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Induction of Non-Diapause Eggs and Manifestation of Quantitative Characters by Low Temperature Incubation of Eggs in the Silkworm, *Bombyx mori* L.

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Induction of non-diapause eggs and manifestation of quantitative characters were studied in different seasons by low temperature incubation of eggs of a low yielding diapause with coloured cocoons silkworm breed, RD1 of *Bombyx mori*. Hundred percent of non-diapause eggs were induced when the egg incubation was carried out at 15°C followed by incubation initially at 25°C and then at 15°C. The diapause-nondiapause relation was found to be reversible. Analysis of variance study demonstrated significant variation in all the quantitative characters whereas significant variation due to different incubation treatments was observed for larval span, fifth instar larval span, cocoon yield/10,000 larvae by weight, cocoon weight, cocoon shell weight, and cocoon shell ratio. Study on hybrid vigour showed significant heterosis over mid parent value for four economic characters *viz.*, cocoon weight, cocoon shell weight, cocoon shell ratio and filament length in a F1 hybrid between RD1 with diapause eggs and Japanese type bivoltine NB_4D_2.

Key words : *Bombyx mori* L., Silkworm, Hybrid vigour, Low temperature incubation, Quantitative characters.

Introduction

Temperature during embryonic development plays an important role in determination of voltinism and moultinism in silkworm (Watanabe, 1918; Kogure, 1933; Itikawa, 1941; Nagatomo, 1941; Morohoshi, 1957). Larval abnormalities have been induced by exposure of silkworm eggs to low temperature (Doira, 1978; Nagaraju *et al.*, 1987). Extensive studies have been carried out on the induction of non-diapause eggs through low temperature incubation in the silkworm (Yamashita and Hasegawa, 1985; Singh and Verma, 1993; Morohashi, 2000). Recently, Kosegawa *et al.* (2000) induced non-diapause eggs by low temperature incubation (15°C) and constant darkness in univoltine and bivoltine breeds and Jayaswal and Raut (2000) by low temperature incubation (18°C) in a polyvoltine silkworm breed. Limited information is available on influence of low temperature incubation on the induction of non-diapause eggs and manifestation of quantitative characters in low yielding with coloured cocoons silkworm breeds. Hence, the present study was undertaken to know the effect of low temperature incubation of eggs on the induction of non-diapause eggs, diapause - nondiapause relation, manifestation of quantitative characters and hybrid vigour in a silkworm breed, RD1 of *Bombyx mori*.

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Materials and Methods

Effect of low temperature incubation on the induction of non - diapause eggs and manifestation of quantitative characters were studied in the eggs of RD1 (a low yielding silkworm breed with coloured cocoons) which lays diapause eggs but occasionally lays non-diapause eggs to a tune of 1 - 2% during summer months. The expriment was initiated during October - November 2000. Initially, the diapause eggs of RD1 were incubated at 15°C and 80 - 85% RH. Rearing was carried out by retaining all the larvae and non-diapause eggs were obtained. In subsequent generations, four sets of 10 - 15 non-diapause eggs were made. One set was incubated at a low temerature of 15°C and 80 - 85% RH in dark condition till blue egg stage (A). Second set was initially incubated at 25°C and 80 - 85% RH in dark condition and on 6th day of incubation, eggs were shifted to low temperature at 15°C till blue egg stage (B). The third set of non-diapause eggs were incubated at 25°C till blue egg stage (C) and the acid treated (diapause) eggs were also incubated at 25°C and 80 - 85% RH (normal temperature incubation) till blue egg stage (D). Layings of all the four sets were brushed cellularly, replicated three times and all the larvae were retained in each batch. Silkworm rearing was carried out at room temperature. Data were recorded for all the sets on percent of hatching, larval span, fifth instar age larval span, yield/10,000 larvae both by number and weight, cocoon weight, cocoon shell weight, cocoon shell ratio and pencent of floss, cocoon filament length, denier and the number of non-diapause and diapause layings laid by moths. The experiment was repeated 3 times during December - January 2000 - 2001, February - March 2001 and April - May 2001. Data were statistically analysed to know the effect of different seasons and manifestation of quantitative characters. Study on hybrid vigour over mid parent value was determined in F1 hybrids between RD1 (non-diapause), RD1 (diapause) and NB_4D_2 (Japanese type) bivoltine silkworm breed.

Characteristics of RD1

Freshly laid egg shell (chorion) colour is deep yellow. Newly hatched larvae are black. Full grown larvae are marked, slender with bluish white. Total larval duration is around 20 days. Larvae spin dark greenish yellow spindle shaped cocoons. A low yielding silkworm breed is highly tolerant to fluctuating eco-climating conditions. It combines well with other Chinese and Japanese type bivoltine silkworm breeds.

Results and Discussion

Analysis of variance study showed significant effect for all the characters due to season whereas all the characters except hatching pencentage and floss pencentage differed significantly among the treatments (Table 1). Average rearing performance of RD1 incubated at different temperatures in different seasons has been presented in Table 2. Quantitative characters showed distinct differences in different seasons. Jayaswal and Raut (2000) have observed similar results.

It is obvious from this experiment that non-diapause eggs can be successfully induced by subjecting the silkworm eggs at 15°C. However, if the induced non-diapause eggs are incubated at 25°C, the resulted progeny reversed to diapause character (Table 3, 4 and 5). The results are in agreement with those of (Kobayashi *et a.l*, 1986; Jayaswal and Raut, 2000; Kosegawa *et al.*, 2000) who observed that low temperature during embryonic development plays major role in controlling egg diapause. Incidence of non-diapause eggs was high in European races following low temperature incubation in constant darkness (Kosegawa *et al.*, 2000) as compared to Japanese and Chinese races. Diapause determination in silkworm is maternal and temperature and photoperiod are the most effective at embryonic stage of the previous generation (Yamashita and Hasegawa, 1985; Kobayashi, 1990).

Results of the present study demonstrated reduction in quantitative characters *viz.*, total larval span, fifth instar larval span, cocoon weight, cocoon shell weight and cocoon shell ratio of induced non-diapause by low temperature incubation. Thus, there is a negative correlationship between quantitative characters and incidence of non-diapause eggs. Similar esults were observed by Morohoshi (1957), Singh and Verma (1993) and Jayaswal (1996) who observed reduction in quantitative characters with the decline in the incidence of diapause eggs.

Hybrid vigour study in F1 hybrids between non-diapause, diapause and a bivoltine strain NB_4D_2, showed significant difference over mid parent value for yield/10,000 larvae by weight, cocoon weight, cocoon shell weight, cocoon shell ratio and cocoon filament length in the F1 hybrid between diapause RD1 and NB_4D_2 (Table 6). The results support the earlier findings of Yamashita and Hasegawa (1985) who observed that in a given strain of silkworm which is programmed to lay diapause eggs are

Table 1. ANOVA for different characters of RD1 incubated at different temperatures and seasons

Source of variation	d. f.	Hatching %	Larval span	Fifth instar	Cocoon yield/10000 larvae by no.	Cocoon yield/10000 larvae by wt.	Cocoon weight	Cocoon shell weight	Cocoon shell ratio	Floss %
Season	2	3.13*	17309.19**	505.53**	3012156.19**	9.75**	0.053**	0.0034**	17.69**	3.89*
Treatment	3	1.29	5345.14**	1759.81**	428362.70	7.80**	0.112**	0.0046**	9.10**	0.41
Season × Treatment	6	8.29**	371.64**	9.31	173180.67	0.83	0.009	0.0002	0.79	0.40
Error	24	0.87	53.78	37.11	288690.69	0.74	0.004	0.00	0.39	0.87

* and **, significantly different at 5% and 1% level, respectively.

Table 2. Performance of RD1 incubated at different tempetatures (mean ± SE) during different seasons

Season	Treatment	Hatching %	Larval span (hr)	Fifth age Span (hr)	Cocoon yield/10000 larvae by no.	Cocoon yield/10000 larvae by wt. (kg)	Cocoon weight (g)	Cocoon shell weight (g)	Cocoon shell ratio	Floss %
Dec.00 - Jan.01	A	98.1±0.8*	438±0.0*	96±0.0*	9047±193	8.511±0.32*	0.899±0.02*	0.093±0.002*	10.3±0.9*	13.3±0.9
--do--	B	94.4±0.7*	489±7.5*	120±6.6	9468±144	10.355±0.42	1.095±0.05	0.128±0.016*	12.0±0.2	13.6±0.5
--do--	C	97.0±0.5	512±7.6	126±5.6	9125±131	10.816±0.31	1.197±0.03	0.131±0.004	11.0±0.4*	12.5±0.3
--do--	D	95.7±0.7	512±0.8	126±6.0	9338±146	11.679±0.37	1.128±0.02	0.144±0.005	12.7±0.3	13.0±0.6
Feb. - Mar.01	A	93.7±0.5*	384±0.0*	90±0.0*	8085±354	7.102±0.30	0.899±0.01*	0.107±0.002*	11.9±0.2*	13.1±0.3
--do--	B	97.2±0.2	432±0.0	114±0.0	8744±320	9.368±0.17	1.130±0.03	0.163±0.005	14.4±0.4	13.2±0.3
--do--	C	96.0±0.5	432±0.0	114±0.0	7988±579	8.808±1.32	1.072±0.03	0.152±0.002	14.2±0.4	12.6±0.6
--do--	D	97.8±0.5	432±0.0	114±0.0	8154±169	8.443±0.32	1.014±0.02	0.147±0.004	13.8±0.4	13.1±0.1
Apr. - May01	A	97.1±0.2	402±5.0*	84±6.0*	8696±534	8.867±0.47	1.010±0.04	0.128±0.004*	12.2±0.4	11.8±0.7
--do--	B	97.3±0.2	432±0.0	114±0.0	8690±137	11.291±0.06	1.104±0.03	0.143±0.005*	12.9±0.2	12.3±0.6
--do--	C	97.3±0.4	432±0.0	114±0.0	8452±217	9.513±0.48	1.218±0.05*	0.165±0.006	13.5±0.3	12.5±0.4
--do--	D	97.1±0.4	432±0.0	114±0.0	9218±338	9.843±0.11	1.103±0.04	0.161±0.009	14.9±0.6	11.8±0.5
CD at 5% (Season)		0.785	6.179	5.133	452.72	0.724	0.051	0.008	0.525	0.785
CD at 5% (Treatment)		0.906	7.135	5.927	522.75	0.837	0.059	0.008	0.606	0.905
CD at 5% (Season × Treatment)		1.570	12.358	10.266	905.44	1.449	0.102	0.015	1.050	1.570

A: Incubation of non-diapause eggs at 15°C upto blue egg stage.
B: Early incubation of non-diapause eggs upto 6 days at 25°C and then shifted to 15°C till blue egg stage.
C: Incubation of non-diapause eggs at 25°C upto blue egg stage.
D: Diapause eggs incubated at 25°C upto blue egg stage.
*, Significantly different from the control (treatment D) at 5% level.

Table 3. Inheritance of non-diapause and diapause eggs in RD1 following different incubation schedules

Treatment	Replications	No. of non-diapause layings	No. of diapause layings	No. of mixed layings	Total no. of layings	% of non-diapause layings	% of diapause layings
First trial (December, 2000 January, 2001)							
	1	142	-	-	142	100.00	0.00
A	2	138	-	2	140	100.00	0.00
	3	143	-	-	143	100.00	0.00
	1	1	112	-	113	0.88	99.12
B	2	15	55	1	71	21.13	78.87
	3	35	46	4	85	41.18	58.82
	1	4	143	-	147	2.72	97.28
C	2	2	121	-	123	1.63	98.37
	3	1	166	-	167	0.60	99.40
	1	-	83	1	84	-	100.00
D	2	-	31	-	31	-	100.00
	3	2	145	1	148	1.35	98.65

Table 4. Inheritance non-diapause and diapause eggs in RD1 following different incubation schedules

Treatment	Replications	No. of non-diapause layings	No. of diapause layings	No. of mixed layings	Total no. of layings	% of non-diapause layings	% of diapause layings
Second trial (February - March, 2001)							
	1	82	-	-	83	100.00	-
A	2	68	-	-	68	100.00	-
	3	62	-	-	62	100.00	-
	1	1	45	13	59	1.69	98.31
B	2	6	65	2	73	8.22	91.78
	3	7	28	22	57	12.28	87.72
	1	6	86	3	95	6.32	90.68
C	2	12	74	5	91	13.19	86.81
	3	3	49	7	59	5.08	94.92
	1	5	94	3	102	4.90	95.10
D	2	6	100	1	107	5.61	94.39
	3	4	108	2	114	3.51	96.49

Table 5. Inheritance of non-diapause and diapause eggs in RD1 following different incubation schedules

Treatment	Replications	No. of non-iapause layings	No. of diapause layings	No. of mixed layings	Total no. of layings	% of non-diapause layings	% of diapause layings
Third trial (April May, 2001)							
	1	101	-	-	101	100.00	0.00
A	2	70	-	-	70	100.00	0.00
	3	49	-	-	49	100.00	0.00
	1	1	51	-	52	1.92	98.08
B	2	-	49	-	49	0.00	100.00
	3	1	50	-	51	1.96	98.04
	1	-	34	-	34	0.00	100.04
C	2	-	41	-	41	0.00	100.00
	3	1	69	-	70	1.43	98.57
	1	1	79	-	80	1.25	98.75
D	2	-	116	-	116	0.00	100.00
	3	-	81	-	81	0.00	100.00

Table 6. Mean performance of RD1 (non-diapause), diapause and their hybrids

Breed/Combination	Yield/10,000 larvae by wt (kg)	Cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell ratio	Cocoon filament length (m)
RD1 (ND)	11.878	1.322	0.183	13.84	470
RD1(D)	11.739	1.339	0.178	13.29	517
NB_4D_2	14.066	1.698	0.335	19.73	834
RD1 (ND) × NB_4D_2	14.344	1.677	0.295	17.59	797
RD1 (D) × NB_4D_2	13.888	1.901	0.336	17.67	840
RD1 (ND) × NB_4D_2 (MPV)	9.45	11.05	13.62	4.52	21.33*
RD1 (D) × NB_4D_2 (MPV)	6.54	25.10*	30.82*	5.78*	23.41*

ND, Non-diapause; D, Diapause; * Significantly different at 5% level.
MPV denotes heterosis percentage over mid parent value.

superior to those that lay non-diapause eggs.

From this study, it is evident that non-diapause could be successfully induced by low temperature incubation in the diapause eggs of low yielding silkworm breed RD1. However, there was a negative relationship between quantitative characters and incidence of non-diapause. It was also observed that the induced non-diapause by low temperature were found reversed to diapause when the eggs from resulted moths were incubated at 25°C.

References

Doira, H. (1978) Genetic stocks of the silkworm. Kodansha Ltd., Tokyo, Japan.

Itakawa, N. (1941) On facultatively occurred trimoulters in the silkworm. *J. Seric. Sci. Jpn.* **12**, 17-23.

Jayaswal, K. P. (1996) Impact of diapause on the silk production in *Bombyx mori*. Ph. D. Thesis, Calcutta Univ., Calcutta.

Jayaswal, K. P. and S. K. Raut (2000) Influence of low temperature incubation on diapause incidence and quantitative traits in *Bombyx morl* L. *Uttar Pradesh J. Zool.* **20**, 233-237.

Kobayashi, J. (1990) Effect of photoperiod on the induction of egg diapause of tropical races of the domestic silkworm, *Bombyx mori* and the wild silkworm, *B. mandarina*. *JARQ* **23**, 202-205.

Kobayashi, J., H. Ebinuma, M. Kobayashi and M. Yoshitake (1986) Effect of temperature on the diapause egg production in the tropical race of the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.* **55**, 343-348.

Kogure, M. (1933) The influence of light and temperature on certain characters of the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.* **12**, 171-183.

Kosegawa, C., G. Vemananda Reddy, K. Shimizu and T. Okajima (2000) Induction of non-diapause egg by dark and low temperature incubation in local variety of the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.* **69**, 369-375.

Morohoshi, S. (1957) Physiogenetical studies on moultinism and moultinism in *Bombyx mori*: a new hormonal antagonastic balance theory on the growth. Jpn. Soc. for promotion of Sci. Tokyo, Japan

Morohoshi, S. (2000) Development physiology of silkworm. Oxford & IBH Publishing Co., New Delhi.

Nagaraju, J., R. Singh and V. Premalatha (1987) Low temperature induced abnormalities in silkworm, *Bombyx mori*. *Curr. Sci.* **56**, 1249-1251.

Nagatomo, T. (1941) Sex-linked inheritance of trimoulting in *Bobyx mori*. *J. Seric. Sci. Jpn.* **12**, 171-183.

Singh, K. and M. Verma (1993) Studies on strengthening non-diapause character in some multivoltine races, *viz*., AP - 5 and G by manipulation of photoperiod and incubation temperature. *Indian J. Entomol.* **55**, 341-350.

Watanabe, K. (1918) Studies on the voltinism in the silkworm, *Bombyx mori* Inhertance of bivoltine vs. quadrivolitine. *Ibid.* **3**, 397-350

Yamashita, O. and K. Hasegawa (1985) Embryonic diapause; in *Comprehensive Insect Physiology Biochemistry and Pharmacolog*. Karkut, G. A. and L. I. Gilbert (eds.), pp. 407-434, Pergamen Press, London.