

Original Research Article

Marker-assisted selection of bivoltine silkworm genetic resources for thermotolerance

ABSTRACT

The 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 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. Therefore, present study has been carried out to screen forty selected bivoltine silkworm germplasm resources exhibiting higher rate of survival and high productivity using SSR markers (LFL329; LFL1123; S0808; S0813) linked to thermo-tolerance in silkworm so as to identify thermo-tolerant breeds. Based on the PCR product resolved in 2% agarose gel and presence of bands corresponding thermotolerant and susceptible markers observed, 10 bivoltine accessions showed 87-100% tolerance and were selected for further validation through field trials in hot zones.

Keywords: Bivoltine, Silkworm, Thermo-tolerance, SSR markers

INTRODUCTION

The 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 (Oommen, 2001) and most of it is produced from cross breeds raised by crossing the multivoltine and high yielding bivoltine silkworm breeds (Datta 1984; Chatterjee 1993). So far, the bivoltine hybrids recommended for large-scale production of cocoons in the field are robust in nature (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 bivoltine breeds especially in the hot and humid climatic conditions of tropics resulted in extensive crop loss, (Ramesha *et al.*, 2009). Many important qualitative characters such as viability and cocoon yield decline sharply due to higher temperatures (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. Hazel, (1995) and Willmer *et al.*, (2004) 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 (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 (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 (Moorthy *et al.*, 2007).

Marker-assisted selection (MAS) has been widely used in breeding programs (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 (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 (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; Zhao *et al.* (2010) identified 5 SSR markers linked to thermotolerance in silkworm and mapped them on 8th chromosome using backcross population of thermo-tolerant variety - Dong 34 and thermo-sensitive variety - Ou17. Kumar *et al.*, (2001) and Srivastava *et al.*, (2007) have studied on the selection of silkworm breeds in respect of thermotolerance by identifying thermotolerant silkworm breeds.

The 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* (Moorthy *et al.*, 2013, 2014; Chandrakanth *et al.*, 2015). The identified breeds will help the breeders to include in the breeding programs aimed for evolving the bivoltine breeds tolerant to abiotic stress.

MATERIALS AND METHODS

The present study was carried out at Central Sericultural Germplasm Resources Centre (CSGRC), Hosur, Tamilnadu, India. A total of 369 bivoltine silkworm accessions (genetic resources) available at this centre were considered for short listing and selection for the screening of thermo-tolerance.

Selection of Silkworm accessions for the screening of thermo-tolerance:

Among 369 silkworm germplasm resources, based on the evaluation data of the characters 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, forty bivoltine accessions were shortlisted (Table 1).

Table. 1: Details of the short listed 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	14.942±1.11 (1.37)	94.877±0.96 (2.47)	1.49±0.07 (8.73)	0.287±0.01 (10.74)	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)

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 37⁰C 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 65⁰ C for 30 min. Equal volumes of Phenol: Chloroform: Isoamyl alcohol mixture (25:24:1) were added and mixed thoroughly and centrifuged at 12000 xg 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 xg 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 xg for 10 min at 4⁰ C. discarded the supernatant and washed the pellet with 70 % alcohol and centrifuged for 5 minutes at 4⁰ 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.

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 (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-AAGTTCCTTTACCAGTTCACAGACAGC	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-ACTTACCCTACACCAGACGGAC		

FP: Forward primer, RP: reverse primer

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.

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.

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% thermo tolerance 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% thermo tolerance (Table 3). However, 18 accessions were detected as thermo susceptible 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% thermo tolerance 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 thermo susceptible (less than 500bp PCR product) (Fig. 3a,b and Table-5). The **S0809** primers showed 100% thermo tolerance (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% thermo tolerance was shown by 14 accessions (Fig. 4) and BHT (BBI-363) and Sanish E1(p) (BBI-30) showed <50% thermo tolerance. The remaining 17 accessions were found to be thermo susceptible (250bp) (Table-6).

The silkworm characters are mostly quantitative and are polygenic in nature. Many of the characters are influenced by environmental factors mainly temperature and humidity (Ramesha et al., 2010). 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 (Chandrakanth *et al.*, 2015). 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% thermotolerance 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 (Gimelfarb and Lande1994). 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 (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 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 themosusceptible banding pattern in five silkworm breeds. 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 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. 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.

CONCLUSION

Out of 369 bivoltine accessions available at CSGRC, Hosur, based on quantitative traits and survivability data, 40 best performing bivoltine accessions were shortlisted for molecular screening. Further, through molecular screening 10 bivoltine accessions were shortlisted *i.e.* 8 accessions that are showing 100% thermo- tolerant and 2 accessions showing >85% thermo tolerant and further grouped into two, *i.e.* 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 at selected test centres *i.e.*, CSR&TI, Berhampore, RSRS, Jammu and REC, Chitradurga. The identified accessions prove to be thermo tolerant and can be utilized for single hybrid and double hybrid preparations for commercial utilization in the respective regions.

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Table. 5: 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. 6: 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. 7: 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%

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

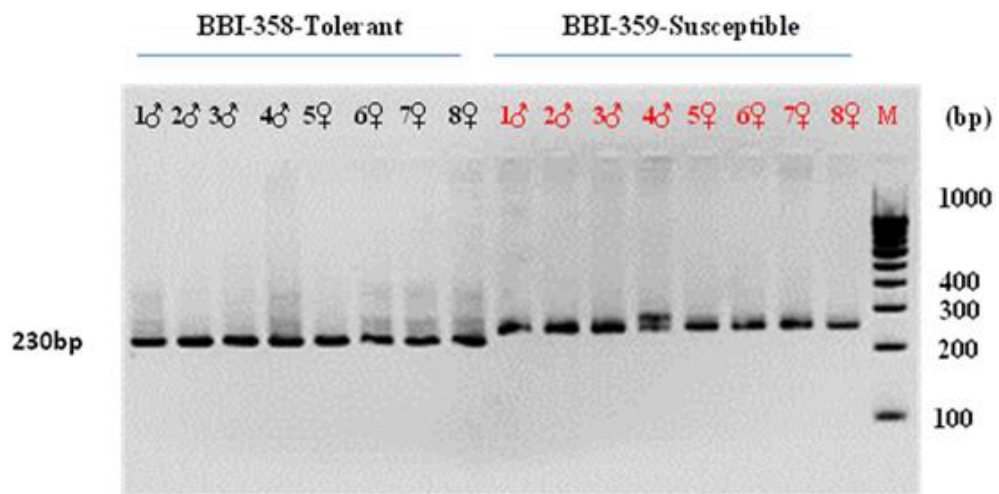


Fig.1(b): Amplification of SSR marker (LFL1123) in thermo-tolerant BBI-363.

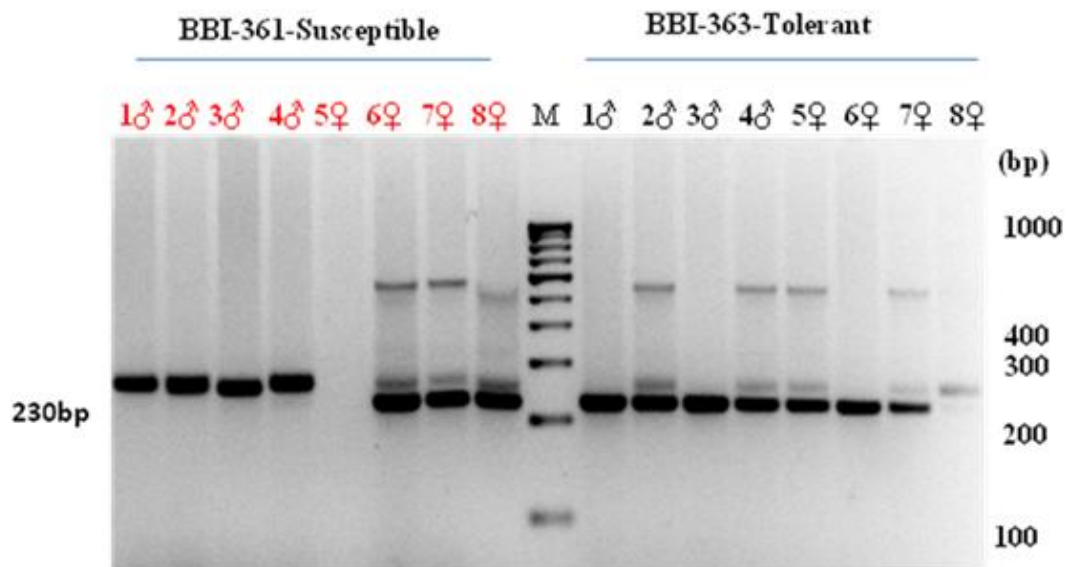


Fig.2(a): Amplification of SSR marker (LFL0329) in thermo tolerant BBI-0044

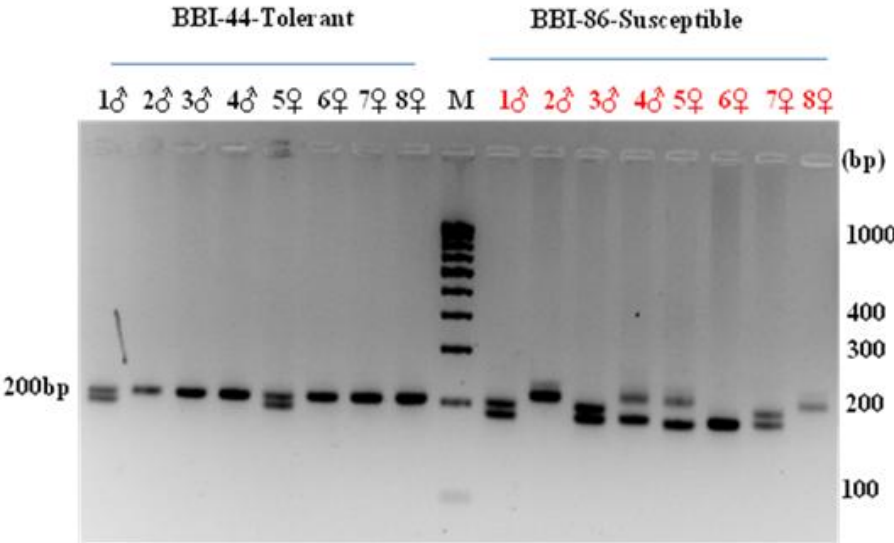


Fig.2(b): Amplification of SSR marker (LFL0329) in thermo tolerant BBI-0184

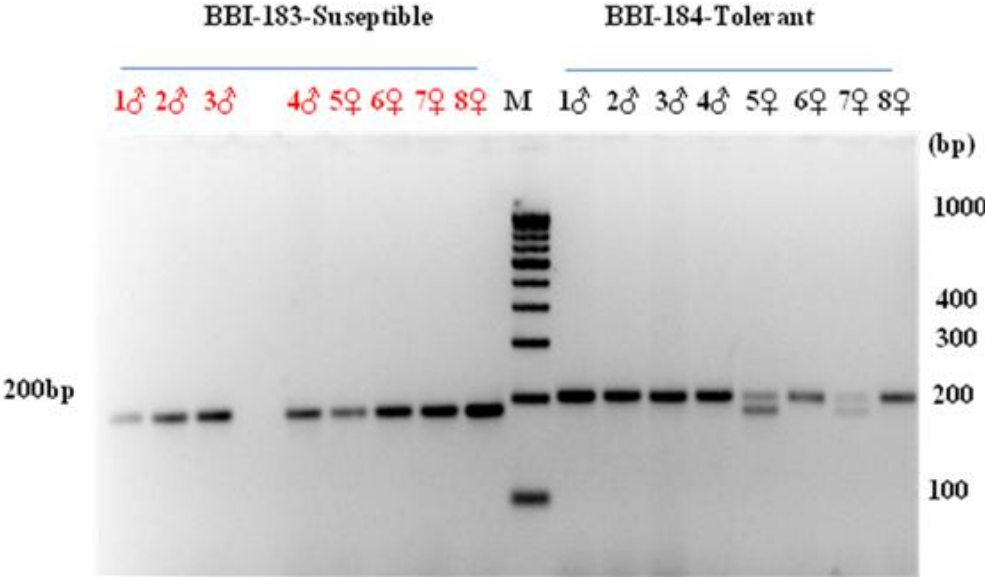


Fig.3(a) Amplification of SSR marker (S0813) in thermo tolerant BBI-0086

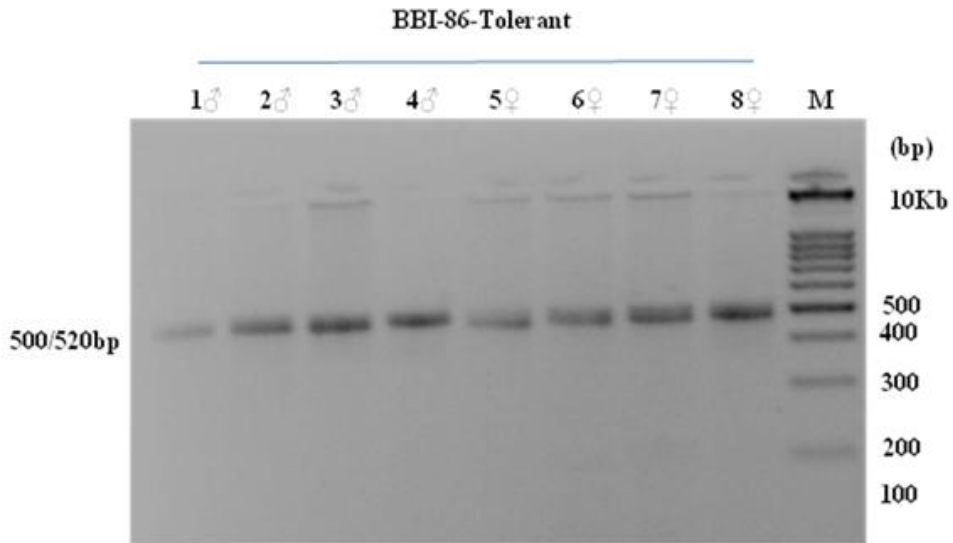


Fig.3(b): Amplification of SSR marker (S0813) in thermo susceptible BBI-0343

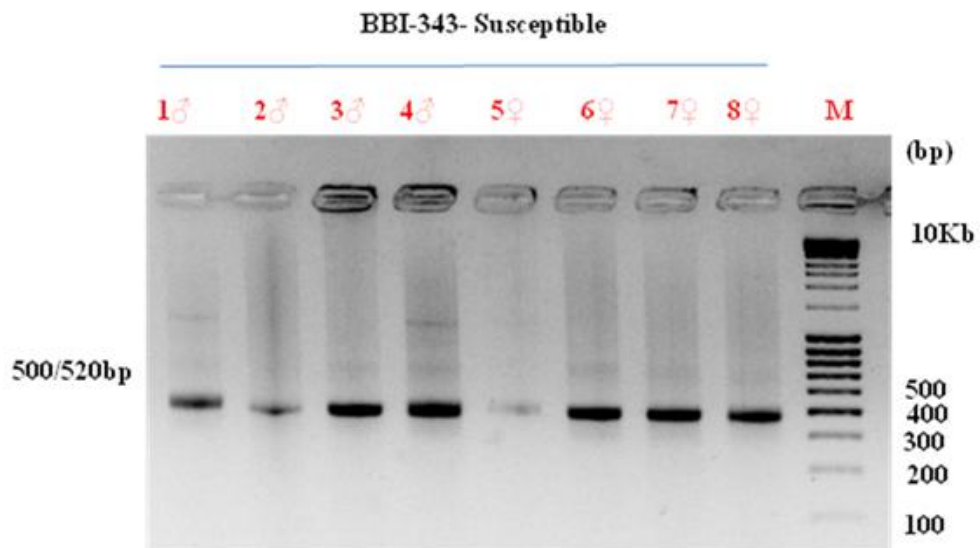
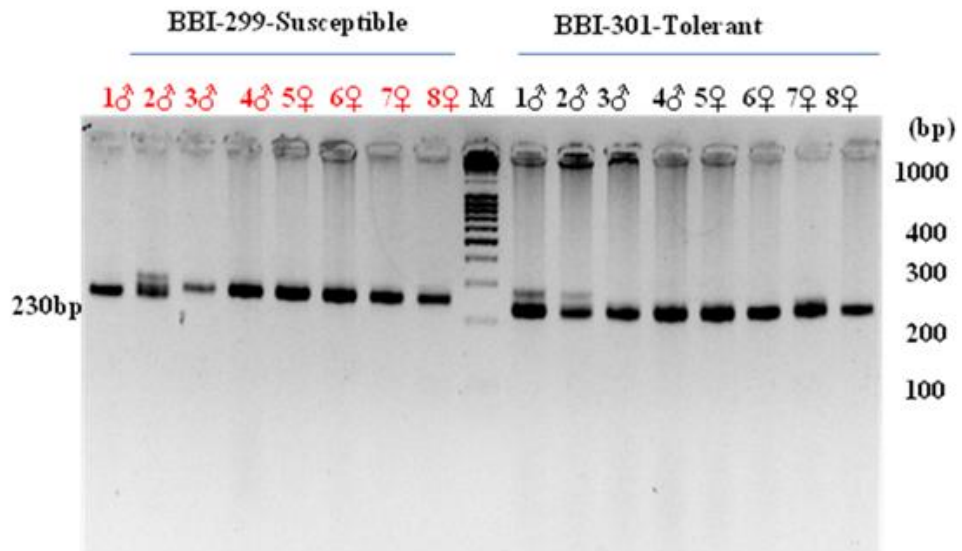
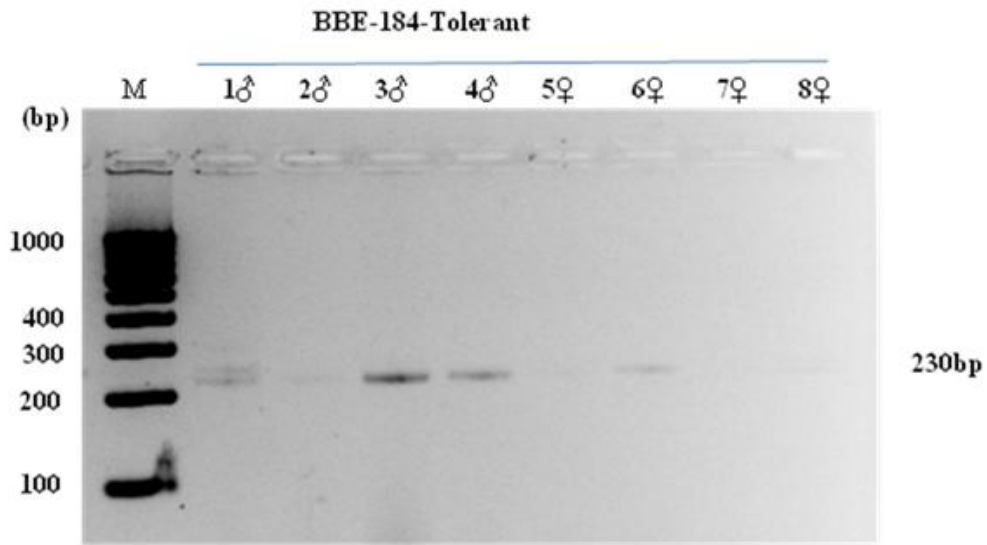
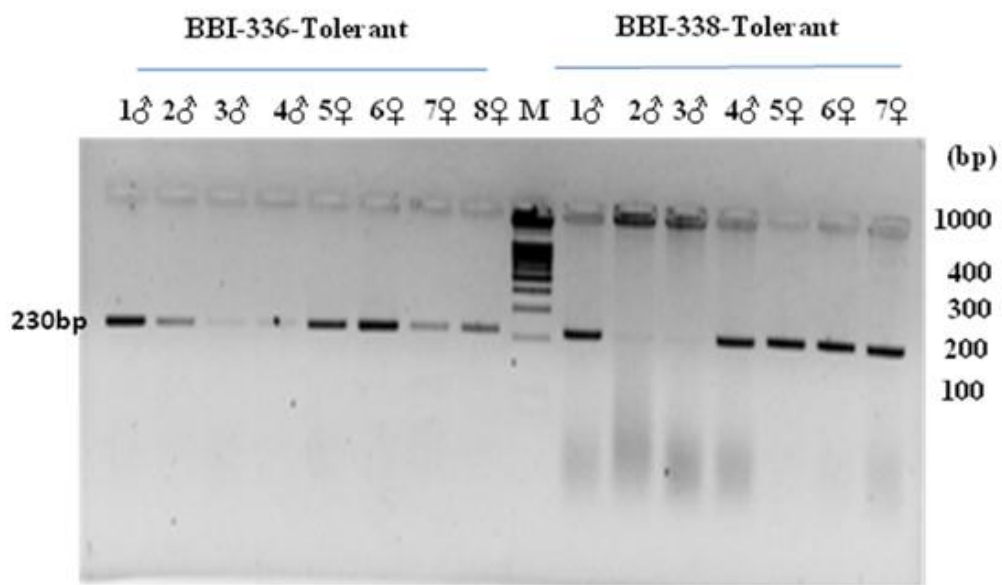
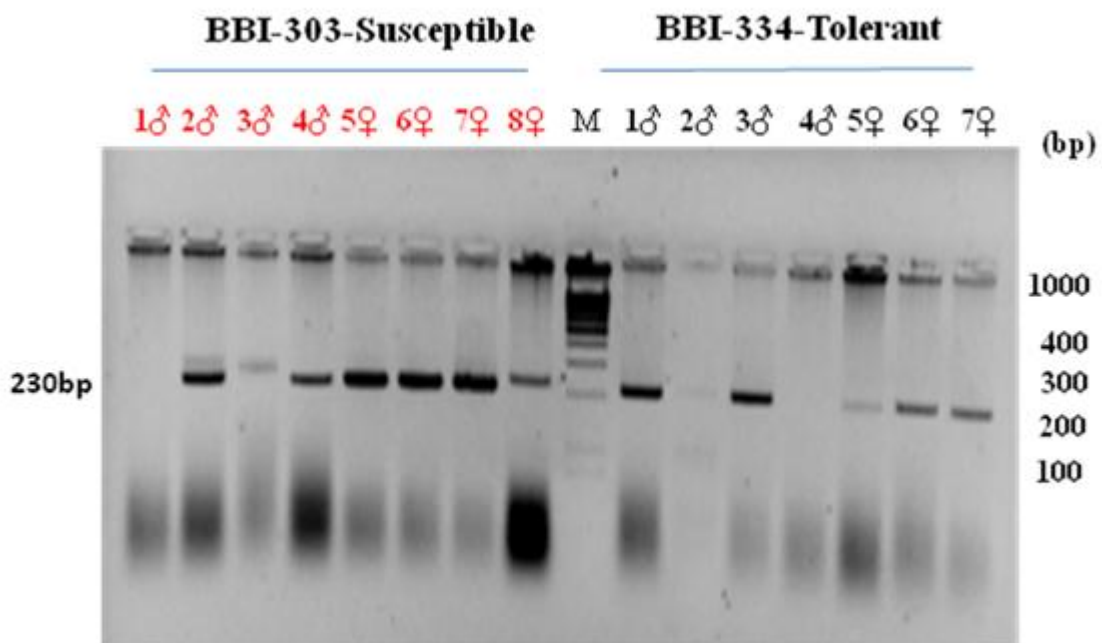
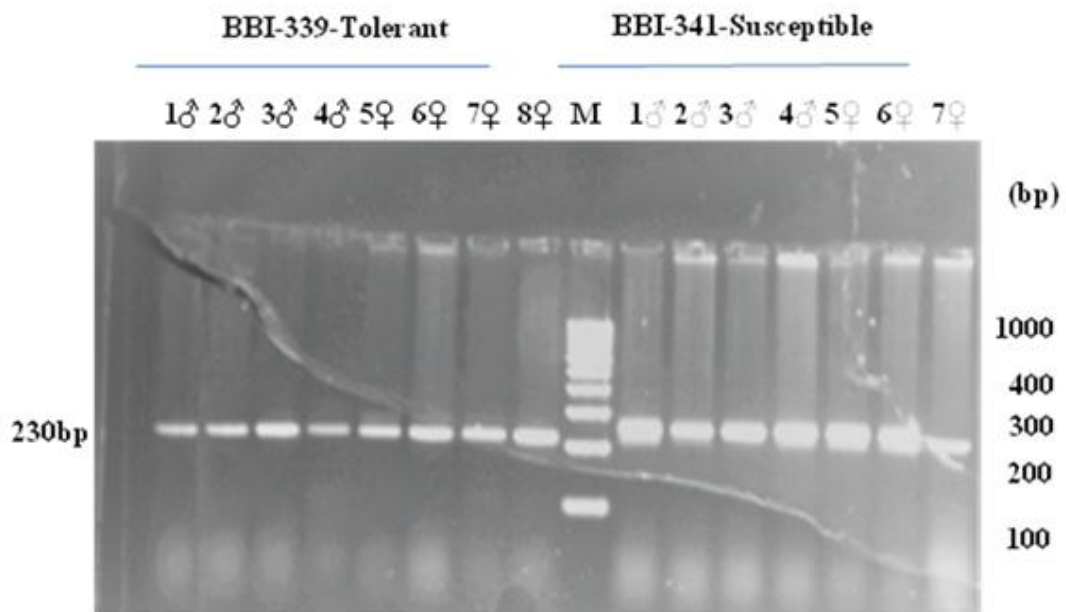
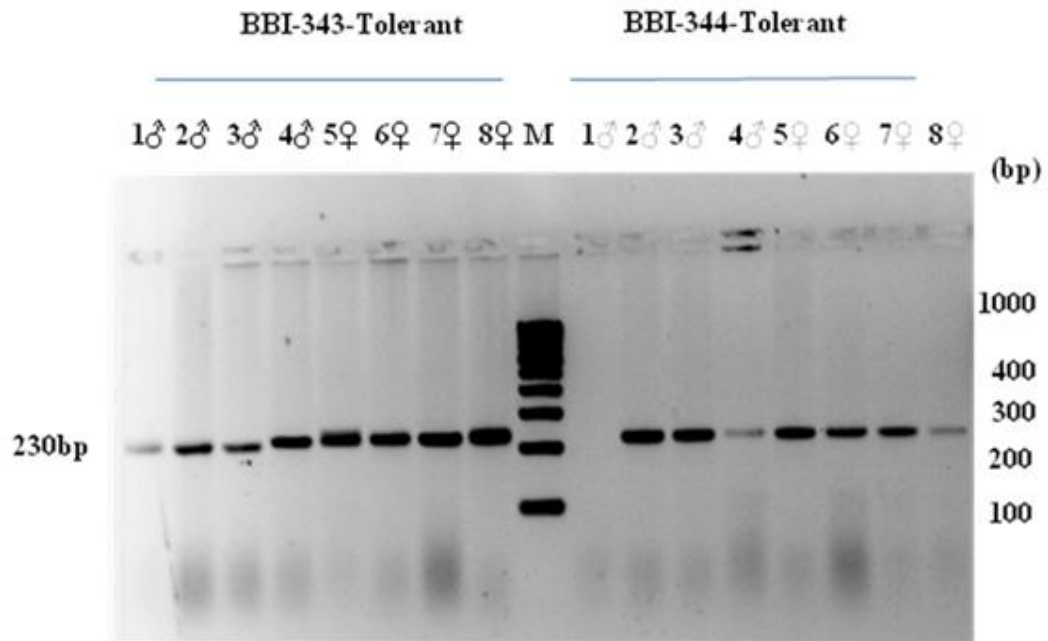


Fig.4(a): Amplification of SSR marker (S0809) in thermo tolerant BBE-0184, BBI-0301, BBI-0334, BBI-0336, BBI-0338, BBI-0339, BBI-0343 and BBI-0344







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