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RESEARCH NOTE

A standard area diagram set for severity assessment of eyespot on rice

Juan F. Rivera[1] · Henrique S. S. Duarte[2] · Emanueli B. Furtado[1] · Leandro J. Dallagnol[1]

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Abstract

This study aimed to develop and validate a standard area diagram set (SADs) to estimate the severity of eyespot of rice caused by *Drechslera gigantea*. For this purpose, a SADs with seven levels of severity (0.3; 1.0; 3.0; 5.0; 10; 15; and 21%) was established. The SADs was validated by 16 raters with no experience in evaluating plant diseases. Both accuracy and precision improved when they used the SADs. The statistical parameters for the 16 raters were: bias coefficient factor - C_b (no SADs = 0.404, with SADs = 0.994); correlation coefficient - r (no SADs = 0.884, with SADs = 0.953); and Lin's concordance correlation coefficient - ρc (no SADs = 0.356, with SADs = 0.947). In addition, estimates were more reliable: inter-rater coefficient of determination - R^2 (no SADs = 0.712, with SAD = 0.873); intra-class correlation coefficient - ρ (no SADs = 0.723, with SADs = 0.924). The SADs proposed here is a useful tool for improving visual assessments of eyespot severity of rice.

Keywords *Oryza sativa* · *Drechslera gigantea* · Disease assessment · Phytopathometry

Rice (*Oryza sativa* Linn.) is the principal food for more than 50% people and contributes about one-fifth to the total calories consumption of the world (Singh et al. 2012). Rice eyespot caused by *Drechslera gigantea* (Heald et Wolf) S. Ito (Ito 1930) is a foliar disease, and it has been reported in several Latin American countries (Ahn 1980; Nunes 2008) and the United States (Kardin et al. 1982). In the south of Brazil, the disease was reported in the 2006/07 season, and since then eyespot has been observed in all crop seasons, in some specific cultivars, mainly after the early dough stages of grain development (Nunes 2013). The pathogen infects the rice leaves causing oval lesions with a grayish center and reddish-brown margin, 0.5–2.5 × 0.2–1.0 mm (Fig. 1). So far, damage in the yield is not clear, especially in Brazil where the disease has been observed occurring in the late stage of plant development. However, the effect of the disease may become significant if it occurs in the vegetative stage and/or the environmental conditions become favorable to the pathogen, such as prolonged leaf wetting, at the beginning of the crop season.

In the case of an emerging disease will be necessary studies to identify resistant genotype, epidemiological requirements to develop an epidemic and evaluation of control measures. For this, it is of great importance to establish a method that allows measure the disease amount, more accurate, precise and with reliable estimative of disease severity in those studies. The use of standard area diagram set (SADs) is tool and a way of visually estimating plant disease severity, an essential variable in phytopathometry (Bock et al. 2010; Del Ponte et al., 2017). Despite the growing relevance of eyespot of rice, there is no SADs published to assess the disease, which will be very useful for several purposes, including breeding for resistance, fungicide screening, and pathotype characterization. The aim of this study was to develop and validate a SADs to estimate the severity of eyespot of rice, providing a useful tool for the evaluation of the disease.

One hundred fifty leaves of rice with symptoms of eyespot were sampled at the experimental area of Federal University of Pelotas, State of Rio Grande do Sul, Brazil, during the 2017/18 season. The symptomatic leaves were arbitrarily collected from epidemical trials of cultivars, which allowed the manifestation of the disease in different levels of severity.

The symptomatic leaves were individually digitized at a resolution of 300 dpi, using a scanner (HP® ScanJet 2400;

✉ Leandro J. Dallagnol
leandro.dallagnol@ufpel.edu.br

[1] Departamento de Fitossanidade, Universidade Federal de Pelotas, Pelotas, RS, Brazil

[2] Departamento de Fitotecnia e Fitossanidade, Universidade Federal do Paraná, Curitiba, PR, Brazil

Fig. 1 Rice leaves of the cultivar BRS Querência affected by eyespot during an epidemic year of the disease (**a**); Details and pattern of eyespot symptoms distribution on rice leaves (**b** and **c**)

Hewlett-Packard, Alto, CA, USA). For each leaf, the proportion of diseased area was determined using the QUANT software (Vale et al. 2003). The SAD illustrations were chosen from these samples with adjustments made by the Paint program (Microsoft® Office 365), establishing a set of seven disease severity levels, in a linear distribution (Nutter Jr and Esker 2006; Bock et al. 2010). Lesions with a grayish center, reddish-brown margin and leaf necrosis were quantified as diseased area. Foliar chlorosis was not considered.

The SADs was validated by 16 raters with no previous experience in plant disease severity assessments. Fifty images of diseased leaves were displayed as a slide show in PowerPoint to the raters. Each image was displayed for one minute and the raters were then asked to write down the estimated percentage of diseased area on a form. For the second assessment, they evaluated the same 50 images again in a different sequence, but with the aid of the SADs developed in this study. The accuracy, precision and inter-rater reliability of the estimates with and without the SADs were calculated as previously described (Dolinski et al. 2017). The statistical analyses were performed using R software (R Core Team 2020). The LCCC statistics were estimated by the epi.ccc function of the epiR package (Stevenson 2012). The built-in boot.sample R function was used for the equivalence test. The ρ was estimated using the icc function of the irr R package (Gamer et al. 2012).

Seven illustrations, covering the minimum (0.3%) and the maximum (21%) of eyespot severity observed in the field, comprised the SADs (Fig. 2). The maximum severity of 21% was the maximum value found in the field in the 2017/18 season, and rice leaf samples collected in other years, the disease severity was always below this value. Furthermore, plant inoculations conduced in controlled environment, providing the ideal conditions for pathogen infection, with concentration of 1×10^4 conidia mL^{-1}, resulted in disease severity below of the maximum limit of the scale.

All statistical parameters (υ, u, C_b, r, and ρc) of Lin's concordance correlation (LCCC) were significantly improved when the raters used the SADs to estimate disease severity, demonstrating that both the accuracy and precision of the estimated values were improved. The statistical parameters values were: scale bias - υ (no SADs = 2.700, with SADs =

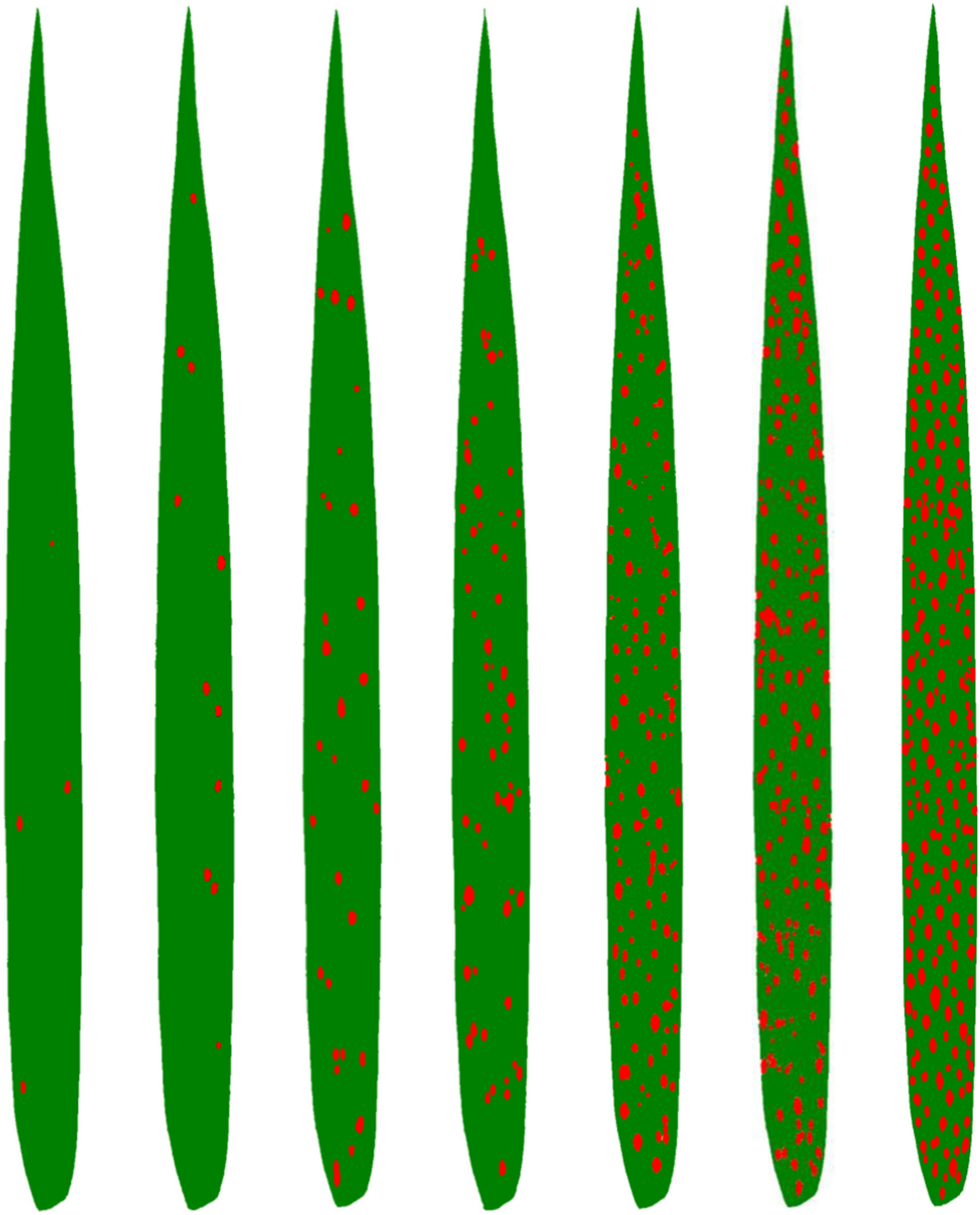

Fig. 2 Standard area diagram set for eyespot (*Drechslera gigantea*) severity on rice leaves. The number below each diagram represent percent leaf area with symptoms (circular spots with dark brown coloring)

0.980) (confidence intervals - CI = -2.000 – -1.441), location bias (u) (no SADs = 1.463, with SADs = −0.024) (CI = -1.718 – -1.260), bias coefficient factor - C_b (no SADs = 0.404, with SADs = 0.994) (CI = 0.533–0.647); correlation coefficient - r (no SADs = 0.884, with SADs = 0.953) (CI = 0.051–0.086); and Lin's concordance correlation coefficient - ρc (no SADs = 0.356, with SADs = 0.947) (CI = 0.541–0.641). As the CI did not embrace zero, the difference was significant ($\alpha = 0.05$). Based on estimated and actual severity, assessments made by the raters were closer to the actual values using

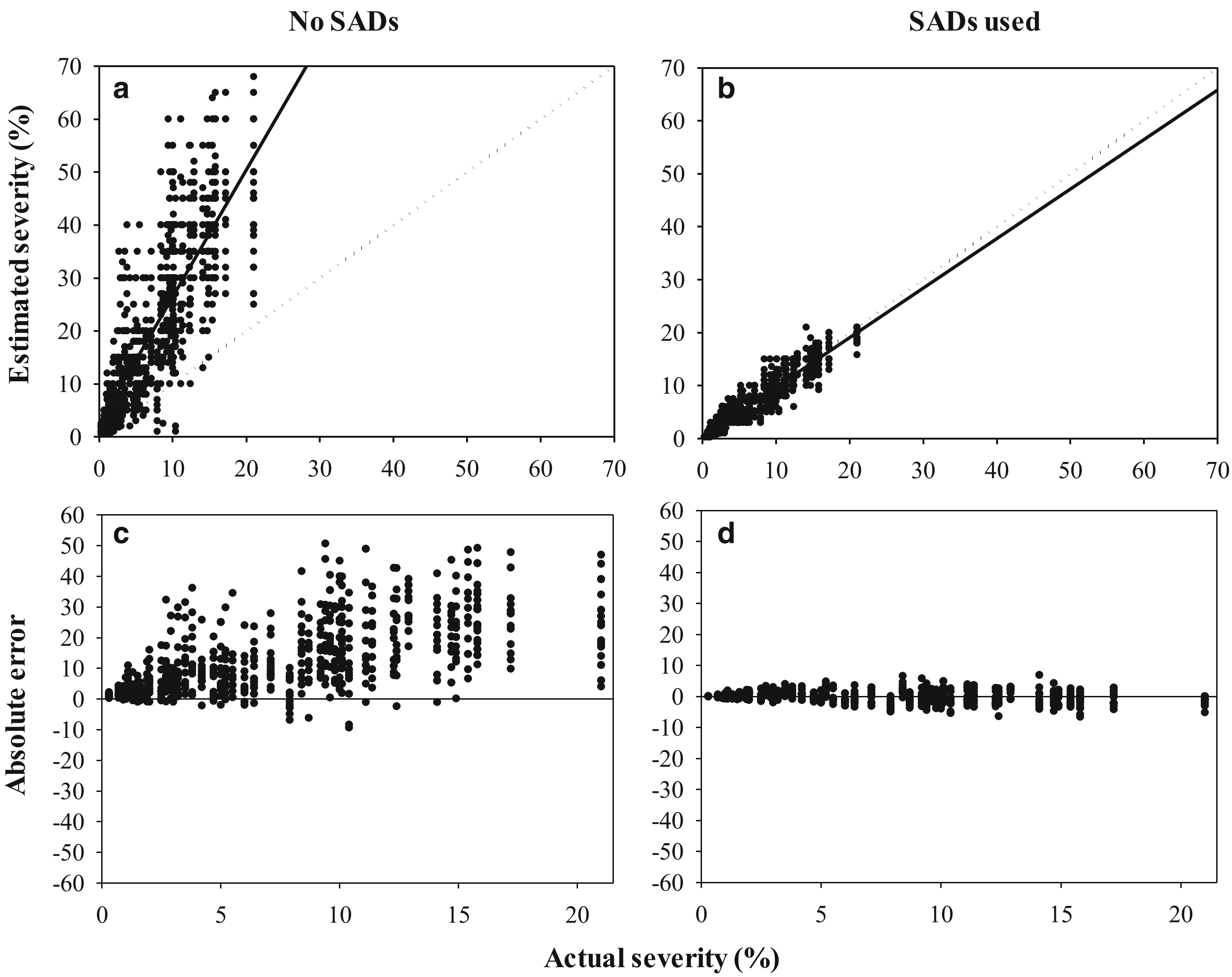

Fig. 3 Relationship between actual and estimated eyespot (*Drechslera gigantea*) severity on rice leaves without (**a**) and with (**b**) the use of a set of standard area diagrams (SADs) for 50 diseased leaves by 16 raters. The solid line represents the best-fitting line. The dashed line is the concordance line, which represents a perfect agreement between actual and estimated severity. Absolute error (estimated minus actual severity) of the estimates without SADs (**c**) and with the help of SADs (**d**) for the 50 diseased leaves

the SADs (Fig. 3 a and b). The absolute error of the estimates reduced significantly when the raters used the SADs (Fig. 3 c and d).

Inter-rater reliability of assessments by 16 raters was significantly improved. Without the SADs, the intra-class correlation coefficient mean (ρ) was 0.723 (confidence intervals = 0.619–0.814), while using the SADs, this value was 0.924 (confidence intervals = 0.893–0.951). In turn, the mean of inter-rater coefficient of determination (R^2) of the pairwise comparisons were 0.712 (minimum = 0.458, maximum = 0.863) and 0.873 (minimum = 0.764, maximum = 0.946) without and with SADs, respectively. The 95% confidence interval (CI) of this mean was 0.145 to 0.177. As the CI did not embrace zero, the difference was significant ($\alpha = 0.05$).

The SADs developed in this study improved accuracy, precision, and reliability of the estimations of eyespot severity. The number of diagrams in this set is similar to that in the SADs developed for brown spot on rice (Schwanck and Del Ponte, 2014), thereby facilitating assessment for the raters. Moreover, each diagram in this study showed the entire leaf, which prevented errors related to patterns of disease distribution on the leaves.

Despite the availability of software with high accuracy and precision in the quantification of diseases severities, such as Quant (Vale et al. 2003), APS Assess 2.0: Image Analysis Software for Plant Disease Quantification, and Leaf Doctor (Pethybridge and Nelson 2015), SADs are still a powerful tool to improve the accuracy and precision of experienced or inexperienced raters (Lage et al. 2015; Nuñez et al. 2017). In addition, SADs does not require specific conditions of image acquisition, nor people with specific training, and it is practical for assessments of large amounts of samples.

SADs for rice eyespot can assist plant pathologists, breeders and extensionists in quantifying the disease, in a way of more accurate and reliable estimates of disease severity in studies involving epidemiological analyses, evaluating disease management strategies and genotypes selection for resistance to the disease.

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