



Assessment of som (*Persea bombycina*) clones for resistance against *Phyllosticta persae*

Soumen Chattopadhyay^{1,2}, Charnenko D. Sangma¹, Amalendu Tikader¹, Rajendra K. Rajan³ & Bharat B. Bindroo²

¹Regional Muga Research Station, Central Silk Board, Boko 781 123, Assam, India; ²Central Sericultural Research and Training Institute, Berhampore 742101, West Bengal, India; ³Central Muga Eri Research and Training Institute, Central Silk Board, Lahdoigarh 785 700, Assam, India

Author for correspondence: S. Chattopadhyay, e-mail: soumenchatto@rediffmail.com

ABSTRACT

Som (*Persea bombycina*) is an economically important tree used as food source by the muga silkworm, *Antheraea assamensis*. *Phyllosticta* leaf spot (PLS), caused by *Phyllosticta persae*, affects all som cultivars during June to September (rainy season) and is responsible for foliage losses up to 26%. Information about the effect of this disease at the farmer level and host resistance is lacking. PLS severity was assessed in six major sericulture areas of Assam, India. Furthermore, eight clones were evaluated for resistance in experimental field trials. Disease incidence and severity index (DSI), area under disease progress curve (AUDPC), apparent infection rate, lesion diameter and frequency all indicated differences among clones. DSI and AUDPC values were 3.6 fold and 4.2 fold higher in the most susceptible accession M-8 compared to M-5. High broad sense heritability ($h^2 = 0.80$) of DSI suggested an additive nature of resistance. Correlations of DSI (r range: 0.55 to 0.91) with other parameters provided a good empirical evidence of obtained PLS responsiveness. M-5 showed partial resistance on the basis of all measures and appeared as the most diverse accession based on Euclidean distance. These results suggested that clone M-5 may be a potential source of resistance for use in PLS breeding programs.

Key words: *Persea bombycina*, *Phyllosticta* leaf spot, multi-parametric screening, resistant clone.

INTRODUCTION

Som (*Persea bombycina* Kost.), the primary food plant for the Indian golden muga silkworm (*Antheraea assamensis* Helfer; Lepidoptera, Saturniidae), grows abundantly in the northeastern parts (latitudes, 29°40'N to 21°59'N; longitudes, 89°29'E to 97°25'E) of India (Choudhuri, 1982). The importance of this interaction is that the muga silkworm is being reared commercially for three to four lifecycles each year on the som plant for better yarn quality (Thangavelu et al., 1988). Som is infected by various pathogens that affect both the quality and quantity of leaves and in turn reduces silkworm cocoon yield. *Phyllosticta* leaf spot (PLS) is caused by the necrotroph *Phyllosticta persae* (Coelomycetes) and is becoming an increasingly important disease of som in northeastern India (Das et al., 2003). The pathogen infects a wide range of woody trees of Assam (Ali & Saikia, 1997).

Fungi in the genus *Phyllosticta* (teleomorph: *Guignardia* or *Mycosphaerella* spp.) cause plant diseases such as leaf blight (Mukunya & Boothroyd, 1972; Silva et al., 2008), gummy stem blight (Wehner & Amand, 1993) and fruit blotch (Fischer, 2000) of various plants. The pathogen may cause serious damage to the host plant through reduced photosynthetic ability and premature leaf fall (Su & Kai, 2012). However in som, the sexual stage of *P. persae* has not yet been identified. Nonetheless, the disease is easily

recognized by small, irregular shaped brown or grayish brown spots scattered on the dorsal side of the moderate to full mature som leaves. As the disease progresses, the spots become collapsed and malformed. PLS does not kill the plants immediately, but foliage losses increase gradually with the age of plants by decreasing the functional leaf area. Because of abundant rainfall and humid tropical temperature, PLS is predominant during June-September in most of the production regions in northeastern India. Cultivars of som are very susceptible to PLS and recorded foliage loss goes up to 26% (Das & Das, 2008). In addition, the loss in foliage quality and the adverse impact on silkworm cocoon production are even greater (Bindroo et al., 2008).

Muga sericulture is mainly practiced by marginal/poor growers using forest and forest fringe lands (Jolly et al., 1981) and contributes significantly to the livelihoods of tribal peoples (Singh et al., 2012). Therefore, a management strategy based on using fungicides is not a viable option. Besides, very little is known about the impact of PLS on som foliage quality at the farm level except for some basic information on the predisposing factors of the infection process (Das & Das, 2008). Overall there is great need then to understand the impact of this disease in the field as well as identify resistance sources of som as the most cost-effective and economical way of managing PLS.

This study was undertaken to evaluate the degree of severity of PLS in two main seed rearer's fields at Kamrup

and Goalpara districts, Assam, India, and to screen available clones of som for resistance to PLS.

MATERIALS AND METHODS

Survey of *Phyllosticta* leaf spot severity in farmers' fields

Natural open pollinated hybrid seed orchards of som of uniform age (8-10 years) were selected. Individual sites (n=3 from each district) were selected in the districts of Kamrup (Singra, Bolkona and Khatolpara) and Goalpara (Agia, Baida and Durapara) of Assam, along with three distinct patches of trees (0.2 ha each) at the Regional Muga Research Station, Boko. These locations represented the major seed crop rearing areas of muga silkworm. In each field, 20 trees were examined. The tree to tree spacing was 3 m x 3 m in all fields. Two evaluations each of PLS severity were obtained during June and August of 2009 and 2010. These evaluations coincided with the 'Aherua' and 'Bhodia' muga silk moth seed crop rearing seasons in Assam that are particularly affected by PLS.

Screening of som clones in experimental field

Eight available som clones were evaluated in the experimental garden of RMRS in Boko (92 m above msl; 25°5'31"N and 91°24'00"E). The experimental design was a randomized complete block with plants placed at a 3 m by 3 m spacing (Chattopadhyay et al., 2011b). Number of test plants were 20 with four replications per clone. Soil type was classified as alluvial (entisol) and having a sandy clay loam in texture with pH 7.6, organic carbon 0.72%, available N=230 kg ha⁻¹, available P=30.2 kg ha⁻¹ and available K=210.7 kg ha⁻¹ at the time of initiation of the experiment. Two split doses of inorganic N as urea (87 g plant⁻¹), inorganic phosphorus as P₂O₅ (125 g plant⁻¹) and inorganic potassium as K₂O (33 g plant⁻¹) were applied after each commercial harvest (June and November); application dose of farm yard manure was 10 kg plant⁻¹ during May (Chakravorti & Barah, 2007).

Disease assessment

Twenty plants of each clone were evaluated visually using the modified logarithmic scale of Horsfall-Barrett (Horsfall & Cowling, 1978). The scale was 0 = 0%, 1 = 0-3%, 2 = +3-6%, 3 = +6-12%, 4 = +12-25%, 5 = +25-50%, 6 = +50-75%, 7 = +75-88%, 8 = +88-94%, 9 = +94%-97% and 10 = +97-100%. The leaf spots were scored four times between 80 and 140 days after ground level pruning. Disease incidence (DI) was calculated as the number of leaves on the main branch with necrotic spots on a percentage basis (Gawande & Patil, 2003). Disease severity index (DSI) was calculated for each accession according to Chattopadhyay et al. (2010). The DSI of the accessions falling in the grades 1 and 2-3 were considered as highly and moderately resistant, respectively, while those falling into 4 and ≥5 were classified as intermediate and susceptible, respectively.

PLS development over time was assessed as the area under the disease progress curve (AUDPC) and the apparent infection rate (AIR). The AUDPC was calculated according to the method of Chattopadhyay et al. (2011a) using the following formula:

$$AUDPC = \sum_{i=1}^n [(x_i + x_{i+1}) / 2] (t_{i+1} - t_i)$$

where, n is the number of evaluation times, x_i is the disease intensity at evaluation time i and t_{i+1} is the time between two disease scores. Whereas, AIR was estimated according to the method of Rao et al. (2008) as follows:

$$AIR = [1/(t_2 - t_1)] \log_e \left[\frac{\{n_2 (1 - n_1)\}}{\{n_1 (1 - n_2)\}} \right]$$

where, t₁ = time during the 1st observation; t₂ = time during the 2nd observation; n₁ = DSI at time t₁ and n₂ = DSI at time t₂.

Lesion sizes (LD) and lesion frequency (LF) of PLS infection were given in units of mm² (length x width) and per leaf, respectively and measured 140 days after pruning from the 8-12th leaf positions from the top according to Chaerani et al. (2007).

Data analysis

Analysis of variance was performed by using 'Statistica' version 8.0 (Statsoft Inc.). Heterogeneity in the variances was observed in the data from disease screening methods. Therefore arcsine-square root transformation was applied before analysis. Recorded data were averaged across the replicates and resulting mean scores were used for analysis. When F values were significant (P<0.05), Fisher's least significant differences (LSD), correlation matrix and broad sense heritability (h²) were calculated in appropriate cases according to Gomez & Gomez (1984) and Jhonson et al. (1955), respectively. Furthermore, mean values for tested parameters of clones from the leaf spot screening were clustered using the same software. The Euclidean distance based on complete amalgamation rule was used to construct dendrogram using hierarchical clustering. A linkage distance of 100 was arbitrarily chosen to separate the clone into two major clusters and four subclusters in the dendrogram (Berdahl et al., 1999).

RESULTS

The severity of PLS was considered high, and also varied significantly (DSI range: 12.3 to 29.8) across the six villages of Kamrup and Goalpara districts, as well as in the RMRS fields of Boko over the two years of assessments (Table 1). In particular, PLS severity was significantly higher (18.5% over respective mean seasonal DSI values) during Aherua compared to Bhodia in all fields assessed. PLS was 32% (Aherua) and 8% (Bhodia) higher, respectively, in Kamrup district compared to Goalpara.

Som resources were screened multi-parametrically using qualitative and quantitative parameters like DSI, DI, AUDPC, AIR, LD and LF values. All clones showed PLS symptoms and DSI variation was 3.6-fold. The mean DSI value was 25.2, compared to a severity index of 9.8 for M-5 (moderately resistant in H-B scale: 3) and 36.0 for M-8 (susceptible in H-B scale: 5) clones, respectively (Table 2). The DI variation was 4.0-fold across the clones and the majority of them (63%) had incidence levels over 47%. The disease progress, in terms of leaf area under disease progress curve (AUDPC) between 80 to 140 d was also analyzed to combine multiple observations of PLS development into a single value. There was a 3.2-fold difference between the maximum AUDPC (M-8) and the minimum (M-5), but the variation of AIR values was 1.4-fold across the clones. Significant variations were recorded for quantitative parameters like LD (3.2-fold) and LF (3.5-fold) across the clones and like qualitative parameters, M-5 exhibited minimum values of both lesion features (Table 2).

The magnitude of variation of DI, DSI, AUDPC, LD and LF were identical (range: 3.2 to 4.1-fold) and higher than AIR (1.4-fold). Nonetheless, all tested attributes significantly ($P < 0.05$) exhibited that M-5 and M-8 as the

most resistant and susceptible clones to PLS, respectively.

Correlation analysis revealed significant associations between the parameters evaluated. A highly positive correlation of DSI was observed (Table 3) with DI ($r = 0.91$; $p > 0.01$) and AUDPC ($r = 0.86$; $p < 0.01$). However, the associations of AIR with DSI and AUDPC were moderate (range: $r = 0.44$ to 0.56 ; $p < 0.05$) and non-significant with DI. Moreover, two measures of disease reactions, DSI and AUDPC values also showed highly significant ($p < 0.01$) correlations with LD ($r = 0.76$ and $r = 0.68$) and LF per leaf ($r = 0.79$ and $r = 0.70$).

The strong association of DSI with DI, AUDPC, LD and LF parameters was partitioned further through hierarchical cluster analysis (Figure 1). The entries were grouped into two major clusters (A and B) with a wide linkage distance of 610.5. The sub-cluster A was further divided into three sub-groups (A1 to A3). Relatively low responsive clones (M3, M6 and M5) were partitioned into B-1 and B-2 subgroups.

DISCUSSION

A consistently high intensity of PLS (DSI range: 18.0 to 29.8) in major muga silkworm seed crop rearing

TABLE 1 - Estimates of *Phyllosticta* leaf spot severity index (DSI), mean square sum (MSS), variance components (VC) in different areas of Assam during 'Aherua' and 'Bhodia' seed crops. Data are mean of four field observations collected from three villages per location over two years.

Season	Location	DSI	VC	MSS	$P < F$
'Aherua' (June -July)	Kamrup	29.8	5.28	296.64	0.001
	Goalpara	20.1	3.17		
	RMRS ¹	16.3	2.04		
'Bhodia' (August- September)	Kamrup	23.2	4.06	153.6	0.01
	Goalpara	18.6	2.54		
	RMRS	12.3	1.85		
	LSD ($P_{0.05}$)	3.2			

¹RMRS: Regional Muga research Station.

TABLE 2 - Responses of som clones to *Phyllosticta* leaf spot disease at the experimental field of RMRS, Boko. Average of twenty values per clone with a total df = 160 for each parameter. Values are back transformation of arcsine [$\sqrt{(x/100)}$].

Clone	DSI ^a	DI	AUDPC	AIR	LD	LF
M-1	25.9	46.9	752.3	0.030	37.1	6.7
M-2	30.4	56.3	823.9	0.034	43.4	8.6
M-3	22.3	41.0	580.8	0.037	19.9	4.2
M-4	30.9	58.1	868.4	0.036	41.3	7.2
M-5	9.8	17.0	287.2	0.026	14.8	2.9
M-6	14.2	21.9	411.4	0.035	19.0	4.0
M-7	31.8	59.2	909.0	0.038	45.6	9.1
M-8	36.0	68.9	920.4	0.037	47.4	10.1
LSD ($_{0.05}$)	3.1	5.2	60.1	0.007	4.5	1.1
h^2	0.80				0.76	0.61
CV= 15.3%						

^aDSI, disease severity index; DI, disease incidence (%); AUDPC, area under the disease progress curve (accumulative %); AIR, apparent infection rate (unit progress/day); LD, lesion diameter (mm²); LF, lesion frequency (no. 50 cm²) in the 7th-10th positioned leaves from the top after 140 days of pruning.

TABLE 3 - Pearson correlation coefficients among qualitative and quantitative parameters of *Phyllosticta* leaf spot assessment.

	DSI ^a	DI	AUDPC	AIR	LD
DI	0.91 ^{**b}				
AUDPC	0.86 ^{**}	0.79 ^{**}			
AIR	0.56 [*]	0.44 ^{ns}	0.54 [*]		
LD	0.76 ^{**}	0.67 ^{**}	0.68 ^{**}	0.43 ^{ns}	
LF	0.79 ^{**}	0.76 ^{**}	0.70 ^{**}	0.49 ^{ns}	0.86 ^{**}

^aDSI, disease severity index; DI, disease incidence (%); AUDPC, area under the disease progress curve (accumulative %); AIR, apparent infection rate (unit progress/day); LD, lesion diameter (mm²)

^b***, * and ns, significant at $p > 0.01$, $p > 0.05$ and non-significant, respectively.

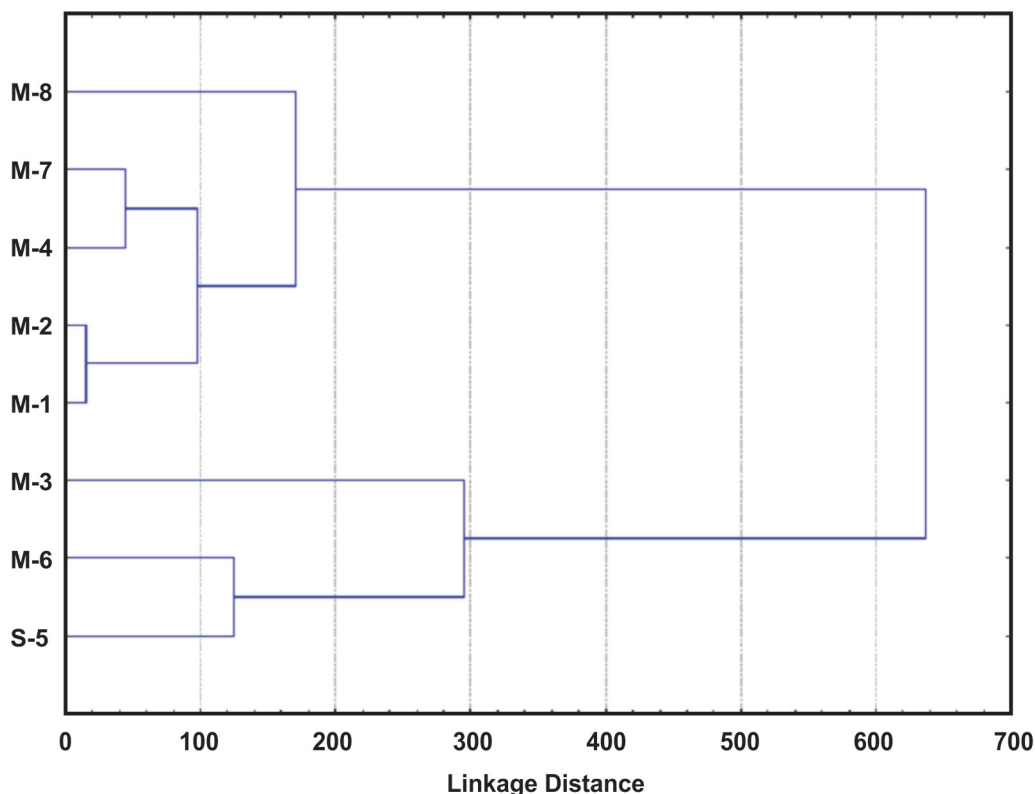


FIGURE 1 - Dendrogram of eight som clones obtained by analysis of disease severity index, disease incidence, area under the disease progress curve, apparent infection rate, lesion diameter and lesion frequency values during leaf spot infection at the experimental field of RMRS, Boko.

areas of Goalpara and Kamrup districts (total: six locations) were recorded in both seasons. It has been shown that the estimated leaf yield loss of a som garden is about 4900 kg due to a PLS severity of 33% during July in northeastern India (Das & Das, 2008). We assessed PLS severity using Horsefall-Barrett (H-B) scale and recorded DSI values within the range of intermediate to susceptible grades of PLS. The relationship between the nearest percent estimate of any disease severity with H-B scale data is linear especially within the range of 0 to 60% (Bock et al., 2010). Therefore the recorded DSI values indicated empirically that having a control measure for the disease is essential. Selected plant ages, cultural practices and climatological factors were very similar. In spite of this, the extent of symptom expression was different in two districts possibly due to differential pathogen loads in the two districts. This

is the first study to document PLS severities of som across the muga sericulture villages.

Considerable levels of damage (based on DSI) due to PLS in muga seed crop rearing areas necessitated research to identify resistant clones among the available resources. A combination of qualitative (DSI, DI, AUDPC, AIR) and quantitative (LD, LF) parameters were used to assess PLS resistance. These parameters are widely used for assessment of foliar fungal disease reactions (Campbell & Madden, 1990). Clones were ranked based on the mean PLS rating averaged over two seasonal data of two consecutive years. Although the disease reaction was assessed four times between 80 to 140 d after pruning (DAP) at an interval of 15 d (data not shown), but the magnitude of the variation of PLS response at 140 DAP justified the use of evaluating clonal differences principally at this data point. Results

indicated that there was substantial clonal variability across the different disease parameters assessed, and while no clone was asymptomatic to PLS, clone M-5 consistently had the lowest disease ratings, indicating a potential source of resistance for further study.

From a breeding perspective, it has been suggested that the use of moderate or rate-reducing resistance may be more durable than complete resistance (Calenge et al., 2004). In the present study this was evaluated in terms of AUDPC and AIR. Although significant clonal differences were indicated by AIR (1.4-fold), a measure of the rate of disease symptom change, the difference was not as distinct as that of AUDPC (3.2-fold), a measure of cumulative disease progress over a specific study period. These findings agree with those of Meles et al. (2004) and Sillero et al. (2000) in indicating that AUDPC is more informative than AIR when assessing the level of resistance of various diseases in the field. As the rate of disease increase is jointly proportional to the level of disease and healthy tissue (Madden, 1980; Kushallappa & Ludwig, 1981), differential leaf growth of various clones may be responsible for less variation in AIR (Pandey et al., 2003). Overall, the results showed a good agreement in the trends of all qualitative and quantitative disease parameters.

The resistant clone M-5 had the lowest DI, DSI, AUDPC and AIR values. These results are in agreement with findings in other pathosystems (Adhikari et al., 1994). However, DI and DSI revealed more distinct differences (4.1-fold and 3.7-fold) among the clones than other parameters. AUDPC and AIR take into account disease severity in different time spans, while DI and DSI values calculated from the single point data (here 140 d after DAP) are often representative of the extent of disease for a specific host-pathogen interaction (Vanderplank, 1982; Silva et al., 2013). Mean DSI and DI values indicated that adequate disease pressure was attained during field screening, and these parameters may be useful for PLS screening in som. Whereas, the AUDPC value reflected the slower development of PLS in the M-5 clone.

Additionally, two other quantitative parameters, LD and LF, were also in good agreement with the measured qualitative features. It has been shown that smaller values for LD and LF are characteristic of partial resistance (Wang & Hartman, 1992; Twizeyimana et al., 2008). In the current study, it has been shown for the first time that the M-5 clone, which had the lowest measures of disease intensity during the entire experiment, possibly possesses partial resistance. Adding to this hypothesis is the fact that results were also consistent across two years in which natural disease intensity was relatively high. Further evidence was based on a high broad sense heritability (h^2 : 80.6 to 60.1) of DSI, and quantitative disease assessment parameters like LD and LF indicating the additive nature of the resistance. Moreover, DSI was strongly correlated with DI ($r = 0.91$; $P > 0.01$) and AUDPC ($r = 0.86$; $P < 0.01$). On the other hand, correlations of AIR with DSI and AUDPC were moderate ($r = 0.56$ and

0.54 ; $P < 0.05$) and non-significant with DI ($r = 0.44$; $P < 0.05$). Highly positive correlation of DSI especially with DI and AUDPC indicates that PLS could be studied by either one of these three parameters with equivalent accuracy. As the AUDPC represents the integration of all host, pathogen and environmental effects during the epidemic (Campbell & Madden, 1990), assessment of AUDPC would be a better option than DSI and DI. Moreover, the importance of the LD and LF as indicators of resistance have not been studied so far in PLS. Highly significant positive correlations between DSI and AUDPC and quantitative parameters like LD ($r = 0.76$; $P < 0.01$) and LF per leaf ($r = 0.79$; $P < 0.01$) confirmed these results and provide additional information that these two quantitative parameters could help in the selection of resistant clones.

Strong association of all disease parameters with LD and LF were further partitioned through hierarchical cluster analysis. Euclidean distance showed that the moderately resistant clone M-5 was also the most diverse accession among the tested lines. The cluster analysis largely showed good agreement among obtained disease reactions and correlative morphological features.

In conclusion, the identification of moderate resistant in accession M-5 through multi-parametric features of high heritability and sufficient genetic divergence indicate the potential use of this clone in a som breeding program for PLS resistance.

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