

Revised Research Article

Evaluation and Identification of Silkworm (*Bombyx mori* L) Genetic Resources Tolerant to Temperature and Humidity

ABSTRACT:

Aim:

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

Study Design:

In this regard the present study has been taken up with ten bivoltine accessions (4 oval and 6 dumbbell) linked to thermo tolerance of SSR markers and evaluated under different agroclimatic conditions namely, (a) high temperature and low humidity; (b) moderate temperature and high humidity and (c) moderate temperature and high humidity.

Place and duration of the study:

The study has been taken up for 2 years, where the trials were conducted for abiotic stress conditions at CSR&TI, Berhampore (West Bengal), RSRS, Jammu (J&K) and REC, Chitradurga (Karnataka)

Methodology:

The ten shortlisted bivoltine accessions (oval and dumbbell) showing thermo tolerance evaluated at hotspots were collected and reared at different locations for data collection and analysis of rearing parameters viz. larval wt.(g), ERR/No.,(survival) ERR/wt (kg), pupation rate (%), single cocoon weight (g), single cocoon shell weight (g), shell ratio (%) along with control CSR2 (oval) and CSR4 (dumbbell).

Results:

Among the ten bivoltine accessions, the performance of BBI-0086 [KPG-A], BBI-0339[DD-2], BBE-0184[SMGS-2] and BBI-0338[DD-1], BBI-0336[APS-8] were found to be stable in all locations respectively.

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: Silkworm, evaluation, abiotic stress, temperature, humidity

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1 INTRODUCTION

India being predominantly a tropical country is characterized by high temperature, scanty rainfall, inadequate mulberry leaf, poor management practices, and extensive disease incidence in the silkworm resulting in crop losses by the farmers [1]. Upscaling quality bivoltine silk production under fluctuating high-temperature regimes necessitates the development of high-temperature tolerant silkworm accessions. The success of the sericulture industry depends on several factors including the quality and quantity of silk production, of which the impact of environmental factors such as biotic and abiotic factors are considered to be vital. Besides, China has successfully developed region-season-specific bivoltine silkworm accessions for sustainable sericulture under different climatic conditions. However, in India, bivoltine silkworm rearing is restricted only to certain seasons (seasonal variations in tropical regions are not distinct when compared to sub-tropical and temperate regions) in some parts of the country because of the negative impact of high temperature, humidity, and rainfall. Basavaraja *et al.* [2] reported that due to optimum climatic conditions in the southern states viz., Kamataka, Tamil Nadu, Andhra Pradesh, and Kerala, silkworm rearing is conducted throughout the year except during April and May. On the other hand, in Uttar Pradesh and Jammu regions, bivoltine silkworm rearing is carried out during the spring and autumn seasons and polyvoltine hybrids during the summer season [3]. As silkworm is poikilothermic in nature, in abiotic factors such as temperatures and humidity plays a major role on the growth and productivity in silkworm. It is also clear that the late age of the silkworm prefers relatively lower temperature than in chawki stage [4]. The recommended temperature regime of 22-27°C for different stages of larval development is found to be favourable for growth and development of larvae and quality cocoon production. However, the warm climatic conditions of tropical regions particularly in summer and moderate temperature with high humidity are contributing to the poor performance of the bivoltine accessions [5]. In contrast the multivoltine races reared in tropical regions are known to tolerate higher temperature regimes.

Bivoltine silkworms are highly prone to abiotic stress especially in the late age of larval stage [6]. Previous studies have identified that many quantitative characters such as survival and cocoon traits decline sharply when temperature is above 28°C during larval development. Therefore, it is highly pertinent to identify bivoltine accessions which can withstand abiotic stress. In order to introduce bivoltine races in a tropical country like India, it is necessary to have stability in cocoon crop under high temperature environments. 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 [7].

While studying the performance of robust and productive bivoltine hybrids under two temperature conditions, Kumar *et al.*, [8] reported that the deleterious effect of high temperature was more pronounced in productive hybrids than the robust hybrids. Hence, development of a productive breed with high-temperature tolerance becomes a challenging task to increase bivoltine silk production. It is also understood that, one more abiotic factor that has significant impact on the performance of insects in terrestrial environments is humidity. The seasonal changes, atmospheric humidity, and soil moisture percentage have profound effect on the growth and quality of mulberry leaves, which in turn influence the silkworm growth and cocoon yield. Further, it is also identified that the different temperature regimes affects the spinning period, cocoon quality and reeling parameters [9].

Latest technologies on using Marker assisted selection (MAS) for genetic improvement in silkworm is very much needed to find out the presence of allelic variation of genes linked to

the economic traits. It is believed that selection based on trait linked DNA marker would be a better option than phenotypic selection [10]. Thus, using SSR markers will make convenient for molecular breeding programmes which allows for genome based characterization and also it will be more precise. Once, the SSR markers linked to the characters required are identified, it could serve as molecular tags to identify the traits specific breeds via markers in silkworm improvement programme. Recently SSR markers (LFL329; LFL1123; SO808; SO813) linked to thermo tolerance in silkworm [11, 12] were identified and used to develop silkworm breeds exhibiting thermo tolerance.

Therefore in the present study, we have emphasized on screening of the bivoltine germplasm resources maintained for thermo tolerance with the help of the SSR markers identified linked with thermo tolerance. Based on screening, the shortlisted bivoltine accessions showing thermo tolerance was evaluated at hotspots to identify the bivoltine accessions exposed to abiotic stress conditions viz. high temperature and low humidity as well as moderate temperature and high humidity. These accessions can be utilized as parental resource material in the breeding programme to develop bivoltine breeds/hybrids which can survive in abiotic stress conditions.

2. MATERIALS AND METHODS

2.1 Selection of Bivoltine accessions

A total of forty bivoltine accessions among 369 bivoltine silkworm genetic resources available at Central Sericultural Germplasm Resources Centre, Hosur, India were shortlisted based on pre and post cocoon parameters viz. ERR/No., ERR/wt., pupation rate %, single cocoon weight, single shell weight, shell ratio%, average filament length (m) and denier. These accessions were further screened for thermo tolerance using SSR markers linked with thermo tolerance viz. LFL1123, LFL0329, S0813 & S0809 reported by [13]. Based on molecular screening, ten bivoltine accessions viz. 8 accessions with 100% thermo tolerance viz. BBI-0086, BBI-0301, BBI-0334, BBI-0336, BBI-0338, BBI-0339, BBI-0343 and BBI-0358 and 2 accessions with 87% thermo tolerance viz. BBI-0044, BBE-0184 (Table 1) were shortlisted for hotspot evaluation at test centres viz. Central Sericultural Research and Training Institute (CSR&TI), Berhampore (West Bengal State, India located at 24.1°N 88.25°E), Regional Sericultural Research Station (RSRS), Jammu (Union Territory of Jammu & Kashmir, India located at 32.73°N 74.87°E), Regional Extension Centre (REC), Chitradurga (Karnataka State, India located at 14.23°N 76.4°E) and Central Sericultural Germplasm Research Centre (CSGRC), Hosur (Tamilnadu State, India located at Elevation 880 m [2,890 ft]).

Table 1 Details of the bivoltine accessions with presence of thermo-tolerance corresponding to the primers

SI No.	Accn No	Accn. name	1	2	3	4	5	6	7	8	% of tolerance
Marker - 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-S0329											
3	BBI-0044	NB4D2	AA	AA	AA	AA	AA	AA	AA	AA	87%
4	BBE-0184	SMGS-2	AA	AA	AA	AA	AA	AA	AA	AA	87%
Marker-S0809											
5	BBE-0184	SMGS-2	AA	AA	AA	AA	AA	AA	AA	AA	100%
6	BBI-0301	YS-7	AA	AA	AA	AA	AA	AA	AA	AA	100%

7	BBI-0334	APS-4	AA	AA	AA	AA	AA	AA	AA	AA	AA	100%
8	BBI-0336	APS-8	AA	AA	AA	AA	AA	AA	AA	AA	AA	100%
9	BBI-0338	DD-1	AA	AA	AA	AA	AA	AA	AA	AA	AA	100%
10	BBI-0339	DD-2	AA	AA	AA	AA	AA	AA	AA	AA	AA	100%
11	BBI-0343	NK-3	AA	AA	AA	AA	AA	AA	AA	AA	AA	100%
Marker-S0813												
12	BBI-0086	KPG-A	AA	AA	AA	AA	AA	AA	AA	AA	AA	100%

2.2 Experimental design

The above said ten bivoltine accessions were evaluated under different agroclimatic conditions temperature, humidity viz. (a) high temperature and low humidity (RSRS, Jammu (June-July'2021) and REC Chitradurga (April-May'2020 and March-April'2021); (b) moderate temperature and high humidity (RSRS Jammu- Sept-Oct'2021) and (c) moderate temperature and high humidity (CSRTI, Berhampore- October-November'2021 and February-March'2022) (Fig.1)

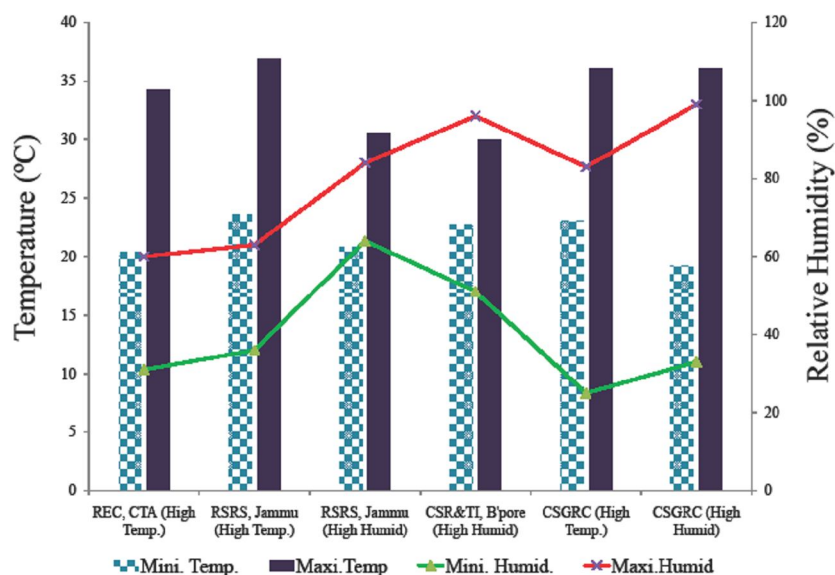


Fig.1 Meteorological data of Test centres

Rearing trials were conducted simultaneously at CSGRC, Hosur corresponding to each rearing trials conducted at different centres. The silkworm rearing trial was carried out in three replications by following the standard method (Krishnaswami, 1978). After completion of rearing, the data pertaining to pre and post cocoon parameters viz., larval weight (g), ERR/No., ERR/weight (kg), pupation rate (%), single cocoon weight (g), single cocoon shell weight (g), shell ratio (%) and the reeling parameters viz. Average filament length (m), Filament size (d), Reelability (%), Renditta (kg) and Raw silk (%) was collected.

2.3 Statistical analysis

The data on the rearing performance of the oval and dumbbell bivoltine accessions along with control CSR2 (oval) and CSR4 (dumbbell) collected from the test centres were compiled and subjected for ANOVA (Three-way factorial analysis) followed by Principal component analysis (PCA). The data on the reeling performances were analyzed by subjecting to general statistics.

3. RESULTS AND DISCUSSION

The analysed data clearly indicated the effect of abiotic stress i.e high temperature with low humidity and moderate temperature and high humidity conditions. The performance of oval and dumbbell bivoltine accessions in selected hotspots varied considerably, where growth and development of larvae, pupation rate, cocoon weight, shell weight and shell ratio was found to be influenced by temperature and humidity. With regard to P value, the data of both oval and dumbbell bivoltine accessions analysed with ANOVA revealed significant among locations, accessions as well as locations x accessions.

In location wise comparison, all the economic traits recorded in oval bivoltine accessions in Hosur was considerably high with exception to larval weight and shell ratio recorded in Jammu. In accession wise comparisons, highest larval weight, pupation rate, yield, single cocoon weight was recorded in YS-7, whereas highest single shell weight and shell ratio percent was recorded in CSR2. On the contrary, lowest larval weight, single cocoon weight, single shell weight, shell ratio percentage was recorded in KPG-A, whereas lowest pupation rate and yield was recorded in CSR2.

Table 2 Performance of the oval bivoltine accessions across the locations/seasons

Locations	Larval wt. (g.)	ERR/No.*	ERR/wt (kg)	Pupation rate (%)	SCW (g)	SSW (g)	SR (%)
Berhampore	25.72	3852 (3.47)	4.62	38.52	1.276	0.227	17.83
Chitradurga	38.15	8559 (3.93)	11.67	83.05	1.418	0.238	16.52
Jammu	42.16	6674 (3.75)	9.73	61.51	1.466	0.275	18.94
Hosur	40.75	9204 (3.96)	13.54	91.46	1.610	0.305	18.93
CD @5% (Locations)	0.793	478.0	0.599	4.506	0.042	0.008	0.364
STD	3.75	1200.15	0.11	1.92	11.85	0.07	0.02
SE	7.50	2400.30	0.23	3.84	23.71	0.14	0.04
Accessions							
KPG-A	34.40	7266 (3.82)	9.65	70.36	1.286	0.219	17.05
SMGS-2	36.09	7416 (3.81)	10.13	72.00	1.444	0.260	17.85
YS-7	38.57	7703 (3.88)	11.22	74.76	1.545	0.271	17.52
DD-2	36.54	7247 (3.79)	10.37	70.40	1.468	0.274	18.55
CSR2 (c)	37.88	5728 (3.59)	8.08	55.64	1.470	0.284	19.30
CD @5% (Accessions)	0.886	534.4	0.670	5.037	0.046	0.009	0.407
CD @ 5% Location X Accessions	1.772	1068.9	1.339	10.075	0.093	0.019	0.814
P value	0.00						
STD	1.63	773.26	0.11	1.16	7.48	0.10	0.03
SE	0.73	345.81	0.05	0.52	3.35	0.04	0.01

*values in parenthesis are log transformed values

On the other hand in dumbbell accessions, the performance of DD-1 was relatively high in pupation rate (DD-1), yield (DD-1), single shell weight (DD-1), shell ratio percent (DD-1), followed by CSR4 in larval weight and NK3 in single cocoon weight. On the contrary, yield, lowest single cocoon weight, single shell weight, shell ratio percentage was recorded in NB4D2, followed by lowest larval weight and pupation rate in CSR26 and CSR4. In location wise comparison, the performance of all dumbbell bivoltine accessions in Hosur was considerably high with exception to larval weight recorded in Jammu. The location wise and accession wise comparative of performances of the oval and dumbbell bivoltine accessions are presented in the table 2 and 3.

Table 3 Performance of the dumbbell bivoltine accessions across the locations/accns.

Locations	Larval wt. (g.)	ERR/No.*	ERR/wt (kg)	Pupation rate (%)	SCW (g)	SSW (g)	SR (%)
Berhampore	28.36	5312.0(3.68)	7.35	52.68	1.228	0.222	18.13
Chitradurga	35.04	8177.6(3.91)	11.48	78.82	1.411	0.245	17.18
Jammu	41.57	7640.0(3.85)	10.91	70.96	1.457	0.263	18.15
Hosur	40.38	9247.6(3.97)	13.72	91.95	1.657	0.319	19.25
CD@5% (Locations)	0.504	0.021	0.432	2.908	0.020	0.006	0.346
P value							
STD	6.03	1661.79	0.13	3.15	16.41	0.18	0.04
SE	3.01	830.90	0.07	1.57	8.21	0.09	0.02
Accessions							
NB4D2	36.26	7445.6(3.83)	9.82	71.72	1.287	0.227	17.69
APS-4	36.07	8080.2(3.90)	11.22	78.75	1.443	0.260	17.95
APS-8	35.87	7996.7(3.89)	11.14	77.68	1.461	0.271	18.48
DD-1	36.68	8360.0(3.91)	11.86	81.39	1.480	0.279	18.78
NK-3	36.22	7420.8(3.85)	10.44	71.65	1.505	0.273	17.99
CSR26	34.96	7369.7(3.84)	11.26	70.66	1.408	0.253	17.84
CSR4	38.31	6487.2(3.73)	10.32	63.36	1.482	0.275	18.51
CD@5% (Accessions)	0.667	0.028	0.572	3.847	0.027	0.008	0.458
CD @ 5% Location X Accessions	1.334	0.056	1.144	7.694	0.053	0.015	0.916
P value	0.00						
STD	1.02	620.84	0.07	0.90	6.12	0.07	0.02
SE	0.38	234.65	0.02	0.34	2.31	0.03	0.01

*values in parenthesis are log transformed values

Further, location wise evaluation of each economical traits of oval and dumbbell bivoltine accessions subjected for Principal Component Analysis carried for CSR&TI, Berhampur. The analysed rearing data of the bivoltine accessions revealed that, the accession of DD1 was similar quadrant with NB4D2, APS-8, and YS-7. These accessions associated with single cocoon weight (g), single shell weight (g), shell ratio(%), and larva weight (g). The APS-4 and SK6 accessions was associated with ERR/No., ERR/weight (kg), and pupation rate (%). The accessions of DD-2, SMGS-2, NK-3, SK-7, and KPG-A in the different quadrant and didn't showed associate with parameters (Figure 2).

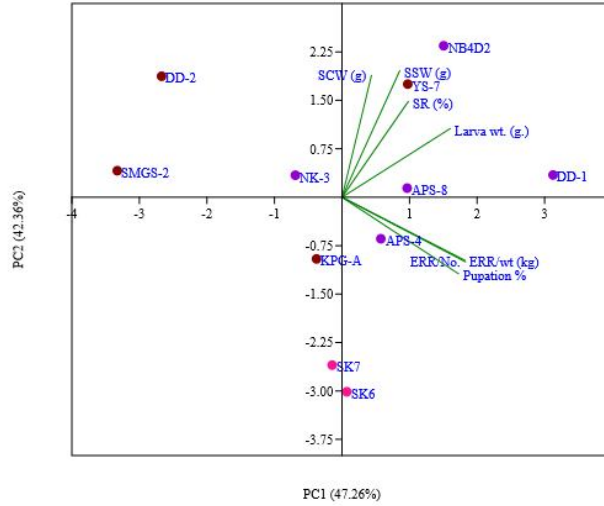


Fig. 2 PCA of the bivoltine accessions tested under CSR&TI, Berhampore

Oval bivoltine accessions Dumbbell bivoltine accessions Control

In case of REC, Chitradurga among the oval bivoltine accessions, SMGS-2 followed by CSR2 was positively correlated with ERR/No. and pupation rate in the first quadrant whereas YS-7 and DD-2 was found to correlate with single cocoon weight, single shell weight, shell ratio (%) and ERR/weight. Whereas the accession KPG-A in the different quadrant has not shown any significant parameter (Fig.3). Similarly among dumbbell bivoltine accessions, CSR-4 has positively correlated with ERR/weight and larval weight in the first quadrant and APS-4 followed by NB4D2 was correlated with ERR/No. and pupation rate (%) in the second quadrant whereas in other quadrant APS-8, NK3, CSR26 and DD-1 were not showed significance (Fig.4).

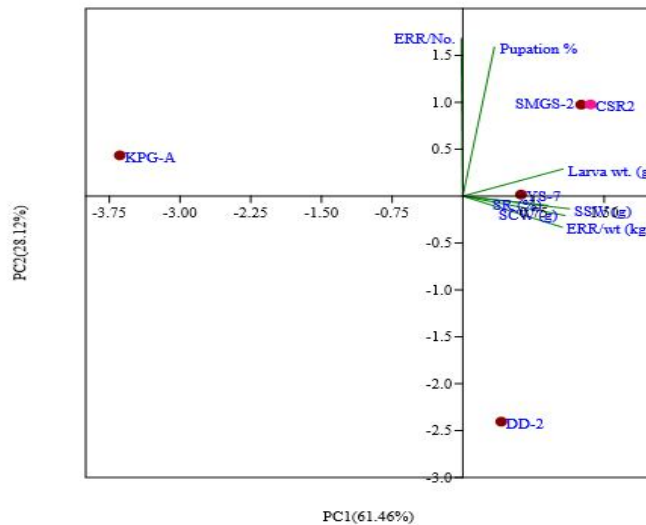


Fig 3. PCA of the oval bivoltine accessions tested under REC Chitradurga

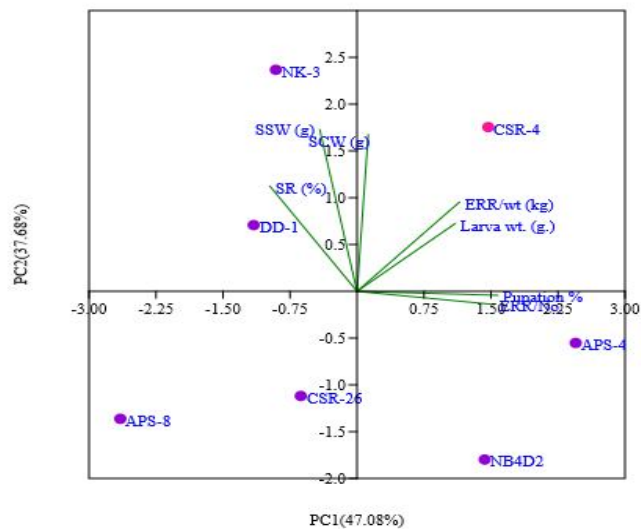


Fig. 4 PCA of the dumbbell bivoltine accessions tested under REC, Chitradurga

Among the oval accessions evaluated at RSRS Jammu, YS-7 followed DD-2 was positively correlated with larval weight, single cocoon weight, single shell weight in the first quadrant. In the second quadrant, SMGS-2 has correlated with ERR/No., ERR/weight and pupation rate (%). Whereas the accessions KPG-A and CSR-2 were not showed significance in third and fourth quadrant. With regards to dumbbell bivoltine accessions, CSR-26 and CSR4 in the first quadrant were positively correlated with larval weight, single cocoon weight, single shell weight and shell ratio (%). The accessions viz. DD1, APS-4 and APS-8 were correlated with ERR/No., ERR/weight and pupation rate (%) in the second quadrant. The accessions viz. NK-3 and NB4D2 in different quadrant were not recorded significant performance (Fig-6).

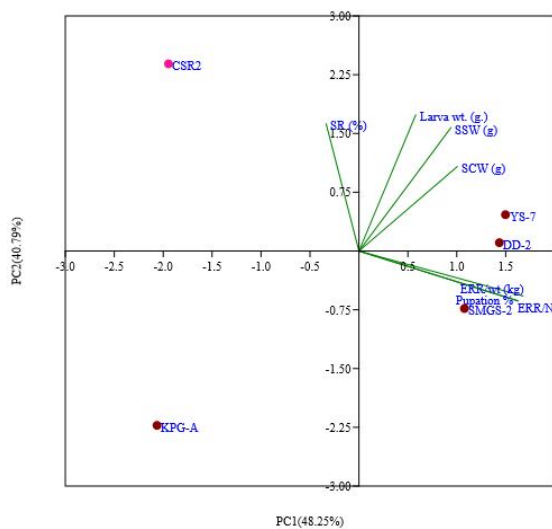


Fig. 5 PCA of the oval bivoltine accessions tested under RSRS Jammu

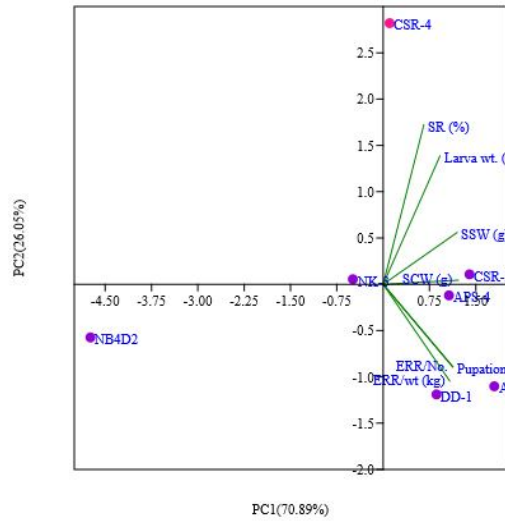


Fig.6 PCA of the dumbbell bivoltine accessions tested under RSRS Jammu

At CSGRC, Hosur, among the oval bivoltine accessions, in the first quadrant CSR2 has positively correlated with larval weight, single cocoon weight, single shell weight and shell ratio (%). Whereas in the second quadrant, the performance of the accession DD-2 was positively correlated with ERR/weight, ERR/No. and Pupaion Rate. In other quadrants KPG-A and SMGS-2 has not showed any significance (Fig-7). Among dumbbell bivoltine accessions, DD-1, NK-3 and APS-8 has positively correlated with larval weight, ERR/weight and single cocoon weight in the first quadrant. And in second quadrant, APS-4 followed by CSR-26 were correlated with ERR/No, pupation rate, shell ratio (%) and single shell weight. In third as well as in fourth quadrant, the accessions NB4D2 and CSR4 has not significant performance (Fig-8).

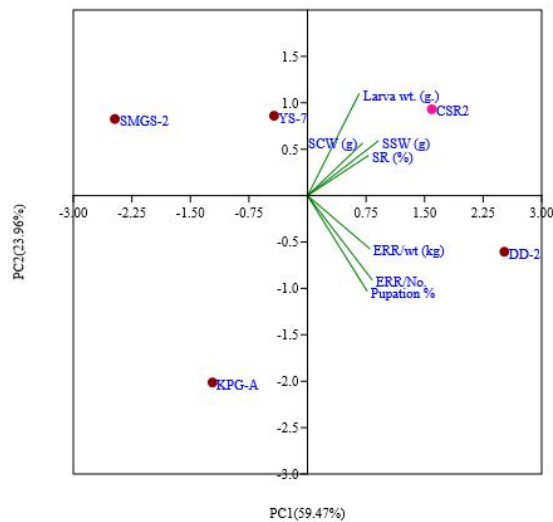


Fig.7 PCA of the oval bivoltine accessions tested under CSGRC, Hosur

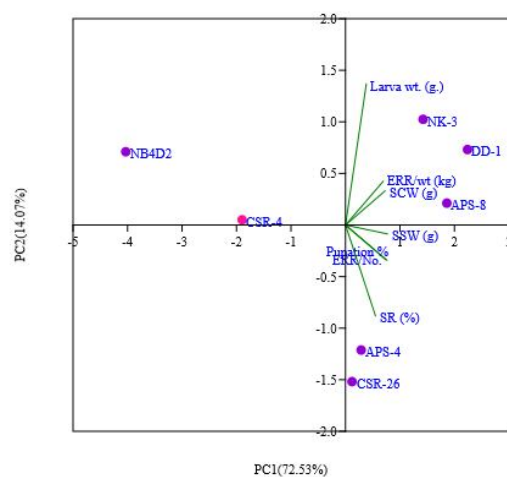


Fig. 8 PCA of the dumbbell bivoltine accessions tested under CSGRC, Hosur

Based on the analyzed data of the 10 bivoltine accessions evaluated at CSR&TI, Berhampore, RSRS, Jammu, REC, Chitradurga and CSGRC, Hosur, the location wise better performing oval and dumbbell bivoltine accessions are listed in Table.4. Further, it's worthwhile to mention that, the performance of few oval and dumbbell accessions such as BBI-0339[DD-2], BBE-0184[SMGS-2] and BBI-0301[YS-7] (oval), BBI-0338[DD-1], BBI-0336[APS-8] (dumbbell) was stable across the locations. The five mentioned accessions can be utilized for commercial exploitation through preparation of single and double hybrids

Table.4 Better performing oval and dumbbell bivoltine accessions among the locations

Locations	Accn.name	Larval wt. (g)	ERR/ No.	ERR/ Wt. (kg)	Pupa tion rate (%)	SCW (g)	SSW (g)	SR (%)
BERHAM- PORE	BBI-0301(YS-7[O])	29.52	5633.3	6.90	56.33	1.365	0.244	17.90
	BBI-0338 (DD1[D])	29.13	7680.0	9.14	76.80	1.261	0.243	19.29
JAMMU	BBI-0339 (DD-2[O])	41.99	8273.3	12.18	77.07	1.501	0.284	18.90
	BBI-0336 (APS-8[D])	41.39	8866.6	12.93	83.27	1.571	0.283	18.00
CHITRA- DURGA	BBE-0184(SMGS-2 [O])	39.80	8753.0	11.62	85.17	1.475	0.259	17.13
	BBI-0338(DD-1[D])	33.87	7897.0	11.58	76.73	1.415	0.258	18.03
HOSUR	BBI-0339(DD-2[O])	40.91	9543.3	13.93	94.81	1.670	0.340	20.01
	BBI-0336 (APS-8[D])	41.26	9436.6	14.30	93.10	1.710	0.336	19.65
SD		5.51	1254.1	2.48	12.05	0.15	0.04	1.00
SE		1.95	443.41	0.88	4.26	0.05	0.01	0.35

The analysis of variance (ANOVA) results depicted in Table 3 and 4 showed significant differences among the locations, accessions as well as locations x accessions (G x E) for larval weight, ERR/no., ERR/weight, pupation rate (%), single cocoon weight ,single shell

weight, and shell ratio(%). This shows that there is environmental effect on the accessions performance.

The present study revealed that the performance of ten shortlisted accessions varied considerably according to the climatic conditions of tested locations. This is in concurrence with Pandey *et al.* [14] who reported that rise in temperature from 24 to 36 °C, produces considerable decline in larval duration affecting the later stages of the silkworm. In another study, Pandey and Tripathi [15] identified role of relative humidity (55-80% RH) on survival rate and larval mortality of the silkworm. The growth and development of silkworm is greatly influenced by environmental conditions. The biological as well as cocoon characters are influenced by temperature, rearing seasons, quality mulberry leaf, and genetic constitution of silkworm strains. The seasonal differences in the environmental affect the genotypic expression in the form of phenotypic differences such as cocoon weight, shell weight, and cocoon shell ratio Rahamathulla[16]. Furthermore, Benchamin *et al.* [17] and Tazima [18] also recorded similar observations on effect of temperature and humidity on growth and development of silkworm. According to Tazima *et al.*, [19], thermotolerance in silkworm is genetically heritable character identified based on the study where pupation rate related to temperature maintained during fifth instar of the larval period.

Suresh Kumar *et al.*, [20] reported the effect of higher temperature ($35 \pm 1^\circ$) on the pure races as well as on the F1 hybrids between polyvoltine and bivoltine silkworm races indicated that the hybrids are more tolerant than the pure races and there was maternal effect regarding temperature tolerance.. The bivoltine viz. CSR2, are promising having high shell weight, SR%, filament length but by introgressing the target gene of thermo-tolerance from Nistari (Indigenous breed of West Bengal) and Sarupat (Indigenous breed of Assam) so as to develop sustainable breed for fluctuating high temperature and humid regions of tropics [21]. Similarly the bivoltine accession NB4D2 is also a productive breed but by crossing with PM, it was productive hybrid in adverse climatic conditions. Rahamathulla *et al.*, [22] has reported the higher pupation rate for CSR6, CSR26, CSR2 and CSR27 during rainy season whereas the pupation rate for CSR4 and NB4D2 was higher during winter season.

In accordance with the previous observations, the present study showed the combined effect of temperature and humidity influence on physiological functions of silkworm affecting growth, survival and production of quality cocoons. This observation necessitates the need for development of region and season specific silkworms and importance of maintenance of temperature and humidity during the rearing of silkworm for a successful crop.

4. CONCLUSION:

The outcome of the present study indicates that the plasticity of selected bivoltine accessions in three varied abiotic stress conditions. The analyzed results of the locations clearly revealed the better performing oval and dumbbell bivoltine accessions namely BBI-0339[DD-2], BBE-0184[SMGS-2] and BBI-0301 [YS-7], BBI-0338[DD-1], BBI-0336[APS-8] (dumbbell). The five mentioned accessions can be utilized for commercial exploitation through preparation of single and double hybrids.

ETHICAL APPROVAL: The authors confirm that they follow the rules of good scientific practice and all ethical standards requested by the journal.

5. REFERENCES:

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