***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

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**Comment [L6]:** Bombyx mori (Linnaeus), as per Phalaena mori Linnaeus, 1758, Syst. Nat., 1: 499. Therefore, Linnaeus should be in Brackets ()

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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 8$^{th}$ 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

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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.

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**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).

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**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) |

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|---|---|---|---|---|---|---|---|---|
| 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±131.19 (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±194.54 (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±136.05 (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±195.05 (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±184.20 (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±150.47 (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±107.47 (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 $37^0$C 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.

**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; SO809; SO813 (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

**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

**Comment [L18]:** This part may include some statistical analaysis showing comaparision

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).

Comment [L19]: ????

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% 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 (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

Comment [L20]: Should be elaborated in perspective to future scope and research

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 3. 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% |

**Table. 4: 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. 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**

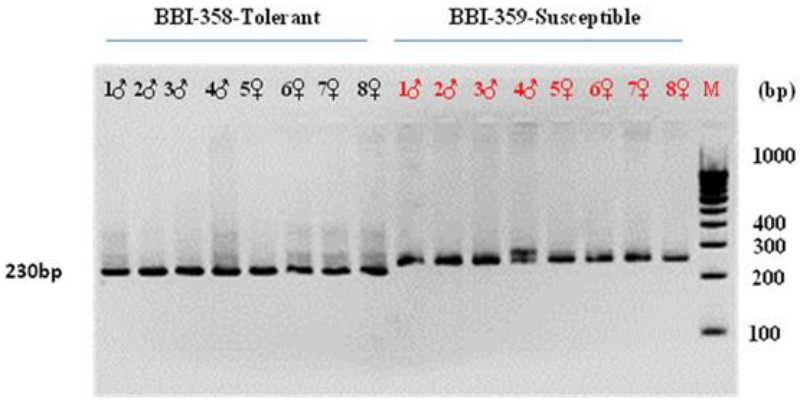



**Fig.1(b): Amplification of SSR marker (LFL1123) in thermo-tolerant BBI-363.**

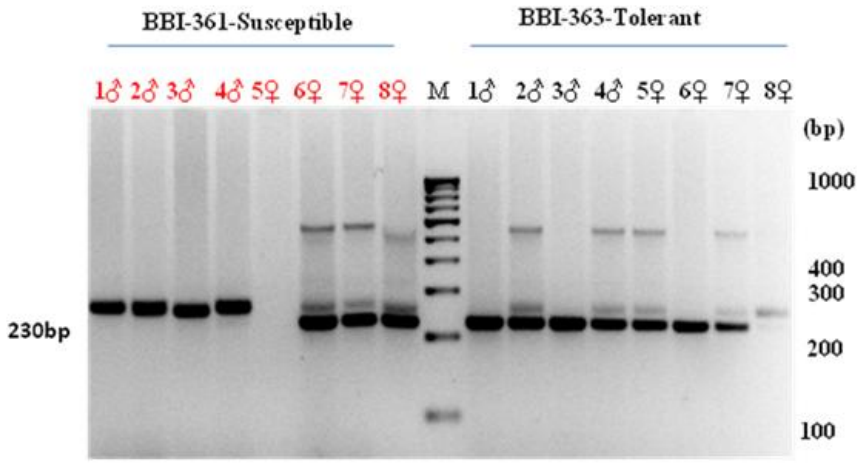


**Fig.2(a): Amplification of SSR marker (LFL0329) in thermo tolerant BBI-0044**

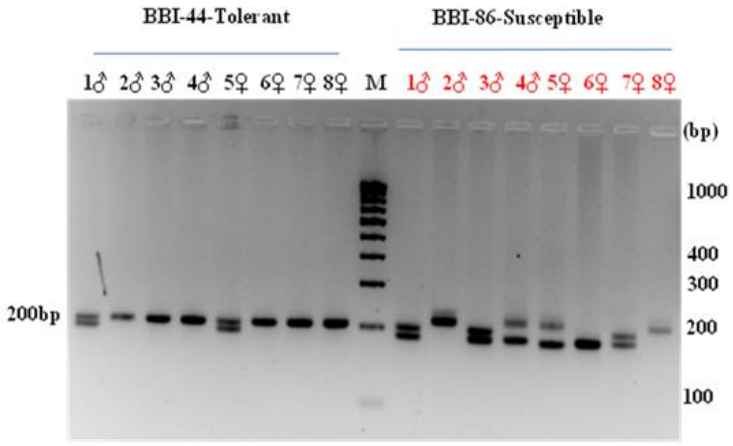



**Fig.2(b): Amplification of SSR marker (LFL0329) in thermo tolerant BBI-0184**

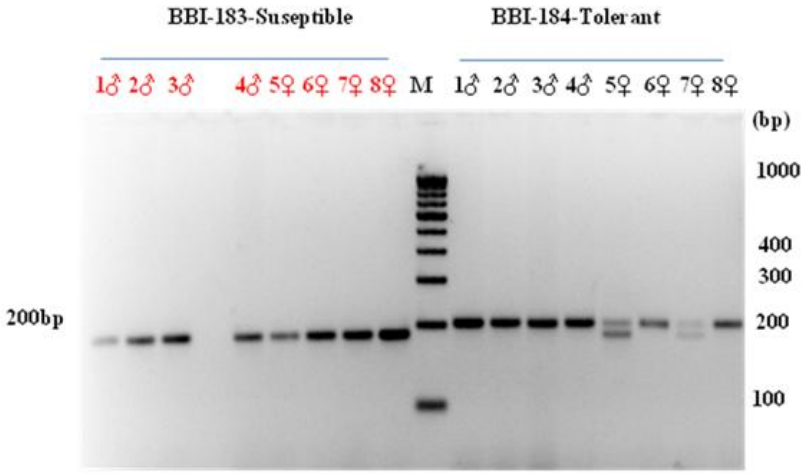


**Fig.3(a) Amplification of SSR marker (S0813) in thermo tolerant BBI-0086**

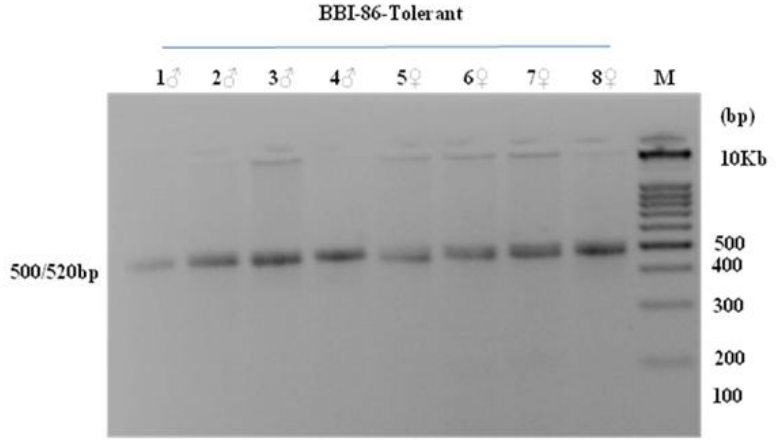



**Fig.3(b): Amplification of SSR marker (S0813) in thermo susceptible BBI-0343**

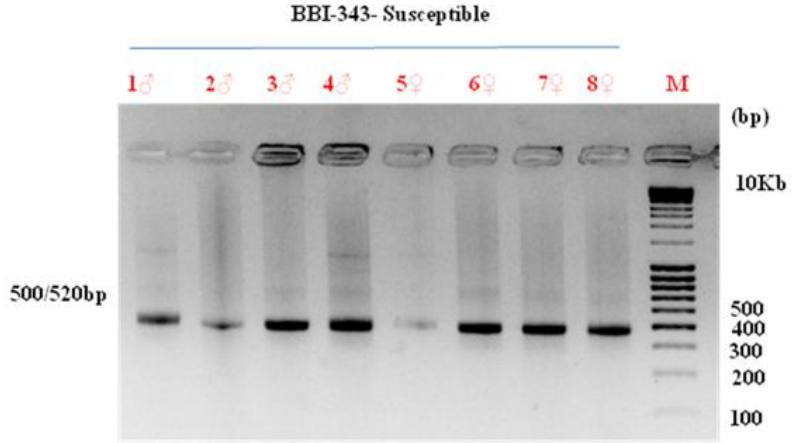
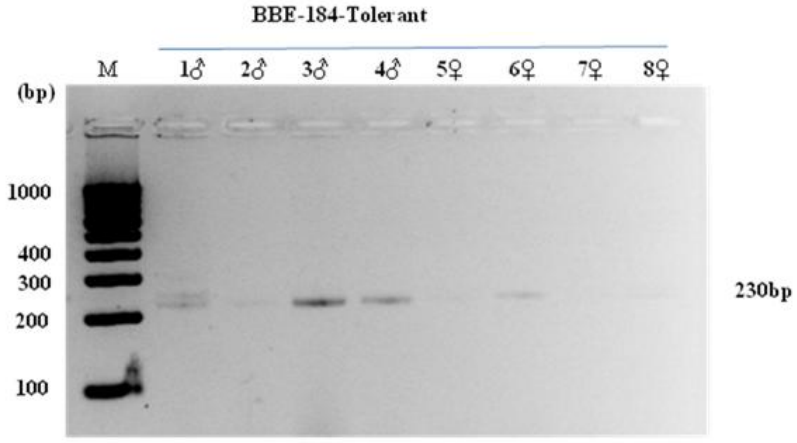
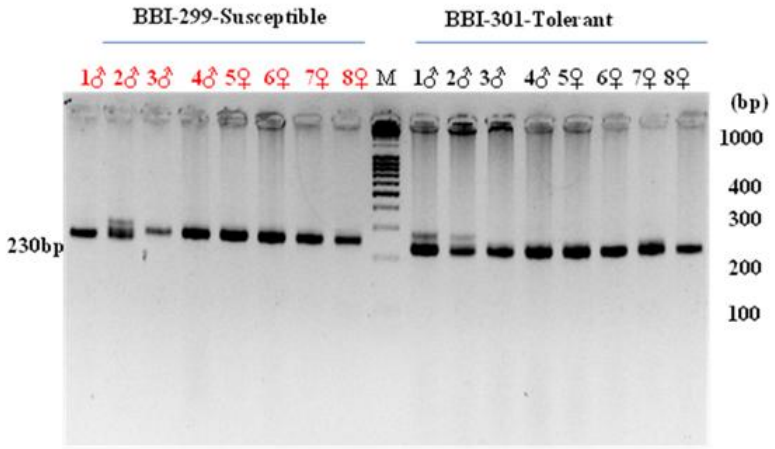
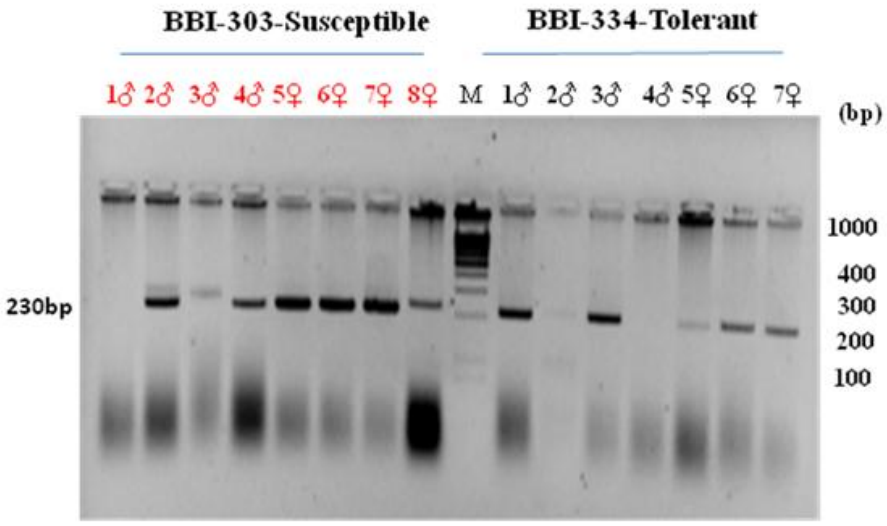
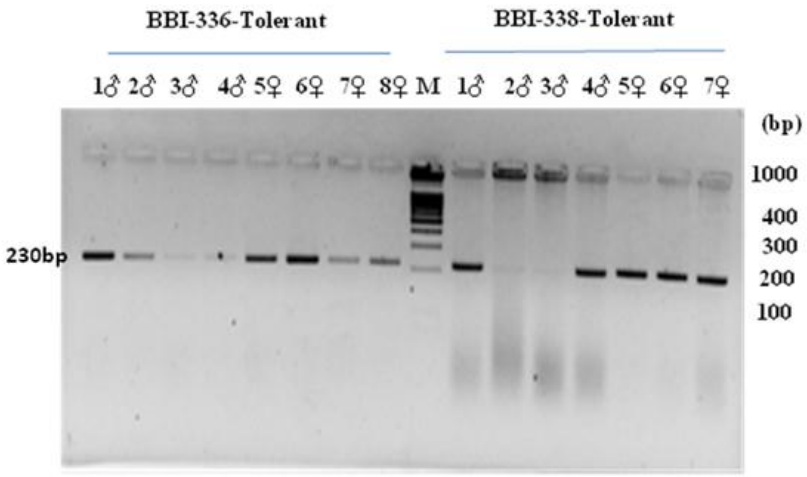


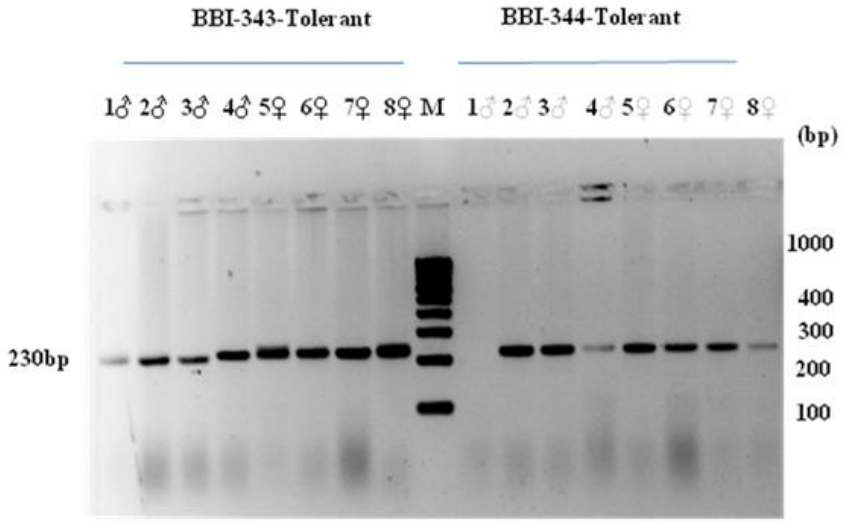

**Fig.4(a): Amplification of SSR marker (S0809) in thermo tolerant BBE-0184, BBI-0301, BBI-0334, BBI-0336, BBI-0338, BBI-0339, BBI-0343 and BBI-0344**

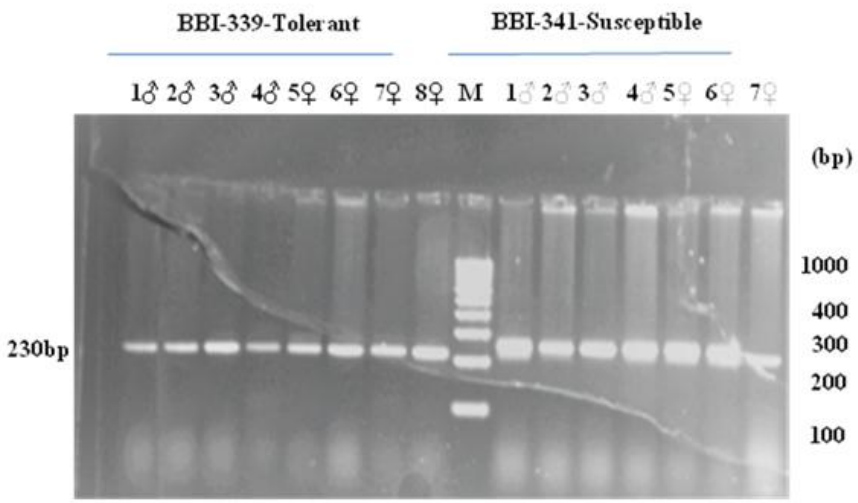

BBE-184-Tolerant
M
1♂
2♂
3♂
4♂
5♀
6♀
7♀
8♀
(bp)
1000
400
300
200
100
230bp


BBI-299-Susceptible
BBI-301-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-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