Marker-assisted selection of bivoltine silkworm genetic resources for thermotolerance

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

The aim of the study is identification of bivoltine breeds showing thermotolerance to evaluate for regional abiotic stress conditions to achieve successful bivoltine cocoon production.

Study Design: Lepidopteran insect, *Bombyx mori* silkworm is more prone to crop losses due to hot climatic conditions during summer seasons. In order to produce bivoltine cocoons consistently throughout the year without affecting productivity during hot summer season, high temperature tolerant silkworm breeds are indispensable. The vast silkworm *B.mori* (L.) germplasm available needs to be screened using reliable molecular markers to select efficient productive breeds with high temperature tolerance.

Marker assisted selection (MAS) has been widely used in selection of desired traits for breeding programs. It is believed that selection based on trait linked to DNA marker would be better option than phenotypic selection which is laborious and time consuming.

Place and Duration of the Study: The study has been taken up for 3 years in collaboration with Seribiotech Research Laboratory, Kodathi

Methodology: The present study has been carried out to screen forty selected bivoltine silkworm *B.mori* germplasm resources exhibiting higher rate of survival and high productivity using SSR markers (LFL329; LFL1123; S0808; S0813) linked to thermotolerance in silkworm so as to identify thermotolerant breeds.

Results: Based on the PCR product resolved in 2% agarose gel and presence of bands corresponding thermotolerance and thermosusceptible markers observed, 10 bivoltine accessions showed 87-100% thermotolerance were selected viz. BBI-0086 (KPG-A), BBE-0184 (SMGS-2), BBI-0301 (YS-7) and BBI-0339 (DD-2) and the accessions spinning dumbbell cocoons- BBI-0044 (NB4D2), BBI-0334 (APS-4), BBI-0336 (APS-8), BBI-0338 (DD-1), BBI-0343 (NK3) and BBI-0358 (CSR26) for further validation through field trials in selected hotspots *viz.* Tropical - High temperature and low humidity at RSRS, Jammu and REC Chitradurga; Sub tropical - High temperature and high humidity at RSRS Jammu and Temperate at CSRTI, Berhampore.

Conclusion: The present study elucidate the impact of temperature and humidity associated stress conditions over economic traits performances of selected bivoltine accessions. The

findings of the present study provide a suitable platform for future bivoltine crop improvement through breeding program.

Keywords: Bivoltine, Silkworm, Thermotolerance, SSR markers

1. INTRODUCTION

Silkworm, *Bombyx mori* (L.) is a poikilothermic insect and it is the main source of quality silk. Till date, India is the second largest producer of silk yarn [1] (Oommen, 2001) and most of it is produced from cross breeds raised by crossing the multivoltine and high yielding bivoltine silkworm breeds [2,3] (Datta 1984; Chatterjee 1993). So far, the bivoltine hybrids recommended for large-scale production of cocoons in the field are robust in nature [4] (Suresh Kumar *et al,* 2001). The majority of the productive silkworm breeds used in sericulture-practicing countries is bivoltines and is of temperate origins. The rearing and cocoon production of polyvoltine cross breeds especially in the hot and humid climatic conditions of tropics resulted in extensive crop loss, [5] (Ramesha *et al*, 2009). Many important qualitative characters such as viability and cocoon yield decline sharply due to higher temperatures [6] (Sugnana Kumari *et al*, 2011). Unlike multivoltine, bivoltine silkworm races have better yield potential and produce superior quality silk but less resistant to extreme climatic conditions. [7] Hazel (1995) reported that the high temperature affects nearly all the biological processes including the rates of biochemical and physiological reactions which in turn affect the quality and quantity of cocoon crops. Many of the silkworm characters are not only controlled by genes but also influenced by environmental factors such as temperature and relative humidity. In India, productive and qualitatively superior bivoltine hybrids have been developed by utilizing Japanese commercial hybrids as breeding resource material [8] (Basavaraja *et al*, 1995). However, these breeds/hybrids are difficult to rear in summer climate. This has led to the development of compatible bivoltine hybrids for rearing throughout the year by utilizing Japanese thermo-tolerant hybrids as breeding resource material [9] (Datta, *et al*, 2001). In order to select the breeds with high temperature tolerance, it is necessary to analyze critically the influence of high temperature on post cocoon parameters of silkworm breeds. In this regard, parental selection based on both phenotypical as well as genotypical screening will be more effective [10] (Moorthy *et al*, 2007).

Marker-assisted selection (MAS) has been widely used in breeding programs [11] (Steele *et al*, 2006), although, to date, it has not been much applied in silkworm breeding. It is believed that selection based on trait linked to DNA marker would be a better option than phenotypic selection [12] (Knapp, 1998). Microsatellites, or simple-sequence repeats (SSRs), are short, tandemly repeated motifs of 1 to 6 bases found in all prokaryotic and eukaryotic genomes. SSRs are widely used in population genetics and linkage map construction because of their high levels of polymorphism and reproducibility [13] (Tautz, 1989), and their genome-wide distribution. SSRs are inherited in a Mendelian fashion and show co-dominant alleles. Once, the SSR markers linked to the characters required are identified, it could serve as molecular tags to identify the traits specific breeds. Many researchers have studied on

thermotolerance in silkworms; [14] Zhao *et al* (2010) identified 5 SSR markers linked to thermotolerance in silkworm and mapped them on 8th chromosome using backcross population of thermotolerant variety - Dong 34 and thermo-sensitive variety - Ou17. [15] Srivastava *et al* (2007) have studied on the selection of silkworm breeds in respect of thermotolerance by identifying thermotolerant silkworm breeds.

Central Sericultural Germplasm Resources Centre, is the national repository for sericultural germplasm include both mulberry and silkworm genetic resources. The centre is mandated to characterize and evaluate the silkworm accessions with set descriptors to identify better performing accessions for various quantitative and qualitative characters. The vast silkworm germplasm with diverse genotypic as well as phenotypic characters have not attempted for screening thermotolerance using molecular markers earlier. Hence, in the present study, some of the productive bivoltine silkworm accessions of the germplasm were selected for molecular screening and identification of thermotolerant silkworm accessions using four SSR markers viz., LFL0329; LFL1123; S0809; S0813 these markers were identified linked with thermo-tolerance in silkworm, *B mori* [16,17] (Moorthy *et al*, 2013; Chandrakanth *et al*, 2015). Further the output of the study on identification of the centre wise bivoltine accessions showing thermotolerance will help in formulation of breeding programmes for abiotic stress and also it can be utilized for commercial exploitation.

2. MATERIALS AND METHODS

2.1 Selection of Silkworm accessions for the screening of thermotolerance

The present study was carried out for a period of three years from 2019-2022 at Central Sericultural Germplasm Resources Centre (CSGRC), Hosur, Tamilnadu, India. Under the study a total of 369 bivoltine silkworm accessions (genetic resources) available were analysed for the economic parameters viz., Effective rate of rearing by number (ERR/No)., ERR/wt. (kg), Pupation rate %, Single cocoon wt. (g), Single shell wt. (g) and Shell ratio %, Average filament length and denier. Based on the performance, forty bivoltine accessions were shortlisted (Table 1) for the molecular screening using SSR markers linked to thermotolerance at Seri-biotechnology Research Laboratory, Kodathi by following the methodology mentioned below.

Table. 1: Details of the shortlisted bivoltine germplasm accessions based on economic parameters

#	Accn. No/ Name	Wt. of 10 larvae (g)	ERR/ No.	ERR/10000 By wt. (kg)	Pupation rate (%)	Single cocoon wt (g)	Single shell wt (g)	SR%
1	BBE-0026 NAN NAUNG 6D	30.278±2.29 (18.49)	9729±85.87 (2.16)	11.842±0.70 (14.53)	95.228±1.14 (2.94)	1.285±0.05 (8.91)	0.196±0.01 (17.87)	15.125±0.58 (9.33)
2	BBE-0030 SANISH E1(P)	31.667±1.92 (14.87)	9660.667±141.9 (3.60)	13.208±0.77 (14.30)	96.292±1.48 (3.77)	1.244±0.04 (6.97)	0.199±0.01 (10.46)	15.973±0.39 (6.01)
3	BBI-0044 (NB4D2)	33.302±1.51 (11.10)	9618.833±85.36 (2.17)	13.275±0.35 (6.46)	94.458±1.14 (2.95)	1.323±0.02 9 (5.46)	0.236±0.01 (5.72)	17.842±0.58 (8.02)
4	BBI-0086 (KPG-A)	29.675±1.59 (13.13)	9705.333±88.18 (2.23)	12.317±1.15 (22.78)	95.945±0.85 92.16)	1.279±0.04 (7.79)	0.212±0.01 (12.05)	16.526±0.43 (6.37)

5	BBI-0133(AT-4)	32.43±1.67 (12.62)	9512.167±177.5 (4.57)	12.425±0.51 (10.14)	96.753±0.56 (1.42)	1.383±0.04 (6.33)	0.258±0.01 (11.29)	18.611±0.44 (5.73)
6	BBI-0137(IB-9)	34.233±1.74 (12.42)	9740.5±49.69 (1.25)	14.625±0.77 (12.83)	94.692±1.18 (3.05)	1.454±0.06 (9.90)	0.277±0.01 (12.70)	19.004±0.40 (5.14)
7	BBE-0167 KYORIESHIM-PAKU (P)	36.142±0.60 (4.06)	9643.667±83.21 (2.11)	13.733±0.50 (8.96)	95.065±1.13 (2.90)	1.446±0.02 (2.69)	0.308±0.01 (7.55)	21.266±0.51 (5.82)
8	BBE-0183 (CSGRC-1)	35.463±1.51 (10.45)	9595±92.89 (2.37)	13.583±0.56 (10.05)	93.828±0.99 (2.57)	1.43±0.03 (4.84)	0.263±0.01 (10.98)	18.357±0.57 (7.57)
9	BBE-0184 (CSGRC-2)	36.585±1.63 (10.91)	9696±153.78 (3.88)	14.258±0.69 (11.93)	97.503±0.57 1.43)	1.483±0.06 (9.28)	0.28±0.01 (11.53)	18.897±0.49 (6.28)
10	BBE-0187 (CSGRC-5)	38.298±2.33 (14.92)	9236±217.38 (5.77)	13.992±0.64 (11.15)	89.132±3.39 (9.32)	1.452±0.06 (9.41)	0.259±0.01 (11.80)	17.827±0.53 (7.21)
11	BBE-0197()A	37.352±1.88 (12.30)	9648.5±82.46 (2.09)	15.333±0.77 (12.32)	94.86±1.13 (2.92)	1.509±0.08 (12.89)	0.274±0.02 (14.93)	18.083±0.34 (4.65)
12	BBE-0222(JC2M)	40.188±1.89 (11.20)	9668.833±129.6 3 (3.28)	16.55±0.64 (9.41)	94.433±1.68 (4.35)	1.564±0.06 (9.58)	0.271±0.01 (8.41)	17.348±0.20 (2.78)
13	BBE-0272(G146)	37.385±1.54 (10.08)	9524.5±153.59 (3.95)	15.517±1.25 (19.70)	91.748±1.86 (4.96)	1.526±0.06 (10.02)	0.309±0.02 (13.33)	20.178±0.28 (3.45)
14	BBI-0299(NS6)	38.523±1.29 9 (8.20)	9722.5±129.22 (3.26)	15.042±0.73 (11.82)	95.085±1.05 (2.70)	1.518±0.08 (12.53)	0.287±0.02 (13.43)	18.895±0.44 (5.73)
15	BBI-0301(YS7)	38.235±1.68 (10.75)	9273.833±240.1 9 (6.34)	15.825±0.69 (10.76)	91.767±3.13 (8.34)	1.535±0.03 (4.85)	0.267±0.01 (11.41)	17.386±0.56 (7.91)
16	BBI-0303(KSO-1)	36.813±1.46 (9.73)	9701.5±103.48 (2.61)	15.15±0.61 (9.93)	95.505±1.31 (3.36)	1.608±0.06 (8.35)	0.299±0.02 (12.73)	18.549±0.48 (6.33)
17	BBI-0334(APS-4)	37.703±1.08 (6.54)	9735.167±54.44 6 (1.37)	16.233±1.05 (15.91)	95.058±1.14 (2.94)	1.48±0.03 (5.20)	0.296±0.01 (8.00)	19.998±0.58 (7.07)
18	BBI-0336(APS-5)	37.842±1.26 (8.14)	9543.833±116.5 6 (2.99)	15.733±0.72 (11.18)	92.022±2.63 (7.01)	1.529±0.04 (5.66)	0.284±0.01 (6.82)	18.544±0.25 (3.36)
19	BBI-0338(APS-8)	39.78±0.97 (5.99)	9432.667±176.2 1 (4.58)	16.417±0.81 (12.13)	92.238±1.94 (5.16)	1.564±0.03 (5.12)	0.317±0.01 (7.44)	20.313±0.68 (8.19)
20	BBI-0339(DD-1)	39.373±1.61 (10.01)	9678±70.69 (1.79)	16.092±0.85 (12.95)	93.915±1.64 (4.29)	1.595±0.03 (4.48)	0.323±0.01 (6.01)	20.255±0.35 (4.21)
21	BBI-0341(NK-1)	38.894±0.96 (5.52)	9452.5±121.26 (3.14)	15.225±0.62 (10.00)	92.848±1.17 (3.09)	1.528±0.04 (6.13)	0.277±0.01 (7.15)	18.152±0.25 (3.38)
22	BBI-0342(NK-2)	38.667±1.61 (10.18)	9672.333±95.82 (2.43)	13.883±1.21 (21.41)	93.253±1.59 (4.18)	1.493±0.04 (7.03)	0.283±0.01 (10.76)	18.921±0.48 (6.26)
23	BBI-0343(NK-3)	40.253±1.54 (9.36)	9721.667±73.62 (1.86)	16.167±0.60 (9.07)	94.235±1.33 (3.47)	1.605±0.05 (8.31)	0.29±0.01 (10.65)	18.064±0.21 (2.90)
24	BBI-0344(NP-4)	41.91±0.94 (5.51)	9601.833±90.27 (2.30)	17.675±1.14 (15.80)	93.775±1.20 (3.13)	1.698±0.03 (4.66)	0.341±0.01 (7.00)	20.112±0.44 (5.31)
25	BBI-0345(NP-5)	39.083±1.02 (6.41)	9493.667±131.1 9 (3.38)	16.733±0.75 (10.94)	92.353±1.63 (4.32)	1.632±0.04 (5.44)	0.293±0.01 (8.95)	17.955±0.44 (5.95)
26	BBI-0346(KSO-2)	36.737±1.17 (7.77)	9668±54.241 (1.37)	14.942±1.11 (18.20)	94.877±0.96 (2.47)	1.49±0.07 (10.74)	0.287±0.01 (8.73)	19.271±0.21 (2.70)
27	BBI-0347(KSO3)	37.397±1.08 (7.08)	9778.833±76.44 (1.91)	14.95±1.04 (17.09)	96.802±0.64 (1.61)	1.528±0.06 (9.16)	0.309±0.01 (10.85)	20.188±0.39 (4.67)
28	BBI-0349(HND)	40.682±1.78 (10.71)	9463.833±194.5 4 (5.04)	16.425±1.38 (20.57)	93.108±2.03 (5.34)	1.613±0.07 (11.31)	0.324±0.01 (10.07)	20.111±0.45 (5.46)
29	BBI-0350(HDO)	37.598±1.14 (7.43)	9544.5±129.93 (3.33)	14.575±0.32 (5.34)	92.942±2.08 (5.48)	1.509±0.03 (5.40)	0.283±0.01 (8.86)	18.721±0.51 (6.65)
30	BBI-0358(CSR-26)	31.097±2.85 (22.33)	9717.667±136.0 5 (3.43)	13.55±0.59 (10.75)	95.222±2.09 (5.36)	1.282±0.10 (18.19)	0.233±0.02 (20.39)	18.122±0.23 (3.12)
31	BBI-0359(CSR-27)	36.205±1.83 (12.38)	9696.667±66.38 (1.68)	13.743±0.83 (14.83)	94.763±1.2 (3.10)	1.387±0.08 (13.26)	0.283±0.01 (12.43)	20.395±0.19 (2.29)
32	BBI-0360(A3)	39.178±1.65 (10.31)	9411.833±195.0 5 (5.08)	14.842±0.55 (9.04)	89.657±3.84 (10.49)	1.604±0.07 (10.76)	0.307±0.02 (15.06)	19.076±0.39 (5.02)
33	BBI-0361(A-CHINESE)	36.59±1.74 (11.63)	9682.667±99.69 (2.52)	15.592±0.65 (10.17)	94.478±1.24 (3.20)	1.535±0.06 (9.80)	0.301±0.01 (10.88)	19.603±0.39 (4.81)
34	BBI-0363(BHT)	37.463±1.48 (9.69)	9234.5±249.40 (6.62)	14.783±0.51 (8.36)	89.505±2.45 (6.70)	1.545±0.06 (9.66)	0.312±0.02 (13.90)	20.123±0.52 (6.33)
35	BBI-0364(GHT)	37.543±1.44 (9.36)	9494.167±184.2 0 (4.75)	15.258±0.87 (13.98)	91.283±2.62 (7.03)	1.511±0.06 (10.35)	0.312±0.02 (12.52)	20.63±0.38 (4.51)
36	BBI-0367(H-281)	38.602±1.42 (9.03)	9320.667±150.4 7 (3.95)	14.217±0.99 (17.07)	89.843±2.55 (6.95)	1.555±0.05 (7.30)	0.295±0.02 (12.29)	18.896±0.54 (7.00)
37	BBI-0369(935 E)	35.935±0.66 (4.48)	9688±67.19 (1.70)	15.092±0.61 (9.85)	94.918±0.51 (1.33)	1.476±0.01 (4.73)	0.291±0.01 (4.72)	19.718±0.40 (5.01)
38	BBI-0370 (SL WU-8)	36.522±0.91 (6.07)	9801.667±28.62 (0.72)	15.692±0.88 (13.77)	96.747±0.87 (2.19)	1.471±0.01 (4.50)	0.299±0.01 (5.04)	20.332±0.36 (4.31)
39	BBI-0377(APS-12)	37.188±1.02 (6.72)	9614.167±107.4 7 (2.74)	15.142±0.89 (14.39)	91.825±2.85 (7.59)	1.564±0.04 (6.16)	0.31±0.01 (7.71)	19.786±0.23 (2.80)
40	BBI-0378(APS-45)	37.012±1.63 (10.78)	9615.667±115.9 (2.95)	16.475±1.30 (19.35)	94.952±1.18 (3.05)	1.564±0.03 (4.25)	0.314±0.02 (13.61)	20.07±1.07 (3.11)

2.2 DNA Extraction

Genomic DNA was extracted from eight individual silk moths (4 each from male and female moths) of forty selected bivoltine silkworm accessions (Table 1) following the method mentioned below: The whole moth tissue (20 -30 mg) was crushed in liquid nitrogen and added 0.5- 1ml of 2 PK buffer (200 mM Tris, 25 mM EDTA, 300 mM NaCl, 2% SDS), and tissue was thoroughly homogenized and centrifuged at 10000 x *g* for 5 min. RNase treatment was done by adding 2µl of 10 mg/ml of RNase-A solution and incubated at 37^0C for 30 min. After this Protease treatment was given to remove the proteins by adding 2 µl of proteinase K (50 mg/ml) solution, incubated at 65^0 C for 30 min. Equal volumes of Phenol: Chloroform: Isoamyl alcohol mixture (25:24:1) were added and mixed thoroughly and centrifuged at 12000 x*g* for 5 min at room temperature. The upper clear aqueous phase was collected into a fresh tube, added equal volumes of chloroform: Isoamyl alcohol mixtures (24:1), mixed thoroughly and centrifuged the samples at 12000 x*g* for 5 min. An aqueous phase was collected transferred into a fresh tube, added 0.8 volumes of chilled isopropanol and mixed. The above mixture was centrifuged at 12000 x*g* for 10 min at 4^0 C. discarded the supernatant and washed the pellet with 70 % alcohol and centrifuged for 5 minutes at 4^0 C. The supernatant was discarded and air dried the pellet. The dried pellet was dissolved in 50 µl MilliQ water. The quality and quantity of DNA was checked by running it on a 0.8% agarose gel and using a spectrophotometer.

2.3 Selection of SSR primers

Four pair of SSR primers from silkworm were selected from the previously well characterized microsatellite repeats representing different gene loci, *viz*., LFL0329; LFL1123; S0809; S0813 [16,17] (Moorthy *et al*,2013; Chandrakanth *et al*, 2015) (Table 2).

Table. 2: Details of the SSR primers and allelic size.

Sl. No	Primer Name	Locus	Sequence 5'- 3'	Tolerant allele size (bp)	Susceptible allele size (bp)
1	1123	LFL1123	FP-AAGTTCTTTACCAGTTCACAGACAGC	230	250
			RP-CGCCATGCAACTGTCGTCAC		
2	0329	LFL0329	FP-GAAATCCGTTTGAAGAATCCACA	200	230
			RP-CATCCGTTGAATGAGTATCGTTTG		
3	S0809	LFL0407	FP-AACATTTGCTTAGGACTGAATTTACAC	230	200
			RP-AATAATAACTTTTACACGCACCTACACTT		
4	S0813	S0813	FP-CCAGGAAATTCCAACCAGTAGCC	520	500
			RP-ACTTACCACTACACCAGACGGAC		

FP: Forward primer, RP: reverse primer

2.4 Polymerase Chain Reaction (PCR)

The PCR amplification was performed on a thermal cycler (Eppendorf- Nexus gradient cycler). The PCR was performed with a final volume of 10 µL containing 5 µL of Emerald Amp GT PCR Master mix (TakaraBio), 1 µl each of 10 pmol forward and reverse primers, 1 µl template DNA (125 ng) and 2 µl of Milli Q water. The PCR program included an initial denaturation step at 94°C for 5 min, a denaturation step at 94°C for 30 sec, annealing at 60°C for 1 min, and an extension step at 72°C for 1 min for 30 cycles followed by a final step at 72°C for 10 min. The PCR products were separated in 4% agarose gel and analyzed for the presence of markers associated with thermo-tolerance.

2.5 Data Scoring and analysis

The size of DNA fragments was estimated by using a web based program called 'INCHWORM' (accessible at http://molecularworkshop.com/ programs / inchworm.html) based on the slope values obtained by plotting migrated distances of DNA fragments of known size. Alleles with different molecular sizes were coded as 'AA' (tolerant) or 'BB' (susceptible) for homozygous conditions and 'AB' for heterozygous condition.

3. RESULTS AND DISCUSSION

In the present study, the DNA extracted from the male and female moths of forty selected bivoltine accessions were PCR amplified using the SSR markers, viz., LFL 1123, LFL0329, S0809 and S0813. Among 40 accessions, PCR amplification using **LFL1123** primers showed 100% thermotolerance evident by the presence of 230 bp PCR product (Figure 1) in KPG-A (BBI-86) and CSR-26 (BBI-358), and three accessions, BHT (BBE-363), H281(BBE-367), and NAN NAUNG 6D (BBI-26) showed >50% thermo tolerance and 17 accessions showed <50% thermotolerance viz. (NB4D2)BBI-0044,(AT-4)BBI-0133, (KYORIESHIM-PAKU(P)BBE-0167,(A)BBE-0197,(JC2M)BBE-0222,(NS6)BBI-0299,(YS7) BBI-0301,(DD-1)BBI-0338,(NK-2)BBI-0342,(NK-3)BBI-0343,(NP-4)BBI-0344,(KSO3) BBI-0347, (HND)BBI-0349,(A-CHINESE)BBI-0361,(GHT)BBI-0364,(935E)BBI-0369 and (SL WU-8)BBI-0370 (Table 3). However, 18 accessions were detected as thermosusceptible by the presence of a 250 bp PCR product. Using **S0329** primers, 100% thermotolerance was observed in NB4D2 (BBI-44) and CSGRC-2 (BBI-184) (200 bp PCR product). Silkworm accessions like DD-2 (BBE-339), APS12 (BBE-377), A-chinese (BBE-361) Kyorie shimpaku (BBI-167) showed >50% thermo tolerance and the remaining 16 accessions showed <50% thermo tolerance and 18 accessions were found thermo susceptible (Table 4) by the presence of less than 200 bp PCR product (Figure 2).

The analysis using **S0813** primers showed 100% thermotolerance (more than 500bp PCR product) only in one accession ie., KPG-A (BBI-86) and accessions CSGRC-5 (BBE-187), YS-7 (BBE-301), showed >50% thermotolerance and BHT(BBI-363), CSGRC-2 (BBI-184) and Sanish E1(p) (BBI-30) showed <50% thermo tolerance and the remaining 34 accessions were observed as thermosusceptible (less than 500bp PCR product) (Fig. 3a,b and Table-5). The **S0809** primers showed 100% thermotolerance (230bp) in nine accessions ie., CSGRC-2 (BBI-184) YS-7 (BBE-301), APS4 (BBI-334), APS8 (BBI_0336), DD1 (BBI-338),

DD2(BBI-339), NK3(BBI-343), BHT (BBI-363), and APS12 (BBI-377). A >50% thermotolerance was shown by 14 accessions (Fig. 4) and BHT (BBI-363) and Sanish E1(P) (BBI-030) showed <50% thermotolerance. The remaining 17 accessions were found to be thermosusceptible (250bp) (Table-6).

The silkworm characters are mostly quantitative and are polygenic in nature. Many of the economic characters are influenced by environmental factors [18] (Falconer, 1990). Therefore, screening of silkworms for tolerance to high temperature is desirable for identifying silkworm resources available at CSGRC and which could be used for silkworm breeding programme to develop breeds with high productivity and viability. Accordingly, 40 productive bivoltine silkworm genetic resources were screened for thermotolerance using SSR markers. The four markers selected for the present study viz., LFL 1123, LFL0329, S0809 and S0813 which were polymorphic between parents and also associated with thermotolerance in silkworms. Presence of these markers are not uniform in the entire silkworm screened, however, PCR amplification showed varied level of thermotolerance based on the markers. Amongst, ten bivoltine silkworms showed 100% thermtolerance in different SSR markers used (Table.7). Similarly, > 50% thermotolerance was observed in many of the silkworms in the present study. The efficiency of markers depends on several factors including marker number and kind (co-dominant/ dominant), the strength of marker association with selection indices, population size, and trait heritability [19] (Gimelfarb *et al* 1994). The selection efficiency of markers, in fact could be superior to phenotypic selection even when the target trait heritability is relatively low and rather small population (>50 individuals) size [20, 21] (Moreau *et al*, 2004; Fan *et al,* 2006). In contrast, thermosusceptible markers were also observed in many of the silkworms screened with different SSR markers. Similar results were recorded by [22] Chandrakanth *et al*., (2018) in ten bivoltine silkworm breeds screened for thermotolerance using two SSR markers viz., S0808 and S0816 showed homozygous thermotolerant banding pattern in four silkworm breeds and thermosusceptible banding pattern in five silkworm breeds. [17] Chandrakanth *et al* (2015), used four tolerant and one susceptible breeds and screened with 85 SSR markers. Only 11 markers able to discriminate tolerant and susceptible breeds based on the banding pattern. Bivoltine and multivoltine silkworms are genetically divergent and it is difficult to generate an allele common to tolerant silkworm breeds. The SSR marker S0809 showed strong correlation and association of thermotolerance in 09 silkworm breeds viz., CSGRC-2 (BBI-184) YS-7 (BBE-301), APS4 (BBI-334), APS8 (BBI_0336), DD1 (BBI-338), DD2(BBI-339), NK3(BBI-343), BHT (BBI-363), and APS12 (BBI-377) in the present study. Concurrent findings were also recorded by [22] Zhao *et al*, (2010), they have mapped 5 markers viz., S0803, S0809, S0816, S0819 and S0820 linked to thermotolerant gene (*KN*) on chromosome 8 using backcross population of Chinese silkworm breeds as parent. [23] Sreekumar *et al,* (2008), reported that the DNA profiling of silkworm using microsatellite markers showed breed-specific profiles for 15 silkworms which indicates the prospects of microsatellite markers for establishment of molecular identities to distinguishing silkworm breeds. This kind of approach is need to adopt for screening the vast bivoltine silkworm germplasm available in the CSGRC through molecular markers which could able to identify better parents for selection to develop thermotolerant silkworm breeds by conventional

breeding programme. In the present study, 10 bivoltine silkworms were shortlisted which showed homozygosity for thermotolerance (Table.7) can be utilized for field trials in hot zone of the country for further validation.

4. CONCLUSION

A total of 369 bivoltine accessions available at CSGRC, Hosur were evaluated for the economic parameters including survival. Based on the analysis, 40 bivoltine accessions were shortlisted and screened with SSR markers linked to thermotolerance. As a result, 10 bivoltine accessions were further shortlisted *i.e.* 8 accessions that are showing 100% thermotolerance and 2 accessions showing >85% thermotolerance. These bivoltine accessions were further grouped into two groups viz. the accessions spinning oval cocoons- BBI-0086 (KPG-A), BBE-0184 (SMGS-2), BBI-0301 (YS-7) and BBI-0339 (DD-2) and the accessions spinning dumbbell cocoons- BBI-0044 (NB4D2), BBI-0334 (APS-4), BBI-0336 (APS-8), BBI-0338 (DD-1), BBI-0343 (NK3) and BBI-0358 (CSR26). These bivoltine accessions were evaluated in selected hotspots *i.e.*, CSR&TI, Berhampore, RSRS, Jammu and REC, Chitradurga. Identified bivoltine accessions with better performance in the hotspots can be recommended to utilize in breeding programme to evolve abiotic stress tolerant breeds/hybrids for its commercial exploitation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES:

1. Oommen PJ. Poised for the big leap.In the World Silk meet. Proceedings of the 23rd International Silk Association Congress, Bangalore,India, 3-6 Dec. 2001. Indian Silk 2001: 19-21.

2. Datta RK. Improvement of Silkworm races, *Bombyx mori* (L)., in India. Sericologia. 1984; 24:393-415.

3. Chatterjee SN, Datta RK. Genetic Variability in mulberry silkworm *Bombyx mori*. L., breeds with low silk yield. Ind. J. Seri. 1993; 32:69-86.

4. Suresh kumar N, Yamamoto T, Basavaraja HK, Datta RK. Studies on the effect of high temperature on F hybrids between Polyvoltine and Bivoltine silkworm races of *Bombyx mori* L. International Journal of Industrial Entomology. 2001; 2:123-127.

5. Ramesha C, Seshagiri SV, Rao CGP. Evaluation and identification of superior polyvoltine crossbreeds of mulberry silkworm *Bombyx mori* L. Journal of Entomology. 2009; 6:179-188.

6. Sugnana Kumari S, Sure Venkata Subbarao, Sunil Misra, Upadyayula Suryanarayana Murty Screening strains of the mulberry silkworm, *Bombyx mori*, for thermotolerance. Journal of Insect Science. 2011; 11(1):116.

7. Hazel JR. Thermal adaptation in biological membranes: is homeo viscous adaptation the explanation". Annual Review of Physiology.1995; 57:19-42.

8. Basavaraja HK, Nirmal Kumar NS, Suresh Kumar N, Malreddy, Kshama Giridhar, Ashan MM, Datta RK. New productive bivoltine hybrids. 1995; Indian Silk: 34:5-9.

9. Datta RK, Suresh Kumar N, Basavaraja HK, Kishore Kumar CM, Malreddy N. "CSR18 x CSR19" A robust bivoltine hybrid for all season rearing in the tropics. Indian Silk 2001; 39: 5-7.

10. Moorthy SM, Das SK, Rao PRT, Rajeurs S and Sarkar A. Evaluation and selection of potential parents based on selection indices and isozyme variability in silkworm, Bombyx mori L. International Journal of Industrial Entomology. 2007; 4:1-7.

11. Steele KA, Price AH, Shashidhar HE, Witcombe JR. Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. Theoretical and Applied Genetics 2006; 112:208–221.

12. Knapp SJ. Marker assisted selection as a strategy for increasing the probability in selecting superior genotypes. Crop science 1998; 38:1164-1174.

13. Tautz,D. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic acids research, 1989; 17(16): 6463-6471.

14. Zhao Y, Zhang J, Wu YC, Zhu YH. SSR marker- based mapping and linkage analysis of *Bombyx mori* thermo tolerance gene. Journal of Food, Agriculture and Environment.2010; 8(1): 338-342.

15. Srivastava PP, Kar, PK, Awasthi AK, Raje Urs S. Identification and association of ISSR markers for thermal stress in polyvoltine silkworm *Bombyx mori*. Russian Journal of Genetics 2007; 43: 858-864.

16. Moorthy SM, Chandrakanth N, Ashwath SK, Nirmal Kumar S, Naseema Begum A, Qadri SMH. Identification of molecular markers (SSR) associated with thermo tolerance in silkworm, *Bombyx mori* .In " 6th BACSA International Conference " "Building Value Chains in Sericulture" "BISERICA" held at Padua, Italy on April 7–12th 2013; PP 40.

17. Chandrakanth N, Moorthy SM, Ponnuvel KM, Sivaprasad V. Identification of micro satellite markers linked to thermo tolerance in silkworm by bulk segregant analysis and in silico mapping. Genetica. 2015; 47:1063-1078.

18. Falconer, DS. Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance. Genetics Research 1990, 56(1):57-70.

19. Gimelfarb A, Lande R. Simulation of marker assisted selection in hybrid populations. Genet Res. 1994; 63:39-47.

20. Moreau L, Charcosset A, Gallais A. Experimental evaluation of several cycles of marker-assisted selection in maize. Euphytica. 2004; 137:111–118.

21. Fan Z, Robbins MD, Staub JE. Population development by phenotypic selection with subsequent marker-assisted selection for line extraction in cucumber (*Cucumis sativus* L.). Theor Appl Genet.,2006; 112:843–855.

22. Chandrakant N, Lakshmanan V, Verma AK, Rahul K, Kanika Trivedy. Identification of potential thermo tolerant bivoltine silkworm breeds through phenotypic and molecular approach. Global journal of Bioscience and Biotechnology. 2018; 7 (4):525-530.

23. Sreekumar S, Ashwath SK, Chitra S, Basavaraja HK, Dandin SB, Subrana P and Kamble CK. DNA profiling of a few indigenous and evolved silkworm breeds of India using microsatellite markers. 2008; Indian Journal of Sericulture. 2008; 47(2): 204-213.

Table. 3: Details on the screening of bivoltine accessions using LFL1123 primer

Sl No.	Accn No.	1	2	3	4	5	6	7	8	% of tolerance
1	26	AA	BB	AA	BB	BB	AA	BB	AA	50%
2	30	BB	BB	BB	BB	BB	BB	BB	BB	0
3	44	BB	BB	BB	BB	AA	BB	BB	BB	12.50%
4	**86**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**100%**
5	133	BB	BB	BB	BB	AA	BB	BB	BB	12.50%
6	137	BB	BB	BB	BB	BB	BB	BB	BB	0%
7	167	BB	BB	BB	AB	AB	AA	AA	BB	25%
8	183	BB	BB	BB	BB	BB	BB	BB	BB	0%
9	184	BB	BB	BB	BB	BB	BB	BB	BB	0%
10	187	BB	BB	BB	BB	BB	BB	BB	BB	0%
11	197	BB	BB	BB	BB	AA	BB	BB	BB	12.50%
12	222	AA	AA	BB	BB	BB	BB	BB	AB	25%
13	272	BB	BB	BB	BB	BB	BB	BB	BB	0%
14	299	BB	BB	AB	AB	AA	BB	BB	BB	12.50%
15	301	AB	AB	BB	BB	nw	AB	BB	AB	37.50%
16	303	BB	BB	BB	BB	BB	BB	BB	BB	0%
17	334	nw	BB	BB	BB	BB	BB	BB	BB	0%
18	336	BB	BB	BB	BB	nw	BB	BB	BB	0%
19	338	AB	AB	BB	nw	BB	AA	nw	BB	13%
20	339	BB	BB	BB	BB	BB	BB	BB	BB	0%
21	341	BB	BB	BB	BB	BB	BB	BB	BB	0%
22	342	BB	AA	BB	AA	AB	AA	AB	AB	37.50%
23	343	AB	BB	BB	BB	AB	BB	BB	BB	25%
24	344	BB	BB	BB	BB	BB	BB	BB	AB	12.50%
25	345	nw	BB	BB	BB	BB	BB	BB	nw	0%
26	346	AB	BB	BB	BB	AB	BB	BB	BB	0%
27	347	AA	BB	AA	AB	AB	AB	BB	BB	13%
28	349	AB	BB	BB	BB	AB	AA	BB	BB	13%
29	350	BB	BB	BB	BB	BB	BB	BB	BB	0%
30	**358**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**100%**
31	359	BB	BB	BB	AB	BB	BB	BB	BB	0%
32	360	BB	BB	BB	BB	BB	BB	BB	BB	0%
33	361	BB	BB	AA	BB	nw	AA	BB	AA	37.50%
34	363	AA	AA	AA	AA	AA	AA	AA	BB	87.50%
35	364	BB	BB	AB	nw	AA	BB	nw	AA	25%
36	367	BB	AA	AA	BB	AA	nw	AA	AA	63%
37	369	BB	AA	nw	BB	BB	nw	AA	BB	25%
38	370	AB	AB	AB	BB	AA	BB	BB	AB	12.50%
39	377	BB	BB	BB	BB	BB	BB	BB	BB	0%
40	378	BB	BB	BB	BB	BB	BB	BB	BB	0%

Table. 4: Details on the screening of bivoltine accessions S0329 primer

Sl No.	Accn No	1	2	3	4	5	6	7	8	% of tolerance
1	26	BB	BB	BB	BB	BB	BB	BB	BB	0%
2	30	AB	BB	BB	BB	AB	BB	BB	BB	0%
3	**44**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**100%**
4	86	AB	BB	AB	AB	AB	AA	AB	BB	12.50%
5	133	AA	BB	BB	BB	BB	NW	BB	BB	12.50%
6	137	AB	AB	AB	AB	AA	AB	BB	AA	25%
7	167	AB	AA	AA	AA	AA	AB	AB	AB	50%
8	183	BB	BB	BB	BB	AB	BB	AB	BB	0%
9	184	AA	AA	AA	AA	AA	AA	AA	AA	100%
10	187	AB	BB	AB	AA	BB	AB	BB	BB	13%
11	197	BB	BB	AA	AB	BB	AB	AA	AB	25.00%
12	222	BB	BB	BB	AB	AB	NW	NW	NW	0%
13	272	AB	AB	AB	AB	AB	AB	AA	AB	13%
14	299	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
15	301	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
16	303	BB	BB	BB	AB	AB	BB	AB	BB	0.00%
17	334	BB	BB	BB	BB	AB	BB	AA	BB	13%
18	336	BB	AA	BB	BB	AB	BB	AA	AA	38%
19	338	BB	BB	BB	AA	AA	BB	BB	BB	25%
20	339	AA	AA	AA	BB	AA	AA	BB	AA	78%
21	341	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
22	342	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
23	343	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
24	344	AA	BB	AA	BB	BB	BB	AB	AB	25%
25	345	BB	BB	BB	BB	BB	BB	BB	AB	0.00%
26	346	BB	BB	BB	BB	BB	BB	AB	BB	0.00%
27	347	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
28	349	AB	AB	BB	BB	BB	AB	AB	AB	0.00%
29	350	AB	AB	AB	AB	AB	AB	AB	BB	0.00%
30	358	BB	BB	AB	BB	BB	AB	BB	BB	0.00%
31	359	AB	AB	AB	BB	AB	AB	AA	AB	12.50%
32	360	AB	AA	AB	AB	BB	AB	BB	BB	12.50%
33	361	BB	BB	AA	AB	AA	AA	AA	AB	50%
34	363	BB	AB	BB	BB	BB	BB	BB	BB	0.00%
35	364	BB	BB	AB	AB	AB	AA	AA	AA	0.00%
36	367	BB	NW	BB	NW	BB	BB	BB	BB	37.50%
37	369	BB	BB	AA	AA	AB	AB	AB	AB	25%
38	370	AB	BB	BB	BB	AB	AB	AB	BB	0.00%
39	377	AA	BB	AA	AB	AA	AA	AA	AA	78%
40	378	BB	BB	BB	BB	BB	NW	BB	BB	0.00%

Table. 5: Details on the screening of bivoltine accessions using S0813 primer

Sl No.	Accn No	1	2	3	4	5	6	7	8	% of tolerance
1	26	BB	BB	BB	BB	BB	BB	BB	BB	0%
2	30	BB	AA	BB	BB	AA	BB	BB	AA	38%
3	44	AB	AB	AB	AB	BB	BB	AB	BB	0%
4	**86**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**AA**	**100%**
5	133	NW	NW	NW	NW	NW	NW	NW	NW	0%
6	137	BB	BB	BB	BB	BB	BB	BB	BB	0%
7	167	BB	BB	BB	BB	BB	BB	AB	BB	0%
8	183	BB	BB	BB	BB	BB	BB	BB	BB	0%
9	184	NW	BB	AA	BB	NW	BB	BB	BB	13%
10	187	NW	NW	AA	AA	AA	AA	AA	BB	63%
11	197	BB	BB	BB	BB	BB	BB	BB	BB	0%
12	222	BB	BB	BB	BB	BB	BB	AB	BB	0%
13	272	BB	BB	BB	nw	BB	BB	BB	nw	0%
14	299	BB	BB	BB	BB	BB	BB	BB	BB	0%
15	301	AA	NW	AA	NW	AA	AA	AA	AA	75.00%
16	303	NW	NW	NW	NW	BB	NW	BB	NW	0%
17	334	NW	NW	BB	NW	NW	BB	NW	NW	0%
18	336	BB	BB	BB	BB	BB	BB	BB	NW	0%
19	338	NW	BB	BB	BB	BB	BB	BB	BB	0%
20	339	BB	BB	BB	BB	NW	BB	BB	BB	0%
21	341	NW	BB	BB	BB	BB	BB	NW	NW	0%
22	342	BB	BB	BB	BB	BB	BB	BB	BB	0%
23	343	BB	BB	BB	BB	BB	BB	BB	BB	0%
24	344	NW	BB	BB	BB	BB	BB	NW	BB	0%
25	345	BB	BB	BB	BB	BB	NW	BB	BB	0.00%
26	346	BB	BB	BB	BB	BB	BB	BB	BB	0%
27	347	NW	NW	BB	BB	BB	NW	NW	BB	0%
28	349	BB	BB	NW	BB	BB	BB	BB	NW	0.00%
29	350	BB	BB	BB	BB	NW	BB	BB	BB	0%
30	358	NW	NW	NW	NW	NW	NW	BB	NW	0%
31	359	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
32	360	BB	BB	BB	NW	BB	BB	AA	BB	0%
33	361	BB	NW	BB	BB	BB	BB	BB	BB	0%
34	363	AA	NW	AA	AA	BB	BB	BB	NW	37.50%
35	364	NW	NW	NW	NW	NW	NW	NW	NW	0%
36	367	BB	BB	AA	AA	BB	AA	BB	AA	0%
37	369	BB	NW	NW	BB	BB	BB	BB	NW	0%
38	370	BB	BB	BB	BB	BB	BB	BB	BB	0%
39	377	BB	BB	BB	BB	BB	BB	BB	BB	0%
40	378	BB	BB	BB	BB	BB	NW	BB	BB	0%

Table. 6: Details on the screening of bivoltine accessions using S0809 primer

Sl No.	Accn No	1	2	3	4	5	6	7	8	% of tolerance
1	26	BB	AA	AA	AB	NW	AA	BB	BB	38%
2	30	BB	BB	BB	BB	BB	BB	NW	BB	0.00%
3	44	NW	BB	AA	BB	AA	BB	AA	AA	50%
4	86	AA	AA	AA	AA	AA	AA	AA	BB	87.50%
5	133	AA	BB	BB	BB	BB	BB	BB	BB	12.50%
6	137	AA	BB	AA	BB	AA	BB	AA	BB	50%
7	167	AA	BB	BB	AA	AA	AA	AA	AA	75%
8	183	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
9	184	AA	AA	AA	AA	AA	AA	AA	AA	100%
10	187	NW	AA	NW	AA	NW	NW	AA	AA	50%
11	197	NW	BB	BB	BB	BB	BB	BB	BB	0.00%
12	222	BB	BB	NW	NW	NW	NW	NW	NW	0.00%
13	272	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
14	299	AA	BB	BB	BB	BB	BB	BB	BB	12.50%
15	301	AA	AA	AA	AA	AA	AA	AA	AA	100.00%
16	303	NW	AA	BB	AA	AA	AA	AA	BB	62.50%
17	334	AA	AA	AA	AA	AA	AA	AA	AA	100%
18	336	AA	AA	AA	AA	AA	AA	AA	AA	100%
19	338	AA	AA	AA	AA	AA	AA	AA	AA	100%
20	339	AA	AA	AA	AA	AA	AA	AA	AA	100%
21	341	BB	BB	BB	BB	BB	BB	BB	NW	0.00%
22	342	BB	AA	AA	AA	AA	AA	AA	AA	87.50%
23	343	AA	AA	AA	AA	AA	AA	AA	AA	100.00%
24	344	NW	AA	AA	AA	AA	AA	AA	AA	88%
25	345	AA	NW	AA	BB	BB	NW	BB	BB	25.00%
26	346	AA	AA	AA	AA	AA	AA	BB	AA	87.50%
27	347	AA	AA	AA	AA	AA	AA	AA	BB	87.50%
28	349	BB	AA	AA	AA	BB	BB	AA	AA	62.50%
29	350	AA	BB	NW	AA	AA	BB	BB	AA	50.00%
30	358	BB	BB	AA	BB	AA	AA	AA	AA	62.50%
31	359	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
32	360	BB	BB	BB	NW	BB	NW	BB	BB	0.00%
33	361	AA	BB	MB	AA	MB	MB	MB	NW	25%
34	363	AA	AA	AA	AA	AA	AA	AA	AA	100.00%
35	364	NW	NW	NW	NW	NW	NW	BB	BB	0.00%
36	367	BB	BB	BB	BB	BB	BB	BB	AA	12.50%
37	369	BB	BB	BB	BB	BB	BB	BB	BB	0.00%
38	370	AB	AB	BB	BB	BB	BB	BB	BB	0.00%
39	377	AA	AA	AA	AA	AA	AA	AA	AA	100%
40	378	AA	AA	AA	NW	AA	NW	NW	AA	62.50%

Table 7. Details of the selected bivoltine accessions with thermo tolerance

Sl No.	Accn No	Accn. Name	1	2	3	4	5	6	7	8	% of tolerance
	Marker – LFL 1123										
1	BBI-0086	KPG-A	AA	AA	AA	AA	AA	AA	AA	AA	100%
2	BBI-0358	CSR-26	AA	AA	AA	AA	AA	AA	AA	AA	100%
	Marker-LFL 0329										
3	BBI-0044	NB4D2	AA	AA	AA	AA	AA	AA	AA	AA	87.0%

4	BBE-0184	SMGS-2	AA	AA	AA	AA	AA	AA	AA	AA	87.0%
	Marker-S0809										
	BBE-0184	SMGS-2	AA	AA	AA	AA	AA	AA	AA	AA	100%
5	BBI-0301	YS-7	AA	AA	AA	AA	AA	AA	AA	AA	100%
6	BBI-0334	APS-4	AA	AA	AA	AA	AA	AA	AA	AA	100%
7	BBI-0336	APS-8	AA	AA	AA	AA	AA	AA	AA	AA	100%
8	BBI-0338	DD-1	AA	AA	AA	AA	AA	AA	AA	AA	100%
9	BBI-0339	DD-2									
10	BBI-0343	NK-3	AA	AA	AA	AA	AA	AA	AA	AA	100%
	Marker-S0813										
	BBI-0086	KPG-A	AA	AA	AA	AA	AA	AA	AA	AA	100%

Fig.1(a): Amplification of SSR marker (LFL1123) in thermo-tolerant BBI-0358

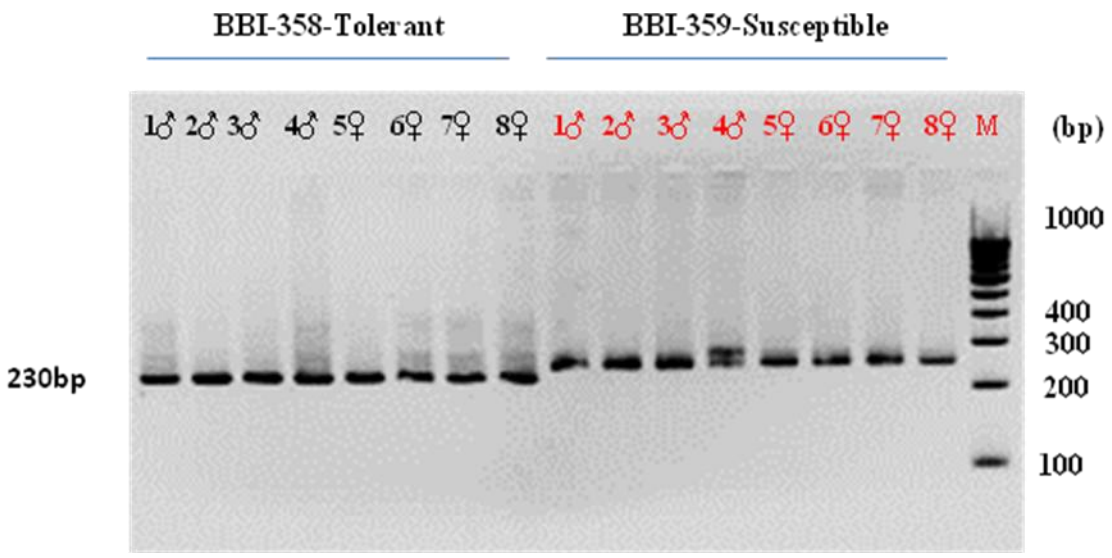


Fig.1(b): Amplification of SSR marker (LFL1123) in thermo-tolerant BBI-363.

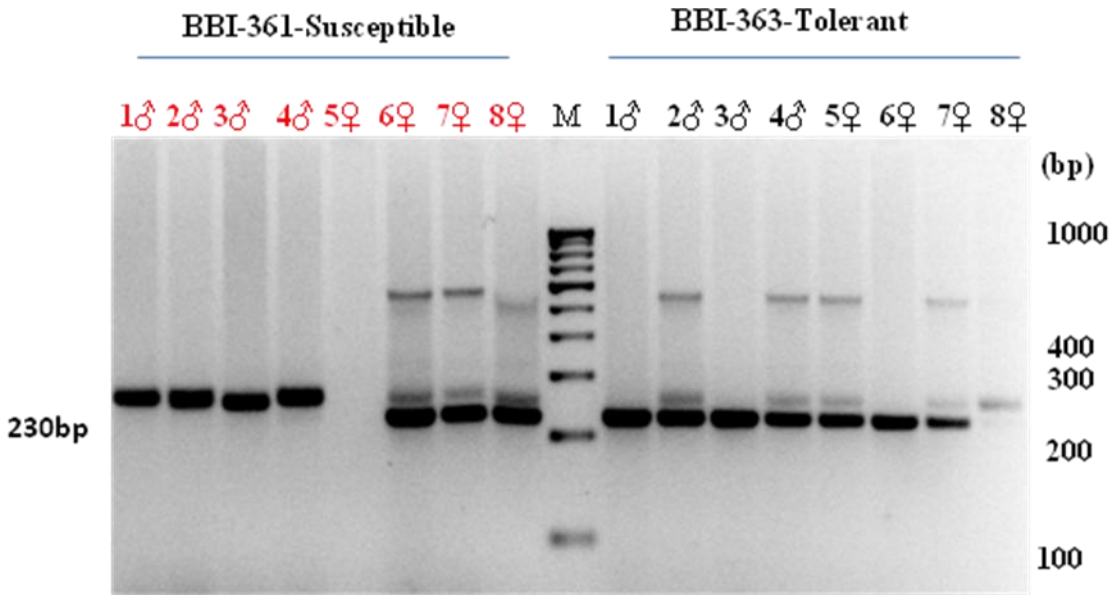


Fig.2(a): Amplification of SSR marker (LFL0329) in thermo tolerant BBI-0044

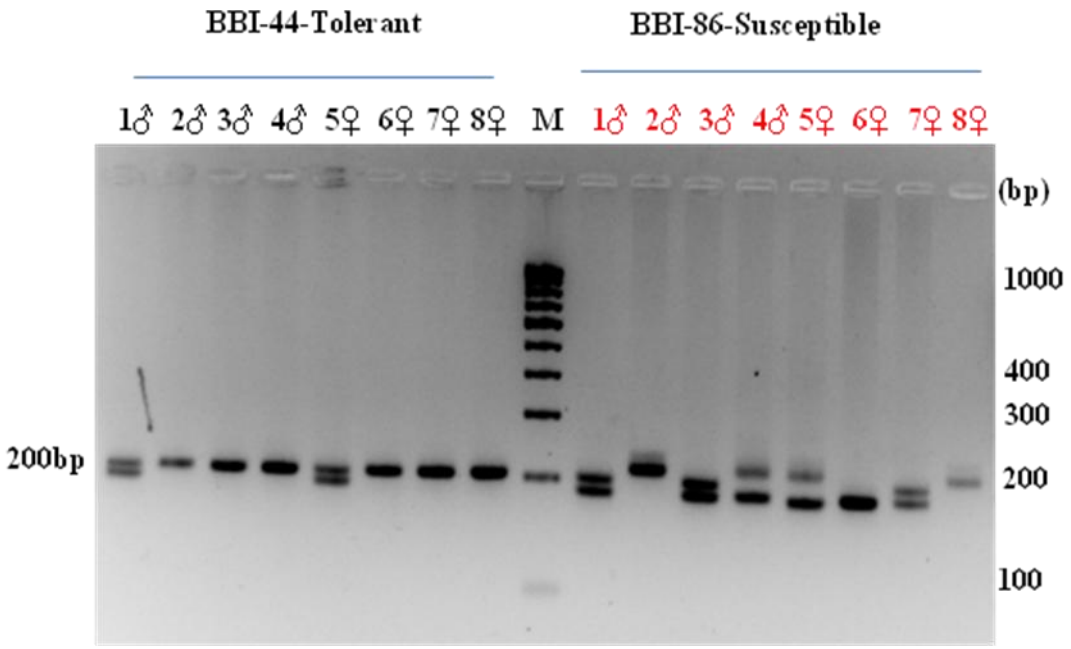


Fig.2(b): Amplification of SSR marker (LFL0329) in thermo tolerant BBI-0184

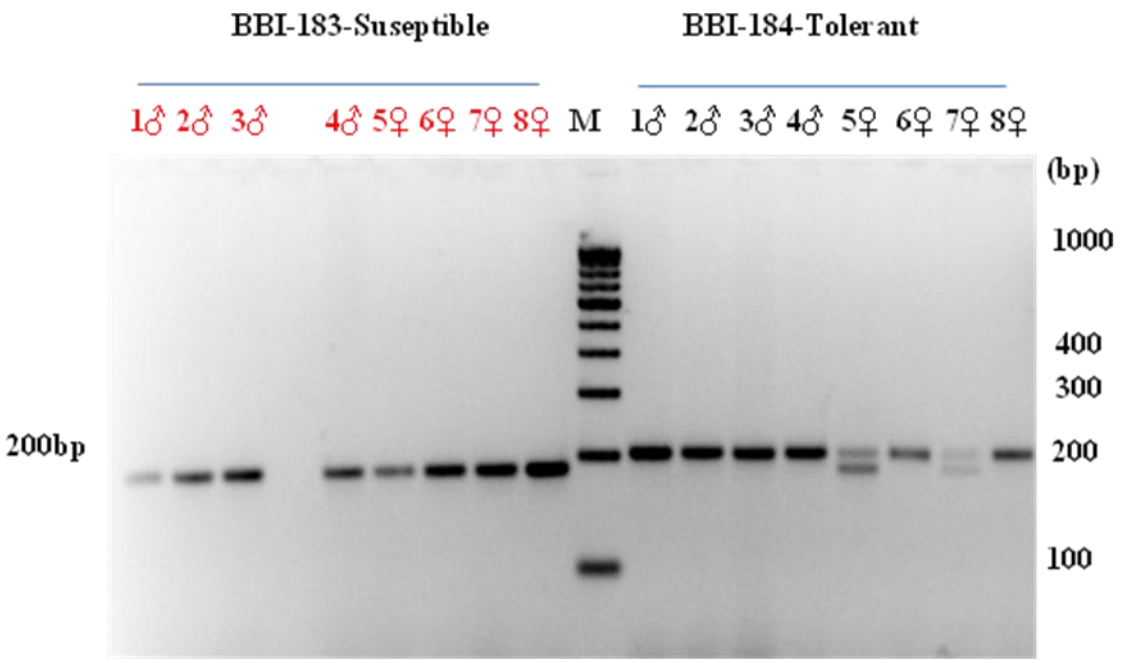


Fig.3(a) Amplification of SSR marker (S0813) in thermo tolerant BBI-0086

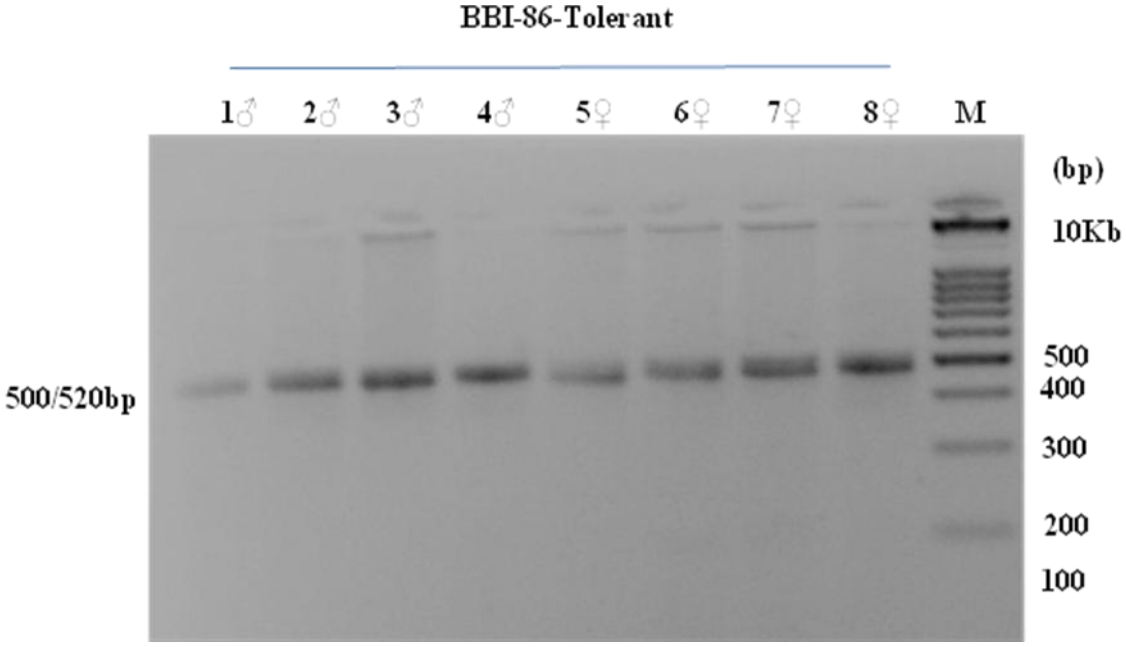


Fig.3(b): Amplification of SSR marker (S0813) in thermo susceptible BBI-0343

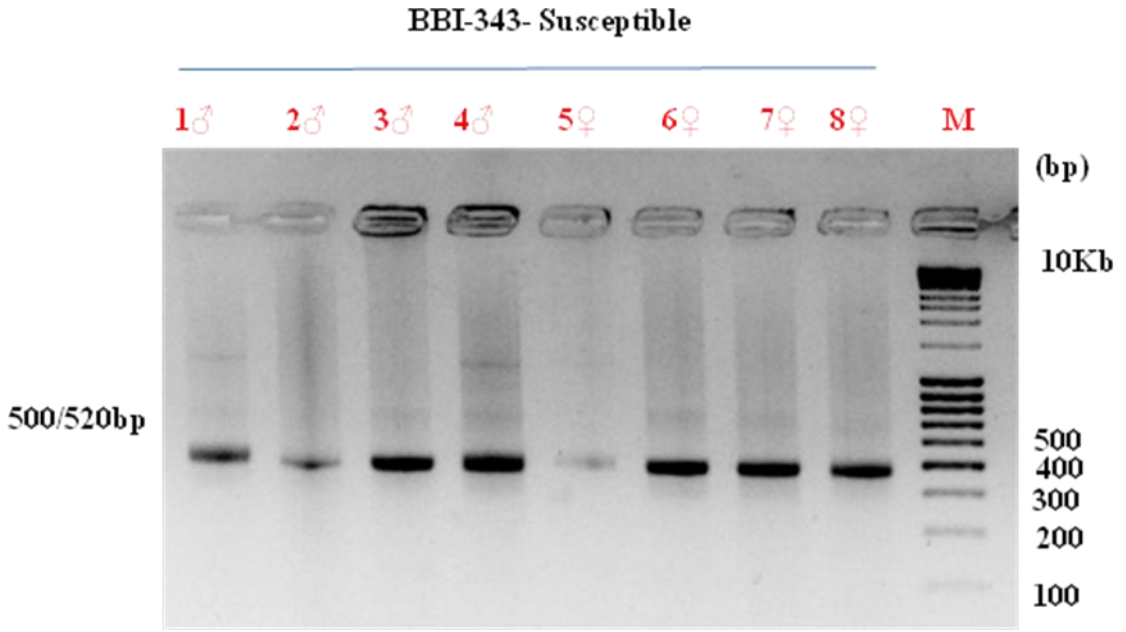
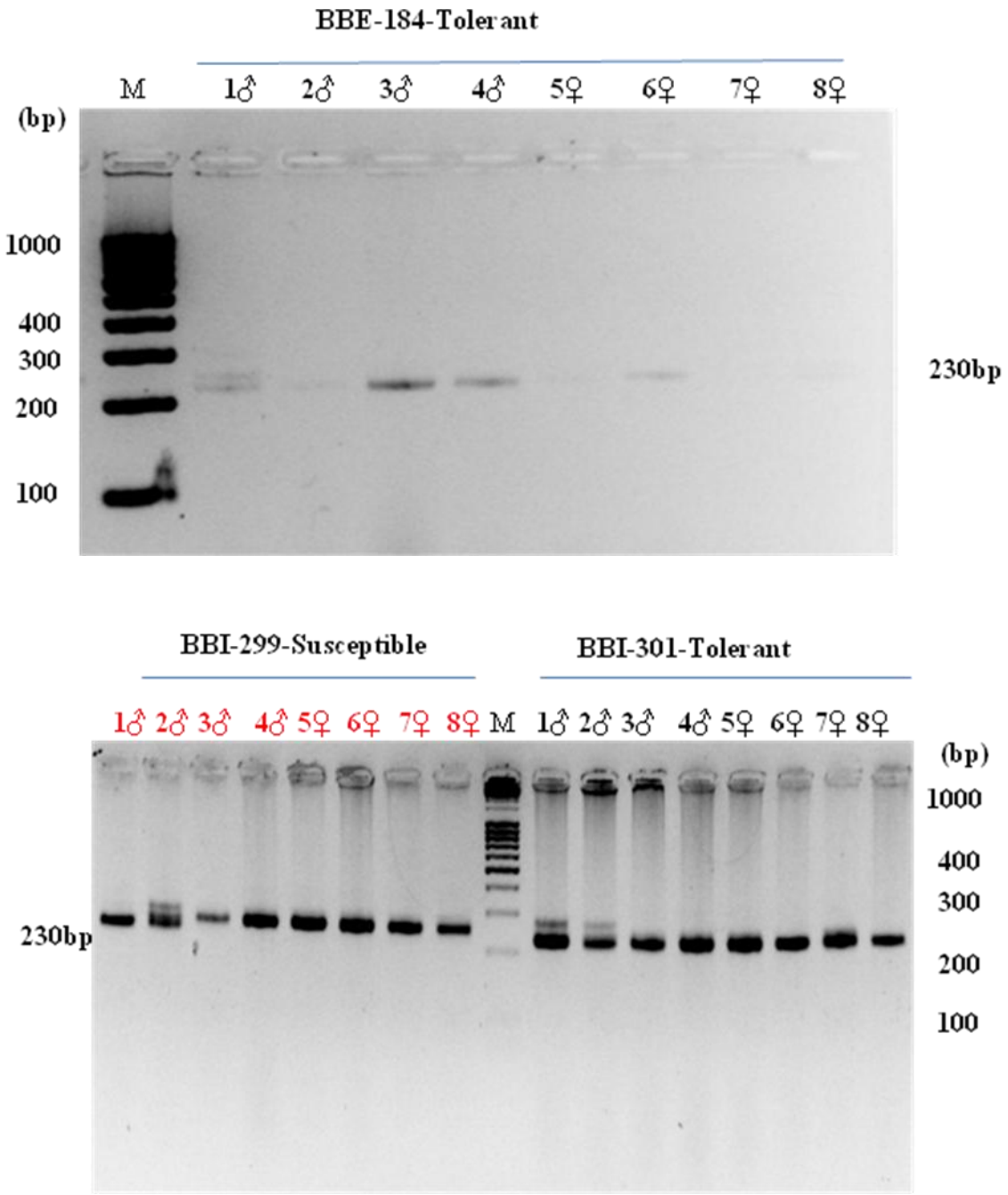
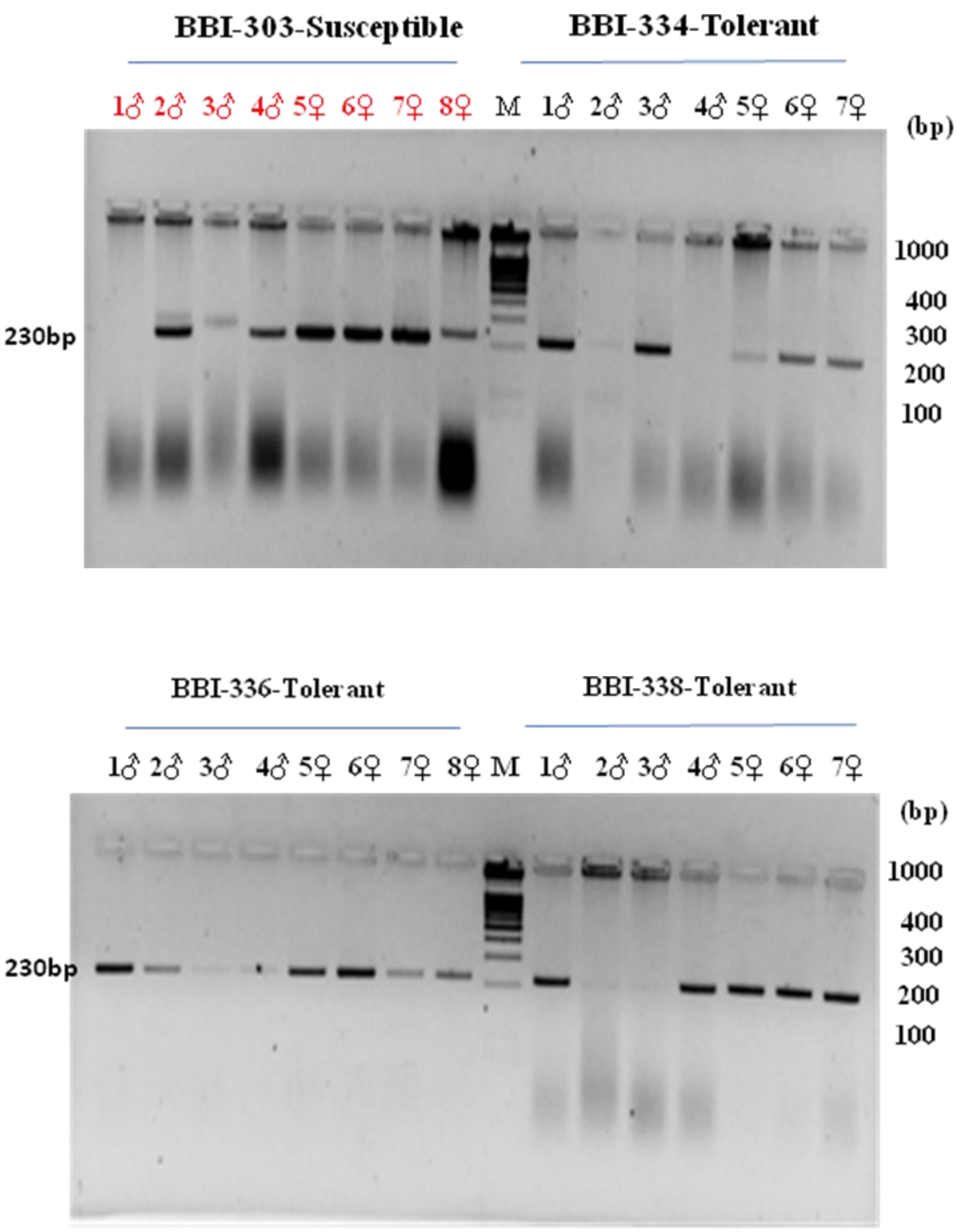
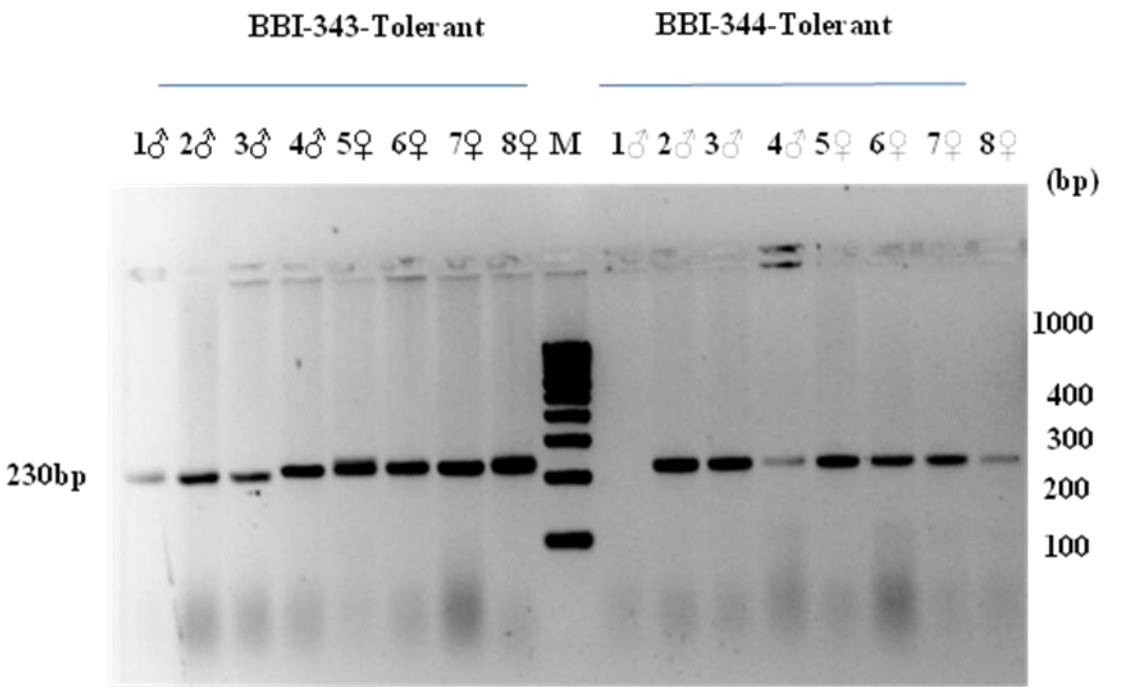
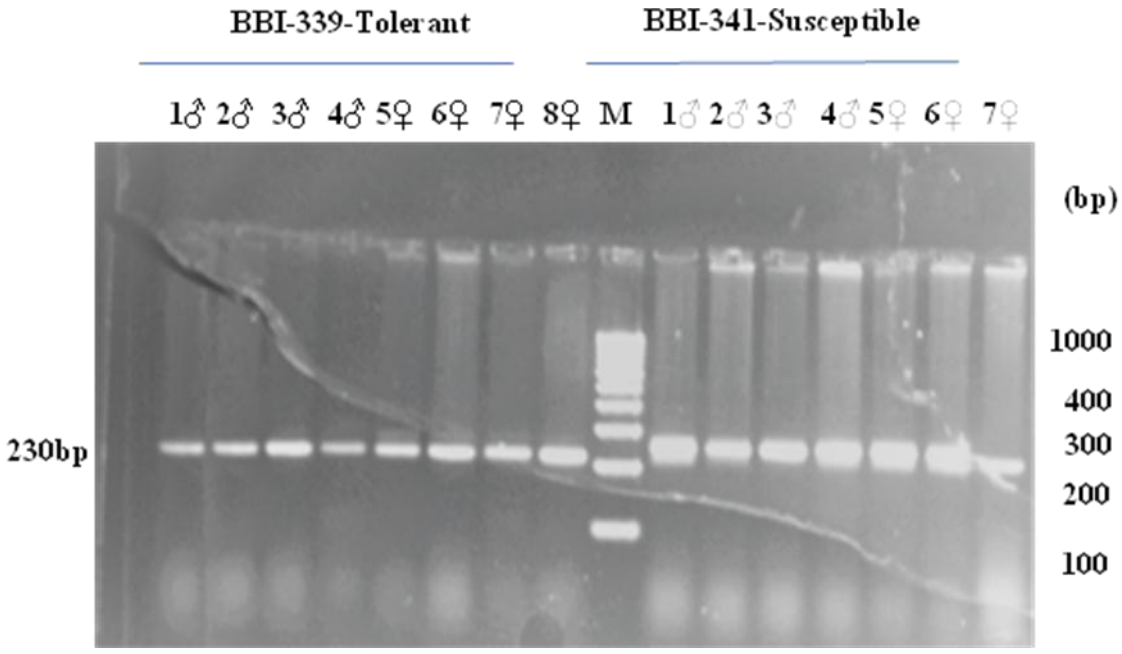


Fig.4(a): Amplification of SSR marker (S0809) in thermo tolerant BBE-0184, BBI-0301, BBI-0334, BBI-0336, BBI-0338, BBI-0339, BBI-0343 and BBI-0344




BBI-303-Susceptible
BBI-334-Tolerant
1♂ 2♂ 3♂ 4♂ 5♀ 6♀ 7♀ 8♀ M 1♂ 2♂ 3♂ 4♂ 5♀ 6♀ 7♀
(bp)
1000
400
300
200
100
230bp
BBI-336-Tolerant
BBI-338-Tolerant
1♂ 2♂ 3♂ 4♂ 5♀ 6♀ 7♀ 8♀ M 1♂ 2♂ 3♂ 4♂ 5♀ 6♀ 7♀
(bp)
1000
400
300
200
100
230bp


BBI-343-Tolerant
BBI-344-Tolerant
1♂ 2♂ 3♂ 4♂ 5♀ 6♀ 7♀ 8♀ M 1♂ 2♂ 3♂ 4♂ 5♀ 6♀ 7♀ 8♀
(bp)
1000
400
300
200
100
230bp


BBI-339-Tolerant
BBI-341-Susceptible
1♂ 2♂ 3♂ 4♂ 5♀ 6♀ 7♀ 8♀ M 1♂ 2♂ 3♂ 4♂ 5♀ 6♀ 7♀
(bp)
1000
400
300
200
100
230bp