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Research Article

Genetic appraisal of frost damage in mulberry germplasm accessions in temperate climate of Jammu and Kashmir, India

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Abstract
Sericulture is an age old traditional occupation in Kashmir which owns a temperate climate and its success is dictated by the quality of mulberry leaves. Like many other plants, Mulberry, *Morus* spp. is also damaged by frost and has been felt as an impediment in expanding the sericulture for two to three seasons from the present one season activity. The impact of winter 2018-19 on the mulberry accessions at Manasbal, Kashmir was measured during February –April 2019 in terms of frost damage and sprouting. Studies revealed large variability in frost damage ranging from 1.21 to 23.71 per cent. *Morus alba* accessions though recorded higher frost damage, offer higher scope and potential for frost tolerance breeding with reliable genetic estimates in terms of high GCV (72.37%), PCV (84.38%) and heritability (73.55%) but, had a low genetic advance (7.84%). A recently developed *M. multicaulis* variety, PPR-1 appeared to be a promising one for frost tolerance breeding as well as commercial cultivation.

Key words
Mulberry, Frost damage, Genetic estimates, Genetic Advance, Heritability, Temperate Kashmir

INTRODUCTION

Sericulture being one of the oldest known occupations in Kashmir and was a prominent player on the silk route with records of exporting 25000 oz of silkworm seeds to Europe during 1855. But with time, the sericulture prospects went on oscillating and distracted the farming and weaving community as it was a state monopoly till the 1990's (Naik, 2017). Since then, efforts are on to bring the industry back on track to the heights of history but, are in vain. It is mainly because of lack of organized mulberry wealth with the farming community who are dependent mainly on the state owned farms, roadsides and bund side have grown unmanaged and unattended mulberry trees for their foliage requirements to feed their silkworms. Low or limited leaf yield of the mulberry genotypes especially under wild tree form is one among the few constraints for sericulture in general, northwestern India and Kashmir in specific compared to the potential leaf yield of 16 to 17 mt/ha/year with improved mulberry genotypes yielding grown as tree form under recommended packages (Dhar *et al.*, 2007). Generally, the majority of the improved mulberry accessions are from *M. indica* background throughout the country, in Kashmir *M. multicaulis* accessions are preferred for late age rearing owing to high fibre content (Dhar *et al.*, 2011). Efforts to enhance the leaf yields to threshold levels are underway to make the Kashmir sericulture occupation a remunerative one. Besides, the extent of damage due to frost/snow in the temperate climate of Kashmir is up to 30 per cent which reduces leaf yield in the spring season and the frost damage to saplings extends the gestation period by a year (Baksh, 2011; Shabnam *et al.*, 2012; Ahanger *et al.,* 2013). So far there are no efforts made to delineate mulberry genetics from the frost aspect even though opportunities are available (Shukla *et al.*, 2016) compared to efforts made for mulberry growth and yield traits (Keshava Murthy *et al.,* 2010; Kala *et al.*, 2016; Chanotra *et al.,* 2019). Mulberry variety resistant to frost damage is a pre-requisite to save time at the sapling stage and to increase the foliage yield under temperate climatic conditions. In this background, an attempt was made to find out the reaction of mulberry germplasm present at Manasbal, Kashmir to the 2018-19 season's winter during the early spring season (March –April) of 2019.

MATERIALS AND METHODS

The frost damage to mulberry accessions in Kashmir valley was measured at the end of winter 2018-19 (February 2019) and the bud sprouting was recorded during early spring 2019 (March –April 2019). Frost damage was measured from mulberry germplasm accessions at P4 Basic Seed Farm, Central Silk Board, Manasbal, Safapora, Kashmir, India located at an altitude of 1583 meters above mean sea level, 74° 40'E longitude and 34° 15' N latitude. The minimum and maximum temperature during winter from November 2018 to February 2019 ranged from -7.7 to 21.6 °C with 292.5 mm rainfall and 18 frost days.

The mulberry germplasm was maintained as high bush at a spacing of 180 × 90 cm; established during March 1992 (age group 1), March 2007 (age group 2) and March 2013 (age group 3). The germplasm comprised of 73 accessions of mulberry comprising of 41 indigenous origins (including 10 Native to Kashmir) and 32 exotic origins (20 Japanese; three Italian, two each of France and Bangladesh and one each from USSR, China, Indonesia, Paraguay and Myanmar). Mulberry germplasm studied comprised of five species of *Morus viz*., *Morus alba* L. (52), *Morus multicaulis* P. (11), *Morus bombycis* K. (5), *Morus indica* L. (4) and *Morus kayayama* L. (1).

Cumulative frost damage was measured in terms of dieback in three branches per tree. Of these three branches observed, one was the tallest; one shortest and the other was a medium in length among all the branches of a tree. Three trees were randomly selected from each germplasm accession excluding those on the exposed sides like road and bund. The method followed for quantifying the frost damage was the extent of dieback in a branch by measuring the total length as well as the dieback diseased length and estimating the frost damage as per cent damage deployed by Ahanger *et al.* (2013) for mulberry frost damage assessment.

Data were subjected to ANOVA (Larson and Hsu, 2010). Genetic parameters *viz.,* PCV and GCV were estimated as suggested by Burton and De Vane (1953) and classified into low (0 - 10%), moderate (10.1% - 20%) and high (>20%) (Robinson *et al.,*1949). Heritability in the broad sense for frost damage was computed (Lush, 1949) and classified into low (0 - 30%), moderate (30.1% - 60%) and high (>60%) (Robinson *et al.,* 1949). Genetic advance (GA) and genetic advance as per cent mean (GAM) was also estimated (Johnson *et al.,* 1955) and categorized into low (0 - 10%), moderate (10.1% - 20%) and high (20% and above). Accessions were grouped under different species of *Morus*; two age groups (27 and 12 years old barring PPR-1 of age group 3, as it was a sole entry) and into four sprouting groups (between March second week to April first week in spring 2019).

RESULTS AND DISCUSSION

Irrespective of the mulberry germplasm the mean frost damage to mulberry at P4 Manasbal during 2018-19 as measured during February 2019 was 6.01 ± 4.43 per cent and it ranged between 1.21 to 23.71 per cent. Among the five species of *Morus* accessed for their frost injury least (4.05 ± 1.41%) was observed with *M. multicaulis* followed by *M. kayayama* (4.07%) and it was maximum with *M. alba* (6.63 ± 4.94%) (**Table 1**). The least frost damaged (PPR-1-1.21%) accession is a *M. multicualis* accession and is a young tree having a high potential for a temperate climate with the least frost damage and relatively an early sprouting character a much desired trait of the days and has been released recently by CSR&TI, Pampore (Shabnam and Sharma, 2016). The present study also offers huge scope for the identification of genetic mechanism associated with cold tolerance in mulberry which has been initiated (Shukla *et al.,* 2019).

The analysis of variance for frost damage has differentiated the mulberry varieties and was significant (**Table 2**) irrespective of the grouping as well as within each subgroup. Genetic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) was high for *Morus* spp. (72.37 and 84.38%) and *M. alba* accessions (73.94 and 86.76%); age class 2 (12 years old) (73.78 & 87.17%) and sprouting group 3 (March 3rd week 2019) (81.32 & 85.98%) and the environmental influence was relatively less in each category considered indicating a huge potential for exploring the genetic potential to fight frost injury in mulberry. There was a narrow or marginal variation between the GCV and PCV indicating the least impact of the environment in the reaction of mulberry accessions to frost at Manasbal during the 2018-19 winter. Among the four species, the values for GCV, PCV and ECV were less with *M. multicaulis* and ECV was moderate for *M. bombysis* (10.89). Heritability was high (8.85) for *M. alba* accessions indicating bright breeding options open for exploring these accessions in breeding for frost tolerance. Though heritability estimates represent the relative genetic strength for frost tolerance in *M. alba* accessions and also indicate the efficiency in measuring the frost damage adopted in the study. Heritability values along with genetic advance and genetic advance as per cent mean was high for all mulberry accession in general and specific to *M. alba* accessions at Manasbal. High to low genetic advance coupled with heritability were documented for fresh leaf weight (77.9 %), the number of leaves per meter twig (68.6%), internodal distance (64.4%), leaf length (61.4%), dry leaf weight, moisture percentage (54.3%), leaf area (48.2%) and leaf width (36.7%) in 44

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mulberry accessions in Kashmir at Udheywalla campus, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (Chanotra *et al.,* 2019). Maji (2011) has identified a huge genetic potential in mulberry against powdery mildew disease. Identification of stress specific genes in the mulberry genome (Shukla *et al.*, 2019) may add strength in harnessing the frost or cold damage resistance potential available with the mulberry wealth at Manasbal.

Table 1. Mean frost damage in mulberry accessions of at Manasbal, Kashmir, India

Accession	Frost damage (%)	Accession	Frost damage (%)	Accession	Frost damage (%)
Morus alba **(52) (6.63 ± 4.94%)**					
ChattatulZaingir[4]	2.52	Muriaso[2]	4.06	Chinese white[2]	5.73
Serpentine[3]	2.60	ME – 58[2]	4.18	Mandalay (S-1)[2]	6.59
Italian sarnal[4]	2.81	FGDTR[1]	4.25	S-30[2]	7.84
Brentul Kashmir[4]	2.86	ME-53[3]	4.31	AR-12[3]	7.93
S-146[3]	3.05	Zagtul[4]	4.31	TR-10[2]	8.12
Chattatul[4]	3.05	ME- 129[2]	4.38	S-1708[2]	8.14
Kokusou -27[4]	3.18	Ichehei[3]	4.41	T-4[2]	8.27
Togowase[4]	3.28	S-1635[2]	4.42	S-1301[2]	8.74
S-54[2]	3.33	C-776[2]	4.55	S-36[2]	9.54
C-1733[2]	3.38	Ichinose[4]	4.62	Victory (V1)[3]	10.08
French[4]	3.39	Zangabad[3]	4.73	TR-8[2]	10.39
S-41[2]	3.49	Botatul[4]	4.84	S-799[1]	13.06
Shrim-8[2]	3.51	ME – 27[3]	4.87	Kanva -2[2]	16.11
Limoncina[4]	3.71	Vishala[2]	5.12	Punjab Local[1]	16.16
BC-259[3]	3.77	T-10[2]	5.32	C-4[2]	18.21
Shrim-5[2]	3.88	Sanish-5[4]	5.50	AR-14[3]	23.49
Tomieso[4]	3.97	Karionizamigashi (KNG)[4]	5.55	S-1531[1]	23.71
Okinowa[1]	7.67				
Morus multicaulis **(11) (4.05 ± 1.41%)**					
PPR-1[2]	1.21	Kokusou-21[4]	3.13	Kousen[3]	6.50
Kairyoroso[4]	3.03	Kokusou -20[4]	4.70	Rokokuyaso[4]	4.71
M. multicaulis[2]	3.07	Kasuga[4]	3.84	Goshoerami[4]	4.78
Kokusou -13[2]	5.29	Tsukasaguwa[4]	4.27		
Morusbombycis **(5) (5.67 ± 3.71%)**		***Morus kayayama*** **(1)**		***Morusindica*** **(4) (4.29 ± 2.04%)**	
Senmatso[4]	3.57	Chinarpati[4]	4.07	Mysore local[2]	2.28
Enshatakosuke[4]	3.96			Himachal local[2]	3.65
Shimanouchi[4]	3.99			Nadigam[3]	4.12[3]
Obawase[3]	4.54			Lajward[4]	7.11
English Black[3]	12.28[3]				

Superscripts indicates the sprouting during 2019:- 1: March 2nd week; 2: March 3rd Week; 3: March 4th Week & 4: April 1st Week

All the six genetic estimates for frost were almost double or more in the age class- 2 (12 years old) indicates brighter scope employing this stage as ideal for the breeding programme for frost damage in mulberry against frost. When sprouting with spring was considered, the mulberry accessions sprouting during the 4th week of March (Sprouting 3) have shown higher and dependable genetic estimates for breeding for frost tolerance in mulberry. This being a first attempt to access the genetic potential of mulberry accessions in temperate climate at Manasbal, Kashmir against frost damage which needs to be explored further with repeated studies besides combining with other growth and yield parameters needed for the temperate sericulture. Besides, the least frost damaged accession, PPR-1, a *M. multicualis* accession in age group 3 needs to be explored further as it is least affected by frost and relatively early compared to the rest is evident in this study too (Shabnam and Sharma, 2016). This may also indicate the hidden potential available with young age plants which need to be revisited to confirm.

Table 2. Genetic estimates of frost damage in mulberry germplasm accessions at Manasbal, Kashmir

Germplasm (Number of accessions)	Mean	Range	CD (p=0.05)	GCV (%)	PCV (%)	ECV (%)	H (BS) (%)	GA	GAM
Morus spp. (73)	6.13	3.08–19.53	4.02*	72.37	84.38	43.39	73.55	7.84	127.85
M. alba (52)	6.81	2.46 - 24.39	5.01*	73.94	86.76	45.38	72.64	8.85	129.83
M. multicaulis (11)	4.06	0.00 - 5.60	1.72*	27.03	36.73	24.86	54.17	1.66	40.98
M. bombysis (5)	4.64	0.00 - 7.59	0.95*	35.88	37.49	10.89	91.56	3.28	70.72
M. indica (4)	4.11	0.00 - 6.35	2.33*	37.64	47.14	28.38	63.76	2.54	61.92
Age class 1 (35) $	4.47	2.46 - 10.97	1.75*	30.56	38.90	24.06	61.73	2.21	49.46
Age class 2 (37)$	7.69	2.63 - 26.82	5.81*	73.78	87.17	46.42	71.64	9.89	128.65
Sprouting 1 (5)	13.88	0.00 - 26.82	10.48*	59.56	71.80	40.09	68.82	14.13	101.79
Sprouting 2 (28)	6.22	0.00 - 19.53	5.19*	56.58	76.17	50.99	55.19	5.39	86.59
Sprouting 3 (13)	6.78	0.00 - 24.39	3.18*	81.32	85.98	27.93	89.45	10.74	158.43
Sprouting 4 (27)	4.00	0.00 - 6.35	1.13*	20.83	27.03	17.23	59.38	1.32	33.06

* Critical difference for differentiating the accessions for frost damage in respective groups
$ Age Class:- 1: 27 years old 2: 12 years old

From the study, it can be concluded that, among 73 mulberry accessions under five species of *Morus*, three age groups and four sprouting groups at Manasbal, Kashmir measured for winter 2018-19 frost damage during early spring 2019 revealed large variability in frost damage. High genetic estimates in the mulberry accessions especially of *M. alba,* accessions, accession in Sprouting-3 group (sprouting during March 4th week 2019) and in age class 2(12 year old) offered a good scope for breeding against frost damage in mulberry for temperate sericulture.

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REFERENCES

Ahanger, M.R., Ramegowda, G.K., Illahi, I., Rizvi, G., Dhar, A. and Sahaf, K.A. 2013. Reaction of Mulberry Germplasm to Frost Damage in Kashmir. *Res. J. Agri. Sci.*, **4**(2): 180-183.

Baksh, S. 2011. Breeding of early sprouting and high yielding mulberry amenable to propagation by stem cuttings with high survival ability for rainfed cultivation in Kashmir and other temperate regions of North India. *Golden Jubilee Conference - Sericulture Innovations: Before and Beyond 2011*, CSR&TI, Mysore, 28-29 January 2011, pp. 1-15.

Burton, G.M. and De Vane, E.M. 1953. Estimating heritability in tall Fescue (*Festuca arundinaceae*) from replicated clonal material. *Agronomy J.,* **45**: 478-481. [Cross ref]

Chanotra, S., Bali, R. K. and Bali, K. 2019. Estimation of Genetic Variability and Heritability in Selected Mulberry Germplasm Accessions (*Morus* spp.). *Int. J. Curr. Microbiol. App. Sci.,* **8**(2): 493-499. [Cross ref]

Dhar, A., Fotadar, R.K., Kaur, R. and Khan, M.A. 2007. Suitable mulberry genotypes for North India. *Bulletin No.* ***11***, CSR&TI, Pampore.

Dhar, A., Fotadar, R.K., Shabnam, A. A. and Khan, M.A. 2011. Catalogue on temperate mulberry germplasm. *Book No.* ***1***, CSR&TI, Pampore.

Johnson, H.W., Robinson, H.E. and Comstock, R.E. 1955. Estimates of genetic and environmental variability in soybean. *Agron. J.,* **47**: 314-18. [Cross ref]

Kala, P., Zargar, S., Bali, R., Gupta, N., Salgotra, R. and Koul, A. 2016. Assessment of Genetic Diversity in Mulberry using Morphological and Molecular markers. *Electronic J. Plant Breed.*, **7**(1): 94-103. [Cross ref]

Keshava Murthy, B.C., Puttaraju H.P. and Hittalmani, S. 2010. Genetic variability and correlation studies in selected mulberry (*Morus* spp.) germplasm accessions. *Electronic J. Plant Breed.*, **1**(3): 351-355.

Larson, D.A. and Hsu, K.A. 2010. Analysis of Variance with summary statistics in Microsoft Excel. *Am. J. Bus. Educ.,* **3**(4): 7-11. [Cross ref]

Lush, J.L. 1949. Heritability of quantitative characters in farm animals. In: *Proc. 8th Cong. Genet. Heriditas,* **35**: 356-375. [Cross ref]

Maji, M.D. 2011. Genetic variability of mulberry (*Morus* spp.) germplasm against powdery mildew (*Phyllactinia corylea*) and identification of high resistance genotypes. *Archives of Phytopath.Pl. Prot.* **44** (6): 513–519. [Cross ref]

Naik, A.H. 2017. An Overview of Sericulture Industry in Kashmir. *Int. J. Res. Humanit. Arts Lit.,* **5**:185-196.

Robinson, H.F., Comstock, R.E. and Harney, P.H. 1949. Estimates of heritability and degree in corn. *Agron. J.,* **41**: 353-359. [Cross ref]

Shabnam, A.A. and Sharma, S.P. 2016. Improved mulberry variety: PPR-1 for temperate region. *Bull. No.* **20**, Central Sericultural Research and Training Institute, Central Silk Board, Pampore (J&K).

Shabnam, A.A., Fotadar, R.K., Dhar A. and Bhat, A.H. 2012. Comparative analysis to Tropical and Temperate Genotypes and behaviour of five species of Mulberry under Temperate conditions of Kashmir. *Res. J. Agri. Sci.,* **3**: 089-093.

Shukla, P., Reddy, R.A., Ponnuvel, K.M., Rohela, G.K., Shabnam, A.A., Ghosh, M.K. and Mishra, R.K. 2019. Selection of suitable reference genes for quantitative real-time PCRgene expression analysis in Mulberry (*Morusalba*L.) under different abiotic stresses. *Mol. Biol. Rep.,* **46**(2):1809-1817. [Cross ref]

Shukla, P., Rohela, G.K., Shabnam, A.A. and Sharma, S.P. 2016. Prospect of Cold Tolerant Genes and Its Utilization in Mulberry Improvement. *Indian Hort. J.*, **6** (Special): 127-129.