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An Assessment Key for Tea Blister Blight: Development and Validation

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ABSTRACT

Blister blight caused by *Exobasidium vexans* Massee is the most problematic foliar disease in tea in Sri Lanka. An accurate, reliable and cost-effective method for epidemiological and disease control studies is a need of the industry. An assessment key with 6 scales was developed considering the lowest and highest limits of severity observed in the field and the development stages of the symptoms. The minimum and maximum severity limits represented in the scales were 0 and >30%, respectively. A software was also developed to train raters on use of the key. The key was validated by six raters consisting of 3 experienced and 3 inexperienced raters who utilized 100 diseased leaf images with different levels of severity. The key permitted assessments to be accurate (intercepts of the regression lines between actual and estimated severity did not differ from 0 and angular coefficients were near 1), and precise ($R^2 > 0.80$). The reproducibility of the assessments, estimated by the coefficient of determination of regression lines between first and second assessment was high ($R^2 > 0.80$). Training of the raters with the software improved the accuracy and precision of the assessments of both experienced and inexperienced raters. The scales proved to be reliable for assessments of blister blight severity in the field and the training software to be useful in training raters.

Key words: Blister blight, disease assessment, *Exobasidium vexans*, training software

INTRODUCTION

Blister blight caused by *Exobasidium vexans* is a destructive foliar disease of tea in almost all tea growing countries in Asia. Since its introduction to the island in 1946, blister blight has been the most problematic disease in Up country, Mid country tea growing regions and some parts of low country tea growing areas as well. Blister blight symptoms on leaves are somewhat complex and changes as the infection progresses over a period of several weeks. The disease first appears as translucent spots about 9 days after infection. These spots become circular blisters after a further 7-9 days and the upper leaf surface becomes indented corresponding to protrusion of a blister from the lower leaf surface. Only young succulent leaves and stems are susceptible to this disease and which reduces the quality and quantity of the leaf harvested (Gadd and Loos, 1949; Balasuriya, 2003).

Quantification of a disease is particularly important to estimate yield loss, assess various germplasm, varieties and/or cultivars for disease resistance and for disease management practices to assess the value of treatments in reducing the economic impact of many diseases (Nutter *et al.*, 2006). The intensity of a disease can be measured as incidence (proportion of affected plants/plant parts in a sample), severity (percentage of the plant area affected), or disease count (number of lesions or colonies) (Madden *et al.*, 2007). There are only few studies to guide researchers on developing disease assessment methods and selecting the best assessment method.

Blister blight disease assessment has been carried out measuring incidence (proportion of affected shoots in a sample) (Webster and Park, 1956), or number of lesions or blisters on a leaf (Balasuriya, 2003). These methods are relatively easy to use and applicable over wide range of conditions. However, the current assessment methods have limited value because they do not take into account the development stages of the disease and disease severity especially for situation in which sizes/development stages of blister vary greatly. Since the counts of infected leaves or number of blisters are unlikely to represent actual disease intensity, the economic impact of the disease and resistant level estimated can vary.

Use of computer based software has been shown to improve overall accuracy and precision of the users in the literature, even though they had previous experience with visual estimation methodologies. Greater improvements after training could be expected for users with no previous experience. Therefore, it is important to develop computer aided training software to improve the accuracy of disease assessments.

In line with the above, experiments were carried out to (1) develop a key to estimate blister blight disease severity considering the stages of disease development (2) quantify the impact of the developed key on accurate and precise of disease estimates by raters and (3) develop a software to train raters to improve their ability of the precise and accurate disease assessment using the developed key.

MATERIALS AND METHODS

Plant materials and image acquisition

Leaves naturally infected with blister blight were collected from fields regardless of cultivar, to represent a wide range of severity. One hundred leaves were chosen arbitrarily to reflect the approximate frequency and range of blister blight severities in the field and photographed with a blue background using Nikon D40 (Japan) camera. For the selected images, "true" disease severity was estimated using ASSESS 2.0® image analysis software (Image Analysis Software for Plant Disease Quantification, APS, St. Paul, MN, USA, 2008). Severity of blister blight is defined as the proportion (percentage) of leaf area with translucent spots, blisters and/or necrotic spots. The experiment was repeated twice.

Development of the key

Based on the maximum and minimum levels of severity observed, a scale with 0-6 levels of severity was developed, based on the form and distribution of the observed lesions (Table 1). The developed key represents all possible disease symptoms in tea following infection of *E. vexans*. This includes the area covered by initial translucent spots and then immature and mature blisters and necrotic spots. Further, it also represents more severe stages of the disease development including stem infections, leaf fall and leaf distortion which have not been considered in the previous assessment methods.

Development of training software

Training software was developed in Visual Basic 6.0. Large number of diseased leaves representing range of severity levels was photographed. The actual blister blight severity of the leaves was determined using Assess 2.0® software, scaled using the key and uploaded into the software.

Validation of the key

For validation of the key, the leaf images were assessed by 3 inexperienced and 3 experienced raters using the developed key. These images were inserted randomly in individual slides for visualization in Microsoft® PowerPoint 2007. To evaluate the repeatability of the accurate and precise estimates using this key, a second assessment was carried out seven days later, with the same raters, as described previously.

The raters were trained with the developed training software on using the disease assessment key. After the training, assessments were carried out with the same evaluators.

The accuracy and precision of each rater was determined through linear regression analysis, with real severity achieved with the aid of the Access 2.0® software.

The accuracy of estimation of each rater, as well as the group of raters, was determined by 't' test applied to the intercept of linear regression (a), to verify the hypothesis $a = 0$ and to the angular coefficient of the straight line (b), $b = 1$ at 5% probability level (P=0.05). The equations of linear regression were calculated where actual severity was the independent variable and estimated severity as the dependent variable. The estimated repeatability by the same rater was determined using these same parameters obtained in regression where the first evaluation was compared to the second (Nutter *et al.*, 1993).

The precision of estimation was determined by the coefficient of determination of the regression (R^2) and by the variance of absolute errors. Repeatability of the key was determined by the regression of the second evaluation compared to the first using same samples.

Inter-rater variability was determined by inter-class correlation coefficient analysis. The regression analyses and graphs were performed using SigmaPlot 10.0 (Systat software, 2006) and interclass correlation analysis using SPSS 10.0.

RESULTS AND DISCUSSION

Blister blight severity ranged from 0.09% to 66% in the field. Forty four percent (44%) of the leaves had 5-15% severity and only 7% of the leaves showed more than 30% infection (Figure 1). The developed percentage scale was elaborated with six levels of severity to quantify blister blight in tea leaves (Table 1). The representation of the symptoms in the key includes hypersensitive reaction (HR), translucent spots, blisters and necrotic spots. HR, the resistant reaction mostly observed in highly resistant cultivars, was given a very lower scale (1) in the key. Considering sequential development order and extend of damage at each stage, the intermediate scales were determined to satisfy the logistic increment of disease severity (Weber-Fechner's law, Horsfall and Barratt, 1945). Values of severity higher than 30% have not been considered in forming intermediate leaves of the scale, because the frequency in the field has been only 7%.

Accuracy is represented by the closeness of the estimate value to the true value and, measured by the intercepts (a) and the slope coefficients (b) of the regression line between actual and estimated severity. Truly accurate assessments are those where the slope of the regression line is equal to 1 and the intercept of the regression line is 0 (Nutter *et al.*, 1993).

Table 2 depicts the severity estimates of six raters including 3 raters who have previous experience in blister blight assessment and 3 raters who do not have any previous experience in blister blight assessment but have substantial knowledge about the disease and the development stages.

Table 1. Key for blister blight severity assessment in tea

Scale	Description
0	No translucent spots/ blisters/necrotic spots (No disease)
1	All translucent spots show hypersensitive reaction (HR)
2	Translucent spots cover <**2%** leaf area
3	Blisters/necrotic spots cover <**5%** of total leaf area. If only translucent spots appear, it covers **2-15%** leaf area
4	Blisters/necrotic spots cover **5-15%** of total leaf area. If only translucent spots appear, it covers >**15%** leaf area
5	Blister(s) necrotic spots cover **15-30%** of leaf area
6	Blisters and/or necrotic spots cover > **30%** leaf area and/or Twig/stem is infected/ die back/ leaf fall/stem breakage

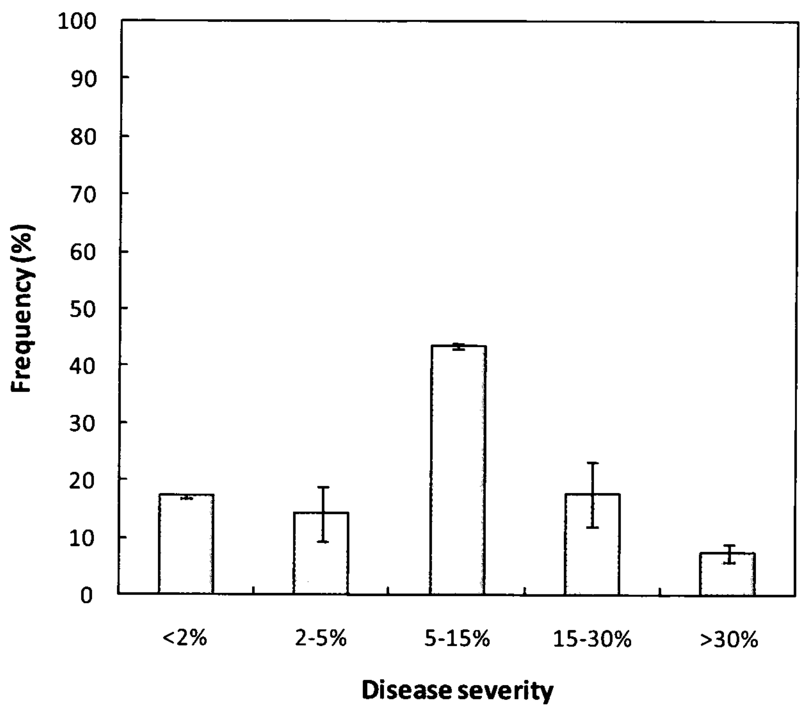

Figure 1. Frequency of blister blight severity (% area diseased) in randomly selected 100 leaves of most susceptible cultivar TRI 2024. Error bars represent standard errors of two experiments

Table 2. Intercept (a), angular coefficient of the straight line (b) coefficient of determination (R^2) of the equations of simple regression line between visual estimation of blister blight using the developed key and actual value determined by image analysis software Assess 2.0.

Rater	Assessment 1			Assessment 2			Assessment after training		
	a	*b*	R^2	*a*	*b*	R^2	*a*	*b*	R^2
Inexperienced									
1	0.629*	0.784*	0.814	0.584*	0.7953*	0.813	0.339*	0.916*	0.862
2	1.313*	0.630*	0.638	1.494*	0.447*	0.563	0.366*	0.772*	0.804
3	-0.457	1.050	0.645	-0.088	1.060	0.693	0.165	0.882*	0.848
			0.699			0.690			0.838
Experienced									
4	0.159	0.988	0.871	0.132	0.966	0.866	0.169	0.942	0.893
5	0.378*	0.912*	0.872	0.583*	0.842*	0.831	0.302	0.842*	0.866
6	0.515*	0.908*	0.807	0.278	0.960	0.852	0.205	0.963	0.924
			0.850			0.850			0.894

*Situations in which the null hypothesis (*a*=0 or *b*=1) was rejected by t-test, $p<0.05$.

Before training there was generalized over estimation of severity (Table 2). The intercept values were significantly different from 0 and angular coefficient significantly differed from 1 in 4 of the six raters in the first assessment and 3 raters in the second assessment. After the training, all trained evaluates made accurate values of intercept equal to 0 for the regression line between actual and estimated severity. After the training, though the values were significantly different from the ideal situation, intercept and angular coefficient of the regression lines of the all inexperienced rates approached 0 and 1 respectively. It is evident that the training with the use of key increases the accuracy of the assessments of both inexperienced and experienced raters and the training software is a useful tool for training inexperienced raters in the blister blight assessment. Similar situations had been reported where rater training had a positive influence on the quality of assessments, as observed by Michereff *et al.*, (2000) in the validation of a scale for yam (*Dioscorea cayennensis* Lam.) leaf blight. Nutter and Schultz (1995) showed that computer software can be used to train raters in order to improve assessments.

Precision is considered to be the repeatability or variation associated with an estimate and determined by the coefficient of determination of the regression line of each rater. R^2 of the inexperienced raters ranged from 0.64-0.81 in the first assessment and 0.53-0.81 in the second assessment. Average R^2 was 0.70 in both assessments. The precision of the assessment was increased after training with R^2 0.80-0.86 with an average of 0.83. The precision of the assessment of the experienced raters were always greater than the inexperienced raters. The training also improved the precision of the experienced raters too. However, for experienced raters, who had had previous experience in the blister blight assessment, improvements were not as significant as for inexperienced raters. Studies have demonstrated that there is great variation in the ability of people in assessing the severity of diseases with precision; the quality of the disease estimate is influenced by psychological stimuli and responses and by factors such as complexity of the sampling unit, size and shape of lesions, color and number of lesions, fatigue, and difficulty to concentrate on the task (Sherwood *et al.*, 1983).

The residual plots (estimated severity-actual severity) (Figure 2) showed under estimation at the lower level of blister blight severity and over estimation at the higher severity. This trend was also obvious after training (Figure 3). By continuous training with the software this trend could be minimized.

The reproducibility of an assessment is the ability of the rater or disease assessment system to repeat a previous assessment both accurately and precisely. The results showed that there was a good relationship between the first and second assessment (Figure 4).

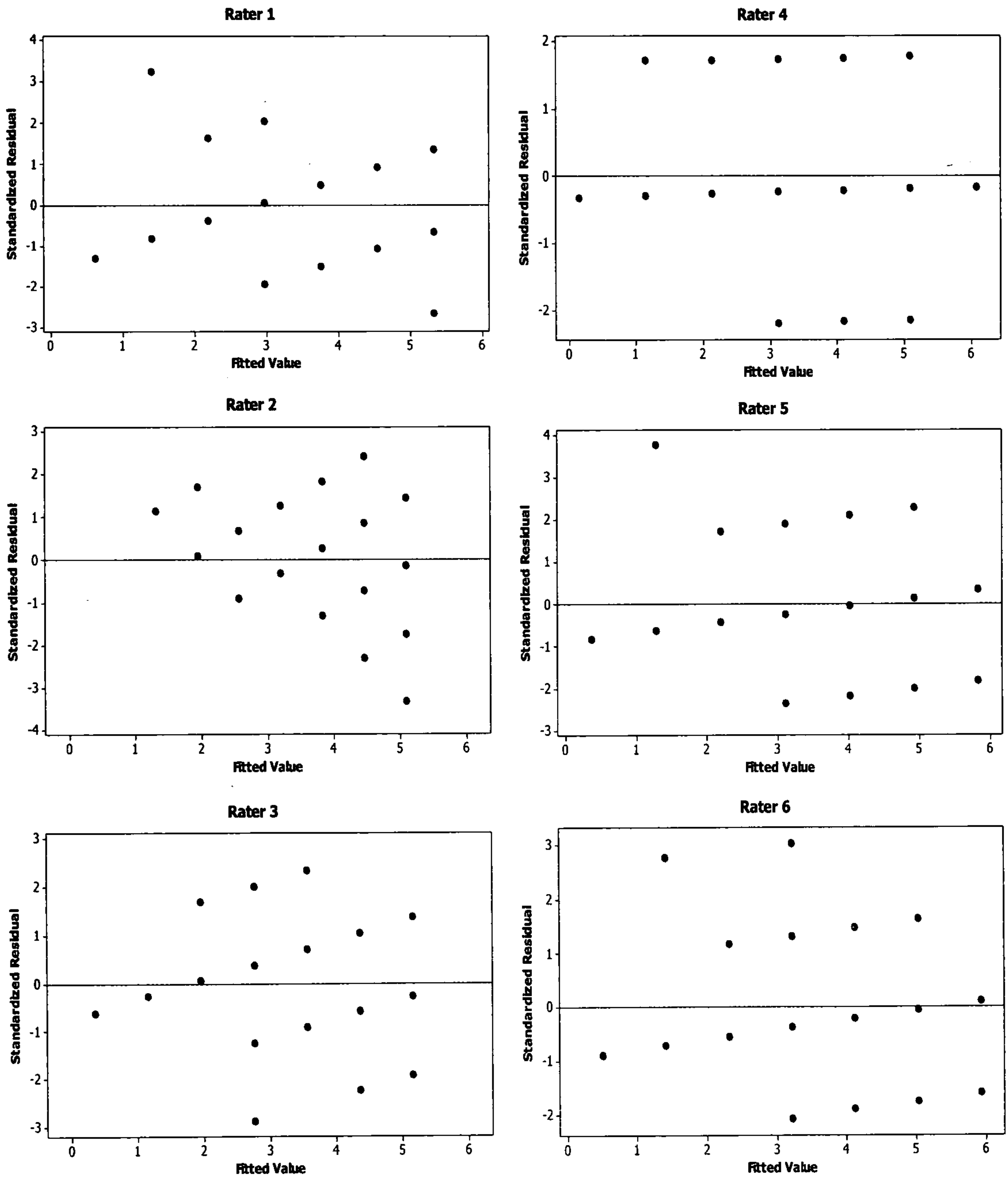

Figure 2. Distribution of the residuals (estimated severity-actual severity) of the blister blight assessment. The assessment was carried out before the raters were trained with the software.

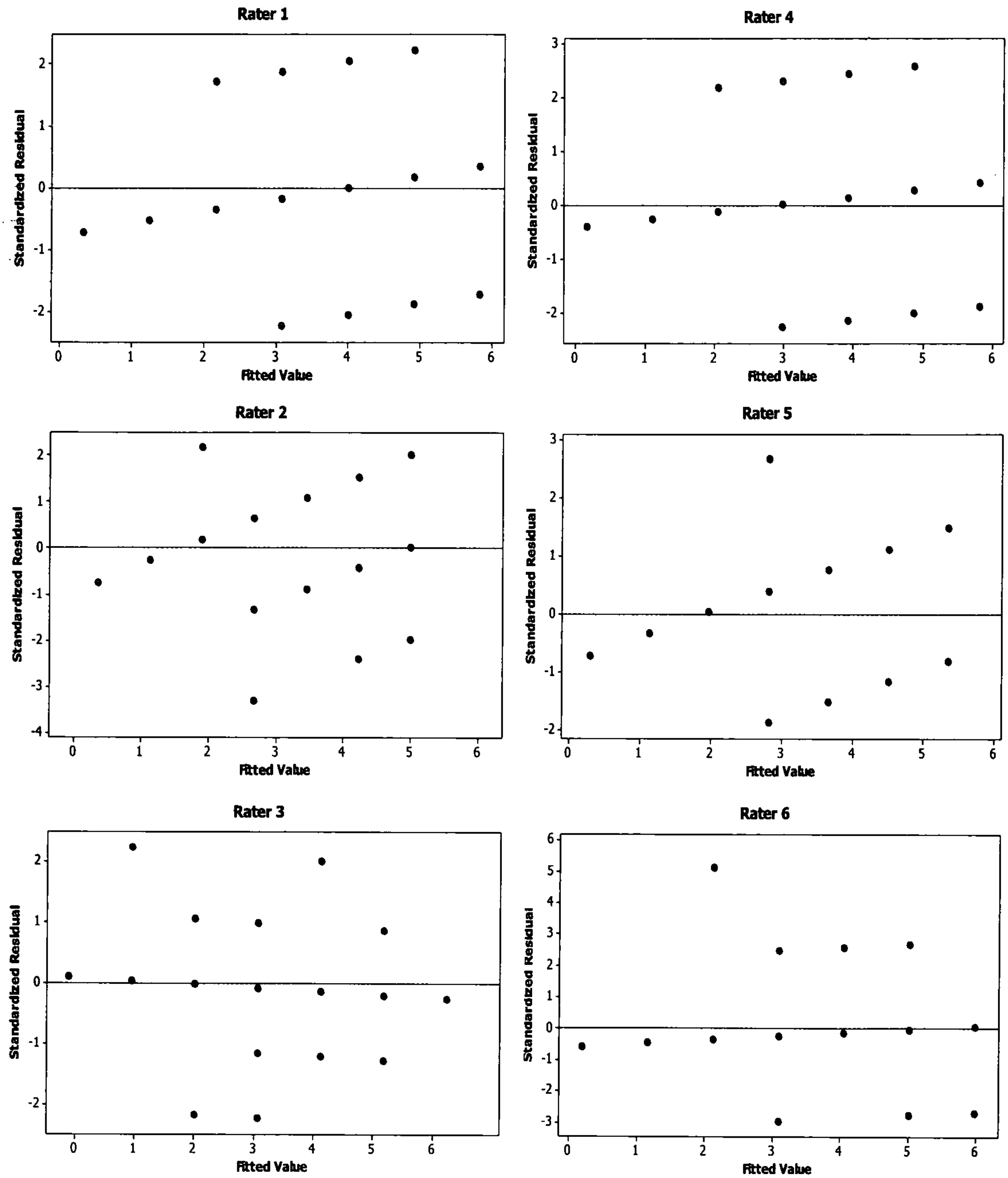

Figure 3. Distribution of the residuals (estimated severity-actual severity) of the blister blight assessment. The assessment was carried out after the raters were trained with the software.

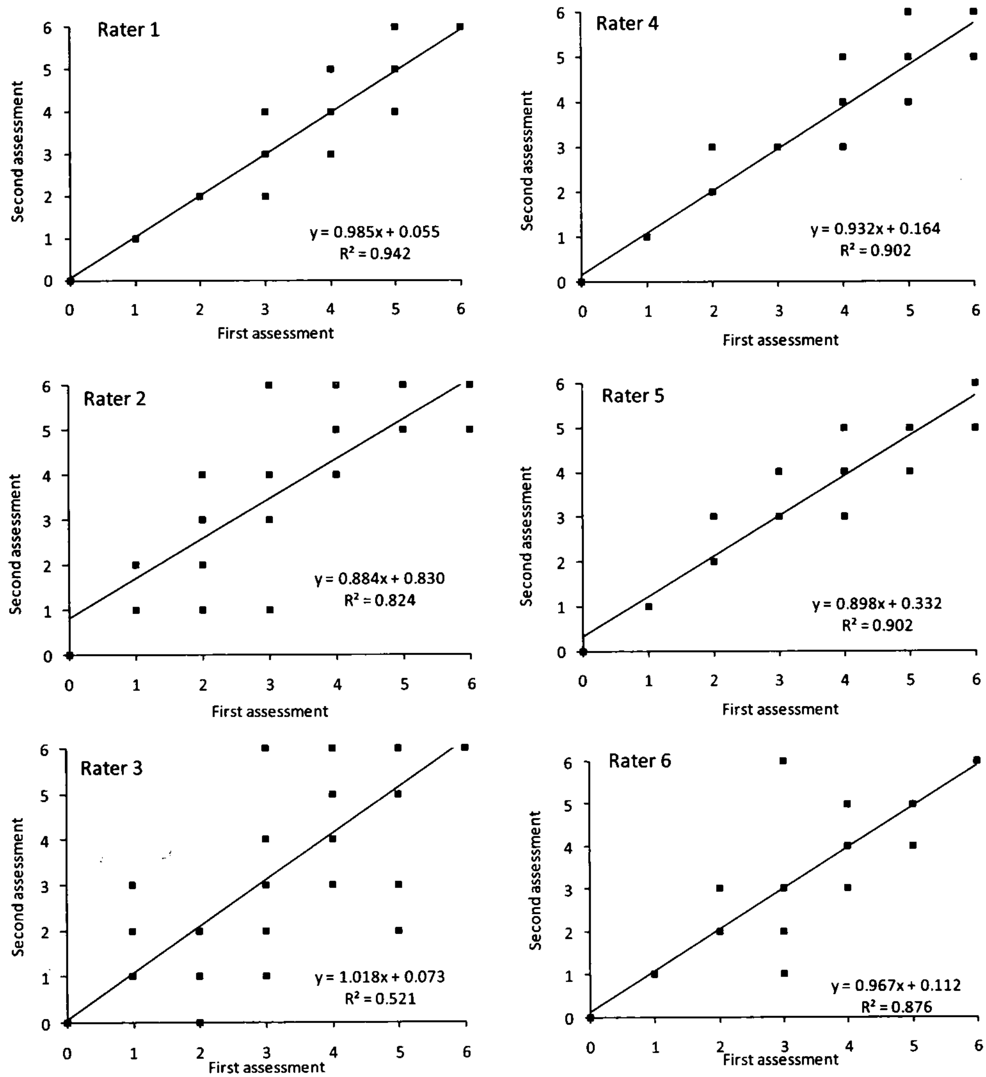

Figure 4. Estimated disease severity of blister blight in tea between the first and second disease assessments by each of the six raters in experiment repetition.

The R^2 ranged from 0.87 to 0.94 except in rater 2 (0.57). Intercept of the linear regression between first assessment and second assessment was statistically equal to 0 in all raters except raters 2 and 3. The angular coefficient was equal to 1 in the assessment made by raters 1, 2 and 6.

Inter-class correlation analysis showed inter-class correlation coefficient 0.85, 0.73 and 0.86 for first, second and the assessment made after the training, respectively suggesting higher inter-rater reliability.

CONCLUSION

The key proposed in this study produced precise, relatively accurate and reproducible estimates and proved to be adequate to assess blister blight severity in the field. The training software seems to be very good tool for training raters and to improve accuracy and precision of the key.

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