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Development of an assessment key and techniques for field screening of tea (*Camellia sinensis* L.) cultivars for resistance to blister blight☆

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ABSTRACT

Blister blight caused by the biotrophic fungus, *Exobasidium vexans* Massee, is the most problematic foliar disease of tea in Sri Lanka. A reliable and accurate method is needed for field assessment of severity of the disease for epidemiological studies, formulating disease control strategies and crop improvement programmes. A field assessment key with 0–6 scores was developed for blister blight, considering the lowest (0) and highest (>30%) limits of disease severity observed in the field and different stages of symptom development. The key was validated by six raters, 3 experienced and 3 inexperienced. The field assessment trials made using the key were accurate and precise ($R^2 > 0.80$). The area under the disease progress curve (AUDPC), calculated using the disease severity levels obtained using the assessment key, was used to combine multiple observations of disease progress into a 0–9 susceptible scale. Ascending numbers in the scale represent increasing susceptibility. The new scale was proposed to discriminate blister blight resistance in tea accessions/cultivars in field screening. Screening trials for validation of the susceptible scale, conducted using tea cultivars of known resistance or susceptibility levels and newly developed accessions of tea, at three locations, revealed that the 0–9 scale is simple to apply, offers a fine discrimination of blister blight resistance levels, and allows objective evaluation.

1. Introduction

Blister blight, caused by *Exobasidium vexans* Massee, is a destructive foliar disease that affects the tea production in terms of quantity (De Silva, 1967) as well as quality (Gulati et al. 1999). Tea plants grown mainly in higher (>1200 m) and mid (600–1200 m) elevations suffer from blister blight during South-West (May–September) and/or North- East (October–December) monsoons every year in Sri Lanka. Cool, wet weather with higher relative humidity (>80%) and long periods of surface wetness are pre-requisites for infection as well as dispersal of basidiospores of *E. vexans* (Kerr and De Silva, 1969). Blister blight affects succulent, harvestable shoots in the plucking fields and the newly emerging shoots of tea bushes, recovering from pruning, thereby causing considerable economic loss. The extent of crop loss is reported to be 32% (Loos, 1950) in Sri Lanka. However, the disease can completely prevent harvest if the cultivars are highly susceptible and the weather conditions are conducive.

Blister blight is a polycyclic disease caused by airborne basidiospores of *E. vexans*. Blister blight symptoms on leaves are somewhat complex and changing as the infection progresses over a period of several weeks. The disease first appears as translucent spots (Fig. 1A) 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 (Gadd and Loos, 1949). Basidiospores are produced generally from the lower surface giving a whitish, powdery coating to the blisters (Fig. 1A). The infected stems often break off or die-back beyond the point of infection. Fortunately, when leaves and stems mature, they become resistant to blister blight (Gadd and Loos, 1949; Balasuriya, 2003).

Tea cultivars exhibit varying levels of resistance to blister blight (Balasuriya, 2003), however, there are no cultivars showing complete resistance to the disease identified in Sri Lanka or elsewhere. The more resistant cultivars express a hypersensitive reaction (HR)

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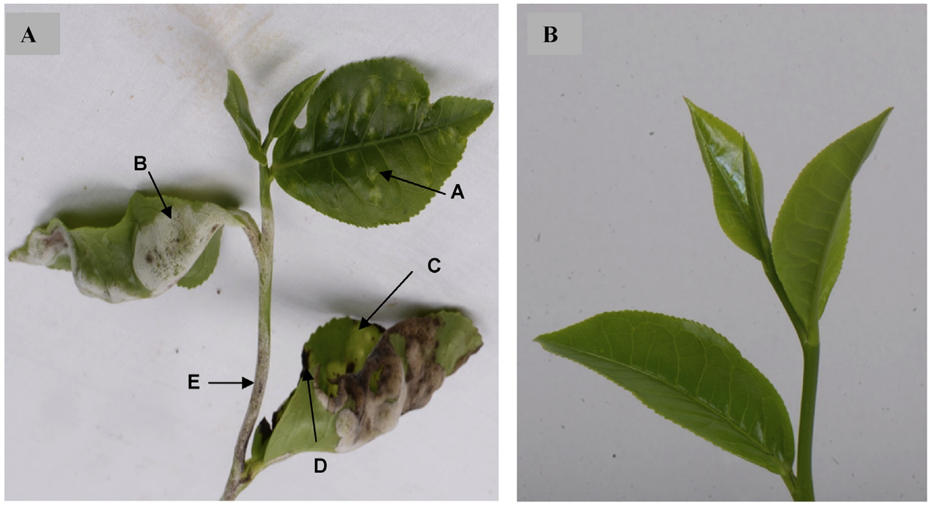

Fig. 1. A tea shoot severely infected with blister blight (A) and a healthy harvestable tea shoot (B). A-Translucent spots, B- Abaxial view of mature sporulating blister, C-Adaxial view of mature blister, D-Necrotic spot and E-Stem blister.

or smaller blisters with limited sporulation. Highly susceptible cultivars produce abundant or larger blisters with copious sporulation resulting in tissue distortion or stem breakage. Host resistance is given priority in disease control strategies, though, there is very little known about the nature of resistance expressed in tea to *E. vexans* infection. Resistant planting materials can provide economical and environmentally friendly disease management. Hence, breeding for blister blight resistance is one of the objectives of crop improvement programmes of tea.

Since *E. vexans* is a biotroph, producing basidiospores through culturing on artificial media is difficult. Use of basidiospores collected from blisters results in inconsistency in symptom development on artificial inoculation, making field screening for blister blight resistance practically impossible. Assessment of blister blight has earlier been carried out by measuring disease incidence i.e. the proportion of affected shoots in a sample (Webster and Park, 1956), or the number of lesions or blisters on a leaf more recently (Balasuriya, 2003). These methods are relatively easy to use and applicable over a wide range of conditions. However, these have limited value because they do not take into account the development stages of the disease and disease severity especially in situations where sizes and 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. An accurate blister blight assessment key for tea is, therefore, necessary for precise selection of resistant lines in breeding as well as evaluating disease management strategies.

In this paper the development of a key to estimate the severity of blister blight disease, considering the stages of disease development and phenotypic variation in resistance expression, is reported. The paper also examines the impact of the key on accurate and precise estimation of the disease by raters and standardizes a field screening technique for tea accessions/cultivars.

2. Materials and methods

2.1. Plant materials and image acquisition

Tea leaves showing blister blight symptoms were collected from fields regardless of cultivar grown, to represent a wide range of severity levels. One hundred leaves were chosen randomly to have a range of blister blight severities and digitally photographed with a blue background. For selected images, “true” disease severity was estimated using ASSESS 2.0® image analysis software (Lamari, 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 conducted twice.

2.2. Construction of the key

Taking into account the maximum, minimum and intermediate levels of severity observed, a key with 0–6 scores was constructed (Table 2). The key included different host reactions to *E. vexans* infection, the HR, the area covered by initial translucent spots and then immature and mature blisters and necrotic spots. Further, it also represents more severe stages of disease development, stem infections and leaf distortion which have not been considered in previous assessment methods.

2.3. Evaluating precision and accuracy of the disease assessment key

For validation of the key, one hundred leaf images were assessed by 3 inexperienced and 3 experienced raters using the developed key. To evaluate the repeatability of the estimates with accuracy and precision, a second assessment was carried out seven days later, using the same images and the same raters, as described

Table 1
Test accessions and ‘checks’ used in blister blight screening in on-farm trials at three different locations. S-Susceptible, R-Resistant.

Plant materials	Dayagama West	Glasgow	Mattakelle
Test accessions	15	15	15
	89	89	89
	101	101	101
	210	210	210
	272	272	–
	497	497	497
	582	582	582
Checks	TRI 4071 (S)	TRI 4071 (S)	TRI 2025 (S)
	DT1 (R)	DT1 (R)	DT1 (R)