# Development and validation of a standard area diagram set to assess blast severity on wheat leaves

**Article** *in* European Journal of Plant Pathology · July 2013

DOI: 10.1007/s10658-013-0191-x



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Eur J Plant Pathol (2013) 136:603–611
DOI 10.1007/s10658-013-0191-x

# Development and validation of a standard area diagram set to assess blast severity on wheat leaves

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Accepted: 14 February 2013 / Published online: 12 March 2013


**Abstract** This study aimed to develop and validate a standard area diagram set (SADS) to quantify the severity of blast, caused by *Pyricularia oryzae*, on wheat leaves. The SADs has ten levels: 0.1, 1, 5, 10, 22, 32, 42, 52, 62 and 72 % blast severity. To validate the SADs, 12 inexperienced raters estimated disease severity on 50 images of leaves from cultivars BR-18 (susceptible) and BRS-229 (partially resistant). Blast severity was first estimated without the use of the SADs on 50 leaves with a range of blast severity. The same raters evaluated the same 50 leaves using the SADs as an aid. The SADs improved accuracy (coefficient of bias, $C_b$=0.88 and 0.99, without and with SADs, respectively) and agreement (Lin's concordance correlation coefficient, $\rho_c$=0.84 and 0.96 without and with SADs, respectively) of the estimates of severity. The absolute error was (-) 52 % without the SADs and (-) 24 % when using SADs as an aid. Severity estimates were more reliable when using SADs ($R^2$=0.87 unaided and $R^2$=0.92 with SAD). The SADs proposed in this study will improve accuracy and reliability of estimates of blast severity on wheat leaves.

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**Keywords** Epidemiology · Disease assessment · *Pyricularia oryzae* · *Triticum aestivum*

## Introduction

Blast, caused by the fungus *Pyricularia oryzae* Sacc. (teleomorph: *Magnaporthe grisea* (Hebert) Barr), is an important disease of wheat (*Triticum aestivum* L.) and can cause yield losses up to 70 % (Goulart et al. 2007). The pathogen infects the leaves, stems and ears of wheat plants (Igarashi et al. 1986). Symptoms of wheat blast include gray-green and water-soaked leaf lesions which have dark green borders. The lesions become light tan in colour with necrotic borders after they have completely expanded (Filha et al. 2011). Spikes and spikelets can be infected by *P. oryzae* and develop several gray-brown lesions (Goulart et al. 2007). Seedling blight, spike tip death, and bright black spots on the rachis are often observed (Goulart et al. 2007). Ear infection by *P. oryzae* decreases nutrient translocation to the grains, which become shrivelled in appearance, small, misshapen, and underweight (Goulart et al. 2007). The progression of blast symptoms is affected by environmental conditions and plant growth stage (Goulart et al. 2007). Severe epidemics of blast in Brazil occur during the rainy season when temperatures range from 21 to 27 °C and the relative humidity is high (Goulart et al. 2007).

A standardized method of disease measurement is important for studying epidemic progress, when comparing the efficacy of different control measures such as fungicide sprays and cultivars with different levels of disease resistance. As a consequence of accurate disease measurement, losses can be minimized due to improved control strategies being implemented (Gomes et al. 2004). In the case of blast of wheat, severity is measured as the percentage of diseased leaf (Bergamim Filho and Amorim 1996). The evaluation of any disease using subjective estimates can be subject to error; therefore, it is necessary to use standardized criteria to measure disease (Lenz et al. 2010).

The use of a standard area diagram set (SADs) has been an important method to accurately and reliably measure disease severity (Bergamim Filho and Amorim 1996; Martins et al. 2004). SADs are based on illustrations of plants or plant organs with symptoms that represent a range of disease severity (Bergamim Filho and Amorim 1996). SADs improve both accuracy (how close the estimated values are to the true values) (Nutter and Schultz 1995) and reliability (the extent to which the same estimate obtained under different conditions yields similar results) (Nutter et al. 1991) during disease severity evaluations by reducing subjectivity (Martins et al. 2004). In order to compare the estimated values with the real values, the concept of agreement is defined as the product of the precision (variability of the estimates) and accuracy (Madden et al. 2007). Desirable characteristics of SADs include: ease of use, reproducibility of results, wide applicability, and the presence of intervals that represent all stages of symptoms (Berger 1980). In developing SADs, it is important to ensure that the minimum and maximum limits of severity observed on the plant are represented; additionally, symptoms should be representative of those seen under conditions favourable for natural infections (Horsfall and Barrat 1945). Before being proposed as a standard method to quantify plant diseases, the SADs must be validated (Martins et al. 2004).

Considering the importance of blast and the absence of a SADs to evaluate blast severity on wheat leaves, this study aimed to develop and validate SADs to accurately and reliably measure blast severity.

## Materials and methods

### Wheat plants

Wheat seeds from cultivars BR-18 (susceptible) and BRS-229 (partially resistant to blast) were surface sterilized in 10 % (v $v^{-1}$) NaOCl for 2 min, rinsed in sterilized water for 3 min, and sown in plastic pots (20-cm-diameter) (Ecovaso, Jaguariúna, SP, Brazil) filled with 1 kg of a 1:1:1 mixture of pine bark, peat, and expanded vermiculite (Tropstrato®, Vida Verde, Mogi Mirim, São Paulo). A total of 1.63 g of calcium phosphate monobasic was added to each pot. Ten seeds were sown per pot, and 5 days after seedling emergence, each pot was thinned to seven seedlings. Seedlings were fertilized with a nutrient solution containing (mg $l^{-1}$), KCl (192 $mgl^{-1}$), $K_2SO_4$ (104.42 $mgl^{-1}$), $MgSO_4.7H_2O$ (150.35 $mgl^{-1}$), urea (61 $mgl^{-1}$), $NH_4NO_3$ (100 $mgl^{-1}$), 0.27 $NH_4MO_7O_{24}.4H_2O$, $H_3BO_3$, 6.67 $ZnSO_4.7H_2O$ (1.61 $mgl^{-1}$), $CuSO_4.5H_2O$ (1.74 $mgl^{-1}$), $MnCl_2.4H_2O$ (4.10 $mgl^{-1}$), $FeSO_4.7H_2O$ (4.08 $mgl^{-1}$), and disodium-EDTA (5 $mgl^{-1}$) (Rodrigues et al. 2001) in deionized water. At sowing and at 20 and 35 days after seedling emergence 50 ml was added to each pot. Plants were watered as needed with deionized water.

### Inoculum production and inoculation procedure

A pathogenic isolate of *P. oryzae* (UFV/DFP-01), obtained from an ear of a wheat plant (cv. BR-18), was used to inoculate the foliage. The isolate was cultured in Petri dishes containing oatmeal agar medium (OAM) in a growth chamber at 25 °C with a 12-h photoperiod for 10 days. Mycelia producing conidia were carefully removed from the Petri dishes using rubber policeman to obtain a suspension of conidia. Each plant was spray inoculated with 25 ml of the conidial suspension of *P. oryzae* ($10^5$ conidia $ml^{-1}$) at 60 days after emergence (growth stage 45) (Zadoks et al. 1974). The inoculum was applied to the adaxial leaf blades of run off using a VL Airbrush atomizer (Paasche Airbrush Co., Chicago, IL). Gelatin (1 %, wt $vol^{-1}$) was added to the suspension to aid conidial adhesion to the leaf blades. Immediately after inoculation, the plants were transferred to a growth chamber with a temperature of 25±2 °C and a relative humidity of 90±5 % and were subject to an initial 24-h dark period, before transfer to a plastic mist growth chamber (MGC) inside a greenhouse for the duration of the experiment. The MGC was made of wood (2 m wide, 1.5 m high and 5 m long) and covered with 100-mm-gorge transparent plastic. The temperature inside the MGC ranged from 25±2 °C (day) to 20±2 °C (night). The relative humidity was maintained at 92±3 %

using a misting system in which nozzles (model NEB-100; KGF Company, São Paulo, Brazil) sprayed every 30 min. Relative humidity and temperature were measured with a thermo-hygrograph (TH-508, Impac, Brazil). The photon flux density inside the greenhouse was measured at noon with a Li 250A light meter (Li-Cor Environmental, Lincoln, NE, USA) and provided a reading of approximately 900 μmol photons $m^{-2}$ $s^{-1}$.

Development of the SAD set

A total of 150 leaves with a range of blast severity were collected from plants of cultivars BR-18 (75 leaves) and BRS-229 (75 leaves) and individually scanned to obtain images with a resolution of 300 dpi. These images were processed and disease severity was measured using QUANT software (Vale et al. 2003). From the minimum and maximum severity of the disease measure on the leaves analyzed and following a linear SADs, an additional eight intermediate disease severity diagrams were established for a set of 10 images. A standard wheat leaf was used as the template and diagrams with the ten severity levels were created using the PAINT.NET image editing software (http://www.getpaint.net/) applying the same patterns of lesions distribution found on the scanned leaves. The typical symptoms of necrosis and chlorosis were included on the SADs.

Validation of the SADs

Twelve inexperienced raters validated the SADs. Fifty images of leaves with a range of severity were pasted on individual slides to be viewed in a PowerPoint presentation file. In the first assessment the raters did not use the SADs as an aid to estimate blast severity. In the second assessment the raters used the SADs as an aid to estimate blast severity using the same set of images. To evaluate each leaf, the rater compared its image with the SADs to new estimate the percentage of diseased area considering both symptom of chlorosis and necrosis were included in estimates of disease severity.

Based on the data obtained during SADs validation, the agreement between estimates and actual values for each rater was determined with Lin's concordance correlation coefficient (LCCC) ($\rho_c$) (Lin 1989). The analysis was performed separately using the data obtained with and without use of the SADs.

The $\rho_c$ is the most appropriate analysis for this type of experiment because it combines the measures of accuracy and precision to assess the relational fit to the line of concordance (45°) represented by $\rho_c = C_b \cdot r$, where $C_b$ = the bias correction factor that measures how far the best-fit line deviates from 45° and is a measure of bias or accuracy; and $r$ = the correlation coefficient between estimated severity ($Y$) and actual severity ($X$), which measures, in this case, the precision of the best fit line.

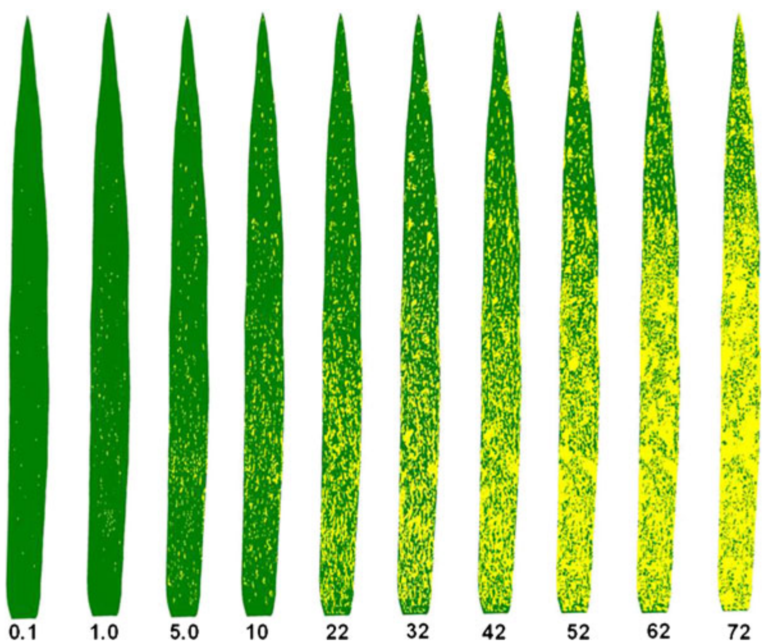


**Fig. 1** The standard area diagram set (SADs) to evaluate blast severity on wheat leaves. Data are presented as the percentage (%) of leaf area with blast symptoms

$C_b$ is the bias correction factor and is derived from: $C_b = 2/[(\upsilon + 1/\upsilon + u^2)]$, where $\upsilon = \sigma_y / \sigma_x$, and $\sigma_y$ and $\sigma_x$ are the standard deviations of *Y* and *X*, respectively; and $u = (\mu_y - \mu_x) / \sqrt{(\sigma_y \cdot \sigma_x)}$, where $\mu_y$ and $\mu_x$ are the mean values of *Y* and *X*, respectively. The term $\upsilon$ measures the SADs difference between actual and estimated values,

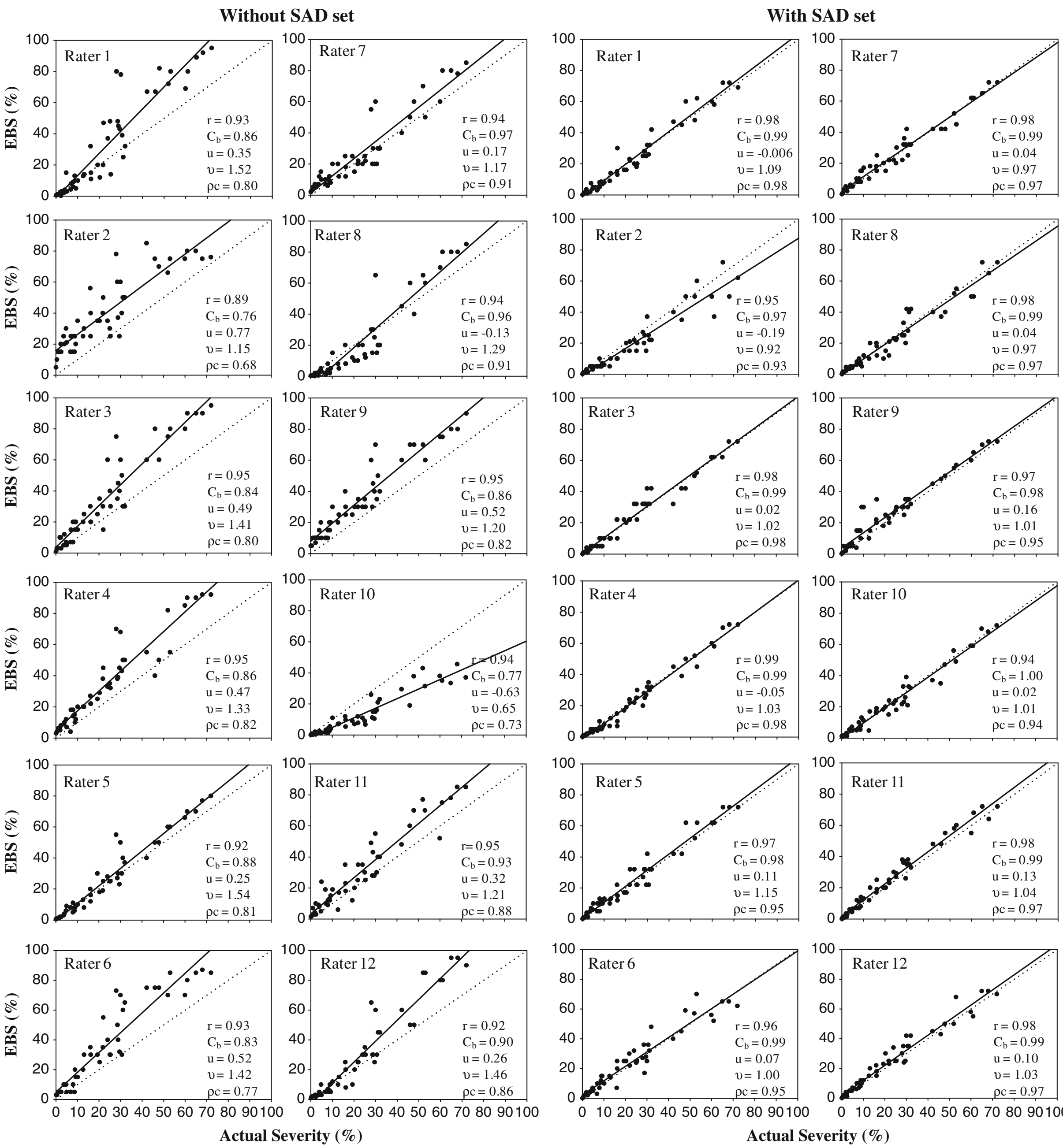


**Fig. 2** Estimates of blast severity (EBS) without and with the use of the standard area diagram set (SADs) showing the relationship between the actual and the estimated severity (*solid line*) for the ten raters. The *dotted line* is the concordance line and represents perfect agreement between the actual and the estimated severity (slope of 1 and intercept of 0). Accuracy was determined with Lin's concordance correlation coefficient ($\rho_c$) and calculated as the product of the correlation coefficient ($r$) and the bias correction factor ($C_b$). $C_b$ is a function of location bias ($u$) and scale bias ($\upsilon$) indicating changes in line height and slope, respectively

which is defined by the difference in slope of the two lines. Equal slopes would have a $\upsilon$ of 1. The term $u$ is a reflection of a location shift relative to the SADs, primarily reflecting height differences in the lines. Equal heights would have a $u$ of 0. A perfectly accurate measurement occurs when the points are on the concordance line (i.e., $r$=1, $C_b$=1 [$\upsilon$=1, $u$=0], and thus, $\rho_c$=1) (Bock et al. 2010; Nita et al. 2003).

The reliability of the estimates was determined by linear regression analysis of the inter-rater estimates for each leaf and using the $R^2$ of each pair of the rater's estimates to judge reliability (Nutter and Schultz 1995). Regression analysis was performed with MiniTab V14 (Minitab Inc., Pennsylvania, United States).

For all parameters analyzed ($r$, $C_b$, $\upsilon$, $u$, and $\rho_c$) and for the inter-rater reliability, the differences between means (i.e. with minus without SADs) was calculated and an equivalence test used to test their significance (Yi et al. 2008; Bardsley and Ngugi 2012; Yadav et al. 2012). The equivalence test was used to calculate the 95 % confidence intervals (CIs) for each statistic (the difference between the means) by bootstrapping using the percentile method (with an equivalence test, the null hypothesis is the converse of $H_0$, i.e. the null hypothesis is non-equivalence). All analyses were based on 2000 balanced bootstrap samples using PROC SURVEY SELECT / PROC UNIVARIATE (SAS institute/ inc., cary, NC, USA). The 95 % CIs were calculated on the difference between the means of the groups. If the CIs embrace zero, the difference was considered non-significant ($P$=0.05). Precision was also determined with analysis os the absolute error (estimated severity minus actual severity).

## Results

The SADs designed in this study has ten levels of blast diseased wheat leaves with a range of severity from 0.1 to 72 % (Fig. 1). Blast symptoms initially appear as individual lesions on leaves, but once a severity of 22 % is reached, the lesions start to coalesce, which was considered in the development of the SADs. For all raters using the SADs, the estimated severity approached the actual severity (Fig. 2). Based on the equivalence test, all statistical parameters ($r$, $C_b$, $\upsilon$, $u$, and $\rho_c$) were significantly improved when the raters used the scale to estimate blast severity on the leaves with unknown amounts of disease, demonstrating, therefore, that both accuracy and precision of the estimated values were improved (Table 1, Fig. 2).

**Table 1** Effect of using a standard area diagram set (SADs) as an assessment aid on the bias precision and agreement of assessments of severity of blast on wheat leaves on 50 diseased leaves estimated by12 raters

| Variables | Means[a] | | Difference[b] between means | 95 % CIs[c] of the difference |
|---|---|---|---|---|
| | No SADs | With SADs | | |
| Scale ($\upsilon$)[d] | 1.25 (0.23) | 1.01 (0.04) | −0.234 (0.0013) | −0.33– −0.10 |
| Location ($u$)[e] | 0.28 (0.36) | 0.03 (0.09) | −0.242 (0.0024) | −0.44– −0.01 |
| Coefficient of bias ($C_b$)[f] | 0.88 (0.07) | 0.99 (0.01) | 0.111 (0.0004) | 0.07–0.15 |
| Correlation coefficient ($r$)[g] | 0.94 (0.02) | 0.97 (0.01) | 0.029 (0.0001) | 0.01–0.03 |
| LCCC[h] | 0.84 (0.08) | 0.96 (0.02) | 0.127 (0.0004) | 0.09–0.16 |
| Inter-rater coefficient of determination ($R^2$) | 0.87 (0.75–0.94) | 0.92 (0.85–0.96) | 0.045 (0.0001) | 0.03–0.05 |

[a] The values for standard deviation are in parentheses

[b] Mean of the difference between each rating. The values for standard errors are in parentheses (bootstrap calculated value)

[c] 2000 bootstrap samples were used to obtain the confidence intervals (CIs). If the CIs embrace zero, the difference was not significant ($P$=0.05)

[d] Scale bias or slope shift ($\upsilon$, 1 = no bias relative to the concordance line)

[e] Location bias or height shift ($u$, 0 = no bias relative to the concordance line)

[f] The correction factor ($Cb$) measures how far the best-fit line deviates from 45° and is a way to measure accuracy

[g] The precision is measured by the correlation coefficient ($r$)

[h] Lin's concordance correlation coefficient (LCCC) combines both measures of precision (r) and accuracy ($Cb$) to measure agreement with the true value

Without use of the SADs, the precision ($r$) ranged from 0.89 to 0.95, with a means of 0.94, but when the SADs were employed, precision ranged from 0.95 to 0.99, with a mean of 0.97 (Table 1, Fig. 2). The most precise evaluation of blast severity using the SADs was also confirmed by reduction in absolute error (Fig. 3).

Agreement ($\rho c$) was higher when the SADs were used (Fig. 2) and the mean value of $\rho c$ increased from 0.84 to 0.96. In general, the values of $u$ and $\upsilon$ (location

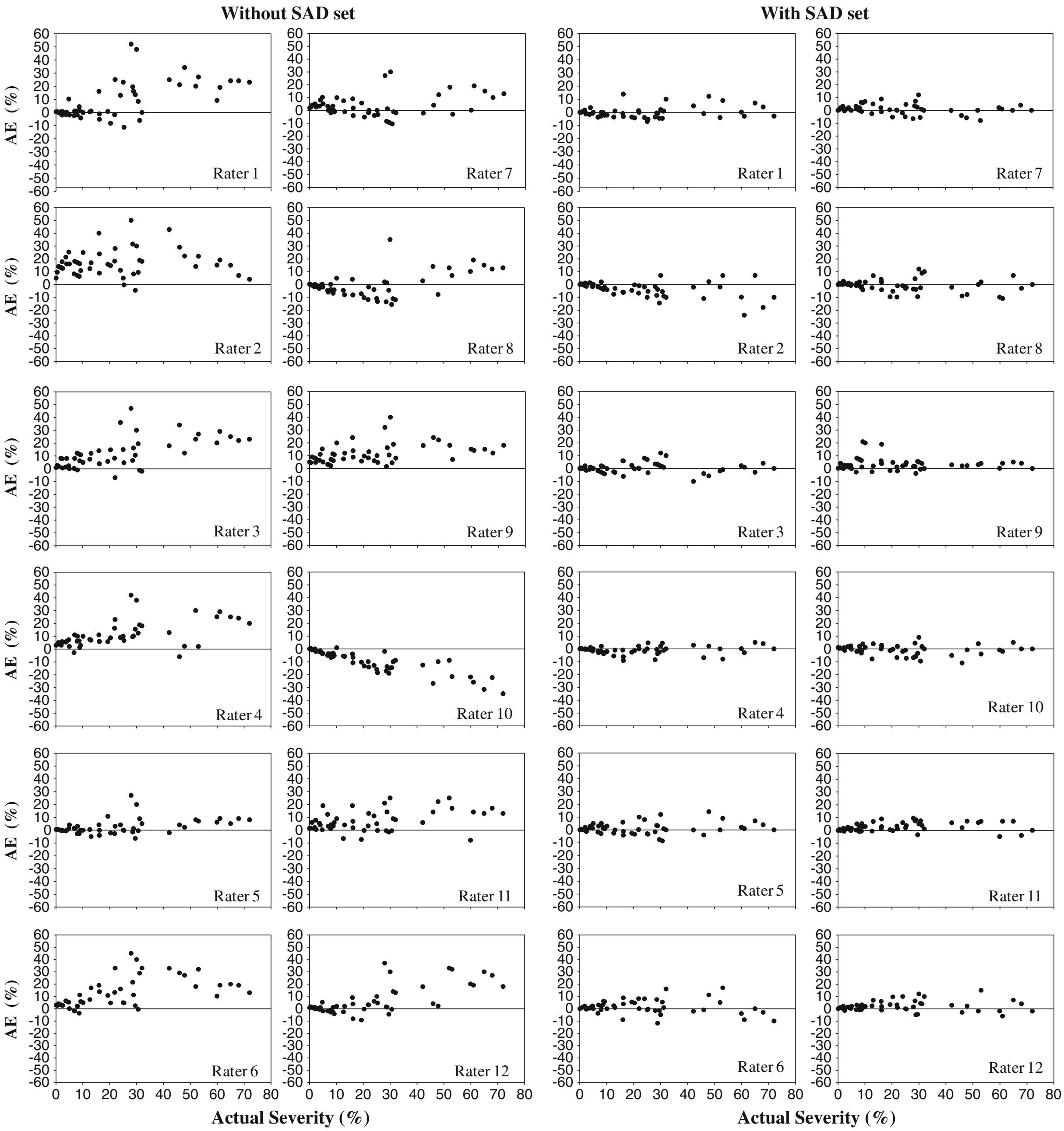


**Fig. 3** Estimates of the absolute error (AE) (estimated severity minus actual severity) for assessment by each of the ten raters both with and without the use of the standard area diagram set (SADs). Low absolute error values indicate that the estimated and the actual severity are similar

and scale bias ), approached 0 and 1 when the SADs were used (Fig. 2). The correction factor ($C_b$) values approached 1 for all raters who used the SADs (Fig. 2).

When using the SADs, the raters showed a tendency to overestimate severity. However the overestimate was lower when using the SADs than without then. This trend can be confirmed by analyzing the positive absolute errors in most of the estimates (Fig. 3) as well as the positive values for location bias ($u$) for 75 % of the raters (Fig. 2).

Without the use of SADs, raters had higher deviations in error, and for 83.3 % of the raters (1, 2, 3, 4, 5, 6, 7, 9, 10 and 12), the errors exceeded 25 %. However, when the SADs was used, 75 % of the raters (1, 3, 4, 5, 7. 8. 10, 11 and 12) had no errors above 15 %, and the 25 % had few cases in which the errors surpassed 15 %. For all raters, the absolute errors were lower than 24 % and were clustered at severities close to10% when the SADs was used (Fig. 3).

The inter-rater reliability was consistently high between pairs of raters. Without the use of the SADs, 70.6 % of pairwise comparisons of the raters had coefficient of determination ($R^2$) values lower than 0.90. The remaining 29.4 % had $R^2$ values > than 0.90 (Fig. 4). With SADs, 18.4 % had $R^2$ values < 0.90, and 81.6 % had $R^2$ values > 0.90 (Fig. 4) indicating that the used SADs provided more reliable results.

## Discussion

SADs are important for quantification of disease severity in epidemiological studies, aimed at obtaining more accurate and reliable data that can help to decide the best method of disease control to be used (Nutter et al. 1993). SADs have been demonstrated to improve the accuracy and reliability of estimates of several crop diseases including soybean rust on soybean (Godoy et al. 2006), coffee leaf rust on coffee (Capucho et al. 2011), white spot on corn (Capucho et al. 2010), *Ramularia* on cotton (Aquino et al. 2008) and brown spot on rice (Lenz et al. 2010). This is first report of a SADs to estimate blast severity for the wheat-*P. oryzae* pathosystem. During SADs validation, the raters tended to overestimate the severity of the disease; a similar phenomenon was observed in previous studies (Newton and Hackett 1994; Parker et al. 1995; Diaz et al. 2001; Capucho et al. 2010; Lenz et al. 2010), although, in some others studies, the severity was underestimated (Gomes et al. 2004; Michereff et al. 2000).

The SADs proposed in this study have ten images with a range of severity linearly distributed from 0.1 to 72 %. The number of diagrams is considered quite sufficient to include the levels of blast severity. A few amounts of diagrams contained in a SADs can compromise the accuracy and the precision of the values of blast severity selected by the raters (Yadav et al. 2012). However, an excessive number of diagrams can be time consuming and affect the efficiency of the assessments (Correa et al. 2009; Yadav et al. 2012). A linear arrangement of SADs has been used in other scales assure more precise and accurate assessments (Yadav et al. 2012), and the use of logarithmic distribution in the SADs has been questioned (Bock et al. 2010).

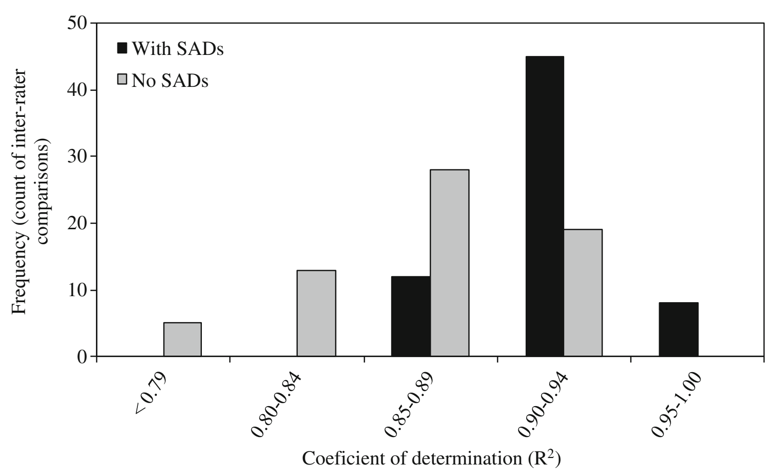


**Fig. 4** Inter-rater reliability determined by the coefficient of determination ($R^2$) without and with use of the standard area diagram set (SADs) disease severity estimates were made by assessment aids by 12 raters who assessed a set of 50 images of blast diseased leaves of wheat

In the present study, accuracy, precision and reliability of SADs to estimate blast severity was determined using an equivalence tests considered to be an important statistical tool for agreement studies (Yi et al. 2008). The equivalence test has been used in the statistical analysis of other SADs improved agreement and reliability (Yadav et al. 2012; Bardsley and Ngugi 2012). In the present study, the equivalent test (based on 95 % CIs by bootstrapping of the difference between the means) demonstrated that the values for all the statistics parameters ($r$, $C_b$, $\upsilon$, $u$, $\rho_c$) were greatly improved for the raters when using the SADs to estimate blast severity compared to when not using them.

The reliability of estimates between raters is another indicator of the effectiveness of SADs (Berger 1980). When different raters use the same SADs to evaluate the severity of diseased leaves, the estimates should be similar (Nutter and Schultz 1995). The inter-rater was measured using the coefficient of determination ($R^2$), when comparing two raters, if their estimates are identical, the $R^2$ will approach 1.00 (Belasque et al. 2005). The reliability of the estimated severities between raters was higher when using the SADs proposed in the present study. For the evaluations made without using the SADs, the $R^2$ values were lower than 0.90 for 70.6 % of the comparisons between raters. Using the SADs, the $R^2$ values were higher than 0.90 for 81.6 %, indicating high reliability of estimates when the SADs is used. The use of the SADs to assess blast severity contributed to a reduction in the absolute errors. Therefore, it to training people who will be involved in blast severity assessments to assess disease using SADs may help to reduce the absolute errors, increase the efficiency of the evaluations, and standardize the results as reported by Stonehouse (1994) when quantifying diseases on Andean bean.

In conclusion, the results from the present study show that the SADs developed to evaluate blast severity on wheat leaves increased the agreement and the reliability of estimates. Given the importance of blast control for wheat production, this SADs may become a valuable tool for epidemiological studies aimed at improving disease control.

**Acknowledgments** F.A. Rodrigues thank the National Counsel of Technological and Scientific Development (CNPq) for his fellowship. Mrs. Jonas A. Rios, D. Debona, and H. S. S. Duarte were supported by CNPq. We are grateful to Dr. C. H. Bock for his help with the statistical analysis. This study was supported by grants from CAPES, CNPq and FAPEMIG to Prof. F. A. Rodrigues.

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