Int. J. Indust. Entomol. Vol. 13, No. 2, 2006, pp. 109~112

[Note]

International Journal of *Industrial Entomology*

Identification of Productive Mulberry Silkworm Hybrids Resistant to Densonucleosis Virus Type 1 (BmDNV1)

P. Sudhakara Rao*, B. Nataraju, M. Balavenkatasubbaiah and S. B. Dandin

Central Sericultural Research and Training Institute, Mysore - 570008, India

(Received 22 June 2006; Accepted 22 November 2006)

The use of commercial silkworm hybrids resistant to important silkworm diseases is economical and better option particularly in tropical areas. This necessitated the evolution of productive bivoltine silkworm breeds non-susceptible to $BmDNV_1$. Non-susceptibility to $BmDNV_1$ infection was found to be controlled by a single recessive gene, nsd-1 or a dominant gene, *Nid*-1. A major dominant/recessive gene confers resistance to $BmDNV_1$ from potent donor parents have been transferred to 10 productive but susceptible bivoltine silkworm strains through conventional breeding methods. By utilizing these breeds prepared 25 hybrids (5×5) and hybrid evaluation was carried out to identify most promising hybrids resistant to $BmDNV_1$. All these hybrids are inoculated with BmDNV1 inoculum along with productive control hybrid CSR2×CSR 4 and reared under standard rearing procedure. Based on inoculated rearing and test reeling results, two most promising hybrids (CSR18DR×CSR29DR and CSR21DR×CSR50DR) were selected for commercial exploitation. The selected hybrids have shown a survival rate of >85% with productive traits, where as control hybrid have shown 11.1% survival with inferior cocoon traits. The methodologies adopted were discussed.

Key words: Hybrid evaluation, *Bombyx mori* L, $BmDNV_1$, productive bivoltine hybrids

Introduction

The utilization of disease resistant varieties is the most economical and effective way of disease management in silkworm cocoon production. A survey conducted by this laboratory in some parts of Karnataka state, which is a major silk producing state in India has revealed that, of the total incidence of flacherie caused by non-occluded virus is about 43.63% mostly due to *Bombyx mori* Infectious flacherie virus (BmIFV) and *Bombyx mori* Densonucleouses virus (BmDNV) (Nataraju *et al.*, 1998). Rearing of resistant silkworm stocks against BmDNV seems to be effective for protection against BmIFV too (Watanabe, 1994). The densonucleousis virus (DNV) infects the midgut epithelium and multiplies in the nuclei of columnar cells of silkworm. The infected columnar epithelium cells with hypertrophied nuclei are readily discharged into the gut lumen and the alimentary canal is found to be pale yellow in colour and almost devoid of any contents (Maeda and Watanabe, 1978; Abe *et al.*, 1993). Non-susceptibility to $BmDNV_1$ infection was found to be controlled by a single recessive gene, nsd-1 (Watanabe and Maeda, 1981) or a dominant gene, Nid-1 (Eguchi *et al.*, 1986). Thus an attempt has been made at this institute to breed productive bivoltine breeds and identify most promising hybrids resistant to BmDNV1 for commercial exploitation resulted in selection of two (CSR18DR×CSR29 DR and CSR21DR×CSR50DR) promising hybrids.

Materials and Methods

Seventy silkworm breeds available in this institute germplasm collection, which includes 30 of diapausing and 40 of non-diapausing group were screened to know their susceptibility status against $BmDNV_1$ and identified 3 resistant donor breeds (H330, C.Nichi and A). Their genetic inheritance pattern was established (Ratnasen *et*

*To whom the correspondence addressed
Central Sericultural Research and Training Institute, Mysore - 570008, India. Tel: +91-821-2362571; Fax: +91-821-2362845; E-mail: parimi_rao@yahoo.com

al., 2004). The resistance character was Introduced to ten productive bivoltine silkworm breeds, which include 5 ovals *viz.,* CSR2, CSR 18, CSR 21, CSR 27, and CSR 35 and 5 dumbbell breeds, CSR 4, CSR 5, CSR 28,CSR 29 and CSR 50 through conventional breeding methods (Ratnasen *et al.*, 2005).

These five oval and five dumbbell breeds resistant to $BmDNV_1$ were utilized to prepare 25 hybrids. Composite layings were brushed in mass. Three hundred freshly moult out III instar larvae each in three replications of 25 hybrids were separated and inoculated with $BmDNV_1$ inoculum along with control hybrid CSR2 × CSR 4 and their parents. $BmDNV_1$ stock solution was prepared by extracting the infected mid-gut of larvae and confirmed its identity by immunodiffusion method using anti $BmDNV_1$ antibody. For utilization of screening the stock inoculum 10^{-1} (10%) was diluted to 10 times to obtain 10^{-2}.

Rearing was conducted twice during March-April and May - June, 2005. The same number of larvae in three replications each of 25 hybrids and control hybrid was continued under normal rearing conditions. Data were recorded on eight important economic traits such as pupation rate, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length, reelability, raw silk percent and neatness. The inoculated rearing results and test reeling data obtained was considered to select promising hybrids through index score method (Singh and Chaudhary, 1979; Suresh Kumar *et al.*, 2005). The range of variation in each character has been studied using suitable class intervals and the rearing of variability with regard to each character is classified in to three groups with suitable index scoring. Hybrid combinations with relatively higher index value were considered to have greater economic value. Thus two most promising hybrids (CSR18DR × CSR29DR and CSR21DR × CSR50DR) were selected for commercial exploitation.

Results

The performance of breeding lines on inoculation is presented in Table 1. The data indicated that the breeding lines have shown >75% survival and with productive cocoon traits of >22% shell ratio when challenged with $BmDNV_1$. The data pertaining to short-listed 5 hybrids were presented in Table 2. All the 5 hybrids have shown >80 % survival on inoculation. The cocoon weight varied from 1.6-1.76 g with a shell ratio of >22%. But the two most promising hybrids (CSR18DR × CSR29DR and CSR21DR × CSR50DR) have shown a survival of >85% and cocoon shell ratio of >23%. The reeling parameters have indicated their productiveness. On contrary the productive control hybrid CSR2 × CSR 4 have shown 11.1% survival with a shell ratio of. >22% where as reeling parameters are inferior to selected hybrids (Table 3).

Discussion

Due to fluctuating climatic conditions, inadequate silkworm disease management practices and poor quality mulberry leaf, frequent crop losses are witnessed especially due to flacherie disease with the farmers in tropical areas. The productive commercial hybrids presently used by farmers are susceptible to flacherie disease and it is felt that there is an urgent need to develop bivoltine silkworm hybrids resistant to these conditions with increased productivity. In the present study, an attempt made to introduce recessive/dominant resistant genes available in the donor parents to productive, but susceptible bivoltine silkworm strains is successful. The resultant lines are resistant to BmDNV1 and productive. As could be seen from the Tables 1 &2 that the survival

Table 1. Performance of breeding lines of sib mated population by BC6S3

Breed	Fecundity (no.)	Pupation rate (%)	Cocoon weight (g)	Shell weight (cg)	Shell ratio (%)	Filament length (m)	Raw silk (%)
CSR2DR	548	75.0	1.668	37.5	22.5	984	16.6
CSR27DR	518	76.2	1.537	35.1	22.9	936	18.0
CSR35DR	529	75.1	1.660	38.0	22.7	941	16.5
CSR21DR	526	76.0	1.614	38.3	23.7	920	16.5
CSR 23DR	508	77.0	1.583	33.0	20.8	968	16.1
CSR4 DR	523	78.0	1.681	38.0	23.5	986	16.9
CSR5DR	520	75.0	1.702	38.4	22.6	911	17.0
CSR28DR	516	76.0	1.599	35.4	22.4	966	15.4
CSR50DR	517	77.4	1.576	36.0	22.8	963	17.1
CSR18DR	516	78.5	1.520	33.0	21.6	836	16.1

Table 2. Performance of 5 selected hybrids under inoculated conditions (Mean of 2 trials)

Hybrid	Pupation rate (%)	Cocoon weight (g)	Shell weight (cg)	Shell ratio (%)	Filament length (m)	Reelability (%)	Raw silk (%)	Neat. (p)
1.CSR18DR × CSR29DR	86.2	1.706	40.0	23.4	991	82.2	17.0	93
2.CSR27DR × CSR29DR	82.3	1.765	41.5	23.5	980	81.8	17.2	93
3.CSR18DR × CSR28DR	81.2	1.623	37.5	23.0	985	85.5	17.2	93
4.CSR21DR × CSR50DR	85.0	1.753	41.0	23.3	977	81.7	17.5	92
5.CSR23DR × CSR50DR	83.5	1.675	38.3	22.8	996	85.0	17.6	92
CSR2 × CSR4 (control)	11.1	1.641	37.7	22.9	885	77.2	16.8	92
CD at 5%	1.29	0.096	2.0	0.9	12.2	2.3	0.7	--
CV %	1.26	3.23	2.93	2.24	0.72	2.01	2.32	1.20

Table 3. Performance of two promising hybrids under inoculated conditions (Mean of 2 trials)

Hybrid	Pupation rate (%)	Cocoon weight (g)	Shell weight (cg)	Shell ratio (%)	Filament length (m)	Reelability (%)	Raw silk (%)	Neat. (p)
1.CSR18DR × CSR29DR	86.2 (66.8)	1.706	40.0	23.4	991	82.2 (65.0)	17.0	93
2.CSR21DR × CSR50DR	85.0 (65.1)	1.753	41.0	23.3	977	81.7 (64.7)	17.5	92
CSR2 × CSR4 (control)	11.1 (19.4)	1.641	37.7	22.9	885	77.2 (61.4)	16.8	92
CD at 5%	1.5	0.155	--	1.1	15.2	1.6	0.539	--
CV%	1.38	3.19	3.28	3.29	5.32	1.09	1.39	1.13

(Values in parenthesis are angularly transformed)

rate under inoculated rearing conditions in both hybrids and their parents are higher when compared to normal control hybrid. Very few reports are available on the breeding of silkworm stocks resistant to densonucleosis virus (Eguchi *et al.*, 1998; Chen keping *et al.*, 2001; Sivaprasad and Chandrasekharaiah, 2003). Since a single recessive/dominant gene controls the complete resistance to BmDNV, it is possible to transfer the genes of disease resistance easily into excellent productive silkworm varieties.

The purpose of breeding with pathogen load is to create the conditions which can make the genotype of disease resistance appear completely in order to select them for rearing environment where inadequate disease management practices are prevailing. The genotype one with disease resistance gene always has much more chance to survive. Most of the small and marginal farmers in India are unable to practice complete disease management practices due to their socio-economical conditions where these breeds are better option and equipped to survive. This will facilitate the introduction of bivoltine sericulture technology in tropical areas more effectively.

Acknowledgement

The authors are thankful to Mr. A. K. Palit, Assistant Director (Economics) of this institute for analyzing data.

References

Abe, H., T. Shimada, K. Kobayashi, S. Maeda, T. Yokoyama, T. Oshiki and M. Kobayashi (1993) Detection of Densonucleosis virus in the silkworm, *Bombyx mori* from fecal specimens by polymerase chain reaction. *J. Seric. Jpn.* **62**, 376-381.

Chen, K., Li, M., Yao, Q., Lin, S. and Hou, C. (2001) Breeding of new germplasms of silkworm (*Bombyx mori*) resistant to Zhenjiang (China) strain of Densonucleosis Virus (DNV-Z). *Sericologia*, **41**, 21-27.

Eguchi, R., Y. Furuta and O. Ninaki (1986) Dominant non-susceptibility to densonucleosis virus in the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.*, **55**, 177-178.

Eguchi, R., W. Hara, A. Shimazaki, K. Hirota, M. Ichiba, O. Ninagi and K. Nagayasu (1998) Breeding of the silkworm race "Taisei" non-susceptible to a densonucleosis virus type

1. *J. Seric. Sci. Jpn.* **67**, 361-366.

Maeda, S. and H. Watanabe (1978) Immunofluoresence observation of the infection of densonucleosis virus in the silkworm, *Bombyx mori. Jpn. J. Appl. Entomol. Zool.* **22**, 98-101.

Nataraju, B., R. K Datta, M. Baig, M. Balavenkatasubbaiah, M. V. Samson and Sivaprasad, V. (1998) Studies on the prevalence of nuclear polyhedrosis in sericultural areas of Karnataka. *Indian J. Seric.* **37**, 154-158.

RatnaSen, B. Nataraju, B., M. Balavenkatasubbaiah, V. Premalatha, V. Thiagarajan and R. K. Datta (2004) Resistance of *Bombyx mori* densonucleosis virus type1 inheritance pattern in *Bomby mori. Ind. J. Indust. Entomol*, **9**, 35-40.

Ratna Sen, B. Nataraju, M. Balavenkatasubbaiah, P. Sudhakara Rao, V. Thiagarajan and S. B. Dandin (2005) Breeding of productive silkworm strains resistant to densonucleosis virus type1 (BmDNV1*). Proc. of Nat. Conf. on Tropical Sericulture for Global Competitiveness* (5-7, Nov 2003) p. 113-117.

Singh, R. K. and B. D. Chaudhary (1979) Classificatory analysis. In *Biometrical methods in quantitative genetic analysis*, Kalyani Publishers, Ludhiana, India p. 210-214.

Sivaprasad, V. and Chandrashekharaiah (2004) Strategies for Breeding Disease Resistant Silkworms. In: *Concept papers in Mulberry Silkworm Breeders Summit.* 18-19th July, 2003; p. 69-76. Printed and Published by Dr. Chandrashekaraiah, Director, APSSRDI, Hindupur. Andhra Pradesh.

Suresh Kumar, N., A. K. Palit, G. V. Kalpana and P. G. Joge (2005) New index method for evaluation of silkworm hybrids of *Bombyx mori* L. *Proc.of Nat. Conf. on Tropical Sericulture for Global Competitiveness* (5-7, Nov 2003) p. 74-78.

Watanabe, H. and S. Maeda (1981) Genetically determined non-susceptibility of the silkworm, *Bombyx mori* to infection with a densonucleosis virus (Densovirus). *J. Invertbr. Pathol.* **38**, 370-373.