Original Research Article

Evaluation and Identification of Silkworm (*Bombyx mori* L) Genetic Resources Tolerant to Abiotic Stress

ABSTRACT:

Aim:

it is necessary to develop a suitable bivoltine breeds for the abiotic stress conditions region 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 accessions) showing the marker linked to thermo tolerance 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 and conducted trials during abiotic stress condition at CSR&TI, Berhampore (West Bengal), RSRS, Jammu (J&K) and REC, Chitradurga (Karnataka)

Methodology:

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

Results:

Though the centre-wise better performing bivoltine accessions are different but few oval and dumbbell bivoltine accessions performed similarly in all the centre viz. BBI-0086 [KPG-A], BBI-0339[DD-2], BBE-0184[SMGS-2] and BBI-0338[DD-1], BBI-0336[APS-8] respectively. These accessions can be utilized in breeding programme and for commercial utilization in the respective regions.

Conclusion:

The present study elucidate the impact of temperature and humidity associated stress conditions over economic traits performances of (4) oval and (6) dumbbell bivoltine accessions, and incite gained herein will provide a suitable platform for future bivoltine crop improvement through breeding program.

Keywords: Silkworm, evaluation abiotic stress

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1 INTRODUCTION

India being 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]. For upscaling bivoltine silkworm and subsequent quality silk production under fluctuating high temperature conditions, development of high temperature tolerant silkworm accessions is prerequisite. The success of sericulture industry depends upon several factors including quality and quantity of silk production, of which the impact of the environmental factors such as biotic and abiotic factors are considered to be most important ones. Besides that, China has been successfully developed region-season-specific bivoltine silkworm accessions for sustainable sericulture under different climatic conditions. But in India, bivoltine silkworm rearing is restricted only to certain seasons (summer, spring and autumn) in some part of the country because of the negative impact of high temperature, humidity and rainfall on the silkworm rearing. Basavaraja *et al*. [2] reported that due to optimum climatic condition, in the southern part of Kamataka, Tamil Nadu, Andhra Pradesh and Kerala, silkworm rearing is conducted throughout the year except during April and May. On the other hand, in U.P and Jammu regions, bivoltine silkworm rearing during spring and autumn seasons and polyvoltine hybrids during summer is carried out [3]. It is understood that among the abiotic factors, temperature along with high and low humidity plays a major role on growth and productivity in silkworm, as the silkworm is a poikilothermic insect [4]. It is also clear that the late age of the silkworm prefers relatively lower temperature than in chawki stage [5] and fluctuation of temperature i.e.22-27°C during different stages of larval development was found to be more favourable for growth and development of larvae and to get good cocoon yield than constant temperature. However, the warm climatic conditions of tropical regions particularly in summer as well as the moderate temperature with high humidity are contributing to the poor performance of the bivoltine accessions [6]. But, multivoltine races reared in tropical countries are known to tolerate slightly higher temperature. It is distributed throughout the world, which is considered as most important insect because of its economic importance. It is a considerable fact that the bivoltine silkworms are highly prone to abiotic stress (high temperature with high humidity and low humidity) especially in the late age of silkworms [7]. Research reports suggests that many quantitative characters such as survival and cocoon traits decline sharply as temperature has increases above 28°C during silkworm rearing. Therefore, it is highly pertinent to identify bivotine 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 [8].

While studying the performance of robust and productive bivoltine hybrids under two temperature conditions, [9] Suresh Kumar *et al.*. 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 tolerant as such becomes challenging task to develop the breed. It is also understood that, one more abiotic factor that has significant impact on the performance of insects in terrestrial environments is humidity. Humidity interacts with the availability of free water and with the water content of the food and it mostly shows indirect effect on growth and development. 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. With regard to reeling parameters, the different temperature during spinning period and its effects on cocoon and reeling parameters of new bivoltine hybrids were studied [10].

Therefore, in present study, we have emphasized on screening of the bivoltine germplasm resources maintained at this centre 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 will be evaluated in hotspots to identify the bivoltine accessions survive in the 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 so as to develop bivoltine breeds/hybrids which can survive in abiotic stress conditions.

1. MATERIALS AND METHODS

2.1 Selection of Bivoltine accessions

Among 369 bivoltine silkworm genetic resources available at Central Sericultural Germplasm Resources Centre, Hosur, India, based on pre and post cocoon parameters viz. ERR/No., ERR/wt., pupation rate %, single cocoon wt., single shell wt., and shell ratio%, average filament length (m) and denier, a total of 40 bivoltine accessions were selected. These accessions were further screened for thermo tolerance using SSR markers identified linked with thermo tolerance *viz.* LFL1123, LFL0329, S0813 & S0809 reported by [12,13] Moorthy *et al.*(2013) and Chandrakanth *et al.*(2015). 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. CSR&TI, Berhampore, RSRS, Jammu and REC, Chitradurga.

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

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

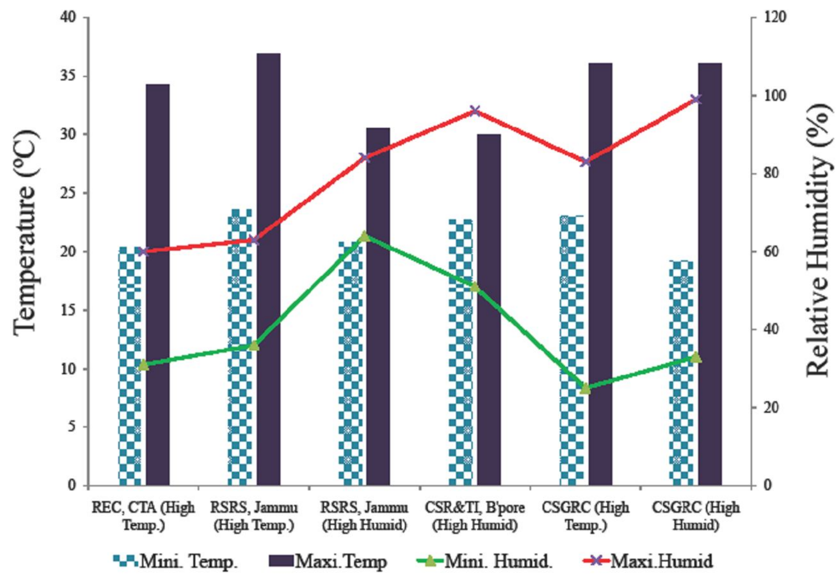

Fig.1 Metereological data of Test centres

The rearing trials were taken up at REC, Chitradurga - April-May'2020 and March-April'2021; RSRS Jammu- June-July'2021 and Sept-Oct'2021, CSR&TI, Berhampore- October-November'2021 and February-March'2022. 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 wt.(g), ERR/No., ERR/wt (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.

2. 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 adverse temperature and relative humidity affected the growth and development of the silkworm larvae in turn it decreases the pupation rate. Even the data clearly indicate the effect of abiotic stress on pupation rate (38.52-61.51%), single cocoon wt. (1.276-1.466 g), single shell weight (0.227-0.275g) and shell ratio% (16.52-18.94%). And the location wise performances of the oval bivoltine accessions as well as accessions wise performance irrespective of the locations are presented in the above Table 2.

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	3852.0 (3.47)	4.62	38.52	1.276	0.227	17.83
Chitradurga	38.15	8559.0(3.93)	11.67	83.05	1.418	0.238	16.52
Jammu	42.16	6674.7(3.75)	9.73	61.51	1.466	0.275	18.94
Hosur	40.75	9204.3(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**
Accessions							
KPG-A	34.40	7266.6(3.82)	9.65	70.36	1.286	0.219	17.05
SMGS-2	36.09	7416.3(3.81)	10.13	72.00	1.444	0.260	17.85
YS-7	38.57	7703.7(3.88)	11.22	74.76	1.545	0.271	17.52
DD-2	36.54	7247.5(3.79)	10.37	70.40	1.468	0.274	18.55
CSR2 (c)	37.88	5728.3(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**

*values in parenthesis are log transformed values

Among the oval bivoltine accessions evaluated at all centres, the accession YS-7 recorded highest pupation rate % (74.76%) with single cocoon weight of 1.545g and single shell weight 0.271g and lowest pupation rate was recorded with CSR2 (55.64%), single cocoon weight 1.470g, single shell weight of 0.284g. In case of dumbbell bivoltine accessions, the performance of the accessions irrespective of the locations and seasons are depicted in Table 3. Here also, the effect of the temperature and humidity has its effect on the bivoltine accessions spinning dumbbell cocoons with the pupation rate % ranged from 52.68-78.82%, Even the single cocoon weight has showed variations from 1.228-1.457 g, single shell weight 0.222-0.263 g.

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**
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**

*values in parenthesis are log transformed values

The performance of the dumbbell bivoltine accessions, irrespective of the test centres revealed that maximum pupation rate (%) was recorded with DD-1 (81.39%) with 1.480g single cocoon wt., 0.279g single shell wt. followed by APS-4 (pupation rate 78.75%), single cocoon wt. 1.443g and single shell wt. of 0.260g.

Further to evaluate the centre wise contributions of each parameter in the rearing performance of the bivoltine accessions, the rearing data of the oval and dumbbell bivoltine accessions were subjected for Principal Component Analysis (PCA) to identify the centre wise better performing oval and dumbbell accessions. Based on the PCA output, the analysed rearing data of the bivoltine accessions of CSR&TI, Berhampore revealed that, the accession DD1 followed by APS-4 and APS-8 were correlated with ERR/No., ERR/wt (kg) and Pupation Rate (%) whereas YS-7 and NB4D2 were positively correlated with single cocoon wt., single shell wt. and larval wt (Fig.2).

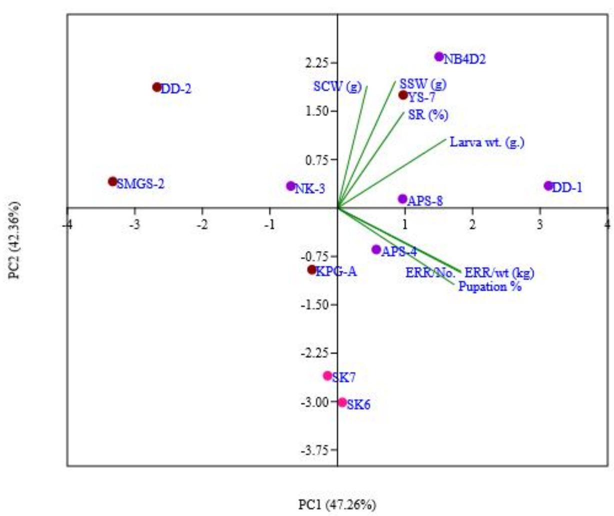

Fig. 2 PCA of the bivoltine accessions tested under CSR&TI, Berhampore

Oval bivoltine accessions Dumbbell bivoltine accessions Control

In case of REC, Chitradurga among the oval bivoltine accessions, SMGS-2 has positive correlation with ERR/No. and pupation rate (%) whereas YS-7 has correlation with single cocoon wt. , single shell wt. and ERR/wt (Fig.3). Among dumbbell bivoltine accessions, APS-4 has correlation with ERR/No. and pupation rate (%) (Fig.4).

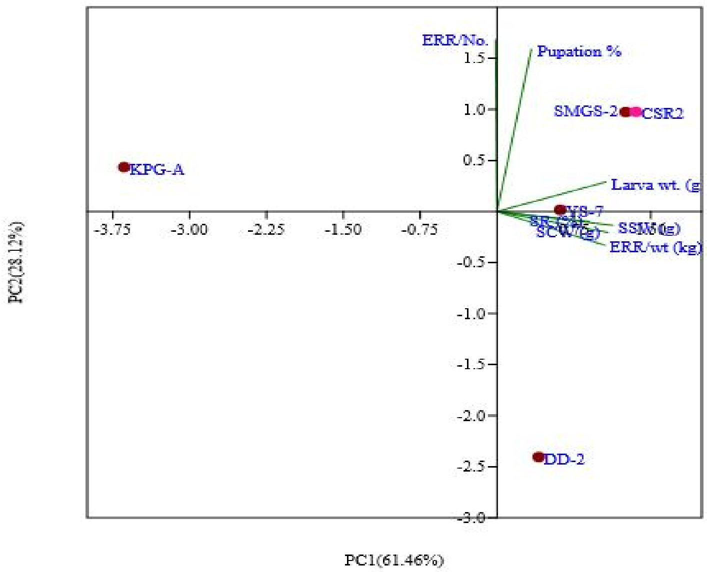

Fig 3. PCA of the oval bivoltine accessions tested under REC Chitradurga

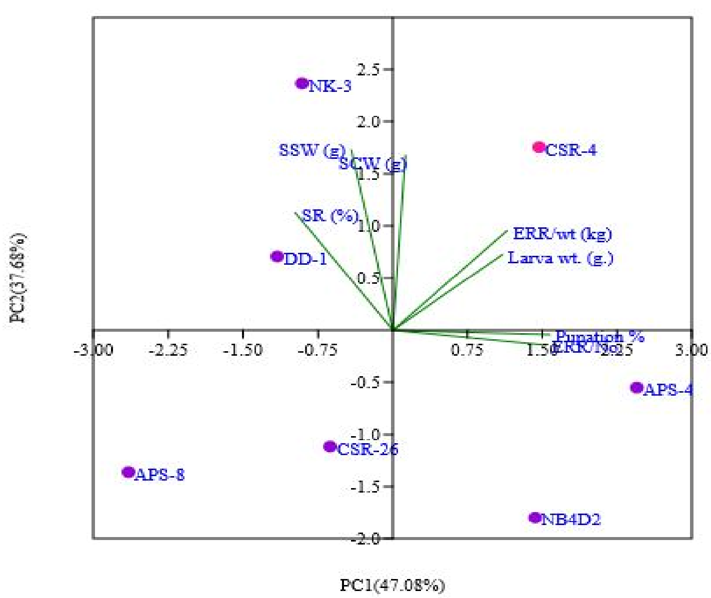

Fig. 4 PCA of the dumbbell bivoltine accessions tested under REC, Chitradurga

Under RSRS, Jammu, out of oval bivoltine accessions evaluated YS-7 has positive correlation with larval wt., single cocoon wt., single shell wt. whereas SMGS-2 followed by DD2 has correlation with ERR/No. and pupation rate (%) (Fig-5). With regard to dumbbell bivoltine accessions DD1 and APS-8 has correlation with ERR/No. and pupation rate (%). However CSR26 has positive correlation with single shell wt. and shell ratio (%) (Fig-6).

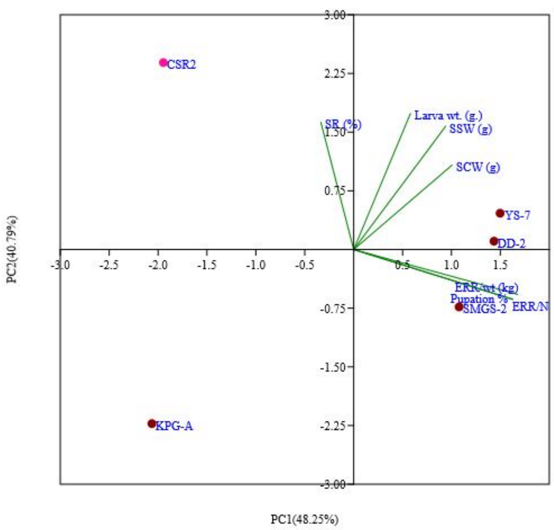

Fig. 5 PCA of the oval bivoltine accessions tested under RSRS Jammu

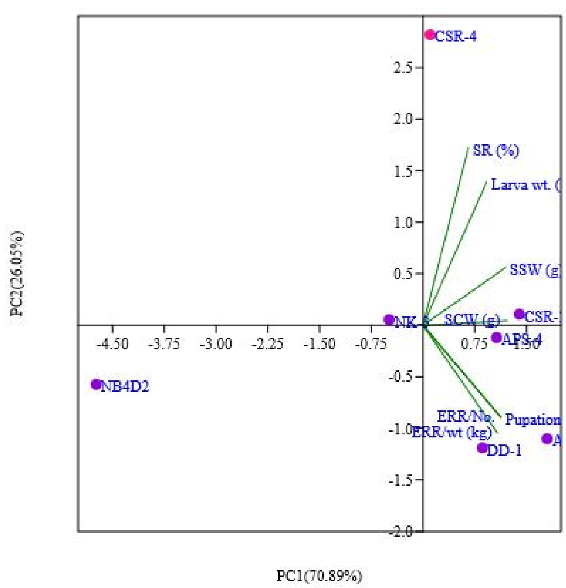

Fig.6 PCA of the dumbbell bivoltine accessions tested under RSRS Jammu

At CSGRC, Hosur, the oval bivoltine accession DD2 performed positive correlation with ERR/wt., ERR/No. and Pupation Rate (%) (Fig-7) whereas among dumbbell bivoltine accessions, APS-4 has correlation with ERR/No. and pupation rate (%) (Fig-8).

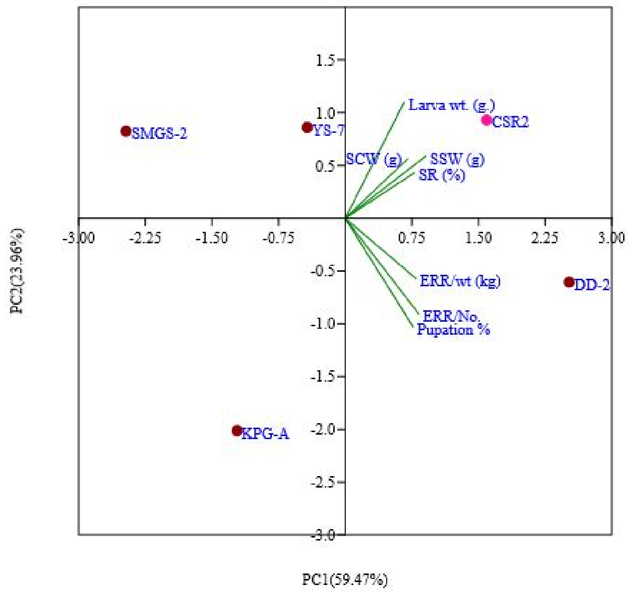

Fig.7 PCA of the oval bivoltine accessions tested under CSGRC,Hosur

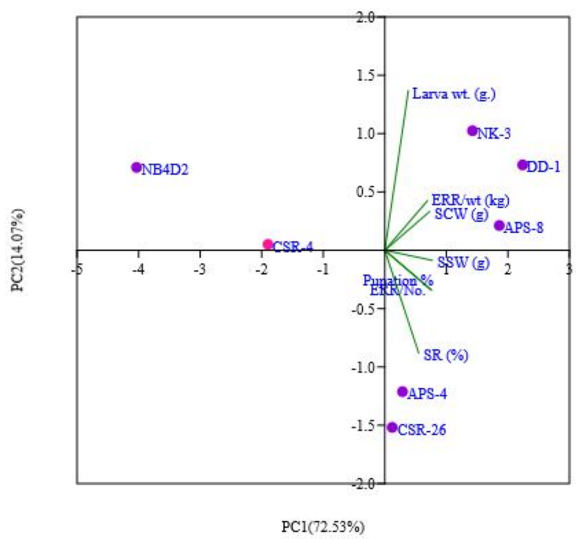

Fig. 8 PCA of the dumbbell bivoltine accessions tested under CSGRC,Hosur

Based on the analyzed data of the 10 bivoltine accessions evaluated at CSR&TI, Berhampore, RSRS, Jammu, REC, Chitradurga and CSGRC,Hosur, the centre-wise better performing oval and dumbbell bivoltine accessions are as follows (Table.4). However, few oval and dumbbell bivoltine accessions were performed similarly in all the centre viz.BBI-0339[DD-2], BBE-0184[SMGS-2] and BBI-0301[YS-7] (oval), BBI-0338[DD-1], BBI-0336[APS-8] (dumbbell). These accessions can be utilized for single hybrid and double hybrid preparation for commercial utilization in the respective regions.

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

Locations	Accn.name	Larval wt. (g)	ERR/ No.	ERR/ Wt. (kg)	Pupation 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 study revealed that the rearing performance of shortlisted bivoltine accessions under different abiotic stress viz. temperature and humidity tested at different test centres was significantly varied. This is in concurrence with [14] Pandey *et al*. who reported that rise in temperature from 24 to 36 °C, produces considerable decline in larval duration which may affect the later stages of the silkworm of *Bombyx mori* L. The findings of this study are corroborated with the previous studies of [15] Benchamin *et al*. (1983). Similar results have been reported by [16] Tazima who had reported that the effect of temperature and humidity on the growth and development of silkworm. It is also in agreement with [17] Pandey *et al* (2008) who observed that the effect of relative humidity (55-80% RH) on survival rate and larval mortality of the silkworm. The study also in agreed with the findings of [18] Tazima *et al* who confirmed that the thermo-tolerance in silkworm is genetically heritable based on pupation rate of silkworm reared under high temperature during 5th instars.

Further, humidity also plays a vital role in silkworm rearing and its manifestation has both direct and indirect effect. The combined effect of both temperature and humidity have shown effect on growth of the silkworms and production of good-quality cocoons, which implies direct influences the physiological functions of the silkworm. Like temperature, humidity also fluctuates widely not only from season to season but also within the day itself. Therefore, it is necessary for the silkworm rearers to regulate it periodically both temperature as well as humidity for their successful crop.

3. CONCLUSION:

The outcome of the present study indicates that the plasticity of performance of bivoltine accessions is varied in three different abiotic stress conditions. The analyzed results of the test centres clearly revealed the better performing oval bivoltine accessions namely BBI-0339[DD-2], BBE-0184[SMGS-2] and BBI-0301 [YS-7] (oval) and BBI-0338[DD-1], BBI-0336[APS-8] (dumbbell). The centre wise identified bivoltine acessions as indicated can be utilized for single hybrid and double hybrid preparation for commercial utilization in the respective regions during abiotic stress

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

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