



Research Article

FIELD EVALUATION OF MULTI-VIRAL DISEASES TOLERANT BIVOLTINE SILKWORM HYBRID OF *BOMBYX MORI* L. (LEPIDOPTERA: *BOMBYCIDAE*)

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ABSTRACT

Bombyx mori Densonucleosis virus (BmDV), *Bombyx mori* Infectious flacherie virus (BmIFV) and *Bombyx mori* nuclear polyhedrosis virus (BmNPV) infection to silkworms causes major sericulture crop loss. The multi-viral diseases tolerant bivoltine silkworm double hybrid, RDIN1, developed through marker-assisted selection was field evaluated by rearing 20,000 dfls at different location and season. RDIN1, had better pupation (94.8%) compared to control, FC1 X FC2 (91.5%) at farmers' level with 4 % significant improvement. Average Cocoon yield of RDIN1 (71.2%) was better compared to FC1 X FC2 (69.3%) in unfavourable conditions. Other parameters such as cocoon weight, shell weight, shell percentage, reelability, filament length, raw silk, denier, neatness had also significant difference between RDIN1 and FC1 x FC2. The RDIN1 is expected to reduce cocoon crop losses invariably due to viral infection and fetch more profit to sericulture farmers.

Keywords: *Bombyx mori*, Densonucleosis virus, Infectious flacherie virus, SSR marker, Nuclear polyhedrosis virus.

INTRODUCTION

With the Climate change the evolution of silkworm diseases causing pathogens and outbreak of silkworm disease are evident (Chen *et al.*, 2016), especially in tropical country like India, where environmental humidity and temperature is frequently favorable for pathogen's survival and infection and occasionally favorable for epizootic outbreaks, resulting in enormous economic losses to the silk industry (James and Li, 2012). Viral diseases are prevalent and are one of the major constraints in almost all the sericultural areas in India. The diseases caused by viruses such as BmNPV (grasserie) and BmDV and BmIFV (flacherie) are more common and account to nearly 70% of reported crop losses (Sivaprasad *et al.*, 2021). India being tropical country and the conditions favour the growth of

viruses, it is highly expected that the silkworm can be infected by multi-virus.

To counteract the danger posed by multi-virus infection, we had developed multi-viral resistant bivoltine silkworm hybrid, RDIN1, through SSR marker (Isocitrate dehydrogenase (IDH216), Glucose dehydrogenase (GDH306), Lipase (LIP283), Protein tyrosine phosphatase (PTP284), Attacin (ATT), Ankyrin (ANK165), Alkaline tyrosine kinase (ATK285) and Dipeptidyl peptidase (DPP150) assisted selection by pyramiding of resistance/tolerance genes (Satish *et al.*, 2022). Validation of the newly developed multi-viral diseases tolerant bivoltine silkworm hybrid, RDIN1 has been carried out at farmer's level to find out the potentiality and performance in the field.

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MATERIALS AND METHODS

A total of 20000 dfls of a Multi-Viral Diseases Tolerant Bivoltine Silkworm double Hybrid (RDIN1) were distributed (ranging from 50 to 450 dfls which is depending on size of rearing capacity) among 80 farmers (Karnataka: Mandya, Malavalli & HD Kote and Andhra Pradesh: Chittoor & Ananthpur) through chawki centres for rearing & cocoon production during rainy (August) & summer (April) crops. Similarly, rearers of FC1xFC2 at the same locality were also monitored and collected cocoon samples for rearing & reeling analysis. Rearing parameters such as Pupation (%), Cocoon Yield (kg/100dfls), Cocoon Wt. (g), Shell Wt. (g) & Shell (%) and reeling parameters such as Reelability (%), Filament length (m), Rawsilk (%), Denier (d) and Neatness (P) were taken into account for statistical analysis (Descriptive, two-factor ANOVA & graphical analysis) & interpretation in comparison with ruling silkworm hybrids FC1 x FC2. Two-factor ANOVA (Season as one factor & Silkworm hybrid as another factor) were employed to know the effect of main factors (season & hybrid) on rearing & reeling parameters for logical inferences.

RESULTS AND DISCUSSION

A total of 20,000 dfls produced were supplied to different farmers in Karnataka and Andhra Pradesh during the period of March 2021 to March 2023. As these tropical locations favours silkworm virus infections because summer season in Andhra Pradesh are congenial for occurrence of Grasserie and rainy season in Karnataka favours flacherie infections (Chopade *et al.*, 2021). The evaluation of test hybrid layings (RDIN1) and control layings (FC1 x FC2) were conducted and the pupation of RDIN1 was found to be 94.8 % compared to 91.5 % of FC1 x FC2 with 4 % significant improve over control (Table 1). Cocoon yield

improvement of 3 % for RDIN1 was found to be that of control. Whereas other cocoon parameters such as single cocoon weight (15 %), single shell weight (4 %), average filament length (2 %) were significantly higher in RDIN1 compared to FC1 x FC2. Shell %, reelability, raw silk, denier, neatness was found to be on par with control compared to test but remained non significant statistically. The overall requirement of RDIN1 hybrid was to have no silkworm crop loss due to viral infections in unfavourable seasons with the better cocoon yield in comparison with popular hybrid FC1 x FC2. These results can be attributed to the SSR markers (Figure 1) used for selection of viral tolerant parental breeds, on which RDIN1 layings were prepared.

The field evaluation of RDIN1 and FC1 x FC2 in the month of March to May (summer season) and June to August (rainy season), in general, the occurrence of viral infections, the RDIN1 performed significantly well with 94.8 % pupation in summer and 94 % pupation in rainy with 4 % improvement in summer and 2 % improvement in rainy. The cocoon weight of control was significantly higher in summer than test and all other parameters remained statistically on par with control. In rainy season, cocoon weight, shell weight, shell %, reelability was found to be significant in test compared to control. The cocoon yield of RDIN1 was also better than FC1 x FC2, and this can be attributed to the combination of productive and tolerant foundation crosses utilized to produce RDIN1 layings (Satish *et al.*, 2022). Rearing performance of RDIN1 finally leads to marketing price of RDIN1. The marketing price is the foremost important factor to farmers and RDIN1 was always satisfactorily better compared to FC1 x FC2. The utilization of molecular techniques in combination with conventional breeding has led to the development of virus diseases tolerant bivoltine hybrid that helps farmers to gain more money.

Table 1. Overall cocoon and reeling performances of RDIN1 & FC1xFC2 at Farmers field.

| Season | Hybrid | Pupation (P) | Cocoon Yield (kg/100dfls) | SCW (g) | SSW (g) | SR (P) | Reel (P) | AFL (m) | RS (P) | Den (d) | Neat (P) |
|--------|---------|--------------|---------------------------|---------|---------|--------|----------|---------|--------|---------|----------|
| Rainy | RDIN1 | 94.06 | 71.26 | 1.872 | 0.405 | 21.63 | 86.64 | 882.78 | 17.00 | 2.84 | 94.43 |
| | FC1xFC2 | 92.33 | 69.32 | 1.659 | 0.356 | 21.45 | 86.47 | 865.43 | 17.02 | 2.81 | 94.14 |
| | CD @ 5% | 0.35 | 0.25 | 0.007 | 0.001 | NS | NS | 3.22 | NS | 0.01 | N/A |
| | SEm± | 0.13 | 0.09 | 0.002 | 0.001 | 0.03 | 0.12 | 1.15 | 0.02 | 0.00 | 0.13 |
| Summer | RDIN1 | 94.87 | 71.23 | 1.794 | 0.388 | 21.62 | 87.17 | 881.29 | 17.02 | 2.84 | 94.44 |
| | FC1xFC2 | 91.52 | 69.35 | 1.707 | 0.354 | 20.71 | 85.94 | 866.94 | 17.00 | 2.81 | 94.13 |
| | CD @ 5% | 0.35 | 0.25 | 0.007 | 0.001 | NS | NS | 3.22 | NS | 0.01 | N/A |
| | SEm± | 0.18 | 0.13 | 0.003 | 0.001 | 0.04 | 0.17 | 1.63 | 0.03 | 0.01 | 0.18 |

SCW: Single Cocoon Weight; SSW: Single Shell Weight; SR: Shell ratio; Reel: Reelability; AFL: Average Filament length; RS: Raw Silk; Den: Denier; Neat: Neatness SE (m): Standard error mean, CD @5%: critical difference at 5% level of significance.

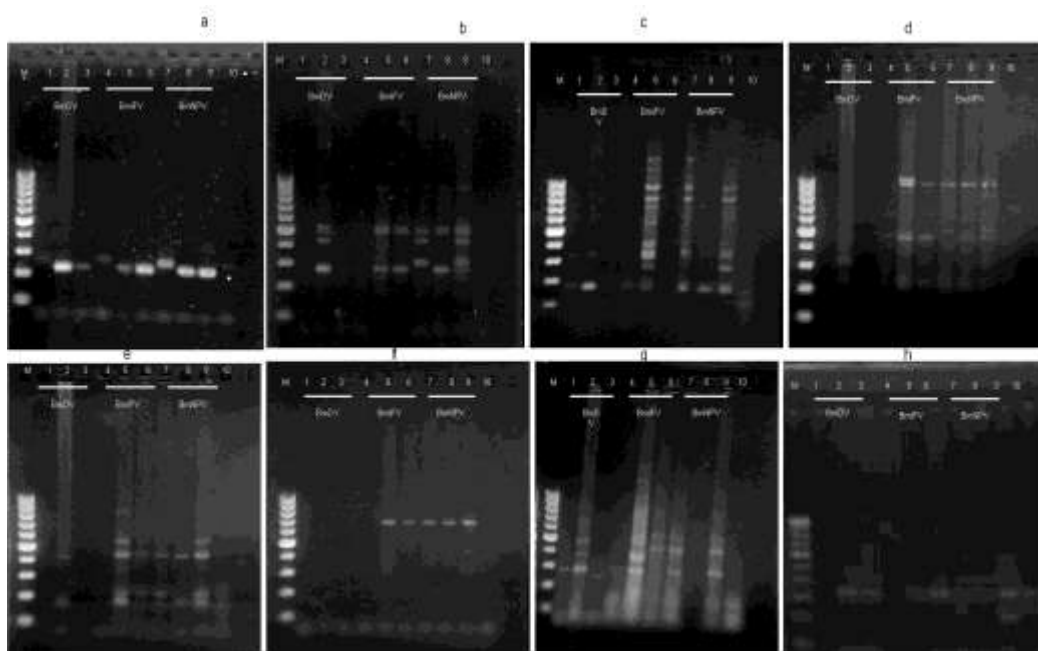


Figure 1. Agarose gel profile of PCR amplicons for 8 SSR marker genes of multi-viral tolerant silkworm breeds. a. IDH216, b. PTP284, c. DPP150, d. ATK285, e. ANK165, f. ATT700, g. LIP285, h. GDH306. M. 100 bp ladder, 1. ♀ HBM10-MVT, 2. ♂ PAM117-MVT, 3. ♀ HBM10-MVT X ♂ PAM117-MVT, 4. ♀ HBM10-MVT, 5. ♂ PAM117-MVT, 6. ♀ HBM10-MVT X ♂ PAM117-MVT, 7. ♀ HBM10-MVT, 8. ♂ PAM117-MVT, 9. ♀ HBM10-MVT X ♂ PAM117-MVT, 10. Double Hybrid - RDIN1.

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