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Multivoltine Silkworm (*Bombyx Mori* L.) Strains for Rearing Exclusively On Artificial Diet During Young Stage, Developed Through Directional Breeding Strategy

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Abstract – **Rearing young stage silkworms on artificial diet for commercial purpose is a relatively new development. Silkworm is a typical monophagous lepidopteran raised on mulberry leaves. As component multivoltine pure strains of existing popular multivoltine x bivoltine silkworm hybrids did not accept the artificial diet, the study was taken up to create a pool of such strains which would feed on the artificial diet. Four strains *viz*., Moria, MAD, 2000K and LMO were short-listed based on the results of an initial screening which recorded feeding response percentage of more than 40. These four strains were further subjected to continuous inbreeding and directional selection for 11 generations for improving the feeding response over the generations and stabilizing it at more than 85 % so that they would form suitable breeding resource materials for preparation of commercial multi x bivoltine hybrids for exclusively rearing on artificial diet during young instar. Care was taken so that breed characters in terms of economic traits were not adversely affected. At the end of eighth generation, the feeding response reached above 88 % and further stabilized at the higher level and thus forming prospective parents for the hybrid combinations with bivoltine parents developed similarly. Data pertaining to G8~G11 were analyzed to check the stability in performance. The traits with particular reference to the diet phase such as feeding response, young instar larval duration and young instar larval weight have reflected non-significant differences in the last four generations indicating the stability in these traits. Other traits such as cocoon weight, cocoon shell weight, survival and cocoon yield also did not vary significantly among the generations in three out of four races. After the strains were stabilized for rearing on artificial diet, they were designated as Moria(A), MAD(A), 2000K(A) and LMO(A) as these strains are different from the normal strains, Moria, MAD, 2000K and LMO. The implications of the improved feeding response and stabilized economic traits in the context of this study are discussed.**

Keywords – **Artificial Diet, Cocoon Traits, Feeding Response, Multivoltine Strains, Silkworm.**

I. INTRODUCTION

Rearing of young instar silkworm on a commercial scale, popularly known in India as chawki rearing has emerged as an attractive enterprise over the past few years. Silkworm is a typical monophagous lepidopteran raised on mulberry leaves. But, rearing young silkworms on artificial diet offers distinct advantages over the existing practice of raising exclusive mulberry garden and then rearing young silkworm on tender mulberry leaves. Artificial diet ensures balanced nutrition and disease free conditions to young silkworm larvae irrespective of the seasons. Despite the care taken for young instar silkworm rearing at the farmers' level, the success of the cocoon is not always assured. This is mainly because, the mulberry leaf grown particularly in tropical conditions is not assured of the required moisture and balanced nutrients for rearing young-instar silkworms throughout the year. The nutritional requirement of young instar silkworm is specific and to meet this, exclusive mulberry gardens are to be maintained. To avoid such prerequisites, an artificial diet was developed for young instar silkworm rearing. Though the existing popular and productive multivoltine x bivoltine hybrid, PM x CSR2 accepted the artificial diet to an extent, the performance was not consistent. It was also obvious that the component multivoltine pure strain, Pure Mysore did not accept the diet consistently well and the feeding response was relatively low. Under such a circumstance, the only option was to modify the selection response of silkworm for the feeding behaviour maintaining the breed characters intact. Thus, commercial breeds destined to be reared on mulberry leaf were converted gradually to feed on artificial diet through directional selection. This would ultimately facilitate to develop a common artificial diet for all the evolved silkworm breeds/hybrids.

When Japan faced a similar predicament, efforts were made to evolve exclusive silkworm strains for rearing on artificial diet and it centered mainly around the abnormal feeding behaviour of the strain, Sawa J. By exploiting the polyphagous nature of Sawa J, exclusive strains and commercial hybrids were developed for rearing on artificial diet [8]. Prior to this study, four multivoltine pure strains and six bivoltine pure strains had been screened in, based on their initial feeding response to artificial diet [2], [3]. In the present study, it was planned to make these pure strains stabilized as maternal component for the hybridization programme through which the better performing hybrid combinations would be evaluated and adjudicated.

II. MATERIALS AND METHODS

Four multivoltine silkworm strains *viz* Moria, MAD, 2000K and LMO which were short-listed based on the results of previous screening with more than 40 % feeding response were subjected to continuous inbreeding and directional selection for 11 generations for improving the trait over the generations and stabilizing it at more than 85

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%. Care was taken so that all other economic traits were within the stipulated level of breed characters. At the end of eighth generation, the feeding response reached above 88 % or above and further stabilized at the higher level and thus forming prospective female parents for the multivotline x bivoltine hybrid combinations.

The rearing of young instar silkworm larvae was accomplished as described by [11] in a rearing room designed for the purpose maintaining a temperature of 30°C and a relative humidity of 90 %. On completion of 48 hours from the time of brushing, the larvae which accepted the artificial diet, grown uniformly and remained healthy were counted. By this time, the number of hatched eggs also was counted. The feeding response (FR) percentage was calculated as described in our earlier studies [2].

On coming out of second ecdysis, the larvae were fed with tender mulberry leaves and transferred to ventilated plastic trays measuring 3 ft x 2 ft and reared on mulberry leaves following standard procedure. On maturation, the larvae were picked up and mounted on plastic collapsible mountages for cocoon spinning. The cocoons were harvested on the 6th day and assessed. Selected cocoons of the strains which recorded more than 40 % feeding response in the screening process, were used to prepare layings for further breeding process.

Larvae of the four short-listed multivoltine strains were brushed on artificial diet as already described. Twelve replications of the larvae of each strain were brushed as cellular batches. On completion of II instar, after careful examination of all the rearing beds, six beds with healthy and uniform larvae were retained for further rearing. Based on FR and the performance of the batches, cocoons conforming to the original characters were selected. Equal number of healthy males and females were selected for the preparation of DFLs for the next generation and the male and female pupae were separately incubated for eclosion. On moth emergence, healthy female and male moths were picked up and half sib-mating was followed. Twenty - five DFLs were prepared for each strain. The selected strains were continuously reared for 11generations and until the FR of the strains stabilized above 80 %. After the strains were stabilized for rearing on artificial diet, they were designated as Moria(A), MAD(A), 2000K(A) and LMO(A) as these strains are different from the normal strains, Moria, MAD, 2000K and LMO.

Data pertaining to each strain for generations 8~11 after the stabilization of FR, were first subjected to descriptive statistical analysis. The mean, standard error (SE) and coefficient of variation (CV) were thus derived. The data on G8~G11 were then analyzed by employing one way ANOVA using *Analyse-It* statistical package to ascertain statistical significance.

III. RESULTS

Table 1 shows that the feeding response in two of the four multivoltine pure strains *viz*., MORIA and MAD was stabilized during the Generations 8~11 because in MORIA the FR was between 87 and 89 % whereas that in MAD was 88~93%. This gives the clear indication that between the two strains, MAD was the better performer in respect of FR, the prime trait in the context of this study with a mean FR of 90.36 %. At the same time the average FR in MORIA was 88.37% which was high by any standard. The CV in both the cases appeared almost same indicating that the variation in FR in the cases of MAD and MORIA was not different from each other. When the mean young instar duration of the two races was compared, it showed that MAD took 10h less time in completing first two instars (184 h) compared to what MORIA took (194 h). The weight of ten young instar larvae on completion of second moult was more in MAD (0.221 g) compared to that in Moria (0.207 g). These three diet-phase traits were largely stabilized as revealed from the fact that the statistical variations in these traits among the generations were non-significant.

The post diet-phase traits such as 5th instar duration, pupation percentage, cocoon yield and the cocoon traits showed consistent results in both the strains in accordance with the breed characters while the FR was stabilized. While MAD recorded mean 5th instar duration of 135 h, MORIA took 10 h more. The average pupation percentage was more in MAD in comparison with MORIA by about 7 percentage points. While the variations in 5th instar duration was non-significant in both the strains, that in pupation was non-significant only in MAD but significant at 5% in MORIA. Cocoon yield in the last four generations ranged from about 9.60 kg to 10.98 kg, with non-significant variations. The mean cocoon weight in MORIA and MAD was 1.278 and 1.266 g, respectively and the shell weight was 0.192 and 0.187g, respectively. These traits conform to the original breed traits along with the shell percentage. Quite understandably, since the trait was stabilized there was no significance in the variations seen in cocoon weight or shell weight among the generations. At the same time, the derivative trait, shell percentage showed significance in the variation at 5% level.

It is clear from Table 2 that the feeding response in the two listed strains *viz*., 2000K and LMO was stabilized during the generations 8~11 as in the case of the first two strains. Both the strains showed consistent FR and the mean FR was same in both the strains with a mere 0.09 % difference between the two (2000K: 89.50 %; LMO: 89.59 %). The duration taken by the strains to complete the young instar on diet also was almost same with just 4 h difference between the two with extremely low CV indicating very low variation among the generation in both the strains. The weight of ten young instar larvae on completion of second moult was slightly more in 2000K than in LMO and the variation was non-significant in both cases indicating stability.

The post diet-phase traits showed that in both the strains, 5th instar duration was 137 h with fair bit of inter generation variation which was significant. At the same time, pupation percentage and the cocoon yield exhibited considerable variation among the generations as evident from the relatively high CV. But this variation was not significant in either of the cases. There was no much

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difference between these two strains in respect of pupation percentage and the cocoon yield. The result showed that between the two strains, 2000K had a higher potential with regard to the cocoon weight with a mean cocoon weight of 1.501 g against 1.393 g in LMO. Shell weight also was relatively high in 2000K with 0.241 g as against 0.231 g in LMO. While the inter generation variations in cocoon and shell weight in the case of 2000K was non-significant in 2000K that was significant in LMO and quite opposite happened in the case of shell percentage. The shell percentage in real term was more in LMO than in 2000K.

IV. DISCUSSION

Silkworm needs a balanced nutrition during young stage for a robust growth. Mulberry leaves consumed by the young instar silkworm is expected to be succulent with high moisture content (>75 %) and rich in proteins (> 30 %) and carbohydrates (>19 %). But the Indian mulberry leaves fed to young instar silkworm is inconsistent in its nutrient level in relation to the dietary requirement [9], [4]. To tide over this constraint, a viable artificial diet for the young instar (up to the second moult) was developed for rearing young instar silkworm. The rearing technology for such a practice was developed simultaneously. The reduction in the labour involvement in the initial stages of rearing has been an added advantage. To develop silkworm strains and hybrids for commercial exploitation which would feed on artificial diet during the young instars as done normally on mulberry leaves remained a major challenge.

All the silkworm strains are not equally good in accepting the artificial diet although the diet contains all the required nutrients in the right proportion. Initially their feeding response (FR) varied from among the four strains and thus a single diet formulated was not suitable for all the silkworm strains and the productive silkworm hybrids as reported by [5] in a similar effort. Thus the strategy of transforming the existing promising pure strains to that with high level of acceptance to artificial diet was resorted to, in the present study. [6] had in fact opined that it was necessary to improve new silkworm varieties suitable for the artificial diet which was different from the mulberry leaves in physical and chemical nature.

In the context of the present work, it was pertinent to make the promising pure strains already in use to adapt to the physiological condition of feeding on artificial diet so that they could be used as breeding resource materials for hybridization resulting in exclusive hybrids for rearing on artificial diet. This was also a model tried by [11] and [10].

The breeding programme concentrated on isolating the better performing pure lines which are components of existing programmes of breeding laboratories of CSRTI, Mysore. An initial screening followed by short-listing based on their inclination towards artificial diet was carried out. The initial response of the identified silkworm strains to artificial diet feeding showed that prominent inter-strain difference exists in silkworm. This can be attributed to the stress placed on several physiological systems while attempting to accumulate different levels of major nutrients available in the artificial diet. It was clear from the results that MAD had the highest mean FR among the races (90.36 %) and MORIA had the lowest FR (88.37 %) Although there is not much to differentiate among these strains with regard to FR, all the three relatively newly evolved strains showed marginally better FR compared to the older strain MORIA. A line of similarity could be drawn between these results and a few in the past. [7] had reported in their effort to breed polyphagous silkworm strains to rear on artificial diet that almost all Japanese strains had accepted the artificial diet and could grow well unlike most of the Chinese strains. [1] also had reported that the feeding ability of Chinese strains on the artificial diet was apparently low and conspicuous. This is important because the new generation multivoltine strains were developed with an object of better productivity and feeding capacity coupled with higher conversion efficiency as manifested in their higher productivity level. This ability is reflected in the marginal improvement of FR in comparison with MORIA.

In a similar effort, many productive breeds having low response to artificial diet were made to adapt to the artificial diet over generations through directional selection. Five multivoltine and six bivoltine strains were thus evolved by [9] and [11]. The hybrids developed from these improved strains accepted the diet and performed well with respect to their economic traits and almost at par with that of mulberry reared counterparts [9], [11]. The present study thus created a larger pool of potential multivoltine strains for rearing exclusively on artificial diet during the young instar which will form breeding resource material for prospective hybrids with these multivoltine strains as potential maternal parents.

One of the main considerations in the present study was that while improving the feeding response over a period through directional selection and inbreeding, the other important economic traits are not adversely affected or rather such traits are maintained as per the breed characteristics. This is fairly clear in the traits of cocoon yield, pupation percentage, cocoon weight and shell weight.

Similarly, the duration for young instar was slightly more in the diet reared batches than that usually reared on mulberry leaf. But this meager extension is offset once the larvae are switched over to mulberry. All the other traits were within the breed traits. Furthermore, there was no deliberate attempt to increase the cocoon traits such as cocoon weight, cocoon shell weight and shell percentage through selection as this could have an adverse impact on the hybrid vigour in the resultant hybrids when these pure strains are used for hybridization.

It is concluded that four multivoltine silkworm pure strains were subjected to continuous inbreeding and directional selection up to 11th generation for improving and stabilizing their feeding response to create a pool of breeding resource material for prospective exclusive hybrids meant for rearing exclusively on artificial diet during young instar. While improving the feeding response, care was taken to retain all the other economic traits conforming to the breed characteristics.

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Table 1: Rearing performance of Moria and MAD during breed stabilization process

Generation	Feeding response (%)	Young instar duration (h)	Larval weight on II moult (10) (g)	V instar Larval duration (h)	Pupation percentage	Yield/ 10000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell per-centage
MORIA									
8	88.01	194	0.2034	137	69.16	9.84	1.235	0.178	14.42
9	87.11	194	0.2101	144	69.20	9.60	1.298	0.190	14.64
10	89.00	194	0.2039	144	84.11	10.08	1.294	0.201	15.53
11	89.37	194	0.2104	153	80.15	9.75	1.284	0.198	15.39
Mean	88.37	194	0.2070	145	75.66	9.82	1.278	0.192	15.00
SE±	0.894	0.74	0.003	1.76	2.492	0.222	0.018	0.004	0.169
CV	3.50	1.30	5.41	4.22	11.41	7.83	4.86	7.31	3.89
Significance	NS	NS	NS	**	*	NS	NS	NS	*
MAD									
8	90.19	184	0.2255	137	79.84	9.97	1.320	0.205	15.49
9	88.51	184	0.2277	144	87.00	10.98	1.249	0.189	15.11
10	89.40	184	0.2177	120	85.44	10.49	1.292	0.180	13.96
11	93.35	184	0.2118	141	81.67	9.95	1.205	0.174	14.41
Mean	90.36	184	0.2210	135	83.49	10.35	1.266	0.187	14.74
SE±	0.930	0.67	0.006	2.98	2.271	0.253	0.021	0.005	0.218
CV	3.56	1.30	8.93	7.62	9.42	8.47	5.68	9.20	5.13
Significance	NS	NS	NS	**	NS	NS	NS	NS	*

* Significant at $P < 0.05$, ** Significant at $P < 0.01$, NS- Non-significant.

Table 2: Rearing performance of LMO and 2000K during breed stabilization process

Generation	Feeding response (%)	Young instar duration (h)	Larval weight on II moult (10) (g)	V instar Larval duration (h)	Pupation percentage	Yield/ 10000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell per-centage
2000K									
8	90.15	188	0.2185	144	85.11	11.08	1.524	0.246	16.16
9	89.92	190	0.2232	136	94.78	13.73	1.514	0.256	16.91
10	89.71	188	0.2269	120	80.89	11.74	1.489	0.232	15.54
11	88.21	188	0.2155	147	83.61	11.42	1.477	0.229	15.48
Mean	89.50	188	0.221	137	86.10	11.99	1.501	0.241	16.02
SE±	0.808	0.62	0.004	3.21	2.210	0.419	0.013	0.005	0.200
CV	3.13	1.10	6.38	8.14	8.89	12.11	2.99	6.48	4.33
Significance	NS	NS	NS	**	NS	NS	NS	NS	**
LMO									
8	89.09	182	0.2182	144	87.17	12.14	1.464	0.250	17.05
9	91.35	184	0.2255	144	85.00	12.02	1.362	0.241	17.67
10	89.72	186	0.2101	120	83.33	11.42	1.484	0.229	15.43
11	88.18	184	0.2094	141	84.60	10.59	1.263	0.206	16.31
Mean	89.59	184	0.216	137	85.02	11.54	1.393	0.231	16.62
SE±	0.599	0.85	0.004	3.12	1.157	0.265	0.029	0.005	0.265
CV	2.32	1.60	6.60	7.88	4.71	7.96	7.17	8.08	5.52
Significance	NS	NS	NS	**	NS	NS	**	**	NS

** Significant at P <0.01, NS- Non-significant.