EXPRESSION PROFILING OF GROWTH SPECIFIC GENES OF *BOMBYX MORI* FOR INCREASED PRODUCTION OF SILK – A REPURPOSED PHARMACEUTICAL AID

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ABSTRACT

Silkworm, *Bombyx mori* has been used for wide variety of application. In the recent past, the various components of silk such as, silk film, functionalized bio-materials, silk thread, silk protein have found their utility in epidermal recovery, bone formation, in surgery and drug delivery systems. This newer findings in pharmaceutical research have added new dimension to the silk production. The genetics of yield expression, shown to be of polygenic nature but it has been given less importance. The current study is aimed to characterize the markers associated with 5 selected yield traits, with selective RFLP (Restriction fragment length polymorphism) derived sequence–tagged site (STS) primers on the

genomic DNA of silkworm germplasm stocks of different origin and diverse yield potential. The molecular characterization directed to recognized two markers exhibits significant association with the yield traits. The markers could classify the stocks according to yield potential, respectful of their origin. The importance of the STS primers is postulated in the situation of applying multiple regression models for recognize markers associated with yield expression and stability for molecular breeding work in *B. mori* for yield improvement. Three restriction banding patterns were recognized among the screened silkworm genetic resources and their scoring revealed high, moderate and low fecundity and growth groups aa, ab banding types were found in Bivoltine high yielding groups and ac, bc found in high yielding Multivoltine groups. The prothoracic gene of *B. mori* naturally is a polymorphic, the respective gene it was well characterized and cloned with PTTH locus encoded PTTH in silkworm. The three alleles which was identified can be diagnosed using the gene amplification as a result of their gene length 3rd and 4th introns varies depending on the genotype. Express Sequence Tag site technique was employed as an important molecular tool to identify productive breeds among promising silkworm genetic resources. Identified productive races based on growth and yield specific genes can be used as high productive/robust breeds by the breeders for marker assisted selection breeding to evolve robust breed for field utilization and augmentation of silk productivity not only in the pharmaceutical field but others as well.

Keywords: Silkworm; EST; RFLP- (STS); molecular markers

INTRODUCTION

Sericulture is an important rural agro-industrial practice in India and all the major commercial silks are produced in the country **[1]**. Sericulture, it's the method of development of silkworms based products like raw silk, has been ongoing for at least 5000 years in China, from where it was spread to Korea and Japan and followed in west region of India. The prestigious silk fiber is produced by the monophagous insect, the silkworm *Bombyx mori*, a Lepidopteron; whose sole food is mulberry. Silkworm is a holometabolous insect, which completes its life cycles of four different metamorphosing phases i.e., egg, larva, pupa and adult 50-55 days. In the recent past, the various components of silk such as, silk film, functionalized bio-materials, silk thread, silk protein have found their utility in epidermal recovery, bone formation, in surgery and drug delivery systems. Silk scaffolds are used in wound healing and in tissue engineering of bone, cartilage, tendon and ligament tissues. This newer findings in pharmaceutical research have added new dimension to the silk production. The genetics of yield expression, shown to be of polygenic nature but it has been given less importance.

The universal collection of more than 4000 *Bombyx mori* strains were collected

maintained by Central Sericultural Germplasm Resources Centre (CSGRC), Hosur, Tamil Nadu, moreover 450 strains of silkworm are maintained and stored in aseptic environment .The gene pool available in the country can be broadly divided in to two groups, the low yielding stocks characterized by high adaptability to tropical conditions and the yielding stocks exhibiting regular diapause, but suffer from the low versatility to the highly variable tropical agro-climatic conditions **[2, 3]** and are mainly developed from Japanese hybrids **[4]**. The proposed 'gene tagged breeding' to combine the best qualities of the temperate high yielding stocks with tropical hardy ones **[5, 6]**. The analyzed many cDNA libraries prepared from various tissues **[7]** on the early stages that mostly enclose the complete genes includes 35,000 ESTs from 36 cDNA libraries. Once generated, they are useful in cloning specific genes of interest and mapping of functional genes in various related organisms. Such markers are obtained by partial sequencing of random clones. ESTs are generally utilized for biomolecular genome sequencing and alignment program and many numbers of organisms were characterized to mine active genes and genome for biological applications and it's a one of the common tools for genome analysis too. ESTs will give biological researchers with fast and precious way for identify a new gens and genomes to recognize the gene over expression and gene regulations for traits mapping. EST database were also associated develop simple sequence repeat (SSR) markers for many species. *Bombyx mori* whole genome characterization and analysis the ESTs database were constructed **[8]** integuments almost 55% of whole genome of silkworm. ESTs are presently being used as markers for genome mapping and identification of expressed genes. The biological method also matched in structure of BAC library and making BAC contigs based on DNA fingerprinting and EST markers substantial to interdependence maps. Vitellogenins, the precursors of major yolk proteins of oviparous animals, Vitellogenin of the silkworm, *Bombyx mori*, is a tetramer with molecular weight of 440K, composed of each two molecules of non-identical subunits termed heavy chain and light chain **[9]**.

The prothoracic gene of *Bombyx mori* is a polymorphic one. The gene for the prothoracicotropic hormone (PTTH) in *Bombyx mori* has been already cloned and characterized. Three alleles a,b,c were identified in the PTTH locus which encodes PTTH in the silkworm, *Bombyx mori*. These three alleles can be easily diagnosed by using the PCR technique because their length of the 3rd and 4th introns varies depending on the genotype **[10]**. Silk

fibroin were secreted on the lumen of the posterior silk gland. Silk fibroin is composed of one heavy chain ($\approx$ 350 KDa) and one light chain (25 KDa) [11, 12]. The characterized a novel mRNA species in the posterior silk gland put into code an gene in a silk protein of 25.197 KDa which was termed as P25. P25 protein is produced in equimolar concentrations to major Fibroin [13, 14]. Therefore, in the present study, organization of yolk protein gene and PTTH gene genetic diversity were analyzed and their abnormalities associated with constructive traits in silkworm germplasm.

MATERIAL AND METHODS

Silkworm Races

The database available silkworm accessions of 105 strains from 30MV and 75BV were used for molecular screening using growth gene and yolk protein gene primers. The chosen primers were from different origin and percentage and donor from silkworm stocks depends on the yield diversity. The prepared strains were well maintained in Central Sericultural Germplasm Resources Centre, Hosur, Tamil Nadu. The data were shown in **Table 1 & 2**.

Genomic DNA isolation

The interested Genomic DNA were isolated from strains which preferred to standardize through phenol chloroform method. Extraction buffer (100Mm tris HCL, pH 8.0, 50Mm NaCl, 50,50Mm EDTA and 1 % SDS) and proteinase k (100 µg/ml) was

added to the ground tissue and incubated at 37°c. Then DNA was extracted with phenol-chloroform-isoamyl alcohol. DNA was dissolved in TE (10Mm Tris-HCL, 1Mm EDTA, pH 8.0). The genomic DNA was quantified in 0.8% Agarose gels and diluted to a uniform concentration (20ng/µL). Gene polymerization reaction were executed using EST tag primers with specifically PTTH and YOLK protein gene, the amplified gene were restricted with EcoRI enzyme. The up and down regulated gene-specific primers were designed for available gene sequence of selective gene using primer3 software programme [15].

PCR condition and analysis of amplified product

The amplification of genomic DNA was performed according to the amplification reaction was carried out in a 20µL reaction volume containing 20ng of template DNA, 10×PCR buffer (10Mm Tris–HCL, pH 8.3,1.5Mm Mgcl2 and 50MmKCL), 0.2µM primer, 100µM each of dATP, dCTP, dGTP and dTTP, and 3units of Taq DNA polymerase (genei) [16]. Amplification was performed in a MJ RESEARCH Peliteir thermal cycler (PTC 200 Controller). PCR schedule was 94°c for 2min, followed by 45 cycles of 94°c for 30 sec, 55°c for 30 sec and 72 °c for 2min and final extension was at 72 °c for 10 minutes. The amplified products were resolved on 1.5% Agarose gels in TBE buffer (89Mm Tris, 89Mm

boric acid and 2Mm EDTA) and electrophoresis was carried out with a constant voltage. gels were stained with ethidium bromide (0.5 ug/ml) and analysis of restriction banding pattern were carried out using 1.5% Agarose gel, and photographed through gel documentation system. The final amplified gene product was confirmed designated 1 as present and 0 as absence. The SPSS/PC-11.5 (MJ Norusis, SPSS Inc., Chicago) package [17, 18] were used for statistical characterization. The PCR profiles were subjected to binary scoring of 0 and 1, Gene clustering were performed to characterize the all 105 genotypes on each genetic distance. The genetic similarity coefficient analysis with selective 20 races were analyzed from the binary and hierarchical clustering analysis by Ward method [19-21]. The characterized 2 major groups are found in the cluster obtained.

RESULTS AND DISCUSSION

The selected 105 silkworm accessions were screened using selective gene primers. The Restriction DNA enzyme patterns were shown three band all the band pattern were carefully characterized among the screened selective silkworm genetic resources and scoring pattern were revealed high, moderate and low functionality and growth groups. The PCR product of selective genes **(Figure 1 & 2)** were designated to confirm the band patterns. The presence and absence of three types were entered in the ward's minimum variance test for all the 75 BV and 30 MV accessions **(Figure 3 & 4)**. Cluster groups were predicted into high, moderate and low productive groups. The presence of allele / band a & b were seen among high productive group and a and c in the moderate and low productive groups **(Table 3 & 4)**. Three restriction banding patterns were recognized among the screened silkworm genetic resources and their scoring revealed high, moderate and low fecundity and growth groups aa, ab banding types were found in Bivoltine high yielding groups and ac, bc found in high yielding Multivoltine groups. The prothoracic gene of *B. mori* naturally it's a polymorphic, the respective gene it was well characterized and cloned with PTTH locus encoded PTTH in silkworm. the three alleles which identified can be diagnosed using the gene amplification as a result of their gene length 3rd and 4th introns varies depending on the genotype [22, 23].
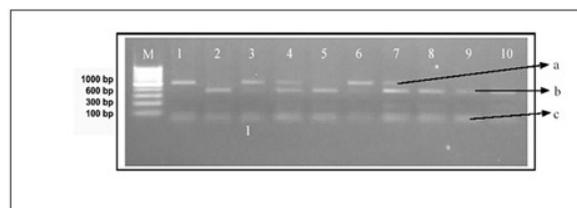

Figure 1: Restriction pattern profiles of yolk protein gene among the selected Bivoltine silkworm races
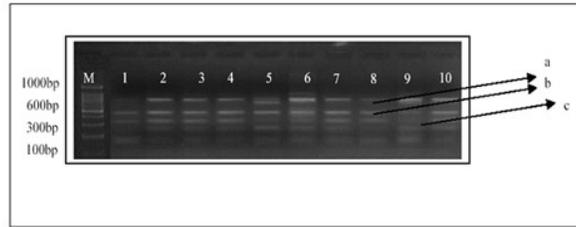
Figure 2: Restriction pattern profiles of PTTH gene among selected Bivoltine silkworm races
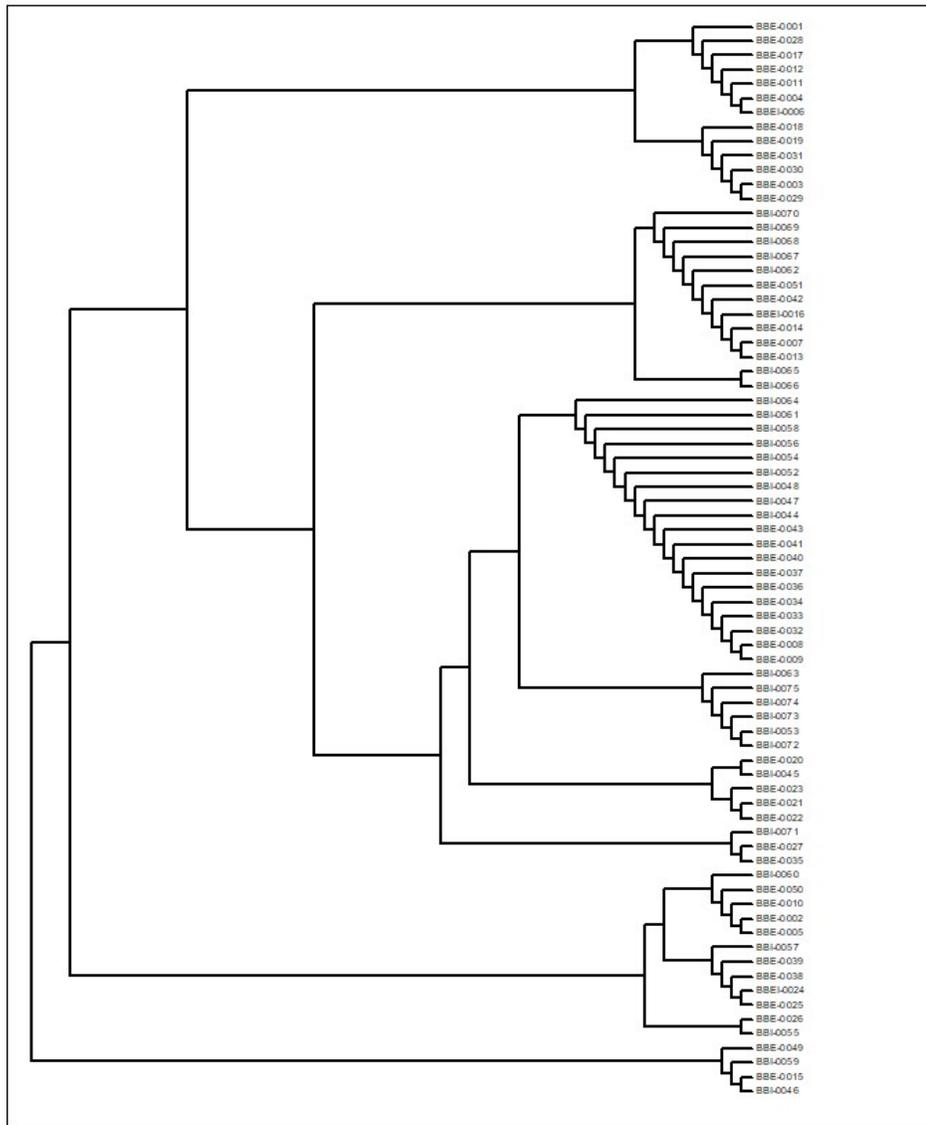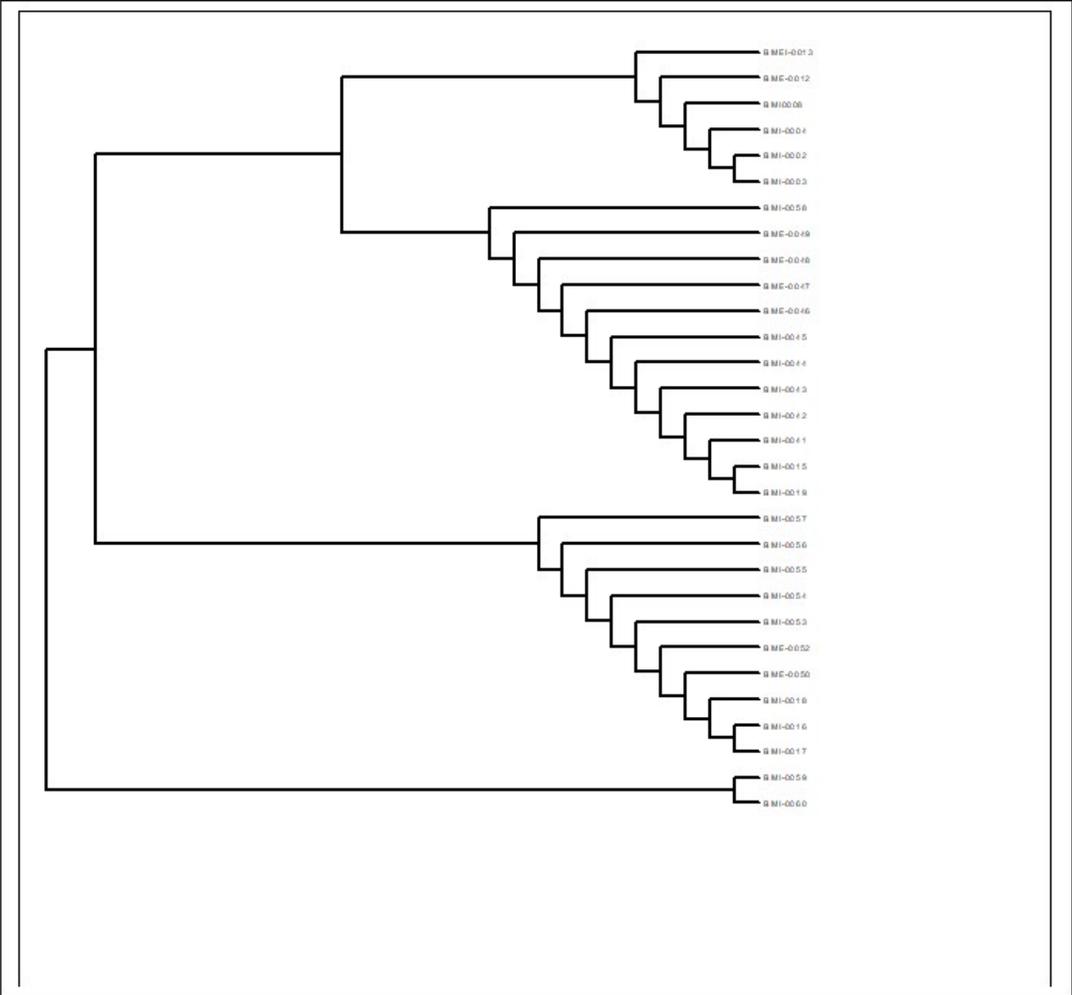

Figure 3: Dendogram – BV based on EST gene promers

Figure 4: Dendogram – BV based on EST gene promers

Table 1: Allelic variations in PTTH & yolk protein gene primers in Bivoltine accessions

ACC. NOS.	Races	PTTH allele	yolk allele	Fec	Hat%	WTG	VLD (h)	TLD (h)	SCW (g)	SSW (g)
BBE-0001	Alps jaunne	ab	aa	368	91.5	34	144	574	1.53	0.24
BBE-0002	Alps Yellow	aa	ab	436	91.4	34.9	172	602	1.63	0.28
BBE-0003	Cevenese Yellow	ab	ab	422	91.2	36.3	154	574	1.72	0.28
BBE-0004	Ascoli Yellow	ab	aa	410	94.6	35.7	148	582	1.56	0.25
BBE-0005	Meigitsu	aa	ab	403	92.5	41.9	147	577	1.77	0.32
BBEI-0006	B-36	ab	aa	486	88.2	41.3	170	597	1.73	0.31
BBE-0007	B-37	aa	aa	450	82.6	36.8	156	612	1.56	0.28
BBE-0008	B-40	ac	aa	425	89.4	35.7	144	578	1.55	0.26
BBE-0009	B-40	ab	aa	466	93.1	41.9	149	582	1.56	0.25
BBE-0010	J-112	aa	ab	467	96.2	41.3	142	568	1.66	0.28
BBE-0011	J122	ab	ab	418	82.2	36.8	154	582	1.91	0.36
BBE-0012	Yakwei	ab	aa	460	92.2	37.9	159	592	1.56	0.28
BBE-0013	Chaung Naung	aa	aa	453	96.5	36	146	576	1.6	0.29
BBE-0014	C-122	aa	aa	467	93.8	40.3	154	582	1.64	0.28
BBE-0015	C-108	aa	aa	394	93.4	46.6	155	584	1.54	0.26
BBEI-0016	C-110	aa	aa	449	96.8	35.6	158	588	1.69	0.3
BBE-0017	Chukwei	ab	aa	458	90.7	40	145	572	1.57	0.27
BBE-0018	Chinese Farmer	ab	bb	402	90.8	38.4	161	590	1.54	0.26
BBE-0019	Chinese Golden-70	ab	bb	440	87.2	38.1	142	572	1.53	0.24
BBE-0020	Chinese Golden-80	aa	bb	408	94.3	36.1	177	630	1.34	0.22
BBE-0021	Chinese Golden -90	ac	bb	425	92.8	27.7	138	573	1.67	0.28
BBE-0022	Haulak	ac	bb	427	78.4	41.8	148	582	1.46	0.26
BBE-0023	King Haung	ac	bb	376	89.9	35	162	592	1.65	0.28
BBEI-0024	Hauchi	ac	ab	458	94.2	40.5	152	588	1.79	0.31
BBE-0025	Nan Naung 6A	ac	ab	375	88.9	38.6	148	571	1.61	0.38
BBE-0026	Nan Naung 6D	bc	ab	382	88.1	32	142	577	1.39	0.24

BBE-0027	Chinese Yellow	bc	aa	365	93.8	37.5	152	575	1.72	0.28
BBE-0028	Azad	ab	aa	419	91.4	38	152	578	1.58	0.26
BBE-0029	AzerbaiJan	ab	ab	383	97	37.5	160	592	1.61	0.27
BBE-0030	Sanish E1(P)	ab	ab	395	91.1	33.9	160	604	1.47	0.27
BBE-0031	Sanish-E1(M)	ab	ab	412	95.2	39.4	163	600	1.7	0.28
BBE-0032	Sanish-E2(M)	ac	ab	529	95.1	38.5	156	587	1.7	0.32
BBE-0033	Sanish-8	ac	aa	464	91.8	34.5	172	610	1.47	0.27
BBE-0034	Sanish-17	ac	ab	455	90.2	32.8	158	604	1.47	0.25
BBE-0035	Sanish-18(M)	bc	aa	390	91.4	39.1	162	596	1.63	0.29
BBE-0036	Sanish-18 (P)	ac	aa	495	94.3	37.7	168	601	1.57	0.3
BBE-0037	Sanish-30	ac	aa	445	95.7	39.1	146	579	1.61	0.28
BBE-0038	Sanish-21	ac	ab	482	92.6	35.8	156	590	1.7	0.29
BBE-0039	Shekhi-1	ac	ab	485	89.5	37.8	152	584	1.56	0.28
BBE-0040	Sheikhi-11	ac	aa	450	86.5	39.2	167	598	1.71	0.32
BBE-0041	Tashkahashi-112	ac	aa	448	89.8	39.1	162	596	1.64	0.29
BBE-0042	Gyandza	aa	aa	516	87.3	35.8	162	594	1.58	0.28
BBE-0043	Belkokona-11	ac	aa	485	93.5	37.8	168	604	1.72	0.29
BBI-0044	NB4D2	ac	aa	479	92.1	34.4	206	646	1.82	0.36
BBI-0045	SH-6	ac	aa	518	95.3	34.8	172	612	1.46	0.26
BBI-0046	YS-3	aa	bb	438	94.4	37.8	156	598	1.5	0.27
BBI-0047	SF-19	ac	aa	504	96	34.4	180	623	1.41	0.27
BBI-0048	JD6	ac	aa	475	95.4	33.9	164	596	1.53	0.27
BBE-0049	UKR-1	ac	bb	499	87.6	32.9	190	628	1.67	0.3
BBE-0050	UKR-2	cc	ab	483	91.6	36.4	185	621	1.79	0.36
BBE-0051	Merefa-6	aa	aa	376	94	35.8	158	596	1.63	0.33
BBI-0052	Jam-1	ac	aa	421	84.1	34.2	172	606	1.6	0.23
BBI-0053	Jam-2	ac	aa	426	86	32.5	166	596	1.56	0.25
BBI-0054	Jam-11	ac	aa	360	85.8	33.6	158	590	1.46	0.24
BBI-0055	Jam-121	bc	ab	432	88.2	38.4	186	616	1.71	0.35

BBI-0056	**Jam-21**	**ac**	**aa**	**407**	**89**	**30.9**	**160**	**606**	**1.55**	**0.27**
BBI-0057	**Jam-23**	**ac**	**ab**	**439**	**84.5**	**33.7**	**158**	**594**	**1.7**	**0.28**
BBI-0058	**Jam-27**	**ac**	**aa**	**435**	**88.5**	**36.4**	**163**	**597**	**1.54**	**0.26**
BBI-0059	**Jam-103**	**aa**	**bb**	**417**	**92.6**	**38.1**	**152**	**586**	**1.53**	**0.26**
BBI-0060	**Jam-110**	**aa**	**aa**	**392**	**92**	**38.7**	**161**	**596**	**1.49**	**0.24**
BBI-0061	**Jam-118**	**ac**	**aa**	**430**	**82.4**	**30.6**	**159**	**588**	**1.75**	**0.31**
BBI-0062	**Jam-119**	**aa**	**aa**	**514**	**91.5**	**35.2**	**164**	**596**	**1.74**	**0.3**
BBI-0063	**Jam-122**	**ac**	**ab**	**411**	**89.7**	**30.6**	**206**	**648**	**1.54**	**0.27**
BBI-0064	**Jam-124**	**ac**	**aa**	**385**	**89.7**	**35.6**	**181**	**616**	**1.54**	**0.27**
BBI-0065	**Jam-125**	**aa**	**ab**	**433**	**95.8**	**34.2**	**166**	**604**	**1.69**	**0.3**
BBI-0066	**Pam-101**	**aa**	**ab**	**467**	**96.3**	**34.4**	**168**	**604**	**1.58**	**0.27**
BBI-0067	**Pam-102**	**ac**	**aa**	**508**	**89.3**	**36.3**	**145**	**577**	**1.65**	**0.25**
BBI-0068	**Pam-103**	**aa**	**aa**	**409**	**82.4**	**36.4**	**144**	**574**	**1.58**	**0.28**
BBI-0069	**Pam-104**	**aa**	**aa**	**430**	**92.5**	**35.9**	**168**	**598**	**1.62**	**0.28**
BBI-0070	**Pam-105**	**aa**	**aa**	**438**	**90.2**	**33.6**	**174**	**611**	**1.65**	**0.31**
BBI-0071	**Pam-106**	**ab**	**aa**	**430**	**88.2**	**37**	**169**	**602**	**1.6**	**0.29**
BBI-0072	**Pam-107**	**ac**	**aa**	**451**	**86.8**	**39.7**	**156**	**590**	**1.66**	**0.3**
BBI-0073	**Pam-108**	**ac**	**aa**	**392**	**85.8**	**38.4**	**189**	**610**	**1.54**	**0.29**
BBI-0074	**Pam-109**	**ac**	**aa**	**413**	**84.1**	**36.6**	**176**	**608**	**1.68**	**0.32**
BBI-0075	**Pam-110**	**ac**	**aa**	**412**	**88.1**	**35.1**	**162**	**594**	**1.58**	**0.3**

Table 2: Allelic variations in PTTH & yolk protein gene primers in Multivoltine accessions

ACC. NOS.	Races	PTTH allele	yolk allele	Fecundity	Hat%	WTG	VLD (h)	TLD (h)	SCW (g)	SSW (g)
BMI-0001	Sarupat	ac	bc	405	85.7	25.21	149	569	1.16	0.17
BMI-0003	Moria	ab	bc	388	86	26.85	145	564	1.13	0.16
BMI-0004	T. white	ac	bc	429	86.8	26.41	147	566	1.21	0.19
BMI0008	Kolar gold	ac	bc	477	87.6	27.85	134	553	1.26	0.19
BME-0012	R. Daizo	ac	bc	433	88.6	29.27	168	572	1.41	0.21
BMEI-0013	G. Plain	ac	cc	417	90.3	24.32	135	558	1.13	0.17
BMI-0015	Raj	ac	bb	383	91	21.06	166	578	1.06	0.15
BMI-0016	G	ac	aa	432	90.4	25.37	149	571	1.22	0.18
BMI-0017	Nistari	ac	aa	381	82.3	20.91	143	557	1.02	0.18
BMI-0018	Nistari (M)	ac	aa	392	90.1	19.64	142	559	0.94	0.13
BMI-0019	Nistari (p)	aa	bb	382	88.8	20.58	134	549	0.97	0.14
BMI-0041	Wai-4	aa	bb	381	85.8	23.53	136	555	1.07	0.15
BMI-0042	MY-23	aa	bb	457	91.6	27.99	141	559	1.26	0.18
BMI-0043	MW13	aa	bb	470	88.6	28.96	138	558	1.33	0.2
BMI-0044	MHMP(W)	aa	bb	407	88	29.27	143	562	1.31	0.19
BMI-0045	MHMP(Y)	aa	bb	479	90.9	31.5	151	568	1.42	0.22
BME-0046	P4D3	aa	bb	461	92.9	27.85	153	571	1.34	0.21
BME-0047	NISTIDI (Y)	aa	bb	426	90.3	23.77	143	558	1.12	0.15
BME-0048	NISTIDI (W)	aa	bb	408	91	23.07	141	557	1.04	0.14
BME-0049	NK4	aa	bb	321	87.2	21.88	151	566	1.03	0.14
BME-0050	Cambodg	ac	ab	315	83.2	22.19	139	556	0.99	0.14
BME-0052	Daizo	ac	ab	393	87.7	21.76	162	564	0.96	0.13
BMI-0053	LMP	ac	ab	390	91.7	23.33	147	562	1.07	0.15
BMI-0054	DMR	ac	ab	410	90.8	24.13	138	549	1.06	0.16
BMI-0055	LMO	ab	ab	439	90.9	25.68	147	566	1.19	0.17
BMI-0056	MY1(SL)	bc	ab	492	89	23.98	153	581	1.18	0.18
BMI-0057	PM(SL)	ac	ab	477	91.9	26.61	144	563	1.23	0.18

BMI-0058	BL23	ac	bb	491	92.9	30.74	151	559	1.4	0.23
BMI-0059	B24	ac	ab	472	82.5	29.07	148	562	1.4	0.23
BMI-0060	MU303	ac	ab	522	91.2	27.87	157	574	1.34	0.2

Table 3: Cluster groups of Bivoltine silkworm accessions based on EST gene primers

ACC. NOS.	No. of acc.	Group	Alleles	Number of qualified parameters
BBE-0001, BBE-0003, BBE-0004, BBE-0006, BBE-0011, BBE-0012, BBE-0013, BBE-0014, BBE-0016, BBE-0017, BBE-0018, BBE-0019, BBE-0026, BBE-0028, BBE-0029, BBE-0030, BBE-0031, BBE-0038, BBE-0041, BBE-0042, BBI-0044, BBI-0047, BBI-0048, BBE-0051, BBI-0052, BBI-0054, BBI-0058, BBI-0061, BBI-0062, BBI-0064, BBI-0065, BBI-0066, BBI-0067, BBI-0068, BBI-0069, BBI-0070	36	High	aa, ab	Fecundity, larval growth, cocoon weight, shell weight and shell ratio
BBE-0007, BBE-0008, BBE-0009, BBE-0020, BBE-0021, BBE-0023, BBE-0027, BBE-0032, BBE-0033, BBE-0034, BBE-0035, BBE-0036, BBE-0037, BBE-0040, BBE-0043, BBI-0053, BBI-0063, BBI-0071, BBI-0072, BBI-0073, BBI-0074, BBI-0075	22	Moderate	aa, ac	Fecundity, larval growth, cocoon weight, shell weight and shell ratio
BBE-0002, BBI-0005, BBE-0010, BBE-0015, BBE-0022, BBE-0024, BBE-0025, BBE-0039, BBI-0045, BBI-0046, BBE-0049, BBE-0050, BBI-0055, BBI-0056 ,BBI-0057, BBI-0059, BBI-0060	17	Low	aa, ac	Fecundity, larval growth, cocoon weight, shell weight and shell ratio

Table 4: Cluster groups of Multivoltine silkworm accessions based on EST gene primers

ACC. NOS.	No. of acc.	Group	Alleles	Number of qualified parameters
BMI-0001, BMI-0007, BME-0015, BMI-0019, BMI-0041, BMI-0042, BMI-0043, BME-0044, BMI-0045, BME-0046, BME-0047, BME-0048, BME-0049, BMI-0061, BMI-0062, BMI-0065, BMI-0074	17	High	ac, bc	Fecundity, larval growth, cocoon weight, shell weight and shell ratio.
BMI-0016, BMI-0018, BME-0050, BME-0052, BMI-0053, BMI-0054, BMI-0055, BMI-0056, BMI-0057	9	Moderate	aa, ab	Fecundity, larval growth, cocoon weight, shell weight and shell ratio.
BMI-0017, BMI-0058, BMI-0059, BMI-0060	4	Low	ac, aa	Fecundity, larval growth, cocoon weight, shell weight and shell ratio.

CONCLUSION

Express Sequence Tag site technique was employed as an important molecular tool to identify productive breeds among promising silkworm genetic resources. Identified productive races based on growth and yield specific genes can be used as high productive/robust breeds by the breeders for marker assisted selection breeding to evolve robust breed for field utilization and augmentation of silk productivity in the field. The utilization of silk in surgery and drug delivery systems have added new dimension to the silk production. Identified productive races based on growth and yield specific genes can be used as high productive/robust breeds by the breeders for marker assisted selection breeding to evolve robust breed for field utilization and augmentation of silk productivity not only in the pharmaceutical field but others as well.

COMPETING INTERESTS

The authors declare that there is no conflict of interest on the manuscript being submitted for publication.

REFERENCES

[1] Krishanswami S, Narasimhanna MN, Suryanaruyana SK, Kumararaj S. Silkworm Rearing Bulletin 15/2 FAO Agricultural Services (1973), United Nations Organizations, Rome pp.53-90.

[2] Chatterjee SN, Mohandas TP. Identification of ISSR markers associated with productivity traits in silkworm, Bombyx mori L. Genome. 2003;46:438-447.

[3] Chatterjee SN, Rao CGP, Chatterjee GK, Ashwath SK, Patnaik AK. A correlation between yield and biochemical parameters in the mulberry silkworm, Bombyx mori L. Theor. Appl. Genet. 1993;87:385-391.

[4] Chatterjee SN, Datta RK. 1992. Hierarchical clustering of 54 races and strains of the mulberry silkworm, Bombyx mori L: Significance of biochemical Parameters. Theor. Appl. Genet. 1992;85:394-402.

[5] Goldsmith MR, Wilkins AS. Molecular model Systems of the Lepidoptera. New York: Cambridge University Press; 1991.

[6] Gupta PK, Varshney RK, Sharma PC, Ramesh B. Molecular markers and their applications in wheat breeding. Plant breeding. 1999;118(5):369-390.

[7] Xing L, Liu XM. Characterization of Betula platyphylla gene transcripts associated with early development of male inflorescence. Molecular biology reports. 2012;39(2):929-935.

[8] Duan J, Li R, Cheng D, Fan W, Zha X, Cheng T, *et al*. SilkDB v2. 0: a platform for silkworm (Bombyx mori) genome biology. Nucleic acids research. 2010;38(suppl_1):D453-D456.

[9] Rapali P, Szenes Á, Radnai L, Bakos A, Pál G, Nyitray L. DYNLL/LC8: a light chain subunit of the dynein motor complex and beyond. The FEBS journal. 2011;278(17):2980-2996.

[10] Gomes FE, Arantes TD, Fernandes JA, Ferreira LC, Romero H, Bosco SM, *et al*. Polymorphism in mitochondrial group I introns among Cryptococcus neoformans and Cryptococcus gattii genotypes and its association with drug susceptibility. Frontiers in microbiology. 2018;9:86.

[11] Wadbua P, Promdonkoy B, Maensir, S, Siri S. Different properties of electrospun fibrous scaffolds of separated heavy-chain and light-chain fibroins of Bombyx mori. International journal of biological macromolecules. 2020;46(5):493-501.

[12] Inoue S, Tanaka K, Arisaka F, Kimura S, Ohtomo K, Mizuno S. Silk fibroin of Bombyx mori is secreted, assembling a high molecular mass elementary unit consisting of H-chain, L-chain, and P25, with a 6: 6: 1 molar ratio. Journal of Biological Chemistry. 2000;275(51):40517-40528.

[13] Mandal BB, Grinberg A, Gil ES, Panilaitis B, Kaplan DL. High-strength silk protein scaffolds for bone repair. Proceedings of the National Academy of Sciences. 2012;109(20):7699-7704.

[14] Zhang YQ. Applications of natural silk protein sericin in biomaterials. Biotechnology advances. 2002;20(2):91-100.

[15] Kasnauskiene J, Ciuladaite Z, Preiksaitiene E, Matulevičienė A, Alexandrou A, Koumbaris G *et al*. A single gene deletion on 4q28. 3: PCDH18–a new candidate gene for intellectual disability?. European journal of medical genetics. 2012;55(4):274-277.

[16] Ramakers C, Ruijter JM, Deprez RHL, Moorman AF. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neuroscience letters. 2003;339(1):62-66.

[17] Frey F. SPSS (Software). The International Encyclopedia of Communication Research Methods, 2017:-2.

[18] Gouda MA. Common pitfalls in reporting the use of SPSS software. Medical Principles and Practice. 2015;24(3):300.

[19] Saraçli S, Doğan N, Doğan I. Comparison of hierarchical cluster analysis methods by cophenetic correlation. Journal of inequalities and Applications. 2013;(1):1-8.

[20] Granato D, Santos JS, Escher GB, Ferreira BL, Maggio RM. Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: A critical perspective. Trends in Food Science & Technology. 2018;72:83-90.

[21] Zolfaghari F, Khosravi H, Shahriyari A, Jabbari M, Abolhasani A. Hierarchical cluster analysis to identify the homogeneous desertification management units. PloS one. 2019;14(12):e0226355.

[22] Gomes FE, Arantes TD, Fernandes JA, Ferreira LC, Romero H, Bosco SM, *et al*. Polymorphism in mitochondrial group I introns among Cryptococcus neoformans and Cryptococcus gattii genotypes and its association with drug susceptibility. Frontiers in microbiology. 2018;9:86.

[23] Sorkheh K, Shiran B, Gradziel TM, Epperson BK, Martínez-Gómez P, Asadi E. Amplified fragment length polymorphism as a tool for molecular characterization of almond germplasm: genetic diversity among cultivated genotypes and related wild species of almond, and its relationships with agronomic traits. Euphytica. 2007;156(3):327-344.