Indian J. Genet., 71(4): 356-362 (2011)

Morphological, anatomical and molecular characterization of full-sib pseudo-F_2 (F_1) progenies in mulberry with resistance to bacterial leaf spot (*Xanthomonas campestris* pv. *mori*)

Rita Banerjee, S. Ghosh, S. G. Doss, A. K. Saha, A. K. Bajpai and R. K. Khatri

Central Sericultural Research and Training Institute, Central Silk Board, Ministry of Textiles: Govt. of India, Berhampore 742 101

(Received: January 2011; Revised: September 2011; Accepted: October 2011)

Abstract

Bacterial leaf spot (BLS), caused by *Xanthomonas campestris* pv. *mori* is a devastating foliar disease of mulberry. Full-sib progenies descendent from control cross between BLS resistant source *'Morus rotundiloba'* and susceptible commercial cultivar 'S-1' were evaluated for BLS disease reaction. The continuous population distribution of BLS disease severity (DSI) across the progenies indicated additive nature of the resistant trait. Besides, morphological and anatomical characterization of the selected resistant and susceptible progenies showed high significant positive correlation between DSI and stomata frequency. Significant negative correlation of DSI was observed with leaf thickness, thickness of upper epidermis and upper cuticle layers. RAPD profiling with Genei primer 17 and 18 generated two unique DNA fragments of 500bp and 450bp for five each of resistance and susceptible progenies, respectively. The dendrogram using the binary statistic of RAPD analysis exhibited distant relationship between the parents as well as resistant and susceptible progeny groups.

Key words: Disease resistance; morpho-anatomical features; mulberry; RAPD; *Xanthomonas campestris* pv. *mori*

Introduction

Mulberry (*Morus* spp.), the solitary food source of domesticated silkworm (*Bombyx mori*) is infested by several foliar diseases. Bacterial leaf spot (BLS) caused by *Xanthomonas campestris* pv.*mori* is a major foliar disease of mulberry and responsible for considerable loss in foliage production potential across the world [1-2]. Occurrence of BLS has been reported in all mulberry growing areas of India [3]. The foliage loss due to the disease is often goes up to 15% [4]. Presently all the commercial cultivars are prone to BLS. Development of host resistance is the most economic and sustainable control strategy, but such attempts are still unexplored in mulberry. Moreover, assessment of BLS disease reaction in the segregating progeny derived from cross between identified resistant source and otherwise improved cultivar is yet to be assessed. Mulberry, being highly heterozygous may be considered as F_1 while exploited in controlled crosses [5]. Progeny derived from these crosses are F_1's but are considered as pseudo-F_2 because the parents are most likely heterozygous at many loci due to cross-pollinated nature [6] of mulberry.

Reports indicated that bacterial pathogen invades leaves through stomata [7]. Besides, it has also been reported that leaf anatomical features like leaf thickness [8], thickness of parenchyma tissues [9] and thickness of cuticle layer [10] are strongly associated with disease resistance in mulberry. However, information regarding association of foliar morphological and anatomical traits with BLS resistance in mulberry is scanty. Similarly, several biotechnological approaches have made since last two decades in regards to phyllogenetic study [11-12], construction of genetic linkage map [13], characterization of stress-related and membrane transporter genes [14] in mulberry, but research on identification of molecular markers for bacterial leaf spot resistance is hitherto unattempted. Therefore, the objectives of the present research were to elucidate BLS disease reaction in the segregating progenies, to determine the correlation between leaf morphological/ anatomical traits and BLS severity and molecular

*Corresponding author's e-mail: rita_csb@rediffmail.com
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

profiling of the selected resistant/susceptible progenies to better comprehend the resistance process to facilitate advanced breeding program in mulberry.

Materials and methods

Planting material

M. rotundiloba, the identified major BLS resistant source (female parent) [15] was utilized in control crossing with otherwise improved cultivar S-1 (male parent) following bagging of female parent and subsequent pollination spanning over 31st January to 10th February 2007 at the Institute Farm. Seeding nursery was raised during May 2007. Sixty full-sib progenies derived from above cross were transplanted in progeny row trial plot with 0.6m interplant distance in a row and 0.9m between rows on 2nd February 2008 along with parents and interspaced with spreader (KPG-1) as source of inoculum. The soil was gangetic alluvium (entisol) clay soil under hot-moist sub-humid agro-ecological sub region of West Bengal with pH 7.6. Recommended inorganic fertilizer dozes of N, P_2O_5 and K_2O @ 360 kg ha^{-1},180 kg ha^{-1} and 112 kg ha^{-1} were applied in the established plantation in five split doses along with FYM @20 mt ha^{-1} 20 days after pruning following five silkworm rearing crop schedule.

Visual symptomatic BLS disease scoring was recorded across 60 full-sib progenies and the two parents during 2008 and 2009 rainy season.

Disease assessment

Bacterial leaf spot disease scoring was recorded using a 0-10 point scale [16]. Progenies with 1 and 2 disease grades (DSI range: 0^+–6) were designated as resistant and a 3 grade (DSI range: 6^+–9) as moderately resistant, grade 4 (DSI range: 9^+–12) as intermediate response, grade 5 (DSI range: 12^+–18) as moderately susceptible and = 6 (DSI range: 18^+ and above) were susceptible. Three branches per plant were rated for disease evaluation, and the highest disease scores were used for qualitative assessment. Disease incidence (DI) was calculated using the formula: DI (%) = (no. of infected leaves on the branch)/(total no. of leaves on the branch) x 100 . A disease severity index (DSI) was calculated for each of 60 full-sib progenies along with parents using the following formula [17]:

$$DSI = \frac{\Sigma \text{ (Ratings for each plant)}}{\text{(10 x Number of plant rated)}} \times 100$$

Foliar anatomical parameters

For estimation of leaf anatomical parameters, microscopic measurements from each progeny (n = 10) in two separate occasions were recorded with a compound microscope (Olympus 276452) at 40X magnification [18].

DNA extraction and RAPD analysis

Mulberry genomic DNA was extracted following the method of DelaPorta [19] with minor modification. The phenolic's activity was avoided by adding 1% polyvinyl pyrrolidone and 1% β-merceptoethanol in the extraction buffer. Equal amount of DNA from *M. rotundiloba* (resistant parent), S-1 (susceptible parent), 5 resistant and 5 susceptible progenies were analyzed for 20 decameric primers (Operon Technologies, USA; Genie, Bangalore, India).

For DNA amplification, 20 µl reaction mixtures contained 2 µl (75 ng/µl) of template DNA, 1.6 µl 100 mM of dNTPs (Genei), 0.1(3U) *Taq* DNA polymerase (Genei), 5µl 5X buffer with 15 mM $MgCl_2$ (Genei), 1.5 µl (100ng/µl) decamaric primer and 9.8 µl double distilled autoclaved water. The amplification reaction was conducted in a Gradient Thermocycler (Eppendorf, Hemburg, USA) under the following temperature conditions: 94°C for 3 mins, 39 cycles of 94°C for 30 s, 37°C for 30 s, 72°C for 2 min with a final extension at 72°C for 10 min. PCR amplification products were separated by standard horizontal electrophoresis in 1.5% agarose (Sigma) gels and stained with 0.05µg/ml ethidium bromide in 1X TBE electrophoresis buffer. The resolved amplification products were visualized under UV light on a UV Transilluminator. Gels were photographed using a gel documentation system (UVP Gel Documentation system, USA).

Statistical analysis

The estimates of descriptive statistic were calculated using STATISTICA AXA 9 software. ANOVA was computed for each parameter separately. To determine if significant correlation existed between DSI and foliar morphological and anatomical features, Pearson's correlation co-efficient and multiple regression analysis were estimated in appropriate cases. The RAPD gels were scored for presence (1) or absence (0) of the amplified fragments and set in a binary matrix and analyzed following NTSYS PC version 2.

Results and discussion

The obtained progenies indicated significant variability for BLS resistance (Table 1and 2). The mean DSI of progeny ranged from 3.0 to 27.2 and mean parental responses were 1.5 and 20.3 for *M. rotundiloba* (resistant source) and S-1 (susceptible source), respectively. It indicated the possibility of further improvement of BLS resistance through transgressive segregation as suggested in brown patch resistance in tall fescue [20]. Five progenies recorded resistant disease reaction (DSI range: 3.0 -5-0). Equal number of progenies (22 each) were classified as moderately resistant and intermediate responsive groups (DSI range: 6.2 – 8.9 and 9.1-12.0, respectively); while, moderately susceptible (DSI range: 12.2-13.1) and susceptible (DSI range: 19.1-27.2) disease reaction groups were represented by 4 and 7 progenies, respectively (Table 1). The population distribution of overall BLS disease severity (DSI) across the progeny was continuous with no discrete classes. Our results corroborate the pattern of segregation of creeping bentgrass progenies to dollar spot [21] and indicating quantitative nature of the resistant trait.

Table 1. BLS disease reaction across the progenies derived from *Morus rotundiloba* and S-1

Disease reaction	DSI range	Progeny (no.)
Resistance	0^{+}-6	5
Moderately resistant	6^{+}-9	22
Intermediate response	9^{+}-12	22
Moderately susceptible	12^{+}-18	4
Susceptible	$>18^{+}$	7

The BLS resistant parents possessed contrasting morpho-anatomical features in respect of leaf thickness, cuticle thickness, stomata frequency, leaf lobation, branching nature and leaf surface [22]. We have selected some of them, as these features are reportedly associated with the resistance of other foliar diseases in mulberry [23, 24]. In our study, stomata frequency, leaf thickness, thicknesses of epidermal and cuticular cell layers of five of each short-listed susceptible (A03-07) and resistant (A08-A12) progenies along with parents (A01and A02) were assessed (Table 2).The

Table 2. Mean values for morpho-anatomical traits and BLS disease severity index of two parents and ten progeny selections across 2008 and 2009

Genotype	DSI[$]	Leaf thickness (µm)[c]	Thickness of epidermal cell layers (µm)[d]		Parenchyma tissue		Thickness of cuticle cell layers (µm)[e]		Stomatal frequency[f]
			Upper	Lower	Spongy	Palisade	Upper	Lower	
Parents									
A01[a]	20.3	109.8±4.3	15.3±1.4	11.1±0.5	38.2±4.5	37.2±2.5	1.2±0.2	0.6±0.1	45±3.2
A02[b]	1.5	98.2±3.6	15.6±1.2	11.3±1.5	32.6±4.0	21.3±3.3	1.0±0.3	0.6±0.1	27±3.4
Susceptible progeny selection									
A03	23.1	92.1±5.3	19.1±1.9	10.8±1.1	24.3±1.4	22.3±2.4	1.4±0.3	1.0±0.3	44±3.8
A04	25.3	98.0±4.2	14.0±1.7	9.6±1.4	21.6±4.0	19.4±4.4	1.2±0.2	0.6±0.1	53±3.8
A05	24.6	93.3±4.4	17.3±1.9	12.1±2.0	27.4±3.0	21.1±4.4	1.1±0.2	0.7±0.3	53±4.1
A06	27.2	101.7±0.7	18.1±2.6	10.2±0.8	33.3±4.2	22.3±3.9	1.0±0.2	0.6±0.1	43±2.9
A07	25.4	100.2±0.9	13.2±2.1	11.4±2.2	35.5±4.7	26.7±2.1	1.2±0.1	1.0±0.3	39±6.0
Resistant progeny selection									
A08	3.3	106.8±2.4	22.9±2.1	10.0±0.8	33.3±4.2	28.2±2.9	1.9±0.3	1.0±0.3	28±4.2
A09	3.1	162.2±11.6	25.7±4.5	17.6±4.1	52.2±5.9	39.7±5.8	1.7±0.3	0.9±0.3	41±2.2
A10	3.0	131.6±8.2	20.3±2.3	14.5±2.1	42.9±3.3	31.6±2.4	1.7±0.3	0.7±0.3	23±2.6
A11	3.3	127.4±9.5	21.8±1.8	16.2±2.1	27.9±2.1	27.9±2.4	1.3±0.3	0.7±0.3	23±2.6
A12	5.0	133.8±7.1	19.6±2.0	12.3±1.6	42.9±1.6	32.1±2.9	1.8±0.2	0.9±0.4	18±1.8
LSD(0.05)	4.49	0.49	0.18	0.16	0.02	0.02	0.31	0.28	0.29

[a]improved cultivar, [b]resistant source; [c]Mean leaf thickness(µm± SD), n=10; [d]Mean thickness (µm± SD), n=10, [e]Mean thickness (µm± SD), n=10, [f]Mean number of stomata per 100 mm^2 ± SD, n=10, [$]DSI = disease severity index

mean stomata frequency of susceptible and resistant progenies to BLS varied from 18 to 53. The leaf thicknesses, upper epidermis and cuticle thicknesses of progeny were in the range of 92.1 to 162.2µm, 14.0 to 25.7µm and 1.0 to1.9µm, respectively.

As the *Xanthomonas campestries* invades mulberry leaf through stomata [25], we have assessed the association between stomata frequency and BLS severity. The stomata frequency exhibited high significant positive (P<0.01) correlation with BLS disease severity (Table 3). On the other hand, leaf thickness had significant negative (P<0.05) correlation with susceptibility to BLS. Moreover, the study revealed that susceptibility to BLS infection increased with decreasing epidermal thickness, which substantiates the previous findings of foliar fungal disease in mulberry [26]. Besides, upper cuticle thickness showed significant negative correlation with BLS susceptibility, but spongy and palisade parenchyma cell layers exhibited negative but non-significant association with DSI values. It has been observed that all selected susceptible progenies (A03, A04, A05 and A07) had high stomata frequency, thin leaf, less upper epidermis thickness with thin cuticle. In contrary, resistant progenies had lower stomatal frequency, higher thickness of leaf, upper epidermis and cuticle cell layers. Most importantly, resistant progeny A-09 exhibited significant superiority for all anatomical attributes.

Regression analysis showed a significant relationship (R^2 = 0.67) between reduced stomatal frequency and increasing resistance to BLS infection (Fig. 1) and decreasing leaf thickness and increasing DSI across the years (R^2 = 0.42). Similarly, decreasing upper epidermal thickness and upper cuticular thickness were observed with increasing DSI over the years. The strong significant association of stomata frequency, leaf

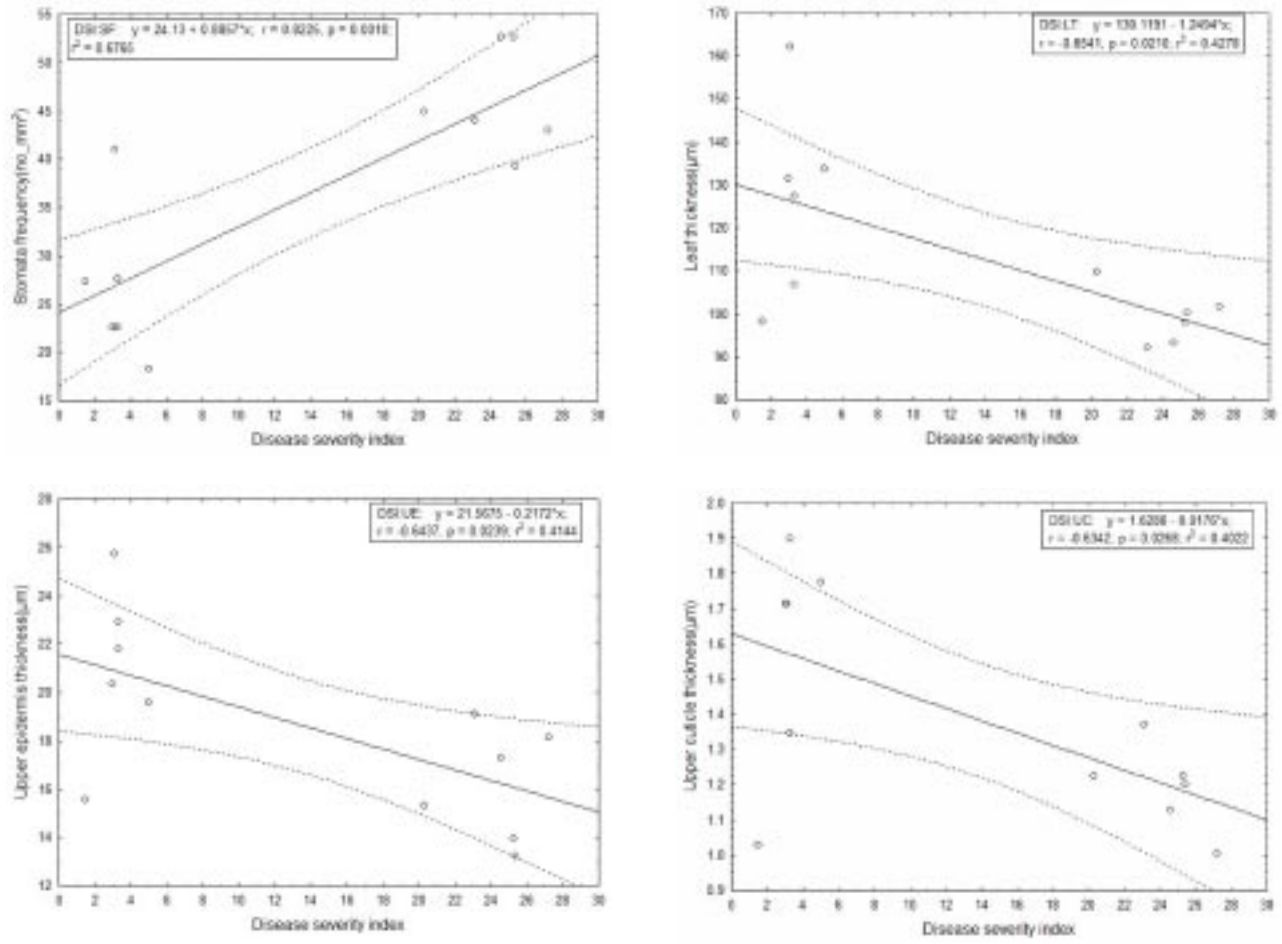

Fig. 1. Regression of stomatal frequency (SF), leaf thickness (LT), upper epidermal thickness (UE). Upper cuticular thickness (UC) and BLS disease severity (DSI). Dashed lines represent 95% confidence intervals in the prediction lines estimated from the data.

Table 3. Correlation of leaf thickness, thickness of epidermal cell layers, parenchymatous tissue, thickness of cuticle layers and stomata frequency with DSI of bacterial leaf spot

Parameter	Leaf thickness	Thickness of epidermal cell layers		Parenchyma tissue		Thickness of cuticle layer		Stomata frequency
		Upper	Lower	Spongy	Palisade	Upper	Lower	
DSI	-0.654*	-0.644*	-0.572^{ns}	-0.511 ^{ns}	-0.464 ^{ns}	-0.634*	-0.12 ^{ns}	0.82**
Leaf thickness		0.721*	0.843**	0.817**	0.810**	0.644*	0.15 ^{ns}	-0.45 ^{ns}
Upper epidermal thickness			0.660*	0.485 ^{ns}	0.510 ^{ns}	0.720*	0.37 ^{ns}	-0.41 ^{ns}
Lower epidermal thickness				0.568 ^{ns}	0.455 ^{ns}	0.364 ^{ns}	0.09 ^{ns}	-0.34 ^{ns}
Spongy parenchyma					0.852*	0.533 ^{ns}	0.19 ^{ns}	-0.38 ^{ns}
Pallisade parenchyma						0.588 *	0.20 ^{ns}	-0.31 ^{ns}
Upper cuticle thickness							0.58*	-0.54*
Lower cuticle thickness								-0.19*

*,** significant at $p<0.05$ and $p<0.01$, respectively; ns: not significant

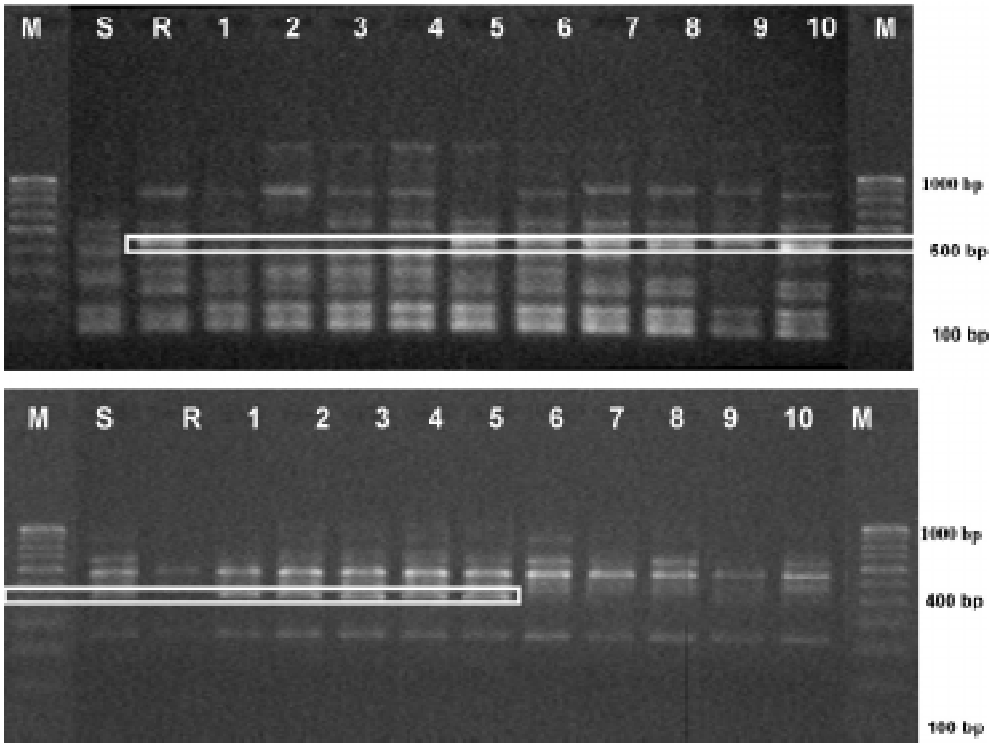

Fig. 2. RAPD profile of contrast responsive progenies for BLS resistance using (a) GP-17 and (b) GP-18. M: Marker, S: Susceptible parent, R: Resistant parent, 1- 5 = susceptible progenies(A03-A07); 6 – 10 = resistant progenies (A08-A12)

thickness, thickness of upper epidermis and upper cuticle layers with BLS disease severity indicated that these variables can be used as indirect selection parameters to increase BLS resistance in mulberry. Presence of lower frequency of stomata in resistant cultivar has also been reported in other vegetatively propagated crop [27].

It seems, BLS resistance in mulberry is the result of interaction of a number of factors. Low stomata frequency, high leaf, epidermis thickness, and cuticle thickness may act as a barrier in resistant genotypes to prevent BLS infection.

RAPD markers linked to disease resistance have

Table 4. Number of amplification products generated with RAPD primers

S.No.	RAPD Primer	Sequence (5'-3')	Annealing temp.	Size (bp)	Polymorphic bands	Monomorphic bands	Total no of RAPD product /primer	Polymorphic bands (%)
1.	GP-14	ACTTCGCCAC	37^0C	350-1100	5	0	5	100
2.	GP-15	AGCCTGAGCC	37^0C	220-900	7	0	7	100
3.	GP-16	AGGCGGCAAG	37^0C	200-800	6	0	6	100
4.	GP-17	AGGCGGGAAC	37^0C	100-900	9	0	9	100
5.	GP-18	AGGCTGTGTC	37^0C	300-950	5	1	6	83.3
6.	GP-20	AGTCCGCCTC	37^0C	300-1000	5	1	6	83.3
7.	GP-24	CCAGCCGAAC	37^0C	250-700	5	1	6	83.3
8.	GP-25	GAGCGCCTTC	37^0C	300-1100	4	0	4	100
9.	OPY2	CATCGCCGCA	37^0C	400-1000	3	0	3	100

been reported in several crop plants [28]. We have analyzed two parents (*Morus rotundiloba* and S-1) and selected contrast responsive progenies (A03-A12) of mulberry to BLS with 20 decamer primers. Of these, 9 primers showed polymorphism among parents and contrast responsive progenies (Table 4). Importantly, a polymorphic DNA fragment (about 500 bp) was obtained in *M. rotundiloba* and all studied resistant progenies with the primer GP-17. Similarly, an exclusive DNA band (about 450bp) was obtained in S-1 and susceptible progenies with primer GP-18 (Fig. 2).

In the cluster tree based on similarity values showed that the resistant parent A02 was out grouped at a similarity coefficient 0.65 (Fig. 3). The main cluster was subdivided into two groups at a similarity coefficient of 0.70. The resistant parent (A02) was found in close proximity with identified resistant progenies namely, A08, A09, A10, A11 and A12. Similarly, susceptible progenies like A03, A04, A05 and A06 were very near to the susceptible parent A01 except A07. RAPD profiling results with GP-17 also supports the view where the susceptible progeny A07 showed DNA fragment (450 bp) identical with resistant lines. In this study, RAPD markers GP-17 and GP-18 were found capable to differentiate resistant and susceptible lines. Perhaps this is the first attempt for identification RAPD fragment linked to BLS resistance in mulberry. However, successful application of linked molecular markers in advanced breeding program requires its validation and subsequent utilization in other genetic background. In conclusion, it can be said that continuous population distribution for BLS disease severity across the developed progeny suggested quantitative nature of BLS resistance in mulberry. Stomata frequency, leaf thickness, upper epidermal thickness and cuticle thickness may be used as additional selection features for BLS resistance/susceptibility . RAPD primer GP-17 and GP-18 amplified 500bp and 450bp DNA fragments may have potential to differentiate BLS responsive resistant and susceptible lines for utilization in mulberry improvement program.

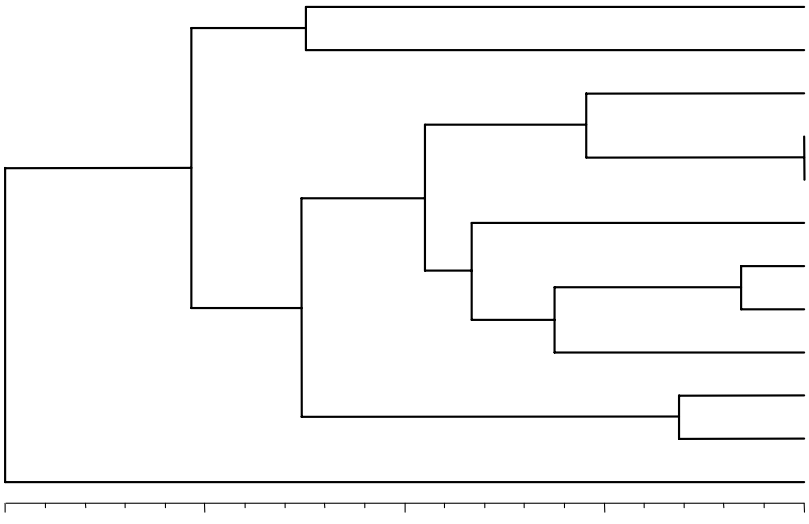

Fig. 3. Dendrogram generated by UPGMA based on 9 RAPD primers

References

1. **Akhtar M. A**. **and Sarwar M.** 1998. Mulberry bacterial blight in Pakistan. Pakistan J. Agri. Res., **9**: 417-419.

2. **Choi S. H., Lee K. G., Choi Y. S., Lee E. I. and Kim Y. T.** 1988. Pathogenicity and bacteriological

characterization of *Xanthomonas campestris* from mulberry. Korean J. Plant Patho., **4**: 143-148.

3. **Pandey P. C. and Singh S.** 1989. Three important diseases of mulberry in India. Indian J. Forestry., **12**: 255-258.
4. **Kore S. S. and Patil P. S.** 1985. Investigations of bacterial blight disease of mulberry (*Morus alba* L. Abstr., 37th Annual Meeting of IPS, January 8-10 Indian Phytopath., **38**: 622.
5. **Leu B. H.** 1998. Introduction to genomics. *In*: Statistical Genomics Linkage, Mapping and QTL analysis.CRC press, LLC : 59-60.
6. **Allard R. W.** 1999. Priciples of Plant Breeding. John Wiley & Sons, New York.
7. **Gudesblat G. E., Torres P. S. and Vojnov A. A.** 2009.Stomata and pathogens warfare at the gates. Plant Signaling and Behavior, **4**: 1114-1116.
8. **Philip T., Govindaiah, Sengupta K. and Naik N.** 1991.Anatomical nature of resistance in mulberry genotypes against *Cerotellium fici* causing leaf rust. Indian Phytopath., **44**: 249-251.
9. **Philip T. and Govindaiah.** 1996. Studies on the factors contributing for disease resistance in mulberry against *Cercospora moricola*. Indian J. Seric., **35**: 39-42.
10. **Gupta V. P., Tewari S. K. and Dutta R. K.** 1995. Surface ultrastructure studies on ingress and establishment of *Pseudomonas syringae* pv. *Mori* on mulberry leaves. J. Phytopath., **143**: 415-418.
11. **Aggarwal R. K., Udaykumar D., Hendre P. S., Sarkar A. and Singh L.** 2004. Isolation and characterization of six novel microsatellite markers for mulberry (*Morus indica*). Molecular Ecology Notes, **4**: 477-479.
12. **Orhan E., Ercisli S., Yildiri N. and Agar G.** 2007. Genetic variations among mulberry genotypes (*Morus alba*) as revealed by Random Amplified Polymorphic DNA(RAPD) markers. Pl. Syst. Evol., **265**: 251-258.
13. **Venkateswarlu M., Urs R. S., Nath S., Sashidhar H. E., Maheswaran M., Veeraiah T. M. and Sabitha M. G.** 2006. A first genetic linkage map of mulberry (Morus spp.) using RAPD, ISSR and SSR markers and pseudotestcross mapping strategy. Tree Genetics & Genomics, **3**: 15-24.
14. **Lal S., Ravi V., Khurana J., and Khurana P.** 2008. Repertoire of leaf expressed sequence tags (ESTs) and partial characterization of stess-related and membrane transporter genes from mulberry (*Morus indica* L.). Tree Genetics & Genomics, DOI 10.1007/s 11295-008-0192-7.
15. **Banerjee R., Maji M. D., Ghosh P. and Sarkar A.** 2009.Genetic analysis of disease resistance against *Xanthomonas campestris* pv. *mori* in mulberry (*Morus* spp.) and identification of germplasm with high resistance. Archives of Phytopath. and Pl. Protect., **42**: 291-297.
16. **Horsefall J. G. and Cowling E. B.** 1978. *In*: Horsefall JG and Cowling EB (eds.). *Plant disease*. Academic Press, New York, USA, 1978. pp 120-136.
17. **Kim H. S., Sneller C. H. and Diers B. W.** 1999. Evaluation of soybean cultivars for resistance to Sclerotia stems rot in field environments. Crop Sci., **39**: 64-68.
18. **Banerjee R., Chakroborty S. P. and Das B. K.** 2006.Morpho-anatomical traits and preliminary screening of mulberry (*Morus* spp) genotypes for moisture stress condition. Indian. J. Genet., **66**:134-136.
19. **Dellaporta S. L., Wood J. and Hicks J. B.** 1983. A plant DNA minipreparation : version II., Plant Mol Biol Rep., **1** : 19-21.
20. **Bokmeyer J. M., Bonos S. A. and Meyer W. A.** 2009.Inheritance characteristics of brown patch resistance in tall fescue.Crop Sci., **49**: 2302-2304.
21. **Bonos S. A., Casler M. D. and Meyer W. L.** 2003. Inheritance of dollar spot resistance in creeping bentgrass. Crop Sci., **43**: 2189-2196.
22. **Central Silk Board.** 1997. Catalogue on mulberry (*Morus* spp.) germplasm. Vol: 1. Silkworm & mulberry germplasm station, Central Silk Board, Hosur.
23. **Chattopadhyay S., Ali A. K., Doss S. G., Das N. K., Aggarwal R. K., Bandyopadhyay T. K., Sarkar A. and Bajpai A. K.** 2011. Association of leaf micro-morphological characters with powdery mildew resistance in field-grown mulberry (*Morus* spp.) germplasm. AOB Plants doi:10.1093/aobpla/plr002.
24. **Sharma D. D., Chowdary N. B., Naik N., Rajan M. V. and Dandin S. B.** 2005. Blight diseases of mulberry (*Morus* spp.) - A review. Sericologia, **45**: 231-245.
25. **Gupta V. P., Vineet K., Rajan R. K. and Dandin S. B.** 2005. Ultrastructure of ingress, infection and sporulation of various pathogens causing foliar disease in mulberry-an appraisal. Sericologia., **45**: 377-392.
26. **Vijaykumar G. C.** 1991. Histopathological and histochemical changes in mulberry (*Morus alba* L.) powdery mildew due to infection by *Phyllactinia corylea* (Pers) Krast. Sericologia., **31**: 703-707.
27. **Bisognin G. A., Segatto F. B., Manforn P. A., Muller D. R. and Rampelotto M. V.** 2006. Leaf morpho-anatomical characteristics of five potato clones. Cinciae Natura, UFSM, **28**: 29-41.
28. **Mondal S. and Badigannavar A. M.** 2007.RAPD markers linked to a rust resistance gene in cultivated groundnut (*Arachis hypogaea* L.). Euphytica, **159**: 233-139.