a]
Cultivar							
IAC-Carioca	2.66[a]	3.00[a]	3.58[a]	4.75[a]	5.41[a]	14.66[a]	31.95[a]
IAC-Milênio	2.41[a]	2.83[a]	3.16[b]	3.75[b]	4.75[b]	9.84[b]	28.20[b]
AND 277	1.25[b]	1.25[b]	1.75[c]	2.25[c]	2.33[c]	0.65[c]	14.51[c]
Interaction							
Iso. × Cult.	0.007	<0.001	0.006	0.679	0.123	0.502	0.006
Iso. × Con.	0.061	1.000	0.006	0.123	0.558	0.927	0.815
Con. × Cult.	0.821	0.002	0.193	0.679	0.105	0.146	0.623

The mean values with the same letter in the same column do not differ significantly by the Tukey test ($p < 0.05$).

chamber was adjusted to 80% humidity, with controlled photoperiod (12 h/12 h) (Monda et al., 2001; Sartorato, 2004), until the end of the evaluation. In addition, two plants of each cultivar were treated with only distilled water and Tween® (mock).

Assessment of angular leaf spot severity was performed at 11, 12, 13, 14, and 15 days after inoculation (d.a.i.) with the aid of the diagrammatic scales (DS) developed by Librelon et al. (2015). The scale has nine levels of severity: 1 to 3 correspond to resistant plants, 4 to 6 to moderately resistant plants, and 7 to 9 to susceptible plants. At 15 d.a.i., ALS severity was also estimated with the Leaf Doctor app on a smartphone with an iPhone Operating System (that is, iPhone 7 model) after the primary leaves of each cultivar were individually photographed against a black background. Statistical analysis of variance (ANOVA) was performed on each evaluation and the mean values were compared by the Tukey test at 5% significance ($p < 0.05$). The area under the disease progress curve (AUDPC) was estimated from each evaluation using the following formula:

$$\mathrm{AUDPC} = \sum_{i=1}^{n_i - 1} (y_i + y_{i+1}/2)(t_{i+1} - t_i)$$

where n is the number of evaluations, y is the severity of the disease, and $(t_{i+1} - t_i)$ is the time interval between two consecutive evaluations (Campbell and Madden, 1990). All data were analyzed using the SANEST software (Zonta and Machado, 1980).

RESULTS AND DISCUSSION

The results were significant for the cultivar factor for all the days of evaluation and approaches used (that is, diagrammatic scale and Leaf Doctor app) due to differences in the degree of resistance of the cultivars. The Tukey mean test showed there was a significant difference between susceptible cultivars (that is, IAC-Milênio and IAC-Carioca) only from 13 to 15 d.a.i., and all cultivars were considered resistant (that is, level < 3) until 12 d.a.i. (Table 1). The ANOVA showed no significance for the isolate factor, showing that the pathogenicity of both isolates (31-31 and 10-59) was similar for the set of cultivars tested. Nevertheless, a significant interaction was observed between isolate and cultivar from 11 to 14 d.a.i., and significance was also found for the isolate × concentration interaction at 12 d.a.i.

Regarding the concentration factor, there was significance only until 14 d.a.i., and a higher degree of severity was found for the 4×10^4 conidia mL^{-1} concentration. The AUDPC showed significance only for cultivar, allowing separation of the three cultivars into three distinct groups, with significance for the isolate × cultivar interaction. The IAC-Carioca cultivar was most susceptible to ALS for both *P. griseola* isolates; IAC-Milênio had an intermediate reaction; and AND 277 was the most resistant cultivar (Figure 1).

Although ALS is a highly significant disease for common bean worldwide, there is no consensus in the literature regarding the best day for assessment after inoculation under controlled conditions of infection. In the V3 stage, there are reports of assessment of degree of

Figure 1. The severity of angular leaf spot disease in primary leaves of AND 277, IAC-Milênio, and IAC-Carioca common bean cultivars at 15 days after inoculation in the V2 phenological stage.

resistance at 7 d.a.i. (Vidigal Filho et al., 2020), 9 d.a.i. (Gonçalves-Vidigal et al., 2011), 12 d.a.i. (Guzman et al., 1995), 15 d.a.i. (Oliveira et al., 2004; Bassi et al., 2017; Almeida et al., 2020, 2021b), 18 d.a.i. (Almeida et al., 2021a), 21 d.a.i. (Nay et al., 2019b), and 28 d.a.i. (Carvalho et al., 1998). However, one of the advantages of considering the V2 stage for this assessment is reduction in the number of days necessary to complete the experiment. Pereira et al. (2011) proposed the use of the V2 stage and evaluated plants at 14 d.a.i. Librelon et al. (2015) evaluated 15 d.a.i. for validation of the V2 DS, while other authors used the same number of days for ALS assessment at the V2 stage (Pereira et al., 2019a, b, Librelon et al. 2020). Our results showed that, in the case of the V2 stage, at least 15 days after inoculation is necessary for reliable evaluation, since only on the last day of evaluation (day 15), could the cultivars be well discriminated. Before that, IAC-Milênio showed ALS severity similar to IAC-Carioca.

Another reason to use 15 d.a.i. for assessment of resistance to ALS is that from that day on, the inoculum concentration used was no longer significant, so the difference between the concentrations did not affect the degree of severity in the cultivars tested, and there was no significant interaction among the factors (that is, cultivar, concentration, and isolate). Just as for number of days for assessment, there is no consensus regarding the inoculum concentration to be used for inoculation 1×10^4 conidia mL^{-1} (Oblessuc et al., 2012; Perseguini et al., 2016), 1.2×10^4 conidia mL^{-1} (Gonçalves-Vidigal et al., 2020; Vidigal Filho et al., 2020), 2×10^4 conidia mL^{-1} (Carvalho et al., 1998; Guzman et al., 1995; Keller et al., 2015; Pastor-Corrales and Jara, 1995), and 4×10^4 conidia mL^{-1} (Pereira et al., 2011; Almeida et al., 2020) are most used. Pereira et al. (2011) reported that the concentration of 2×10^4 conidia mL^{-1} did not discriminate resistant bean lines from susceptible ones, and that the 4×10^4 conidia mL^{-1} generated a greater degree of severity. However, the authors evaluated the inoculated plants at 14 d.a.i., and the results also showed significance regarding the concentration used for 14 d.a.i. Librelon et al. (2015) used the concentration of 2×10^4 conidia mL^{-1} for development of the V2 DS, and Pereira et al. (2019a, b) and Librelon et al. (2020) used the same concentration for evaluation of resistance in advanced bean lines.

The main problem with the DSs proposed for evaluation of ALS severity is not only the subjectivity of the evaluators, but also the difference between the number of scores and the specific range of score variation in terms of leaf area affected by the disease. Schoonhove and Pastor-Corrales (1987) proposed a DS ranging from 1 to 9, with score 3 representing plants with up to 2% of leaf area affected. Godoy et al. (1997) also proposed a DS ranging from 1 to 9, but score 3 represented plants with up to 0.9% of leaf area affected. Moreno (1977) proposed another DS, ranging from 1 to 5, with score 3 representing up to 25% of leaf area affected; and Inglis et al. (1988) adapted the same DS, and score 3 was now understood as plants with up to 50% of leaf area affected.

A more reproducible and accurate way to assess resistance to ALS is through digital image analysis. However, in a study carried out by Rezende et al. (2014) under field conditions for evaluation of ALS using DS (Schoonhove and Pastor-Corrales, 1987) and the image analysis software Quant (Vale et al., 2001), the authors reported that the use of the DS was the most practical way to discriminate the bean lines. Software like Quant proposes a considerable problem in that it is not practical at all; leaves need to be detached and scanned one by one and images must be transferred to the computer for processing and quantification. In contrast, Leaf Doctor is a smartphone app, and digital image quantification of leaf area affected by the disease can be performed in the field and/or greenhouse through simple capture of images and fast processing, without the need to detach leaves or scan them, and/or even transfer the images to computers. The Leaf Doctor approach is quantitatively more sensitive, precise and accurate than the DS, as observed in the present study, allowing the definition of greater differences among the genotypes through the app. This enhancement in precision of assessment could improve the initial steps of bean breeding programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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