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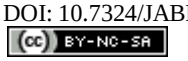

Effect of polyamines on parental and hybrid strains of *Bombyx mori*

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ABSTRACT

The silk produced by *Bombyx mori*, mulberry silk, is of very high economic importance. Many hybrid strains of *Bombyx mori* were developed to improve silk production and to withstand different environmental conditions. Polyamines are low molecular weight polycations present in all living cells. These are very crucial for the growth and survival of organisms. Previous results from our lab showed positive effect of spermidine treatment on CSR2 × CSR4 hybrid strain. The present study was taken up to know the effect of spermidine and spermine on different parental and hybrid strains of silkworm. Treatment of these molecules increased body weight, gland weight and cocoon weights. Results showed that both spermidine and spermine enhanced silk production. Our study shows a method for enhancement of silk production by parental strains.

1. INTRODUCTION

Silkworm breeds or hybrids play a very important role for the development of sericulture industry. Bulk of the silk is produced by Polyvoltine × Bivoltine cross in India. Bivoltine hybrids were successful to some climatic conditions. Many techniques were developed to produce the silkworm strains to withstand the environmental conditions. Cross-breeding strains, Multivoltine × Multivoltine (PM × C Nichi, PM × C110) were developed mainly for rainy seasons; Multivoltine × Bivoltine (PM × CSR2, MH1 x CSR2) for average climatic conditions and Bivoltine × Bivoltine (CSR2 × CSR4) for all climatic conditions. Silk produced by bivoltine silkworm is of very high quality. These also produce more amount of silk. These productive strains have been developed by Central Sericulture Research and Training Institute (CSRTI), Mysore. The five strains selected for the present study are the popular strains of South India reared for their superior qualities. Pure Mysore (PM) strain is suitable for all conditions. Their cocoon weight ranges from 0.8g to 1g and filament length ranges from 325M to 375M. CSR2 strain is reared from August to February. Its cocoon weight ranges from 1g to 1.8g and filament length from 1000M - 1100M. CSR4 strain is reared from August to February. Its cocoon weight ranges from 1.50g to 1.075g and filament length ranges from 950M to 1000M. The hybrid, PM × CSR2 is reared in all seasons. Its cocoon weight is approximately 1.0g and filament length ranges from 758M to 760M. The well known hybrid, CSR2 × CSR4 is widely used strain in South India. Rearing of these worms is suitable from September to February. Its cocoon weight ranges from 1.6 to 1.8g and approximate filament length is around 1000M [1, 2]. Polyamines (PA) were shown to be important regulators of cell growth and differentiation were shown to modulate gene expression in a variety of organisms [3, 4, 5 and 6]. PAs were interact with nucleic acids directly [6]. They were shown to play role in regulating the structure of RNA and in translation [7]. Under physiological conditions spermine (Spm) and spermidine (Spd) are better than putrescine in promotion of growth [8]. Previously many works have reported improvement in economic parameters by supplementation with Vitamins [9], Riboflavin and Ascorbic acid [10, 11]. Recently, genetic approaches were also made to improve silk production by developing transgenic silkworms [12]. Previous results from our lab showed enhancement in silk production on feeding 5th instar silkworms, CSR2 × CSR4 strain with Spd. The present investigation was undertaken to evaluate the effect of polyamine treatment on parental and hybrid strains. PM, CSR 2, CSR 4, PM × CSR2 and CSR2 × CSR4 were selected. Results showed that the PA fed larvae showed better growth in both parental and hybrid strains. Economic parameters like silk gland weight, cocoon weight and filament length improved in both parental and hybrid strains.

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2. METHOD AND MATERIALS

2.1 Standard Polyamines

Spermidine free base (RM 5438), Spermine (RM 7506) were purchased from Hi media chemicals.

2.2 Collection of larvae

Disease free laying (dfls) of CSR2, CSR4, PM, CSR2 × CSR4 and PM × CSR2 were brought from Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Hindupur, Andhra Pradesh, India. The eggs were incubated for hatching and hatched larvae were grown into adult larvae at Government Sericulture farm at Srikakulam. After third moult, the larvae were acclimatized to laboratory conditions by rearing them in plastic trays by providing sufficient ventilation, spraying disinfection and reared at RH 70 ± 10 and temperature 28 ± 2 °C. The larvae were divided into 5 groups in all breeds; one group is set as control and the other four groups are set as treated groups in triplicates. Minimum 30 worms were kept per group. 50 µM and 100 µM concentrations of each drug were selected. Fresh mulberry leaves were sprayed with PA of different concentration by swab method and fed to the larvae three times per day. Control worms were fed with normal leaf. During fourth moult the larvae were placed on mountage and left undisturbed to form cocoons and on day 13 cocoons were harvested and subjected for estimating cocoon parameters.

2.3 Estimation of body weights

Body weights were recorded every alternate day (1, 3, 5 and 7) for 30 larvae from each group by using digital weighing balance. Day 1 weights are taken as initial weights before the drug treatment.

2.4 Estimation of gland weights

On day 7, end of the 5^{th} instar larval stage, worms were scarified for gland weights. Worms were dissected and total silk gland was isolated and weighed. Minimum 3 larvae were scarified per group. Both control and PA treated group larvae silk gland weights were recorded.

2.5 Estimation of cocoon parameters

Larvae were allowed to spin and left undisturbed. On day 13 cocoons were harvested and subjected for cocoon weight analysis.

2.6. Degumming process and silk reeling

Cocoons of both control and treated groups of different concentration were used for reeling purpose. The cocoons were boiled in hot water for more than 10 minutes to remove the raw silk and boiled in hot water till single fiber is obtained. Single filament from each cocoon was reeled on hand by using epprouvettor of circumference 1.125 m/ rotation with same reeling speed. The number of rotations was multiplied to the circumference to obtain the total filament length.

3. RESULTS

3.1 Effect of PA on body weights

PA feeding to the larvae showed an increase in the body weights in both parental and hybrid strains (Table 1). Spd 50 µM, Spd 100 µM, Spm 50 µM and Spm 100 µM showed significant increase in body weights of CSR2, PM × CSR2, CSR4 and CSR2 × CSR4.

Of all the strains tested for PA feeding, maximum increase in body weights was observed with PM × CSR2 strain. On the other hand, PM strain did not show any effect on body weights upon PA feeding.

3.2 Effect of PA on gland weight

Silk glands were taken for weight measurement from both control and PA fed larvae on day 7 of 5^{th} instar larval stage. PA effect on gland weight was checked for all the strains (Fig 1A). The PM strain showed no effect on silk gland weight upon treatment with polyamines. CSR2 strain showed positive effect on gland weight in all treatments. Significant increase was observed with Spm 100 µM treatment. PM × CSR2 strain showed positive effect at higher concentrations of PAs. CSR4 showed no positive effect with PA treatment. CSR2 × CSR4 stain showed positive effect with Spd 50 µM and Spm 100 µM concentrations. Thus from the results it can be found that the response to PA was different with different strains. Both parental and hybrid strains responded to the treatment.

3.3 Effect on cocoon parameters

Control and PA fed larvae were allowed to form cocoons and cocoon weights were analysed on day 13 of the pupal stage (Fig 1B). Parental strain, CSR2, showed better response in cocoon weight with Spd 50 µM, Spd 100 µM and Spm 100 µM treatments. The images of cocoons of all strains at different treatments were given in Fig 2.

3.4 PA treatment on filament length

The total amount of silk reeled from single cocoon of the strains at different concentrations were analysed (Fig 1C). Spd 50 µM, Spd 100 µM, Spm 50 µM and Spm 100 µM showed significant increase in filament length in CSR2 and CSR2 × CSR4 strains. CSR4 strain showed positive repose in for filament length with Spd 50 µM treatment.

In the present study we found that parental and their hybrids strains were influenced by the PA treatment. PA feeding showed positive effect on body weights, gland weights, cocoon weights and filament length. This result shows correlation to administration of folic acid [13, 14) and thiamine on different silkworm races [15]. Both Spd and Spm showed similar effect on silkworm for both growth and economic parameters.

Table 1: Effect of Spd and Spm on body weights of different *B. mori* strains.

			Body weights (gm)				
Drugs	**Concentrations**	**Strains**	**Day-1**	**Day-3**	**Day-5**	**Day-7**	**Percentage increase in body weight**
Control	---	**PM**	0.56 ± 0.001	1.41 ± 0.04	1.88 ± 0.049	2 ± 0.052	72
		CSR2	0.78 ± 0.02	2.6 ± 0.109	3.04 ± 0.133	3.57 ±0.067	78.15
		PM* CSR2	0.67 ± 0.001	2.5 ± 0.041	2.91 ± 0.052	3.51 ± 0.062	80.91
		CSR4	0.97 ± 0.002	2.07 ± 0.053	2.69 ± 0.122	3.28 ± 0.140	70.42
		CSR2* CSR4	0.95 ± 0.001	3.11 ± 0.041	3.50 ± 0.025	4.26 ± 0.051	77.69
Spd	50 µM	**PM**	0.42 ± 0.002	1.42 ± 0.141	1.9 ± 0.141	2.01 ± 0.083	79.1
		CSR2	0.8 ± 0.01	3.24 ± 0.049*	3.68 ± 0.056*	4.29 ± 0.060	81.35
		PM* CSR2	0.67 ± 0.02	2.81 ± 0.034*	3.32 ± 0.052*	3.89 ± 0.127*	82.77
		CSR4	0.92± 0.004	2.94 ± 0.073*	3.2 ± 0.10*	3.54 ± 0.161	74.011
		CSR2* CSR4	0.92 ± 0.001	3.52 ± 0.117*	4 ± 0.08*	4.60 ± 0.096*	80
	100 µM	**PM**	0.53 ± 0.01	1.59 ± 0.288	1.8 ± 0.075	2.12 ±0.136	75
		CSR2	0.78 ± 0.02	3.19 ± 0.049*	3.74 ±0.11*	4.26 ± 0.051	81.69
		PM* CSR2	0.64 ± 0.01	2.81 ± 0.034*	3.28 ± 0.063*	3.89 ± 0.051*	83.54
		CSR4	1± 0.03	2.65 ± 0.075*	3.04 ± 0.11*	3.56 ± 0.136	71.94
		CSR2* CSR4	0.93 ± 0.001	3.54 ± 0.136*	4.02 ± 0.06	4.59 ± 0.139*	79.73
Spm	50 µM	**PM**	0.5 ± 0.010	1.11 ± 0.10	1.37 ± 0.04*	1.69 ± 0.15	70.41
		CSR2	0.69 ± 0.02	2.2 ± 0.067*	3.31 ± 0.118	3.61 ± 0.111	80.88
		PM* CSR2	0.59 ± 0.001	1.82 ± 0.27	3.19 ± 0.09*	3.47 ± 0.053	82.99
		CSR4	1 ± 0.002	2.55 ± 0.07*	3 ± 0.23	3.13 ± 0.11	68.05
		CSR2* CSR4	0.85 ± 0.004	2.44 ± 0.378*	3.28 ± 0.082	4.02 ± 0.185*	78.75
	100 µM	**PM**	0.43 ± 0.001	1.25 ± 0.042	1.98 ±0.182	1.88 ± 0.100	77.12
		CSR2	0.77 ±0.001	3.37 ± 0.053*	3.61 ± 0.037*	4.32 ± 0.029	82.17
		PM* CSR2	0.63 ± 0.002	2.71 ± 0.102	3.23 ± 0.071*	3.84 ± 0.035*	83.59
		CSR4	0.95 ± 0.003	2.56 ± 0.10*	2.80 ± 0.17	3.48± 0.129	72.7
		CSR2* CSR4	0.94 ± 0.001	3.76 ± 0.80	4.42 ± 0.03	4.95 ± 0.101*	81.01

Average larval body weights (n=20) of parental and hybrid strains treated with different concentrations of Spd and Spm. '±' indicates the standard error values. '*' indicates the significantly different with ($p < 0.05$).

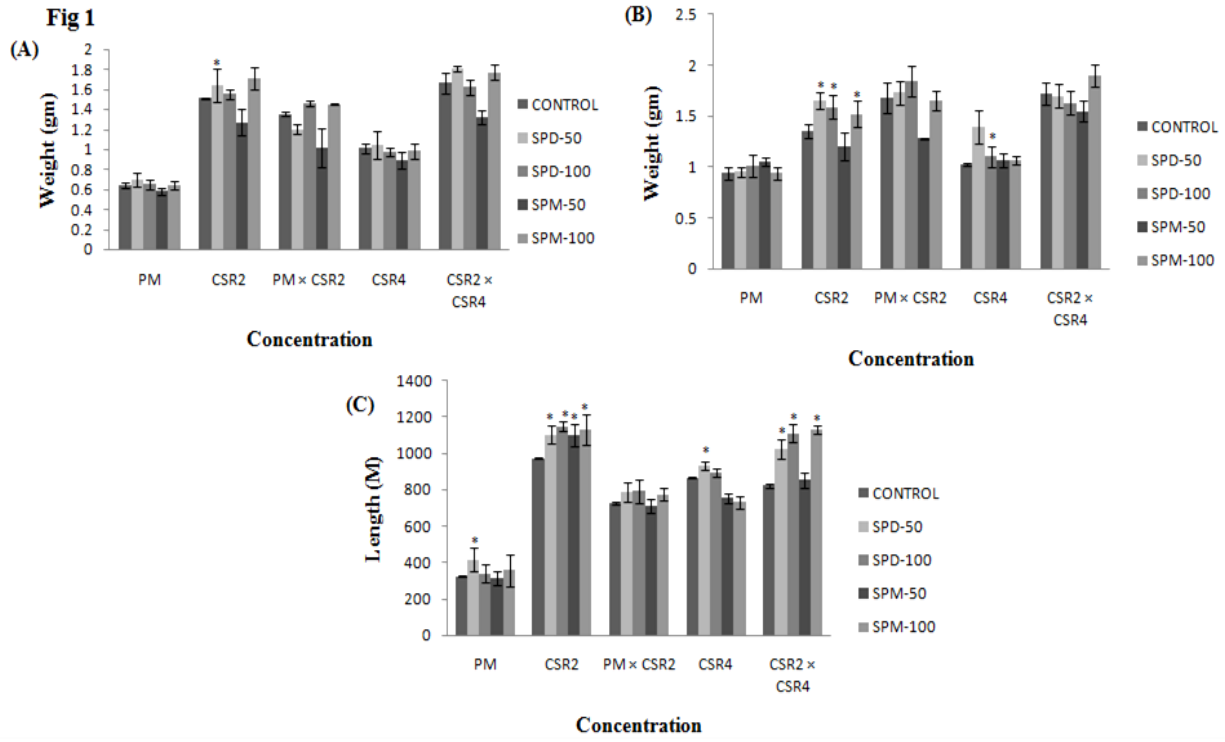

Fig. 1: Effect of Spd and Spm on economic parameters of parental and hybrid strains of *B. mori*. (A) represents average gland weight and (B) represents average cocoon weights (n=5). X-axis depicts different concentrations and Y-axis depicts average weight in grams (gm). (C) represents average length of silk reeled per cocoon (n=9). Concentrations were given on X-axis and average length of silk reeled in meters (M) was given on Y-axis. '±' indicates the standard error values and bars labelled with '*' are significantly different ($p < 0.05$)

Fig. 2: Cocoons of parental and hybrid strains of *B. mori* with different treatments of Spd and Spm. Representative cocoon images of treated and control of parental and hybrid strains of *B. mori*.

4. CONCLUSION

Present study concluded that PA treatments every day to silkworm in their 5th instar stage can enhance the silk production both in parental and hybrid strains.

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Conflict of Interests: There are no conflicts of interest.

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