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Screening of mulberry genotypes for disease resistance in different seasons to bacterial leaf spot

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Abstract

Eighty two mulberry germplasm accessions were evaluated for stable resistance to Bacterial leaf spot (BLS) pathogen *Xanthomonas campestris* pv.*mori* in field condition. Disease reaction was scored by 0-5 scale at 60 days after pruning. Germplasm with low disease severity, regression coefficient nearer to one and regression deviations variance nearer to zero were considered highly stable. Accordingly, *Morus rotundiloba* and MS-8 were observed to be stable accessions. Besides, these two accessions represented as unique entries in clustering of genotypes and showed maximum divergence. Therefore, *M.rotundiloba* and MS-8 may be considered as useful sources of stable resistance to bacterial leaf spot for utilization in mulberry improvement programme. The genotype Surat with maximum disease severity, regression coefficient nearer to one and regression deviations variance nearer to zero may be exploited as susceptible source in BLS resistance breeding programme.

Key words: Bacterial leaf spot; genetic divergence; germplasm; mulberry; resistance and stability

Introduction

Bacterial leaf spot [BLS] (*Xanthomonas campestris* pv. *mori*), is a common foliar disease causing losses upto 15% in mulberry [1-4]. Development of a disease resistant variety is the most effective and economic control measure towards sustainable production [5-8]. Though resistance to BLS in mulberry is known [9], BLS resistance genotypes were not identified. Also, information on the genetic divergence of genotypes with BLS resistance is not available. The present research was initiated in 2003 to identify stable source(s) of resistance against this pathogen in a mulberry depository at the Central Sericultural Research &Training Institute, Berhampore, India.

Materials and methods

About eighty two germplasm accessions from 13 different countries (Table 1) were grown at the experimental field of the Institute (88º 15'E and 24º 6'N) in 2002. Five rows, with five plants in a row (3m long and 0.6m wide) of each accession were grown in 0.6m interplant distances in a row. Recommended doses of fertilizers were applied at the rate of 336Kg ha^{-1} N, 180Kg ha^{-1} P and 112Kg ha^{-1} K along with 20 MTha^{-1} FYM at five split doses 20-25 days after pruning (DAP) in the five-crop schedule. BLS severities were visually estimated as percentage of foliage with infection or killed as a result of infection in three consecutive years (environments) i.e. 2003, 2004 and 2005. Three independent tests were made for three consecutive years between 2003 and 2005. During 2005, summer was very hot from mid April to third weak of June and 659mm precipitation was measured from June to September. For the same period in the preceding years (2003 and 2004), precipitation was 872 mm and 1060 mm respectively. Average temperature for four consecutive BLS evaluation months of 2005 were 38°C, 34°C, 34°C, 35°C, while in 2003 and 2004 corresponding temperatures were 35°C, 34°C, 35°C, 25°C and 35°C, 34°C, 34°C and 33°C, respectively. The disease was rated following 0-5 scale on 60 DAP [9-12]. A rating of 0 was assigned with no visible bacterial leaf spot symptom and that of 1 indicated 0^{+}-5% infection of leaf area. Similarly, 2, 3, 4 and 5 ratings indicated 5^{+}-10%, 10^{+}-25%, 25^{+}-50% and $>50^{+}$ % infected leaf area, respectively. In this study observations were recorded from three randomly selected branches of three plants considering each as a replication of individual accession forming a total of (82 x 54) 4428 observations. BLS resistance ratings were articulated as of percent disease

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Table1. Mulberry germplasm by origin and their mean disease severity for bacterial leaf spot resistance evaluated across three years in West Bengal

S.No.	Germplasm	Origin	Mean disease severity (PDI)	S.No.	Germplasm	Origin	Mean disease severity (PDI)
1	China black	China	3.13	42	K-2	India	14.19
2	Multicaulis	France	9.66	43	MS-1	India	8.27
3	Berhampore-B	India	4.26	44	OPH-1	India	10.33
4	Kolitha-7	India	3.08	45	Calabrasa	Paraguay	8.64
5	Tollygunge	India	5.29	46	MS-9	India	6.98
6	Sujanpur	India	15.33	47	Farnandodias	Paraguay	12.45
7	M.rotundiloba	France	0.04	48	MS-5	India	5.56
8	Berhampore-6	India	9.93	49	Monlai	Burma	8.19
9	Koliyha-8	India	6.13	50	Thailand unlobed	Thailand	6.88
10	Bush Malda-A	India	8.83	51	MR-1	India	14.38
11	Sultanpur	India	1.95	52	M.cathyana	Indonesia	12.93
12	Phillipines	Phillipines	4.27	53	Monla-1	Burma	6.74
13	Cyprus	Cypras	4.13	54	ACC-165	India	4.15
14	Berhampore-20	India	14.37	55	Nagaland Local	India	13.34
15	Kolitha-9	India	1.07	56	Jatinuni	India	13.97
16	Black cherry	India	2.81	57	Thailand lobed	Thailand	11.31
17	Burma-8	Burma	7.17	58	M.australis	Australia	11.94
18	M.indica X	India	7.05	59	M.indica HP	India	1.37
19	Dudhia red	India	2.12	60	Shrim 8	Bangaladesh	2.93
20	Bush Malda-B	India	13.08	61	Molai	India	7.86
21	KPG-1	India	24.11	62	Punjab local	India	4.36
22	Italian Mulberry	Italy	10.74	63	MS-7	India	6.13
23	Bogura-1	Bangladesh	12.10	64	Miuraso	Paraguay	1.90
24	CSRS-2	India	9.54	65	Kaliakothai	India	12.51
25	Dudhia white	India	14.90	66	MS-6	India	3.40
26	Matigara white	India	8.89	67	FGDTR-9	India	2.70
27	Okiniwaso	Japan	2.50	68	Kokuso-13	Japan	3.08
28	Bogura-4	Bangladesh	14.43	69	M.nigra	Indonesia	9.69
29	Assambola	India	11.54	70	Shrim-2	Bangaladesh	2.43
30	Bishnupur-4	India	8.27	71	Golaghat	India	1.20
31	Matigara black	India	8.89	72	Mysore local	India	13.48
32	M.indica black	India	10.89	73	MS-8	India	0.99
33	Australia	Australia	8.93	74	M.multicaulis	Italy	1.55
34	Kajli	India	5.48	75	Sujanpur 5	India	6.46
35	Bishnupur-9	India	2.56	76	Shrim-5	Bangaladesh	3.07
36	Tista valley	India	2.33	77	Almora L	India	10.29
37	KPG-2	India	11.72	78	Surat	India	13.86
38	Mandalaya	Burma	2.45	79	RFS-175	India	8.04
39	Berhampore-A	India	2.38	80	China white	China	1.60
40	Kolitha-3	India	1.64	81	Kakpilla	India	3.02
41	Kurseong	India	8.17	82	Jodhpur	India	1.50
	Popn. Mean PDI = 6.51						

index (PDI) using standard procedure [13]. Stability analysis of tested germplasm was conducted according to Eberhart and Russell [14]. The relationship among 82 accessions based on the disease severity was portrayed graphically in the form of Dendrogram using Genetic Model of INDOSTAT software.

Results and discussion

The mean disease severity index (PDI) of 82 mulberry germplasm across three years ranged from 0.04 to 24.11 (Table 1). The difference in response of the genotypes in the various environments of three years indicated G x E interactions (Table 2). This was confirmed by the highly significant effect of the genotype x environment interaction in the joint analysis of variance and indicated the need to assess the response of the genotypes to environmental variation. The results of the estimates of adaptability and stability of mulberry genotypes indicated that Shrim-5, MS-6, FGDTR-9, Shrim-2, *M. multicaulis* and China white are adapted to favourable environments (minimum pathogen load) with low predictability, while ACC-165, Punjab local, Miuraso, Kukpilla and Jodhpur had average capacity for adaptation to all the environments and were highly predictable. On the other hand, *M.rotundiloba*, Kolitha-9, Kolitha-3, *M. indica* HP and MS-8 with less mean disease severity, regression coefficient less than one and regression deviations variance nearer to zero indicated adaptability of these genotypes to unfavourable environments (maximum pathogen load) and highly stable performance. However, accessions *M.rotundiloba* and MS-8 had least disease severity (PDI <1), regression coefficient nearer to one and regression deviation variance nearer to zero indicated better adaptability of these accessions to unfavourable environments and high predictability for BLS resistance.

Table 2. Joint analysis of variance for BLS disease severity (PDI) in 82 mulberry germplasm across three years

Source of variation	df	mean squares
Genotype (G)	81	93.26**
Envoronment (E)	2	1476.83**
G x E	162	15.58**
E+ (G x E)	164	33.40**
E(Linear)	1	1.88
GXE (Linear)	81	51.91**
Pooled deviation	82	15.50**
Pooled error	492	3.54

** P<0.01(F-test)

The adaptability and stability of a genotype are useful parameters for recommending it for crop improvement programme. Eberhart and Russell [14] proposed an assessment of genetic response to environmental changes using linear regression co-efficient and variance of the deviations from linear regression. The genotypes are grouped according to the degrees of regression co-efficients as well as to the size of variance of the regression deviations. The genotypes with regression co-efficient greater than one would be more adapted to favourable growth conditions, those with regression co-efficient less than one would be adapted to unfavourable environmental conditions and those with regression co-efficient equal to one would have an average adaptation to all environments. Again, genotypes with variance of deviation from linear regression nearer to zero would be highly predictable. More it deviates from zero; the predictability of genotypes would reduce.

The study revealed that Shrim-8, MS-6, FGDTR-9, Shrim-2, *Morus multicaulis* and China white exhibited less mean disease severity and regression coefficient >1 while, the levels of variances in the stability regression deviations were >0 (Table 3). In contrary, ACC-165, Punjab local, Miuraso, Kuckpilla and Jodhpur had regression coefficient of unity and non-significant regression deviation variances. While, five genotypes namely, *Morus rotundiloba*, Kolitha-9, Kolitha-3, *M. indica* HP and MS-8 showed minimum disease severity (Fig. 1), regression co-efficient <1 and regression deviation

Table 3. Estimates of stability parameters of minimum responsive mulberry germplasm evaluated for bacterial leaf spot resistance across three years in West Bengal

Genotype	b_i	S^2d_i	Genotype	b_i	S^2d_i
Kolitha-7	0.38	0.14	M.indica HP	0.58	3.45
Rotundiloba	0.17	1.22	Shrim 8	1.47	2.93
Sultanpur	0.67	2.42	Punjab local	1.02	0.21
Cyprus	0.34	3.26	Miuraso	0.94	2.91
Kolitha-9	0.55	7.11	MS-6	1.70	5.26
Black cherry	0.61	1.03	FGDTR-9	1.70	4.99
Dudhia red	0.71	1.06	Shrim-2	1.26	3.80
Bishnupur-9	0.12	1.25	MS-8	0.51	1.01
Tista valley	0.20	5.29	M.multicaulis	1.46	4.91
Mandalaya	-0.12	2.02	China white	1.48	4.79
Berhampore-A	0.74	4.96	Kakpilla	1.09	3.06
Kolitha-3	0.44	5.31	Jodhpur	0.97	0.64
ACC-165	0.84	1.56			
SE $b_{i\pm}$	0.66				

variances nearer to zero. Similarly, *M. australis* had disease severity greater than population mean of 6.51 with regression co-efficient >1. The variance of regression deviations was more than zero (Fig. 2). Genotypes Sujanpur, Bush Malda–B and Nagaland local had more disease severity, regression co-efficient equal to one and non-significant regression deviation variance. However, the genotype Surat showed maximum disease severity, regression co-efficient less than one and regression deviations variance very near to zero respectively.

The genotypes when grouped on the basis of BLS disease severity, maximum number of accessions were grouped in cluster I, II and III in order (Fig. 3). Besides, cluster IV, V and VI each represented by a single genotype. Grouping of genotypes having exotic and indigenous origin was found in cluster I and II, while genotypes of cluster III were from indigenous origin. Genotypes of cluster III showed maximum mean disease severity (PDI 15.3) followed by I (PDI 10.0), IV (PDI 6.7),

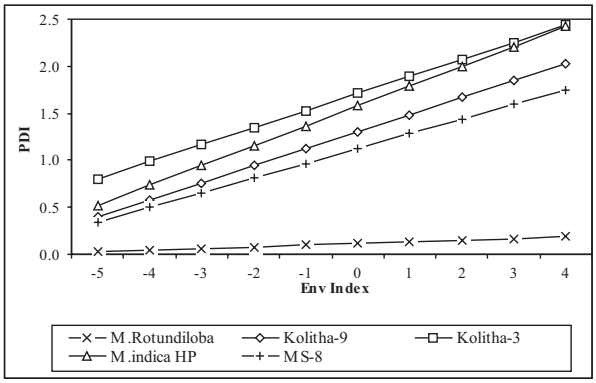

Fig. 1. Regression between PDI and environmental index on the resistant mulberry germplasm tested, 2003-2005

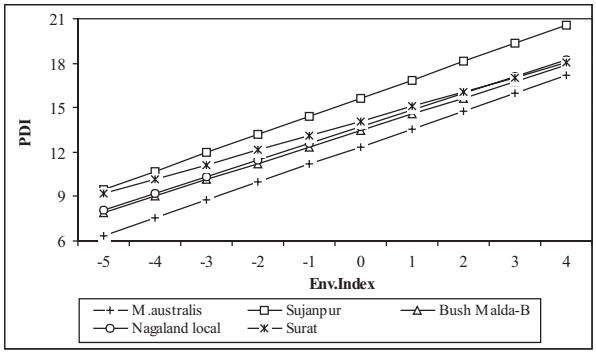

Fig. 2. Regression between PDI and environmental index on the susceptible mulberry germplasm tested, 2003-2005

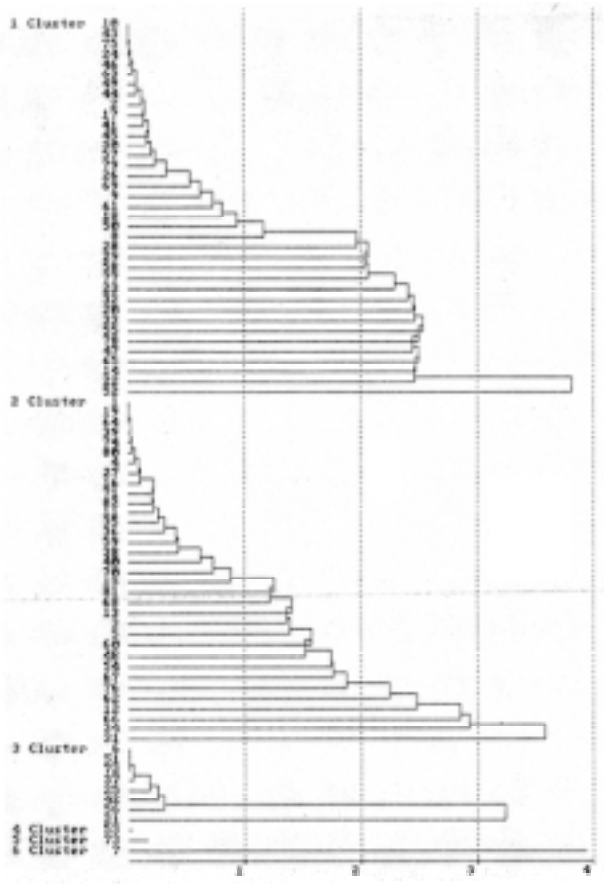

Fig. 3. A dendrogram obtained by INDOSTAT for 82 mulberry genotypes based on disease severity

II (PDI 3.1), V (PDI 0.9) and VI (PDI 0.04) in order. The dendrogram obtained using disease severity trait showed that three accessions Monla-1 (53), MS-8 (73) and *M. rotundiloba* (7) each form a separate cluster showing their uniqueness.

Clustering of genotypes indicated that indigenous and exotic accessions were grouped together without any geographic boundaries. The grouping pattern did not show any relationship between genetic divergence and biodiversity in mulberry for BLS disease severity. Moreover, maximum mean disease severity showed by the indigenous accessions of cluster III may be due to the prevalence of the particular pathosystem in that region. Though genetic diversity for disease resistance had been well established in grain cereals [15, 16], however, this is the first report about genetic interrelationship among mulberry germplasm accessions for bacterial leaf spot resistance.

In conclusion, tremendous genetic variability was available among the germplasm resources for BLS

disease severity in mulberry. Most of the genotypes responded differentially to BLS in different environmental conditions, while a few exhibited considerable adaptability and stability for BLS reaction irrespective of environments. Considering mean disease severity as the first parameter for classification of genotypes, accessions *M. rotundiloba* and MS-8 showed least value. Besides, these accessions also had better adaptability and stability in unfavourable environments and also exhibited maximum divergence. Therefore, *M.rotundiloba* and MS-8 may have the potential for their utilization in crop improvement progrmme towards sustainable production and productivity of mulberry. In addition, the genotype Surat had maximum disease severity along with suitability to unfavourable environments may be used as susceptible source. The assessment of genetic diversity may be useful in genome mapping in mulberry for BLS resistance. Thus the regression technique coupled with divergent analysis was considered valuable tool for identification of stable resistance to bacterial leaf spot in mulberry.

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References

1. **Kore S. S. and Pati P. S.** 1985. Investigations of bacterial blight disease of mulberry (*Morus alba* L). Abstr., 37th Annual Meeting of IPS, January 8-10, Indian Phytopathology. **38**: 622.
2. **Maji M. D., Qadri S. M. H. and Pal S. C.** 1998. *Xanthomonas campestris pv. mori*, a new bacterial pathogen of mulberry. Sericologia, **38**: 519-522.
3. **Sharma Y. R., Singh G. and Kaur L.** 1995. A rapid technique for Ascochyta blight resistance in chickpea. International Chickpea and Pigeonpea Newsletter, **2**: 35-36.
4. **Maji M. D., Qadri S. M. H. and Pal S. C.** 2000. Control of bacterial leaf spot of mulberry caused by *Xanthomonas campestris* pv. *mori*. Indian Journal of Sericulture, **38:** 81-83.
5. **Laterrot H**. 1998. Disease resistance in tomato present situation and hopes. World Conference on Horticultural research. June 17-20.Rome, Italy (ISHS).
6. **Swaminathan M. S.** 2004. Stocktake on cropping and crop science for a diverse planet .http://www.cropscience.org.au/icsc2004/plenary/0/2159_swaminathan.htm.
7. **Rao M.V.** 2006. Technology missions as a way to achieve set goals. Indian J Genet., **66**: 89-94.
8. **Datta S. K.** 2007. Genomics and safe use of genetic engineering for plant protection. *In*: Key Note Address. National Symposium on Plant Protection-Technology Interface held at Bidhan Chandra Krishi Viswavidayaya, Kalyani, Nadia From 28-29th, December, 2007. pp. 23-14.
9. **Maji M. D., Sau H., Das B. K. and Urs S. Raje.** 2006. Screening of some indigenous and exotic mulberry varieties against major foliar fungal and bacterial diseases. Int. J. Indus. Entomol., **12**: 35-39.
10. **Gangwar S. K. and Thangavelu K.** 1998. Varietal and seasonal occurrence of powdery mildew (*Phyllactinia corylea* Pers.Karst.) disease of mulberry in Tamilnadu. Sericologia, **38**: 357-362.
11. **Srikantaswamy K., Gupta V. P. and Resnukeswarappa J. P.** 1999. Incidence,severity and yield loss due to leaf spot of mulberry caused by *Cercospora morocola*. Indian J.Seric., **38**: 8-11.
12. **Pratheesh Kumar P. M., Maji M. D., Gangwar S. K., Das N. K. and Saratchandra B.** 2000. Development of leaf rust (*Peridiopsora mori*) and dispersal of urediniospore in mulberry (*Morus* spp.). Int. J. Pest Management, **46**: 195-2000.
13. **F.A.O**. 1967. Crop loses due to diseases and pest. Food and Agricultural Organization, Rome.
14. **Eberhart S. A. and Russell W. A.** 1966. Stability parameters for comparing varieties. Crop Sci., **6**: 36-40.
15. **Zhu Y., Chen H., Fan Y., Wang Y., Li Y., Chen J., Fan J., Yang S., Hu L., Leung H. Mcw T. W., Teng R. S., Wang Z. and Mundtt C. C.** 2000. Genetic diversity and disease control in rice. Nature, **406**: 718-722.
16. **Thakur R. P., Subba Rao F. V., Williams R. J., Gupta S. C., Thakur D. P., Nafade S. D., Sundaram N. V., Frowd J. A. and Guthrie J. E.** 1986. Identification to stable resistance to smut in pearl millet. Plant Disease, **70**: 38-41.