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RESEARCH ARTICLE

EFFECT OF LONG TERM EGG PRESERVATION ON MORPHOLOGICAL, REPRODUCTIVE AND MOLECULAR CHARACTERS OF SILKWORM (BOMBYX MORIL.)MUTANT GENETIC STOCKS

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ABSTRACT

Studies on long term egg preservation schedule from 180 days to 300 days was taken up with 20 germplasm accessions of mutant silkworm genetic stocks of Bombyx mori L. to reduce the cost of conservation and minimize the genetic erosion leading to reduced crop cycle. Phenotypic stability was confirmed qualitatively by morphological characterization and quantitatively by growth and reproductive traits. To confirm the genetic stability molecular markers *viz;* SSR (simple sequence repeat) markers were used. It was found negligible polymorphism existed between control and treatment batches. The overall genetic similarity ranged from 0.9 to 1 in the selected mutant silkworm genetic stocks. Without genetic divergence in all the twenty silkworm races, the control and treatment batches aligned together. Since the longterm preservation in mutant silkworm genetic stocks did not alter their phenotypic and genetic stability, it is quite possible to reduce the number of crop cycles from two to one per year and providing a way for successful conservation of 19 mutant silkworm germplasm accessions.

INTRODUCTION

The domesticated mulberry silkworm, *Bombyx mori* L. represents itself as various mutants evolved both from spontaneous and induced mutation. These mutants are maintained by fanciers and breeders in the closed line culture system for many years and serve as a basic tool for genetic analyses including phylogenetic, physiological, ethological, biochemical and molecular studies since systematic linkage studies have been successfully carried out (Doira, 1992). In order to reduce the cost of conservation and to avoid the genetic erosion due to repeated conservation rearing, an attempt was made to develop longterm egg preservation for 20 mutant genetic stocks of *B.mori* conserved at CSGRC, Hosur by minimizing the crop cycle from two to one per year as practiced in the case of bivoltine silkworm genetic resources. The study aimed to confirm the morphological, economical and molecular characters of mutant silkworm under longterm preservation schedule.

The mutant silkworm races shows different phenotypic characters, such as variation in egg color, larval duration, larval marking, cocoon shape, cocoon color and haemolymph colour. Morphological characterisation has direct or indirect relation with various quantitative and qualitative traits. These races also show wide diversity in the yield and economic parameters. Further, morphological traits along with correlation parameters help to identify and group similar performing germplasm for effective conservation in the gene bank. As the mutant silkworm genetic stocks exhibit considerable variations for several heritable characters *viz.*, egg serosa colour, larval markings, cocoon colour and cocoon shape, it is possible to use mutant genetic stocks directly in silkworm breeding for evolving new races (Tzenov *et al.*, 2002) and characterizations of morphological mutant traits were utilized as a basic tool for genetic analysis and were used to study the genetic diversity and distance among the population. However, the morphological characters alone cannot establish the genetic identity of the breeds since the relationships amongst races in different lines have similar characters. Molecular makers have already been used to study the genetic stability of mutant stocks under short term cold preservation and such a stability was further studied under long term cold preservation employing reproducible SSR markers to know whether long term preserved eggs mutant genetic stocks maintain their original genetic make up as per the passport data.

MATERIALS AND METHODS

Location and period of study

This study was conducted for three years at Central Sericultural Germplasm Resources Centre, Hosur which conserves 365 bivoltine,79 multivoltine and 20 mutant silkworm genetic resources and Conservation of silkworm germplasm is the main mandate of this centre.

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Selection Of Genetic Stocks

In the present study Twenty bivoltine mutant silkworm genetic stocks collected from Japan were selected.(Table-1) Mutant genetic resources have diverse morphological features and adds to the aesthetic value . Initially mutant genetic stocks were preserved under 4 months hibernation schedule and in 2005 Muthulakshmi *et al* reported that they can be preserved under 6 months preservation schedule and crop cycle can be reduced from three to two per year without affecting conservation. Preliminary evaluation trials on hatching of eggs preserved under 10 months, 12 months and 14 months hibernation/cold preservation schedule along with control (6months) were conducted. (Table-2)

Growth and reproductive traits

Three sets of rearing trials (six trials -control-3 and experiment-3) were conducted. Eggs preserved for 6 months schedule was kept as control, while eggs preserved for 10 months schedule was considered as treatment.

After the completion of experimental preservation period, the eggs were incubated at 25 ± 1º C and 85 ± 2 % RH. Standard silkworm rearing technique was adopted throughout the rearing period using composited layings (Thangavelu *et al*., 2000), following completely randomised block design (CRBD) with three replications.

Table 1 List of mutant silkworm genetic stock of *Bombyx mori* selected for the study

Sl. No.	Acc.No.	Name	Trait (s) and linkage group
1	BBE-0320	TMS-2	striped larval body markings (pS,2:0.0)..
2	BBE-0306	TMS-12	zebra body markings (Ze, 3:20.8).
3	BBE-0307	TMS-14	red haemolymph (rb,21:0.0).
4	BBE-0321	TMS-17	lemon larval body colour (lem,3:0.0)..
5	BBE-0322	TMS-31	tubby larval body shape (tub,23:6.9)..
6	BBE-0308	TMS-32	stony larval body shape (st,8:0.0).
7	BBE-0309	TMS-33	blind larval body markings (bl,15:0.0), cheek & tail spots (cts, 18:4.6).
8	BBE-0331	TMS-34	egg colour (pe,5:0.0) and brown head & tail spots (bts, 17:30.1).
9	BBE-0310	TMS-35	white egg colour (w-2, 10:16.1) chocolate neonate colour (ch,13:9.6), sooty larval body colour (so, 26:0.0) and melanism larval body markings (mln, 18:41.5).
10	BBE-0311	TMS-38	ursa larval body colour (U-2, 14:40.5), extra-leg body shape (E,6:0.0) and wild wing spot (Ws, 17:14.7).
11	BBE-0312	TMS-61	brownish red egg colour (b-2, 6:8.0), red egg (re, 5:31.7), blind larval body mark (bl,15:0.0) and lustrous eyes in moths (lu, 16:0.0).
12	BBE-0313	TMS-62	ellipsoidal egg shape (elp,18:16.1) and melanism in head & anal plates of larva (mln, 18:41.5).
13	BBE-0314	TMS-64	chocolate neonate colour (la,9:22.1), lemon coloured larval body (lem, 3:0.0) and Chinese translucent skin (oc, 5:40.8).
14	BBE-0315	TMS-65	narrow-brest larval body shape (nb,19:31.2), moricaud larval marking (pM, 2:0.0) and r-translucent integument (or, 22:8.9).
15	BBE-0316	TMS-66	zebra faded larval marking (ZeF, 3:20.8) and wild wing spot on wings (Ws,17:14.7).
16	BBE-0317	TMS-67	yellow haemolymph colour (Y, 2:25.6) and pink and flesh coloured cocoons (Pk, 2:? ; F, 6:13.6) colour and shape.
17	BBE-0323	TMS-69	multi-lunar larval body markings (L,4:15.3).
18	BBE-0318	TMS-75	red egg colour (re, 5:31.7), elongated II & III abdominal segments (e,1:36.4), quail body markings (q, 7:0.0) and sex-linked translucent integument (os, 1:0.0).
19	BBE-0319	TMS-82	knobbed larval body shape (K, 11:23.2)
20	BBE-0333	ODT	translucent larval body cuticle

Table 2 Egg preservation schedule adopted for mutant genetic stocks of *Bombyx mori*

6m		10m		12m		14m	
TEMP(°C)	DAYS	TEMP(°C)	DAYS	TEMP(°C)	DAYS	TEMP(°C)	DAYS
25	20	25	40	25	50	25	50
20	3	20	30	20	30	20	30
15	3	15	20	15	20	15	20
10	3	10	10	10	10	10	10
5	147	5	60	5	65	5	65
2.5	0	2.5	123	2.5	155	2.5	215
5	0	5	10	5	17	5	17
10	0	10	5	10	10	10	10
15	3	15	2	15	3	15	3
20	1	20	1	20	2	20	2
25	R	25	R	25	R	25	R
	180		300		360		420

Hatching trials

Hatching percentage was calculated based on the mean number of larvae hatched out of the total eggs laid by a moth in five layings per accession with three replications.

Based on the results of preliminary studies, a detailed experimentation was undertaken with 10 months preservation schedule to study its effect of prolonged cold preservation on the growth and reproductive traits on the mutant genetic stocks.

From neonate to third moult all the larvae were maintained as such and three hundred fourth instar larvae were maintained in each replication for further data recording. Evaluation data on important economic parameters *viz*., fecundity, hatching percentage, weight of 10 grown larvae, total larval duration, fifth age larval duration, cocoon yield (No.)/10000 larvae and cocoon yield (weight)/10000 larvae, pupation percentage, single cocoon weight, single shell weight and silk ratio were recorded during the experimentation. After cocoon assessment, live cocoons were maintained at 25± 1º C and 85 ± 2 % RH.

Table 3 Details of repeat motifs and primer sequences for the microsatellite loci used

Locus symbol	Repeat motif length	Primer sequence	No. alleles	Allele size range(bp)	Hetero zygosity	T (°C)	MgCl2 (mM)
Sat1411	(GT)8 (GT)5	5'- gaatgttctgctggtgg-3' 5'-taatgtttttatactttattatatg -3'	8*	109–162	0.68	45	3
Sat 892	(GT)10	5′-caataaatgcttacgagtttaa-3′ 5′-tatcggtagttccttgactt -3′	3*	175–187	0.66	47	3
Sat1013	(GT)9	5'-aacagatgctgcggactggt-3' 5'-tgccattcacaatacaacat-3'	5*	135–162	0.8	50	1
Sat1423	(CA)11	5'-ctttcgatcaccgcgttctc-3' 5'-cgctacgaaataccattatctgaca-3'	9*	130–176	0.82	55	2
Sat1893	(CA)10	5'-aatgcagaatcgtaattttt-3' 5'-tttgaccacagacaataagc -3'	7*	98–158	0.85	45	2

Table 4 Comparative performance (t-values) of control (6months) and treatments(10,12 and 14 months) egg preservation for hatching percentage of 20 mutant genetic stocks of *Bombyx mori*

Acc.No.	Details of preservation		
	6m vs 10m	6m vs 12m	6m vs 14m
	t value	t value	t value
BBE-0306	0.297^{NS}	34.90**	14.4**
BBE-0307	-2.914*	21.80**	27.9**
BBE-0308	3.766*	20.80**	30.1**
BBE-0309	-1.821 ^{NS}	23.16**	10.27**
BBE-0310	-0.154 ^{NS}	20.82**	26.07**
BBE-0311	0.776 ^{NS}	11.45**	15.69**
BBE-0312	1.396 ^{NS}	28.94**	31.66**
BBE-0313	5.785**	32.03**	69.46**
BBE-0314	1.373 ^{NS}	22.24**	27.82**
BBE-0315	0.121 ^{NS}	14.297**	15.31**
BBE-0316	0.458 ^{NS}	18.964**	20.89**
BBE-0317	-0.275 ^{NS}	17.66**	20.63**
BBE-0318	2.086*	10.37**	11.77**
BBE-0319	-0.159 ^{NS}	21.52**	27.49**
BBE-0320	2.629*	35.16**	66.32**
BBE-0321	0.114 ^{NS}	9.918**	11.00**
BBE-0322	1.556 ^{NS}	8.915**	11.94**
BBE-0323	1.671 ^{NS}	20.11**	28.74**
BBE-0331	1.256 ^{NS}	6.99**	8.64**
BBE-0333	-0.121 ^{NS}	18.33**	34.54**

Disease free layings produced from the control and treated batches were preserved for 6 and 10 months preservation following the standardized egg preservation schedules (Table 2) developed by (Manjula and Hurkadli 1995). The data recorded for three generations were compiled and statistically analysed using computer Statistical Packages of SPSS Inc.,U.S.A. Data on growth and reproductive traits of control(6 months)and treatment(10 months) were compared by applying 't' test.

Morphological traits

Confirmatory morphological characterization for larva, pupa, cocoon and moth stages were carried out based on standard method and confirmed with passport data.

Serosal colour

The sample (5 DFLs / accession) is observed visually under diffused sunlight on 4th day of oviposition

Larval markings

are observed during V instar under diffused sunlight and represented as dark, faint and absent under each category viz., eye spot, crescent and star.

Haemolymph Colour

Observed in 3rd day old V instar larva. The entire population in an accession is characterised by observing the colour of prolegs and grouped as colourless, yellow and pink.

Pupal colour

For characterising pupal colour entire population is observed. A sample of 25 pupae per replication in each sex is observed with magnifying glass for development of wing pad and legs. They are grouped as brown and black including intermediate hues.

Cocoon colour

The cocoon colour is categorised through visual scoring of entire population immediately after harvest and categorised into white , golden yellow, yellow, greenish yellow and flesh

Cocoon shape

The entire population is studied through visual scoring immediately after deflossing. The cocoon shape is classified into oval, elongated with constriction (EC), elongated without constriction (ENC), spindle, spatulate and dumb-bell (DB)

Molecular traits

To confirm the genetic stability five molecular markers *viz;* SSR (simple sequence repeat) markers were used. Genomic DNA from the moths of 20 mutant silkworm germplasm races from control (6 months cold storage schedule) and treatment batches (10 months cold storage schedule) was extracted from single moth using the phenol chloroform method and purified (Nagaraja and Nagaraju, 1995; Nagaraju.*et al*., 1995). Five primer sequences of PCR-SSR repeat motif in silkworm were selected from the previously well-characterized microsatellite repeats represented differentgene loci(Table 3).The basic program used to amplify PCR-SSR DNA was performed on a thermal cycler PTC 100 (MJ Research). Polymerase chain reaction cycles for the SSR micro satellite loci included (i) an initial denaturation step at 95°C for 3 min, an annealing step at 63°C for 1min and an extension step at 72°C for 1 min followed by (ii) 14cycles of 94°C for 30s denaturation, a 14-step touch down decreasing by 0.5°C at each step to 56°C (30 s) and an extension step at 72°C for 1 min. (iii) conditions for the last 24 cycles were 94°C for 0.5min,

Table 5 Comparative performance (t-values) of 6 and 10 months egg preservation for growth and reproductive characters of 19 mutant genetic stocks of *Bombyx mori*

Name	Fecundity		Hatching %		Larval Weight (g)		Total larval duration (h)		V instar larval Duration (h)		Yield/10,000 larvae (No.)		Yield/10,000 larvae (kg.)		Pupation Rate (%)		Cocoon Weight (g)		Shell Weight (g)		Shell Ratio	
BBE-306	1.900	NS	-0.230	NS	1.781	NS	-0.532	NS	-3.836	**	0.037	NS	-1.359	NS	0.547	NS	-1.145	NS	1.657	NS	-2.365	*
BBE-307	2.474	*	-2.914	*	2.129	*	0.441	NS	-0.848	NS	2.958	*	3.952	**	0.583	NS	3.624	**	1.778	NS	-0.194	NS
BBE-308	1.339	NS	-2.880	*	0.481	NS	-0.281	NS	-2.880	*	1.584	NS	2.429	*	-0.922	NS	0.295	NS	0.849	NS	0.951	NS
BBE-309	-0.790	NS	-1.046	NS	2.086	*	-0.849	NS	-1.795	NS	-1.327	NS	0.100	NS	-2.229	*	1.657	NS	-1.418	NS	-2.800	NS
BBE-310	-2.914	*	-0.154	NS	-0.930	NS	1.835	NS	0.000	NS	-2.057	NS	-1.813	NS	-2.225	*	-1.203	NS	-4.341	**	-2.112	NS
BBE-311	-1.601	NS	1.272	NS	0.207	NS	-0.330	NS	-2.557	*	1.532	NS	-2.378	NS	0.408	NS	-3.370	*	-1.543	NS	-0.548	NS
BBE-312	-1.938	NS	1.729	NS	3.024	*	0.382	NS	-0.887	NS	1.206	NS	1.122	NS	0.138	NS	-0.918	NS	-5.268	**	-7.474	**
BBE-314	-1.296	NS	0.916	NS	-0.377	NS	1.572	NS	-1.808	NS	-3.921	**	0.314	NS	-5.236	**	4.554	**	3.622	*	1.231	NS
BBE-315	1.631	NS	1.681	NS	-0.566	NS	0.735	NS	-1.126	NS	1.819	NS	2.443	*	-0.020	NS	-1.210	NS	-2.804	*	-3.348	**
BBE-316	1.339	NS	-0.604	NS	-2.880	*	0.831	NS	0.553	NS	2.129	*	-0.515	NS	-0.487	NS	-1.285	NS	-3.469	**	-2.184	*
BBE-317	-0.232	NS	1.081	NS	-2.961	*	-0.269	NS	-4.435	**	-0.401	NS	-0.622	NS	0.168	NS	-5.970	**	-1.977	NS	1.157	NS
BBE-318	-1.656	NS	2.086	*	-0.133	NS	-0.010	NS	-1.283	NS	0.727	NS	-0.984	NS	1.217	NS	-2.177	*	2.529	*	-1.661	NS
BBE-319	0.824	NS	-0.255	NS	2.472	*	0.831	NS	-3.758	**	1.960	NS	-2.497	*	0.836	NS	-.5.811	**	-2.016	*	0.486	NS
BBE-320	-3.118	*	2.629	*	1.507	NS	0.370	NS	-1.534	NS	0.218	NS	0.074	NS	0.154	NS	-1.113	NS	-2.459	*	-1.481	NS
BBE-321	0.000	NS	-0.077	NS	-1.524	NS	0.000	NS	-1.692	NS	-0.478	NS	-0.869	NS	0.412	NS	-1.056	NS	-1.750	NS	-1.166	NS
BBE-322	1.35	NS	1.020	NS	1.362	NS	1.153	NS	-0.436	NS	2.073	*	-1.387	NS	-0.294	NS	1.652	NS	-0.238	NS	-1.311	NS
BBE-323	-1.088	NS	0.306	NS	1.491	NS	0.463	NS	-0.680	NS	-1.865	NS	1.130	NS	-2.313	*	-1.608	NS	-1.657	NS	-0.568	NS
BBE-331	0.850	NS	-0.633	NS	-0.318	NS	1.139	NS	0.093	NS	0.448	NS	-1.052	NS	1.126	NS	-1.517	NS	-3.852	**	-2.350	*
BBE-333	-1.548	NS	1.785	NS	1.787	NS	0.526	NS	-0.907	NS	1.138	NS	-0.482	NS	1.426	NS	-2.533	*	-2.109	*	-1.152	NS

Ns – Nonsignificant, *=<.05, **=<.01

56°C for 30 s, and 72°C for 1 min followed by (iv) a final elongation step at 72°C for 10 min extension. The PCR was performed in a final volume of 15 µl containing 10 mmol Tris–HCl/L(pH 8.4), 50 mmol KCl/L, 1.5 mmol MgCl2/L, 0.2 mmol each dNTP/L,0.2 mol each primer/L, approximately 20 ng of each silkworm genomic DNA, 0.5 U of Taq polymerase and distilled de-ionized water.

The PCR amplified product was mixed with 5 µl TE buffer and 2 µl 40% sucrose containing 0.5% bromophenol blue was loaded on to a 2.5% agarose gel along with the 100 bp ladder molecular weight marker and run at constant voltage (100 V) for 2 hours. After electrophoresis, the gels were stained with Ethidium Bromide, clear bands were UV visualized and photographed with digital scientific camera in gel documentation system. Similarity or dissimilarity was calculated as per Standard methods (Nei and Li., 1979).

RESULTS AND DISCUSSION

Eggs preserved under 6 months schedule were kept as control, while eggs preserved for 10, 12, and 14 months schedule (Table- 2) were considered as treatments.(Treatments1,2and3 respectively). Accessions performed better for hatching percentage under different cold preservation schedules (6m,10m,12m and 14m) were identified. Statistical analyses of the data using student t test on hatching collected over three trials revealed tha all the 20 accessions preserved under 12 and 14 months preservation schedule were significantly different from 6 months preservation schedule. (Control).

Table 6 Polymorphic Index Content

Loci Name	H value	PIC value	Number of monomorph loci
sat1411	0.92	0.92	2
sat892	1	1	1
sat1423	1	1	1
sat1893	1	1	1
sat1013	0.94	0.93	2

Whereas, no significant changes in hatching of 15 accessions and less significant changes in few accessions (Table 4) between control (6months) and treatment -2(10 months preservation schedule). Among 20 germplasm accessions 19 performed well in hatching trials (above 60%) and one accession (BBE-0313) showed less hatching (below 30%) under 10 months preservation schedule. Based on hatching percentage of accessions having more than 60% cold storage schedule of 10 months hibernation schedule was selected for conducting rearing trials and 19 mutant genetic stocks were consigned under the selected 10 months cold storage schedule except accession BBE-0313.

The data recorded for three rearing trials for both the control and treatment batches were compared by using the students't' test. (Table-5) The purpose of the study is to compare the rearing performance of the experimental batch (10 months egg preservation schedule) with that of control (6 months egg preservation schedule). Statistical analyses of the data collected over three rearing trials conducted for growth and reproductive characters revealed no significant changes in the quantitative characters and less significant changes in few accessions for some quantitative traits of the genetic stocks between treatment (10 months egg preservation) and control (6 months egg preservation) except for total larval duration where there is no significant difference is observed between control and treatment batches in all the 19 mutant accessions tested. However some mutant genetic stocks showed significant variation between treatment and control for some characters except BBE-0321 which showed no significant difference between control and treatment batches for all the eleven economic parameters followed by BBE-0321 showed significant difference for only cocoon yield (No.)/10000 larvae and BBE-0323 for pupation rate (Table 5) The results indicated that extended schedule of 10 months egg preservation can safely be adopted for 19 accessions which will reduce the cost of conservation and minimize the genetic erosion leading to reduced crop cycle Mutant accessions were characterized using standard morphological descriptors as per the passport data. Studies on the morphological features of the mutants exhibited genetic variations among egg serosa colour, (yellow, white and red) markings of the cuticle (striped, zebra, red haemolymph, lemon, tubby, stony, narrow brest, multilunar, knobbed) colour of the blood, (white, yellow and red) pupal colour(brown and black) and colour of the cocoon, (white, yellow, flesh red) which were reared from the eggs preserved both under 6 months hibernation schedule (control) and 10 months hibernation schedule and very much similar, true to type and on par with passport data and there was no

Table 7 Similarity matrix Table

Acc.No.	BBE0306	BBE0307	BBE0308	BBE0309	BBE0310	BBE0311	BBE0312	BBE0313	BBE0314	BBE0315
BBE0306	1	0.929	0.857	1	1	1	0.8	0.857	0.929	0.929
BE0307		1	0.923	0.929	0.929	0.929	0.857	0.923	1	1
BBE0308			1	0.857	0.857	0.857	0.786	1	0.923	0.923
BBE0309				1	1	1	0.8	0.857	0.929	0.929
BBE0310					1	1	0.8	0.857	0.929	0.929
BBE0311						1	0.8	0.857	0.929	0.929
BBE0312							1	0.786	0.857	0.857
BBE0313								1	0.923	0.923
BBE0314									1	1
BBE0315										1
Acc.No.	BBE0316	BBE0317	BBE0318	BBE0319	BBE0320	BBE0321	BBE0322	BBE0323	BBE0331	BBE0333
BBE0316	1	0.929	0.857	0.733	0.929	0.929	0.929	0.857	0.929	0.929
BBE0317		1	0.929	0.8	1	1	1	0.929	1	1
BBE0318			1	0.857	0.929	0.929	0.929	1	0.929	0.929
BBE0319				1	0.8	0.8	0.8	0.857	0.8	0.8
BBE0320					1	1	1	0.929	1	1
BBE0321						1	1	0.929	1	1
BBE0322							1	0.929	1	1
BBE0323								1	0.929	0.929
BBE0331									1	1
BBE0333										1

variation in morphological traits in all stages of lifecycle. Some of the morphological characters have multiple functions, which also have positive effect on the economic characters. Therefore, it is possible to use mutant genetic stocks directly in silkworm breeding for evolving new races (Tzenov *et al*., 2002). Studies on highly heritable morphological traits *viz*., larval markings, cocoon colour, cocoon shape and moth characteristics, moth emergence pattern and oviposition pattern revealed no significant change between the treatment and control batches throughout the experimental period. This corroborates the results obtained with multivoltine silkworm genetic resources, where extended egg preservation for 45 days against 30 days, did not show significant changes both in the qualitative and quantitative traits (Kumaresan *et al*., 2004). And also, utilisation of mutant silkworm is widely adopted by silkworm breeders.

Based on this identification the silkworm races reared under longterm preservation schedule (10 months) were subjected to genetic analysis test to confirm the original genotypic characters. The genetic fidelity of silkworm reared from dfls of long term preservation schedule (10 months preservation schedule) was compared with control silkworm reared from eggs preserved under 6 months preservation schedule using SSR primers. For molecular characterization of mutants simple Sequence repeats (SSR) markers were used to determine differences between control and treated samples. It was found that negligible polymorphism existed between control and treatment batches. The overall genetic similarity ranged from 0.9 to 1 (Polymorphic Index Content) in the selected mutant silkworm genetic stocks.(Table 6) Without genetic divergence in all the twenty silkworm races, the control and treatment batches aligned together. Genetic distances were measured for twenty individual samples of control and treatment batches and analysis based on SSR profile, the maximum genetic similarity was observed between races. (Table-7). The result clearly indicates that the genetic stability was maintained under long term preservation. This study indicates that the longterm preservation did not altered the genetic distances, banding pattern in the selected mutant genetic stocks.

CONCLUSION

Since the long term preservation in mutant silkworm genetic stocks did not alter their morphological, reproductive and molecular characters, it is quite possible to reduce the number of crop cycles from two to one per year and providing a way for successful conservation of 19 mutant silkworm germplasm accessions. Further, conservation following 10 months egg preservation helps to minimize cost and labour involved in rearing and grainage. This will help to reduce genetic depression/ genetic erosion and minimize the exposure to biotic and abiotic stress factors and it facilitates rearing of mutants in favorable seasons.

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