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Variation in Blister Blight Disease Resistance among TRI 5000 Series Tea Accessions and some Underlying Biochemical Factors

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ABSTRACT

Blister blight disease is a serious economical concern to tea production in Sri Lanka which causes direct crop loss and demand high management cost. Breeding for disease resistance is the economically viable and environmentally friendly long-term strategy. Newly bred TRI 5000 accessions for upcountry region were screened for blister blight resistance under field conditions during 2013-2014. Disease severity was estimated in terms of Area Under the Disease Progress Curve (AUDPC) using a disease assessment key. Accessions 15, 89, 582 and 272 were identified as resistant, 497 and 101 as moderately resistant and 210 as susceptible based on susceptible scale. Biochemical parameters; caffeine, carotenoids, total catechins (catechin (C), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG)) and total polyphenols were analyzed using standard protocols. EC showed strong negative correlation (r^2=0.72) while EGCG had strong positive correlation with AUDPC. Principal component analysis with total polyphenol, carotenoids, caffeine, EC, EGCG and total catechins differentiated resistant accession from others. Possibility of using EC and EGCG as candidate biochemical markers for early screening for Blister blight resistance is discussed.

Keywords: Biochemical markers, disease resistance, epicatechin (EC), epigallocatechin gallate (EGCG), resistant breeding

INTRODUCTION

Plant resistance is an important integral part of integrated disease management of agricultural and plantation crop systems. Resistance is defined as 'inherent capacity of the plant to prevent or restrict the entry or subsequent activities of a pathogenic agent when the plant is exposed, under suitable environmental conditions, to sufficient inoculum of a pathogen to cause disease' (Lenne *et al.,* 1980). A resistant cultivar gets less disease than that of a susceptible cultivar. Plant resistance can also be regarded as a biological control method However for the importance, the plant resistance is always dealt separately.

Natural plant resistance can be divided as vertical (host specific) and horizontal resistance (host non-specific). Vertical resistance is conferred by single R gene and protects a host plant against only specific strains of a pathogen (pathotypes). In vertical resistance many major genes operate in a gene-for-gene manner. For each resistance gene in the host genome there is a corresponding avirulence gene in the pathogen genome (Flor, 1955). Horizontal resistance is under polygenic control and operates against all pathotypes of a pathogen and, referred as partial/quantitative resistance. Therefore, horizontal resistance is more stable and durable than vertical resistance (Keane and Brown, 1997).

Blister blight is the problematic foliar disease in tea in Sri Lanka and other tea growing countries in Asia. It is an economic concern to tea production as it causes direct crop loss, affect quality (Gulati *et al.*, 2009; Ponmurugan *et al.,* 2016) and demand higher investment for disease management contributing to higher cost of production (Jayakody *et al.,* 2006). Recommended Tea cultivars in Sri Lanka have diverse genetic backgrounds and they differ in subtle morphological, anatomical and chemical traits that affect the rate of invasion and sporulation of the blister blight pathogen *Exobasidium vexans* (Sinniah *et al.,* 2015). These differences may be very small, but they could contribute well to differences in the rate of disease in the field. The studies so far suggest that tea cultivars exhibited horizontal or partial resistance against *E. vexans* (Jayaswall *et al.,* 2016; Bhorali *et al.,* 2012). However, hypersensitive reaction is observed in a highly resistant TRI 2043 cultivar.

Plant disease resistance is achieved by two mechanisms: by preformed structures and biochemicals and pathogen induced structural and biochemical responses (Wood, 1972). Punyasiri *et al.* (2005) showed a relationship between the chemical constituents of tea leaves and blister blight disease resistance. The levels of EC were significantly higher in tea cultivars, resistant to blister blight leaf disease than those in susceptible cultivars, while the reverse was true for EGCG. The high resistance of a purple green leafed cultivar, TRI 2043, was attributed to the higher catechin content. Some morphological and anatomical characters also showed correlation with resistance. The higher resistance to blister blight in resistant tea cultivars has been attributed to higher trichome density, leaf length/width ratio and cuticle thickness (Sinniah *et al.,* 2015). Germination of basidiospores of *E. vexans* was higher on the leaf surface and in leaf wax extract of the susceptible cultivars than for those of the resistant cultivars.

Blister blight infection induced changes in levels of pathogenesis-related (PR) proteins, chitinase, β-1,3-glucanase and peroxidase (Chakraborty *et al.,* 2002, 2005; Sinniah *et al.,* 2014). Generation of H_2O_2 and O_2- at the site of infection in the resistant tea cultivar, leading to hypersensitive reaction (HR) and failure in generation of H_2O_2 and O_2- at the early stages of infection cycle in susceptible cultivars, has been reported (Sinniah *et al.,* 2014). Jayaswall *et al.* (2016) reported 25 R genes, 1337 defence-related enzymes, 9

multidrug-resistant transporters (MDR), 66 transcription factors, 9 retrotransposons, metacaspases and chaperons are involved in defense against *E. vexans*.

As tea is an important foreign revenue earning plantation crop in Sri Lanka, crop improvement through breeding is a continuous effort in tea research. In the early days, the importance was given for selection and breeding of tea cultivars with highest yield potential (Anandappa, 1993). However, with time the importance of incorporating stress resistance characters was considered and the effort taken to incorporate stress resistance together with yield, quality and other desirable traits (Kulasegaram, 1978; Gunasekare, 2012). The genetic difference in tea cultivars to blister blight is well established so that breeding resistance cultivars for blister blight is one of the objectives in tea breeding programs. Selection for resistance is by comparative measurements of plant-resistance. It is measured in terms of the amount of pathogen present at a given time compared with the amount present on or in an extremely susceptible cultivar (standard cultivar) (Vale *et al.,* 2001). The amount of tissue affected is, in general, a good estimator of the amount of pathogen present. This strategy is adopted in screening tea cultivars for blister blight resistance and disease assessment key has been modified to improve accuracy (Sinniah *et al.,* 2016).

This study aimed at studying the responses of newly developed TRI 5000 series tea accessions for up country regions to blister blight and identifying underlying biochemical factors contributing to disease resistance.

MATERIALS AND METHODS

Phase III field trials established by Plant Breeding Division at Mattakelle, Dayagama and Glasgow estates in the upcountry region (Agro-ecological zone WU2) were evaluated for blister blight disease resistance. Seven TRI 5000 series tea accessions; namely 15, 89, 101, 210, 272, 497, 582 and recommended tea cultivars DT1 and TRI 2025 were planted (about 150-250 plants per block) and maintained as per TRI recommended estate cultural practices.

Blister blight disease severity in each tea accession was assessed under natural infections during South West monsoon period (May-September) in two consecutive blister seasons in 2013 and 2014. The blister blight disease severity was evaluated as per the method explained by Sinniah *et al.,* (2016). In brief, the accessions were plucked separately at 7-8 days interval between two plucking rounds. One hundred shoots with bud plus three leaves were selected randomly. Blister blight severity was assessed on the 3rd leaf from the bud using blister blight assessment key with the score of 0-6. Disease severity index (DSI) was calculated using the formula given below;

$$\mathrm{DSI} = \frac{Sum\ of\ all\ numerical\ ratings \times 100}{Total\ number\ of\ obervations \times maximum\ score}$$

where, sum of all numerical ratings = [(0xn_1) + (1xn_2) …. + (6xn_7)]. Where n_1 to n_7 are number of infected leaves at each score.

Area Under the Disease Progress Curve (AUDPC) was calculated from DSI using the following formula;

$$\mathrm{AUDPC} = \sum_{n}^{n-1} ([(X_{i+1} + X_i) / 2])\,[t_{i+1} - t_i]$$

where: X_i is the disease intensity at the i^{th} observation, T_i the time (in days) at the i^{th} observation, and n is the total number of observations.

AUDPC summarizes the disease intensity over the experimental period. The susceptible scale (0-9) for each accession was calculated comparing with the reference cultivar TRI 2025. Ascending numbers in the scale represent increasing susceptibility (Sinniah *et al.,* 2016).

Biochemical analysis

Preparation of tea samples for analysis

Tender tea leaves (50 g) from each accession were plucked from Mattakelle estate and brought to the laboratory in ice (4 °C). Samples were immediately pre-frozen at -80°C for 6 h, and then freeze-dried for 24 h (Labconco® Corporation, MS, USA). The freeze-dried leaves were ground in the laboratory mill and passed through a sieve of 500 µm mesh. The fine powder was collected, sealed in triple-laminated aluminum foil packages and stored until use (Punyasiri *et al.,* 2015). The dry matter content was determined by the loss in mass at 105 °C (ISO 1573: 1980).

Analysis of metabolites in freeze-dried extracts using high performance liquid Chromatography (HPLC)

Caffeine, EC, ECG, EGC, EGCG, and C were quantified using the ISO 14502-2 (2005) method by HPLC (Agilent 1260 HPLC Infinity System) on a phenyl-hexyl column (Phenomenex normal phase, 250 mm x 4.8 mm x 5 µm) using elution with solvent systems (9% v/v acetonitrile, 2% acetic acid and 20 µg/ml EDTA and 80% v/v acetonitrile, 2% v/v acetic acid and 20 µg/ml EDTA) and UV detection at 278 nm. All samples were analyzed in triplicate. Caffeine was used as a standard in conjunction with individual catechins considering the Relative Response Factor (RRF) established according to ISO 14502-2 (2005) method.

Quantification of total polyphenols (TPPs)

The TPPs were determined using Folin-Ciocalteu's phenol reagent according to ISO 14502-1 (2005) method using a spectrophotometer (CARY 50Bio, Varian, USA) at 765 nm.

Determination of total carotenoids

The total carotenoid content of fresh leave sample of each tea accession was determined using spectrophotometric method according to the formula of Lichtenthaler and Wellburn (1983).

Statistical analysis

The differences in AUDPC among different TRI 5000 series accessions were analyzed using ANOVA in SAS (Version 9.0, SAS statistical software). The relationship between AUDPC and biochemical parameters were analyzed using correlation analysis. Correlation analysis and Principal Component Analysis (PCA) were carried out in R software (R version 3.4.4).

RESULTS AND DISCUSSION

Blister blight diseased severity of selected tea accessions

The Figure 1 shows blister blight severity in terms of AUDPC in seven TRI 5000 series tea accessions and one recommended tea cultivar at Mattakelle, Dayagama and Glasgow estates in two consecutive blister seasons in 2013 and 2014. The blister blight severity was significantly higher in 2013 when compared to 2014. Blister blight is highly influenced by weather. Relative humidity more than 80%, leaf wetness period 11-13 h and sunshine hours significantly contribute to blister blight infection as well as spore release (Gadd and Loos, 1949). Higher rainy days and less sunshine hours were experienced at the experiment sites during the assessment period in 2013 when compared to that of year 2014 (weather data not shown). The variation in the weather conditions between the years could be reflected in the varied responses of the accessions in 2013 and 2014. It is interesting to note that the 'year * location' and

'year * accession' effects were significant (p<0.001).

The accessions responded to blister blight in significantly different manner (P<0.001). The accessions 101 and 210 showed higher AUDPC compared to other accessions. Accessions 15, 89 and 582 showed significantly lower AUDPC indicating less blister blight severity. AUDPC was converted to susceptible scale as described in Sinniah *et al.,* (2016) (Figure 2). The accessions were categorized as resistance if their susceptible scale is 0-3, moderately resistant and susceptible if their susceptible scale falls between 3-6 and more than 6 respectively. Thus, accession 15, 89, 582 and 272 were identified as resistant, 497 and 101 as moderately resistant and 210 as susceptible accession.

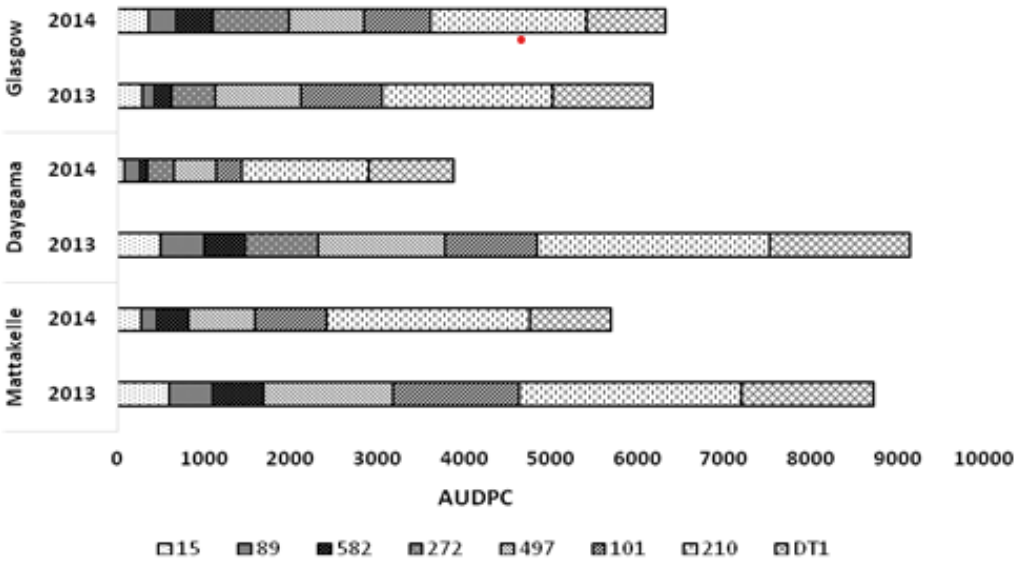

Figure 1. Blister blight severity of TRI 5000 series tea accessions in terms of Area Under the Disease Progressive Curve (AUDPC) at 3 different locations during disease seasons in 2013 and 2014. DT1-recommended tea cultivar

The blister blight severity of accessions significantly varied among all three locations (p<0.001). This is due to variations in topology and microclimatic conditions of the fields. However, when the susceptibility scale was derived comparing to a standard susceptible cultivar, the level of resistance expressed by the accession remained same across the locations. *i.e.* the accessions behaved similarly towards blister blight disease in all three locations. This is also confirmed as the 'accession x location' interaction was statistically non-significant (P=0.152).

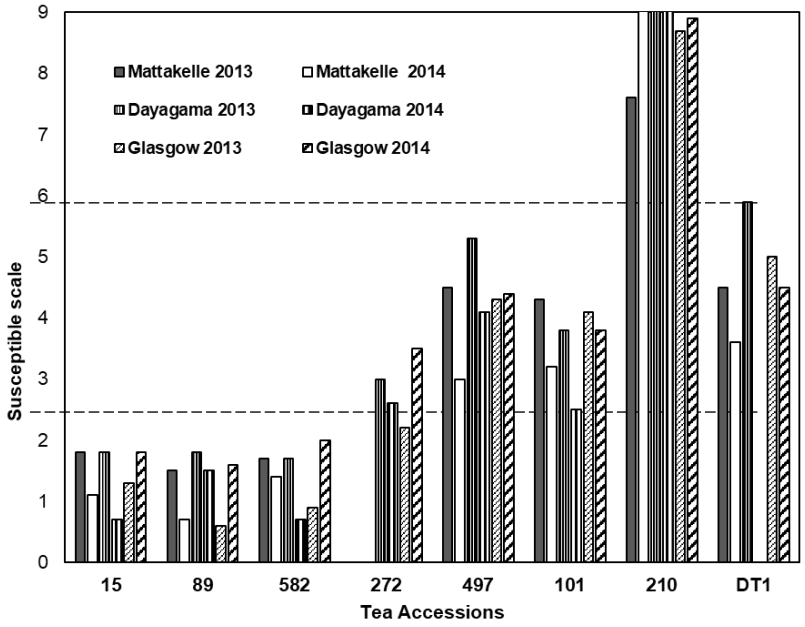

Figure 2. Susceptibile scale of TRI 5000 series tea accessions in terms of Area Under the Disease Progressive Curve (AUDPC) in 3 different locations during disease seasons in 2013 and 2014. DT1-recommended tea cultivar

Biochemical properties of TRI 5000 series tea accessions

The secondary metabolites of tea include polyphenols, alkaloids, volatile oils and others. The major polyphenols are flavanols which include C, EC, ECG, EGC, EGCG, proanthocyanidins, theaflavins, thearubigins. Caffeine is the major alkaloid in tea. Biochemical properties of tea leaves contribute to quality (Engelhardt, 2010), resistance against biotic (Punyasiri *et al.,* 2005) and abiotic stresses (Zeng *et al.,* 2018; Cheruiyot *et al.,* 2007) and health benefits to consumers (Khan and Mukhtar, 2013). Among them polyphenols and alkaloids play major role in defense mechanisms against pathogen infections (Punyasiri *et al.,* 2017).

Table 1 shows the selected biochemical parameters of green leaf of 5000 series accessions and few selected recommended tea cultivars for comparison. Among the studied parameters, EC (r^2 = -0.72) and EGCG (r^2 = 0.80) showed relatively strong correlation with AUDPC (Table 2). This indicates, EC content in resistant accessions is higher than that of the susceptible accessions. ECGC content showed the reverse relationship with blister blight resistance. Similar association of EC and ECGC content of green leaf with blister blight resistance was reported for tea cultivars (Punyasiri *et al.,* 2005). A weak correlation was observed in catechins, EGC and ECG with AUDPC.

A PCA was performed with biochemical parameters which show moderate to high correlation with AUDPC namely TPP, carotenoids caffeine, EC, EGCG and total catechins. Three PCs explained about 79% of observed variations. EGCG, contributed to PC1 (Eigen values 0.60). EC, total catechins and carotenoids mainly contributed to PC2 (Eigen value 0.62, 0.53 and 0.43 respectively). Total polyphenol content contributed to PC3 (Eigen value 0.95).

Table 1. Biochemical parameters of TRI 5000 series accessions

Accessions	TPP%	Caro (mg g^{-1})	Caf (%)	C (%)	EGC (%)	EC (%)	EGCG (%)	ECG (%)	Total Cate (%)
15	28.54	0.937	2.87	0.29	2.97	2.64	6.05	3.80	15.76
89	24.41	1.144	3.64	0.49	3.39	3.02	5.94	3.82	16.66
101	26.51	0.794	3.17	0.36	4.52	2.01	5.95	1.94	14.79
208	28.38	0.642	3.81	0.29	4.52	1.47	7.46	1.94	15.70
210	25.38	0.787	3.05	0.29	2.66	0.93	8.90	2.29	15.07
272	25.41	0.956	3.30	0.51	2.78	1.63	3.85	1.33	10.11
497	26.22	0.687	3.74	0.08	3.56	1.59	7.80	2.88	15.91
582	23.85	0.758	2.93	0.36	3.84	1.57	6.84	1.94	14.55
DT1	24.94	0.745	3.74	0.71	4.19	2.12	8.07	3.48	18.58
2025	27.11	0.975	3.57	0.56	3.28	1.24	9.66	2.75	17.49

*TPP= Total Polyphenols, Caro= Carotenoids, Caf= Caffeine C= catechin, EGC= Epigallocatechin, EC=Epicatechin, EGCG= Epigallocatechin gallate, ECG= Epicatechin gallate

Table 2. Correlation analysis between biochemical parameters and blister blight disease severity in terms of AUDPC

	AUDPC	TPP	Car	Caf	C	EGC	EC	EGCG	ECG	Total Cat
AUDPC	1.00									
TPP	0.27	1.00								
Caro	-0.32	-0.17	1.00							
Caf	0.28	0.07	-0.13	1.00						
C	0.03	-0.32	0.41	0.19	1.00					
EGC	0.00	0.10	-0.53	0.40	0.06	1.00				
EC	-0.72	-0.04	0.54	0.02	0.20	0.10	1.00			
EGCG	0.80	0.12	-0.33	0.27	-0.02	0.08	-0.50	1.00		
ECG	-0.18	0.07	0.38	0.18	0.14	-0.11	0.67	0.23	1.00	
Total Cat	0.33	0.11	-0.08	0.41	0.19	0.35	0.21	0.72	0.72	1.00

*AUDPC= Area Under the Disease Progress Curve, TPP= Total Polyphenols, Caro= Carotenoids, Caf= Caffeine C= catechin, EGC= Epigallocatechin, EC= Epicatechin, EGCG= Epigallocatechin gallate, ECG= Epicatechin gallate

Tea accessions mainly grouped into two clusters (Figure 3) along PC1. All the resistant accession 15, 89, 272 and 582 and a moderately resistant accession 101 grouped in Cluster 1. Cluster 2 included accessions/cultivars moderately resistant and susceptible to blister blight. This grouping reflects the field screening results of blister blight resistance. Therefore, there is a possibility of using biochemical compounds as markers for resistance to blister blight in tea breeding programs. Among the biochemical parameters tested EC and EGCG showed strong negative and positive correlation, respectively with blister blight disease severity in terms of AUDPC and they were the major contributors to PC1 & PC2 in PC analysis Therefore, EC and EGCG content can be proposed as potential biochemical markers for blister blight resistance screening. In support of this, Punyasiri *et al.* (2015, 2017) also reported high EC and low

EGCG content is associated with blister blight resistance in tea cultivars. Further, it has been studied that increasing EC content and decreasing EGCG content with leaf age is related to ontogenic resistance of mature leaves to blister blight resistance (unpublished data).

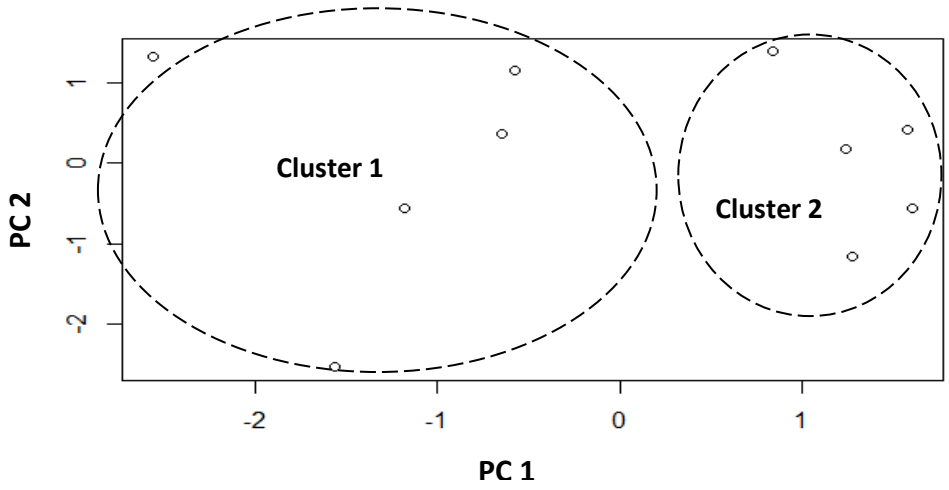

Figure 3. Principal component analysis with biochemical parameters total polyphenols, carotenoids, caffeine, epicatechin, epigallocatechin gallate and total catechins

Biochemical and molecular markers have been identified as effective tools in early identification of disease resistance in breeding programs. Though field evaluation is the most reliable method for disease screening, biochemical markers help identifying putative resistant candidates in early stages in the breeding programs where large number of plant materials is handled. This approach would save cost, manpower and time in the subsequent field screening. Ability of screening independent of natural infection or artificial inoculation is an added advantage in using biochemical markers in blister blight resistant screening. Because blister blight is a strictly obligate pathogen and the disease development is highly influenced by weather conditions. Use of biochemical marker is a suitable method for screening of hybrid progenies.

Biochemical markers have been successfully studied for identifying resistance of banana against *Fusarium oxysporum* f.sp. *cubense*, race 1 (Kavino *et al.,* 2009), damping-off and root-rot diseases resistance of peanut mutants (Khaleifa *et al.,* 2006), Fusarium wilt resistant in *Musa paradisiaca* (L.) cv. (Krishna *et al.,* 2013), resistance against *Diaporthe phaseolorum* var. *caulivora* in *Glycine max* (Simoni *et al.,* 1995). Preformed and induced biochemical compounds such as polyphenols, proteins, PR-proteins, phytoalexins, alkaloids, terpenes have been identified as candidate biochemical markers for predicting disease resistance (Reuveni, 1995, Hernandez *et al.,* 2006) in plants.

For successful use of any marker, it should be present at all growth stages of the plant,

relatively independent of the growth conditions and strong positive correlation with resistance or susceptibility. Thus, validation studies are required to establish EC and EGCG as biochemical markers for predicting blister blight resistance in tea. Mechanism of EC and EGCG in blister blight resistance should also be elucidated.

CONCLUSIONS

Newly developed TRI 5000 series tea accessions mainly grouped as resistant (15, 85, 272 & 582), moderately resistant (101, 497) and susceptible (210) based on blister blight severity in the field. Resistant accessions had lowest disease severity regardless of locations and disease seasons. Principal component analysis using selected biochemical parameters separated resistant accessions from others. Among the studied biochemical parameters, EC, EGCG showed strong negative and positive correlation, respectively with disease severity in terms of AUDPC. Epicatechins (EC) and epigallocatechin gallate (ECGC) were identified as candidate biochemical markers for screening blister blight resistance.

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