Virus resistant transgenic silkworm, the status of its regulatory field trials, and progress towards regulatory approval

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Collaborating Institutes

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Ministry of Textiles
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Sericulture statistics in India

Silkworm

Economics

- 6 million rural people are in sericulture spread over 60,000 villages
- 2nd largest producer of silk in the world
- Silk production is ~18,000 tonnes

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Pathogens affecting sericulture

Staphylococcus

Densonucleosis

Nuclear polyhedrosis

Viral diseases	Nuclear polyhedrosis (BmNPV) Cytoplasmic polyhedrosis (BmCPV) Infectious flacherie (BmIFV) Densonucleosis (BmDNV)
Bacterial diseases	Bacterial disease of digestive organ (*Streptococci sp., Staphylococci sp.*) Septicemia (*Serratia sp.*) Sotto (*Bacillus thuringiensis sp.)*
Fungal diseases	Muscardine (*Beauveria bassiana, Spicaria sp.*) Aspergillosis *(Aspergillus sp.)*
Microsporidian diseases	Pebrine (*Nosema bombycis* and other microsporiadian sp.)

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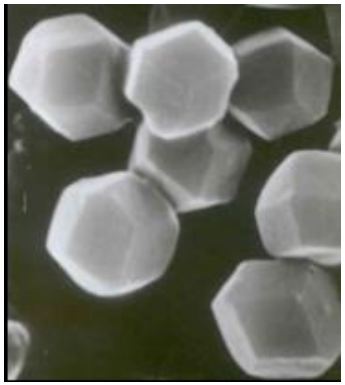
Nucleopolyhedrosis virus causes major crop loss

B. mori **larvae (Control)**



Occlusion Bodies of BmNPV

BmNPV infected larvae

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Life cycle of the domesticated silkworm *Bombyx mori*

Moths

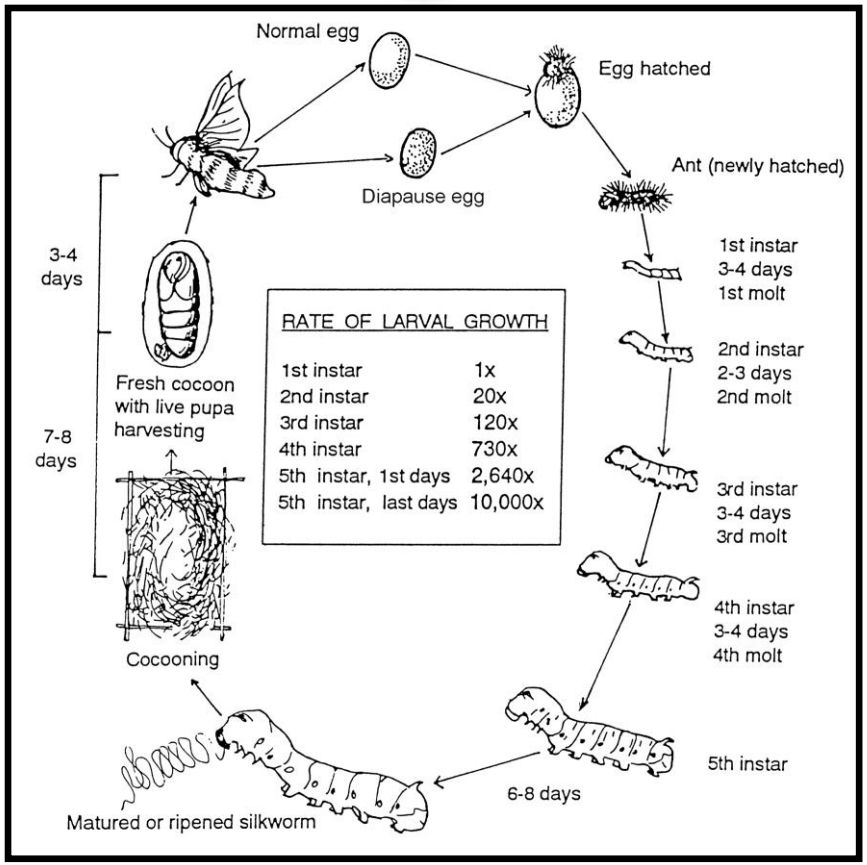
Eggs

Pupa

Larvae

Cocoons

Mature silkworm



Silkworm Life Cycle

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Limitations to conventional breeding methods

- As of now there are no effective methods to control the disease
- Traditional methods have many short comings due to polygenic nature of resistance
- Biotechnological tools are only option to meet the growing demand for quality silk
- **Transgenic methods based on RNAi** were reported to block the viral infection in many cases

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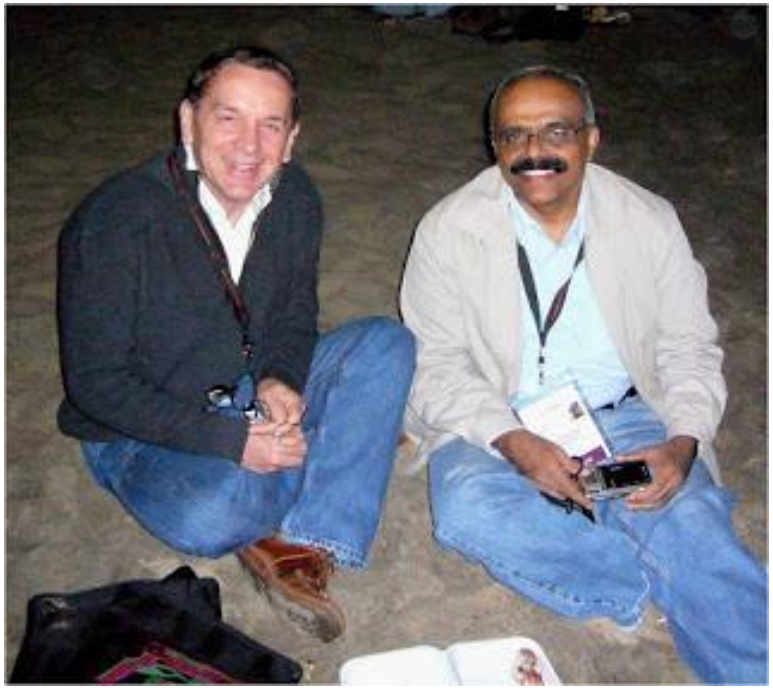
Genesis of RNAi-based Nuclear Polyhedrosis Virus (NPV) resistant transgenic silkmoths

- In 2006, Dr. Nagaraju's group at CDFD developed transgenic silkworms expressing dsRNA for **a single essential viral gene (ie-1)**

- Later, it was speculated that by expressing dsRNA for **multiple essential viral genes** in the silkworm would elicit a more stable defense against the virus



Dr. J. Nagaraju
Prof Pierre Couble
(Indo France collaborative project)

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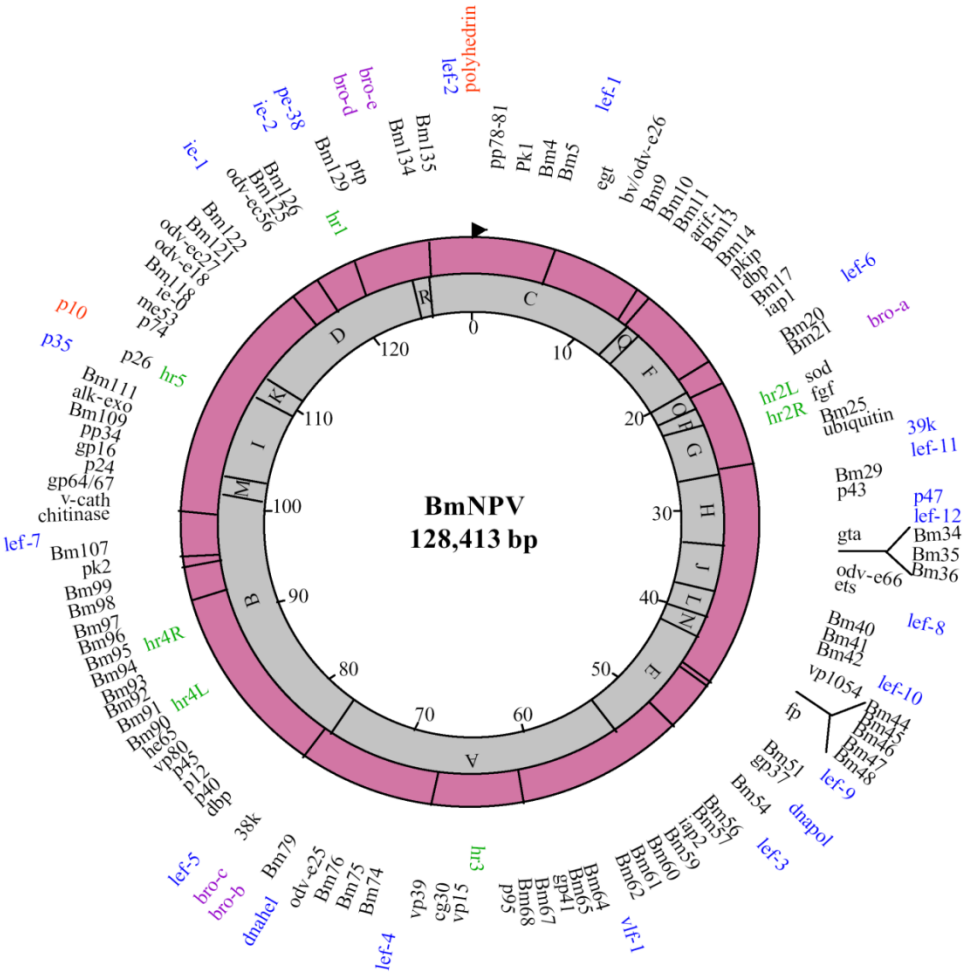
Genome organization of *Bm*NPV



Green – potential origins of replication and/or enhancers for late gene replication

Blue – *Lefs* involved in late gene replication

Orange – required for high levels of protein expression. Ex. Polyhedrin and P10

Viral replication

ie1 : is a transregulator, activates number of late effector factors

lef1: is a DNA primase of the replication complex

lef3 : has ssDNA binding capability

Virus transmission

p74 : structural protein, required for infection (viral infectivity)

Ikeda *et al.* 2006

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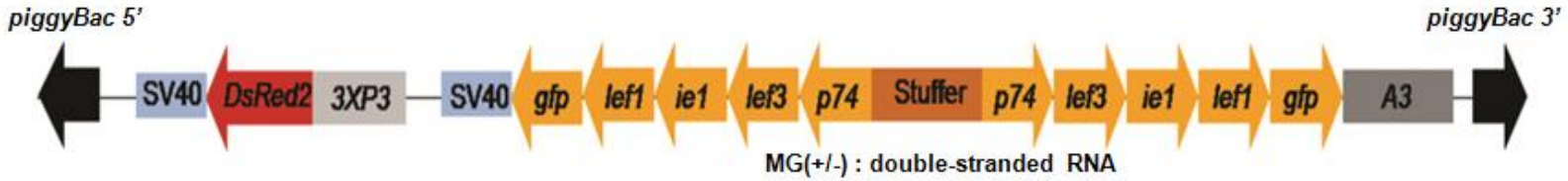
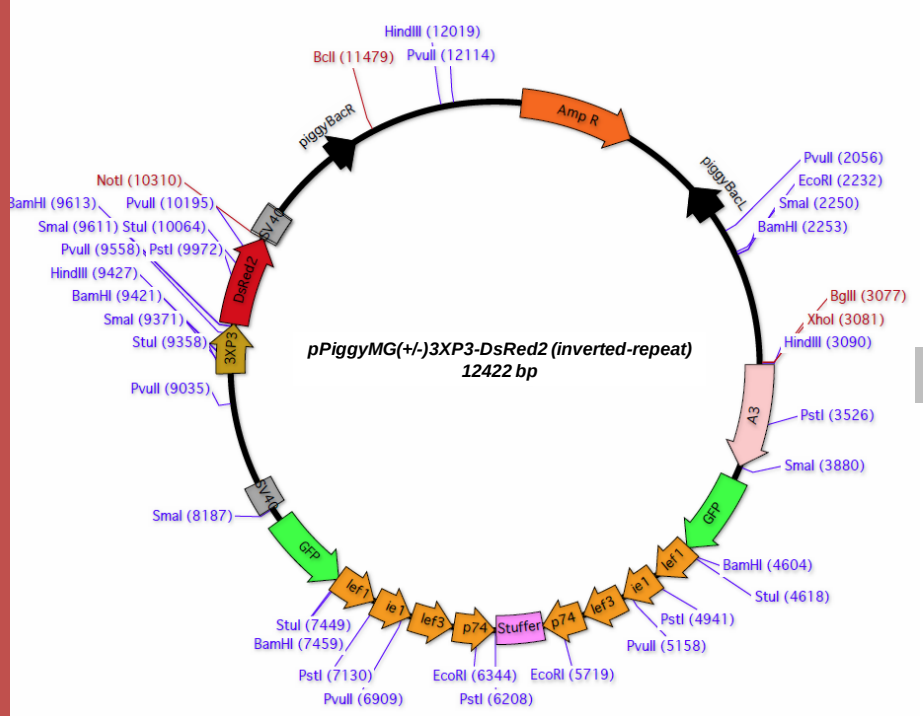
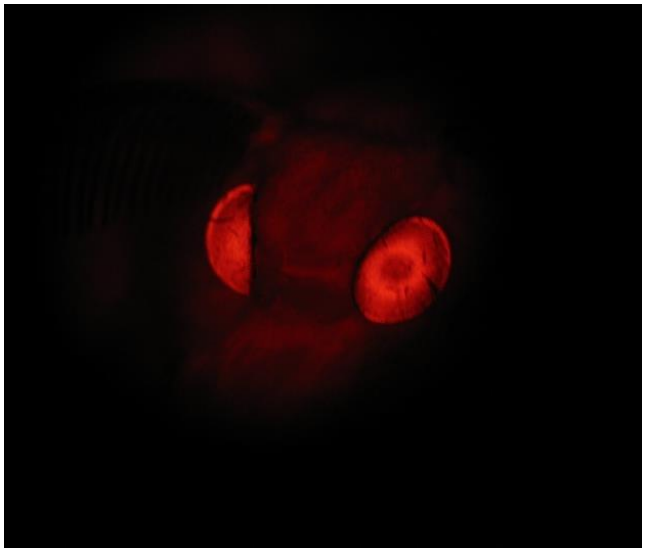
PiggyBac based RNAi vectors with multiple viral target genes

- *PiggyBac* based germline transgenesis was used for the production silkworm lines expressing dsRNA for multiple essential viral genes





Transgenic silkmoth expressing dsRed colour in eyes



Genetics 2013

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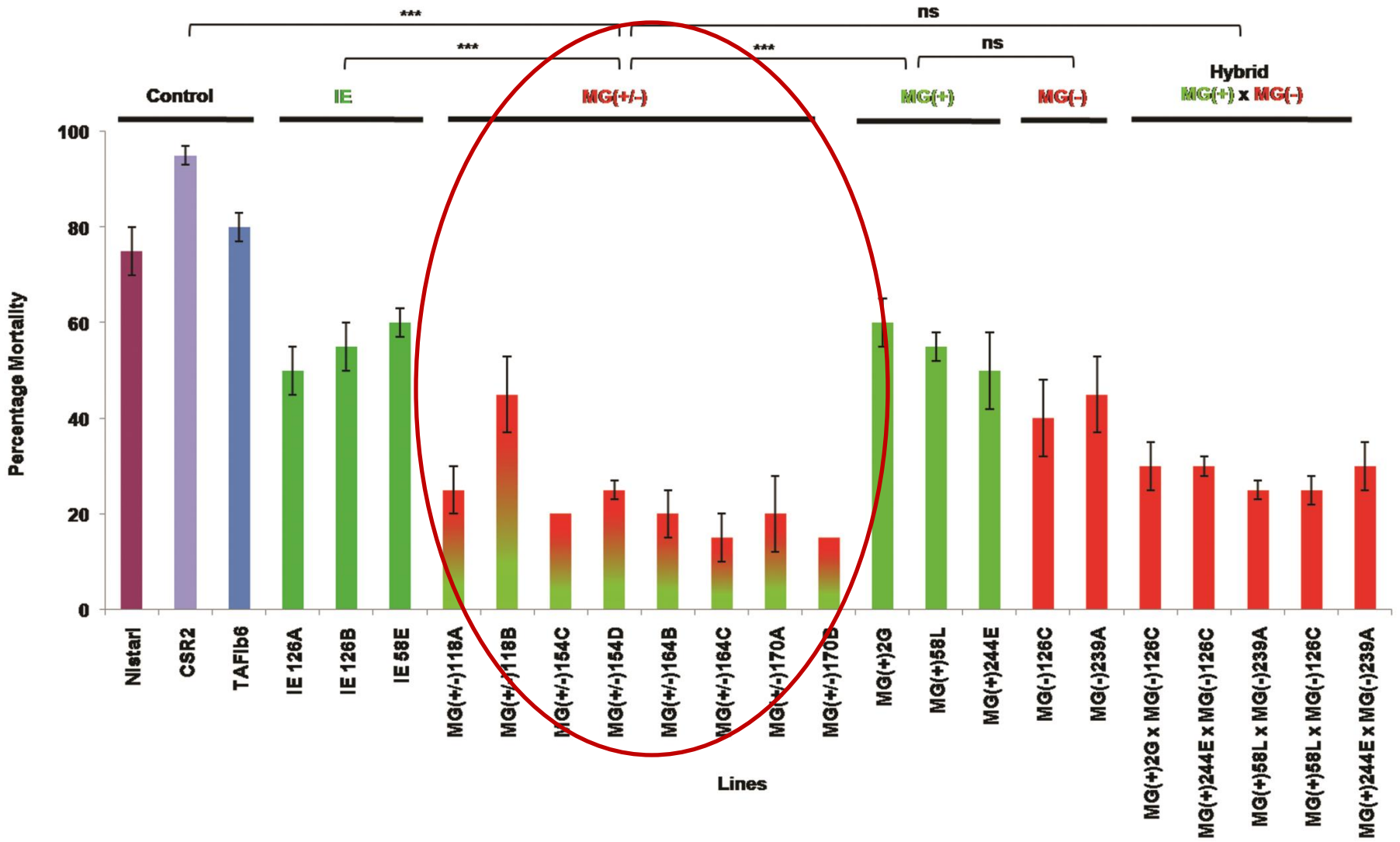
BmNPV resistance in transgenic Nistari lines of the silkworm



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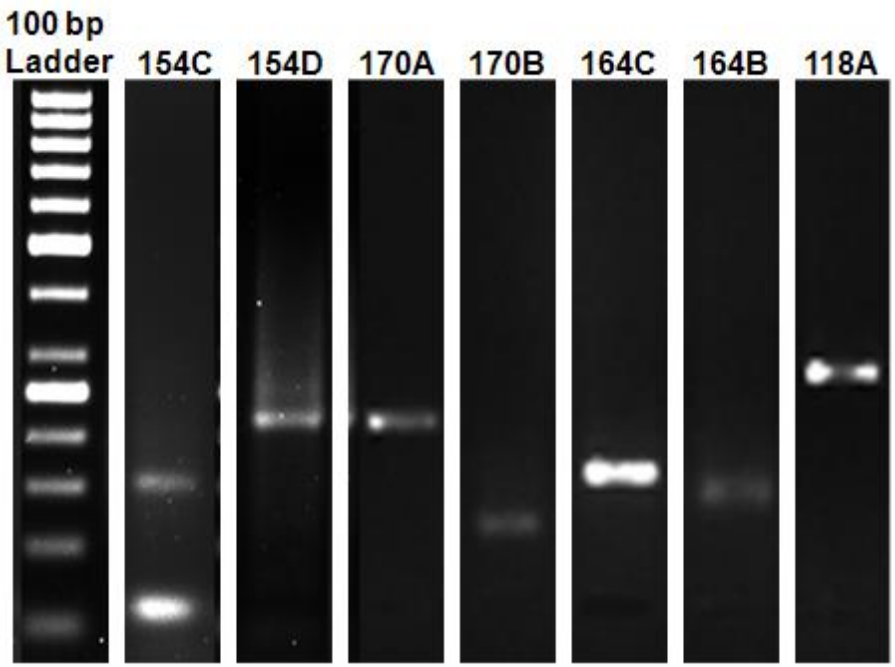
Copy numbers and site of insertion of transgenes in transgenic lines were characterized using Transposable Element Display assay



❑ **Nistari transgenic lines (170A, 170B, 164C, 164B, 154C, 154D and 118A) showing single copy insertions**

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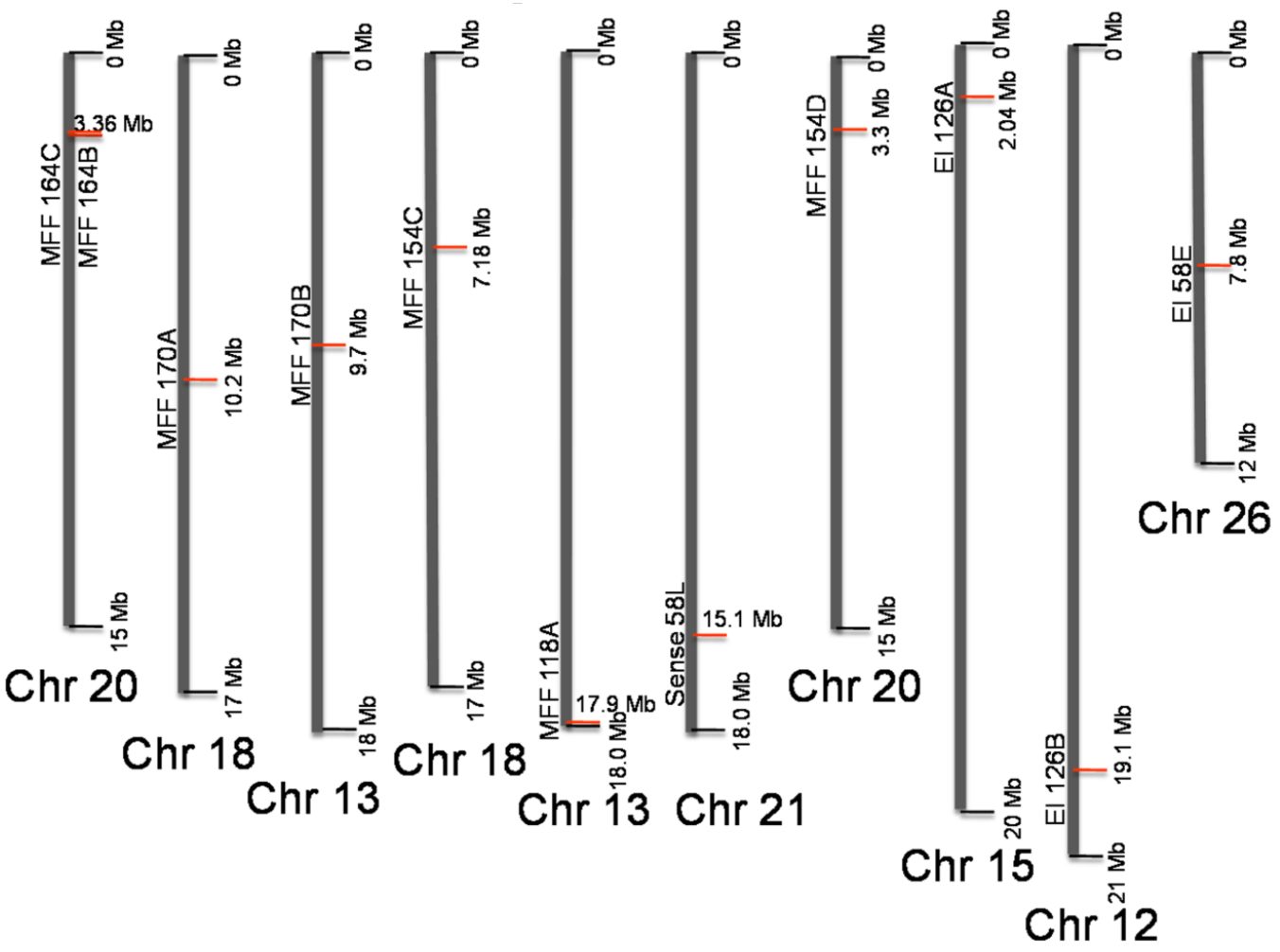
Chromosomal location of the transgene



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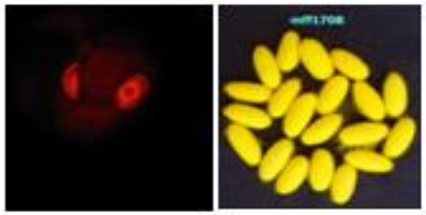
Transfer of BmNPV resistance from transgenic Nistari to commercial CSR2 line



Transgenic Nistari MG(+/-) transgenic line, Baculovirus resistant, polyvoltine with yellow pointed oval cocoons

X

Commercial CSR2 line, Baculovirus susceptible, bivoltine with white oval cocoon

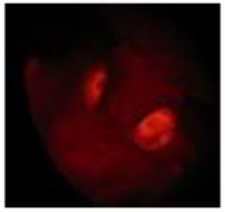
X

Back Cross

F_1 Select for *red eye* individuals

BC_1, BC_2, BC_3, BC_4

BC_4



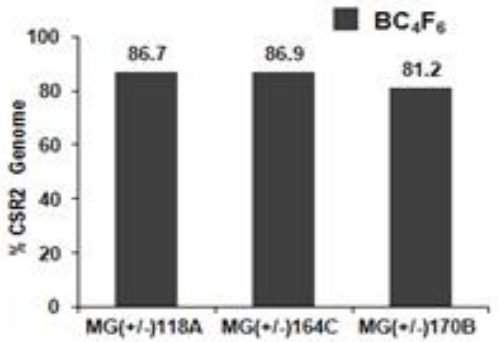
BC_4

Sib-mate

X

BC_4

BC_4F_1 - 1st Inbred generation



Marker assisted breeding

BC_4 F_{27}

Currently at generation BC_4F_{34}

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Transgenic silkworm hybrids

- Nistari and CSR2 transgenic lines were crossed to various ruling breeds to develop baculovirus resistant transgenic silkworm hybrids for field trials

Polyvoltine x Bivoltine

Pure Mysore x Transgenic CSR2 (727)

Transgenic Nistari (164C) x (SK6 x SK7)

Bivoltine x Bivoltine

Transgenic CSR2 (727) x CSR4

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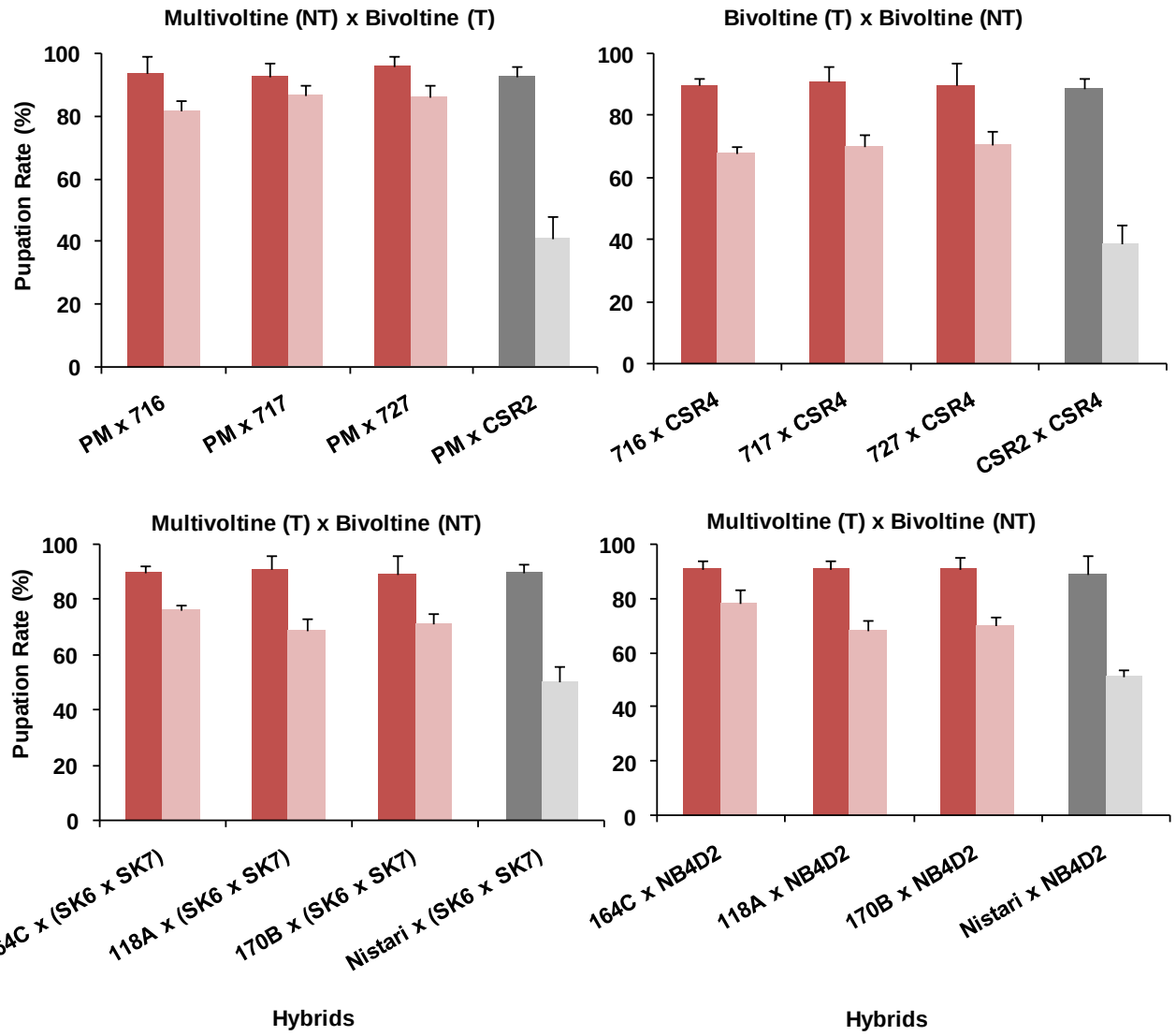
Characteristics of transgenic silkworm hybrids upon BmNPV infection



Transgenic CSR2 lines
716, 717, 727

Transgenic Nistari lines
164C, 118A, 170B

Polyvoltine strain
Pure Mysore - PM

Bivoltine strains
CSR4, NB4D2, SK6 x SK7

Red and light red bars - uninfected and infected transgenic lines

Grey and light grey bars - uninfected and infected control lines

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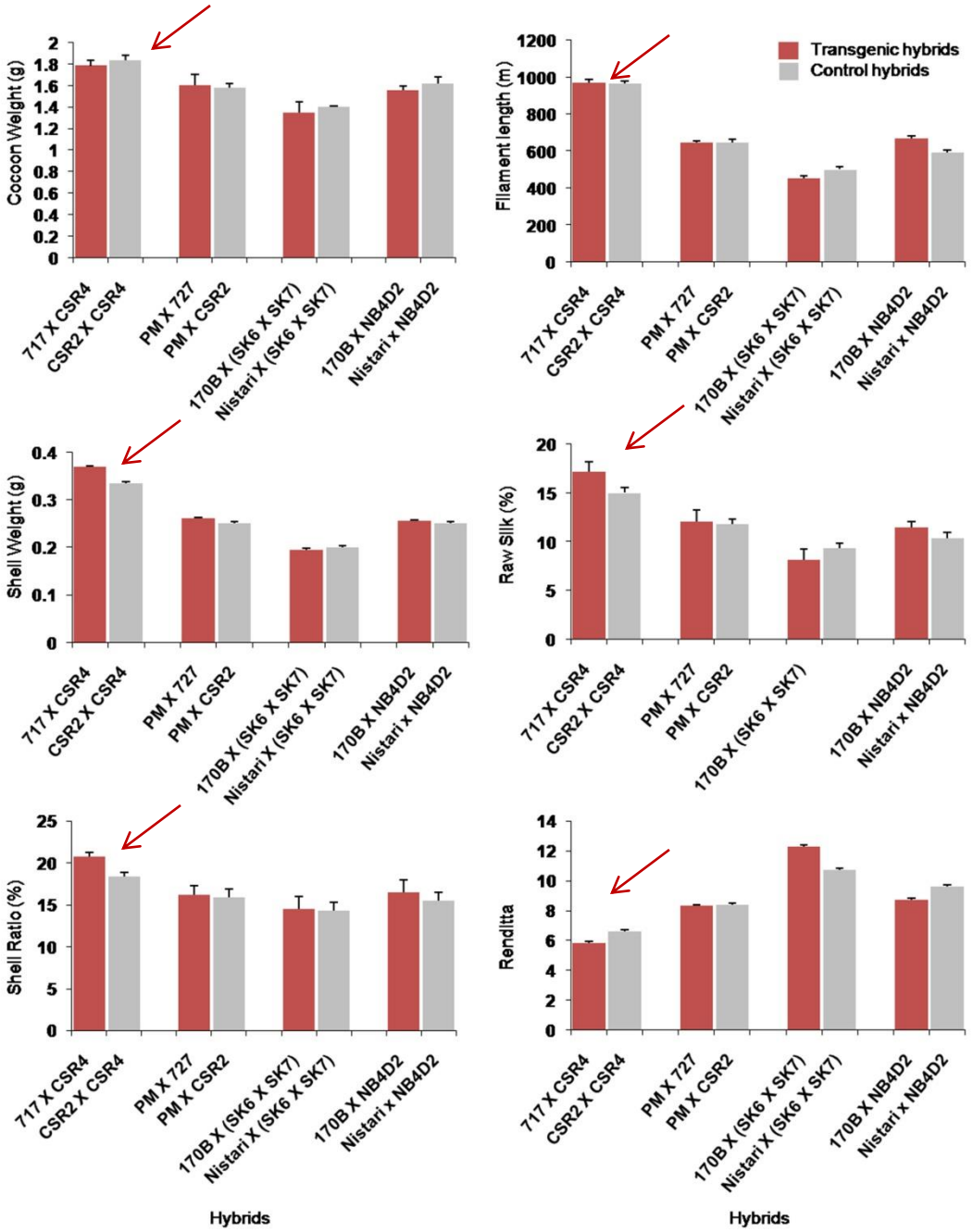
Cocoon and silk characteristics of the transgenic hybrids



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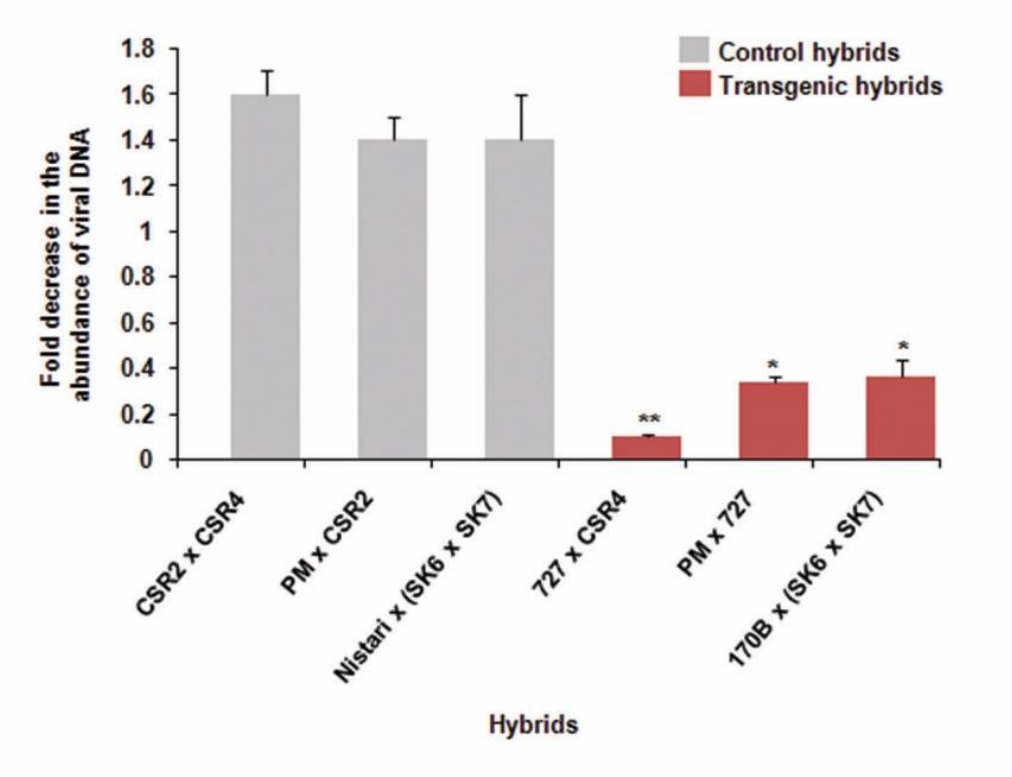
Characterization of transgenic silkworm hybrids upon BmNPV infection

Viral load in the transgenic hybrids



Quantitative PCR analysis of *Bm*NPV using *lef3*

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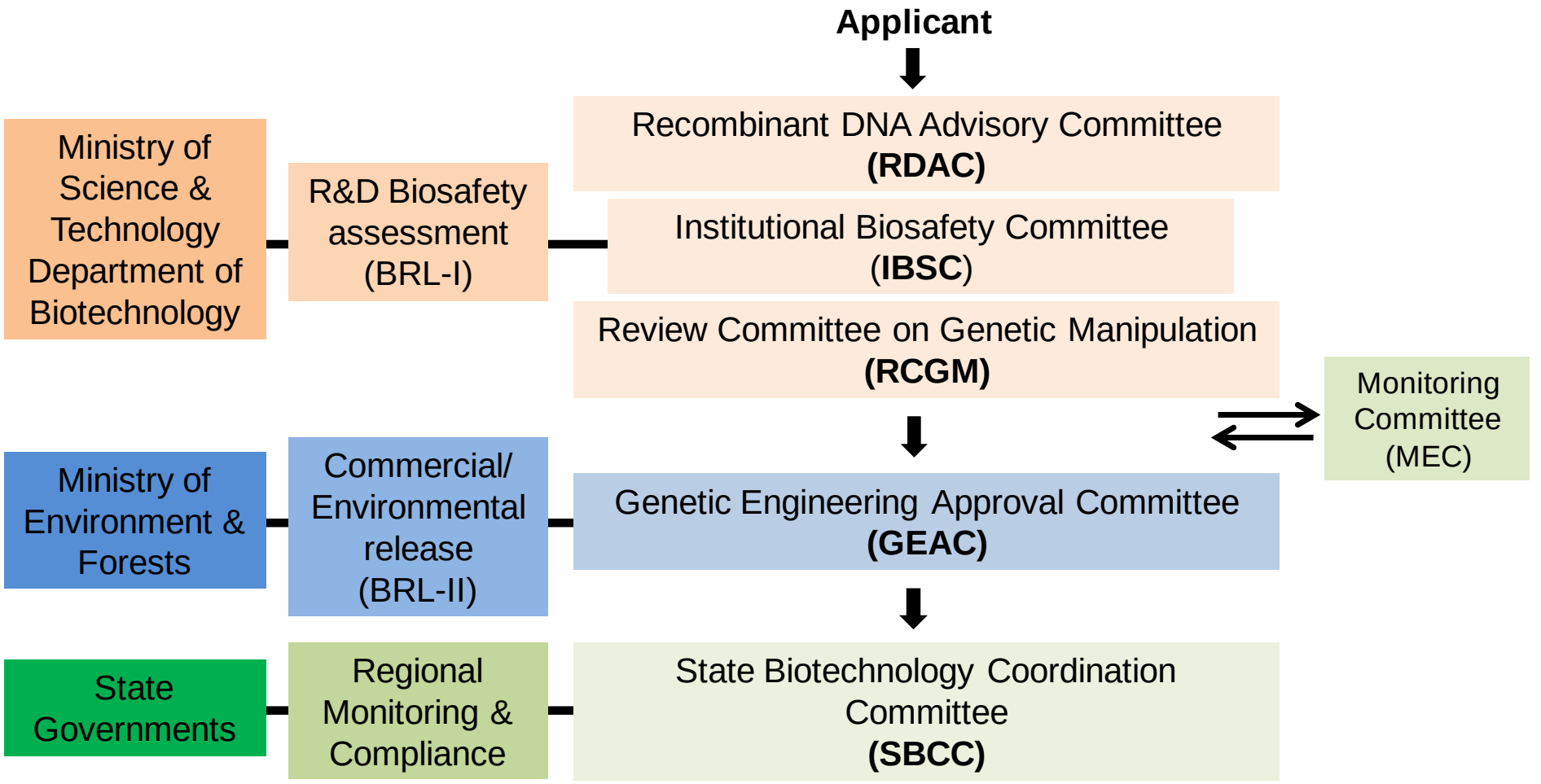
Regulatory committees in India



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Recommendations of RCGM

- In its 132nd meeting held on 25.03.2014, RCGM permitted CDFD to conduct multilocational trials in contained facilities on GE *Bm*NPV resistant *B. mori* at 4 locations in 2 phases,

- Phase I at Institutional level, and

- Phase II at farmer's level

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Standard Operating Procedures (SOPs) for GE silkworms

❑ All SOPs, guidelines, and parameters to be considered during multi-location contained trials have been formulated

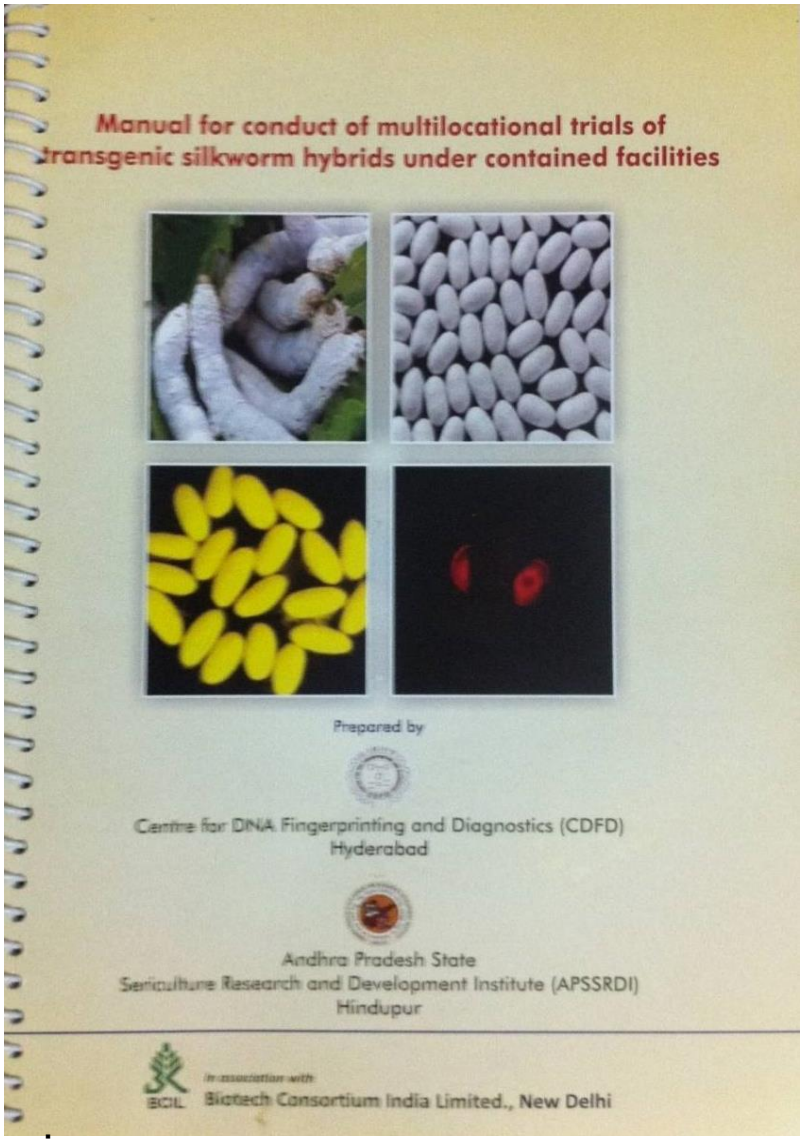
❑ Standard Operating Procedures (SOPs)

❑ Compliance recording formats

- ❑ Record of transport & transport inventory list
- ❑ Record of storage of eggs
- ❑ Record of storage inspection & inventory
- ❑ Record of brushing of eggs
- ❑ Record of rearing
- ❑ Record of harvest/termination
- ❑ Record of post rearing activities
- ❑ Record of corrective action, if any

❑ Data recording formats

- ❑ Brushing record
- ❑ Log sheet
- ❑ Index card
- ❑ Rearing performance
- ❑ Reeling performance
- ❑ Quality performance of raw silk reeled



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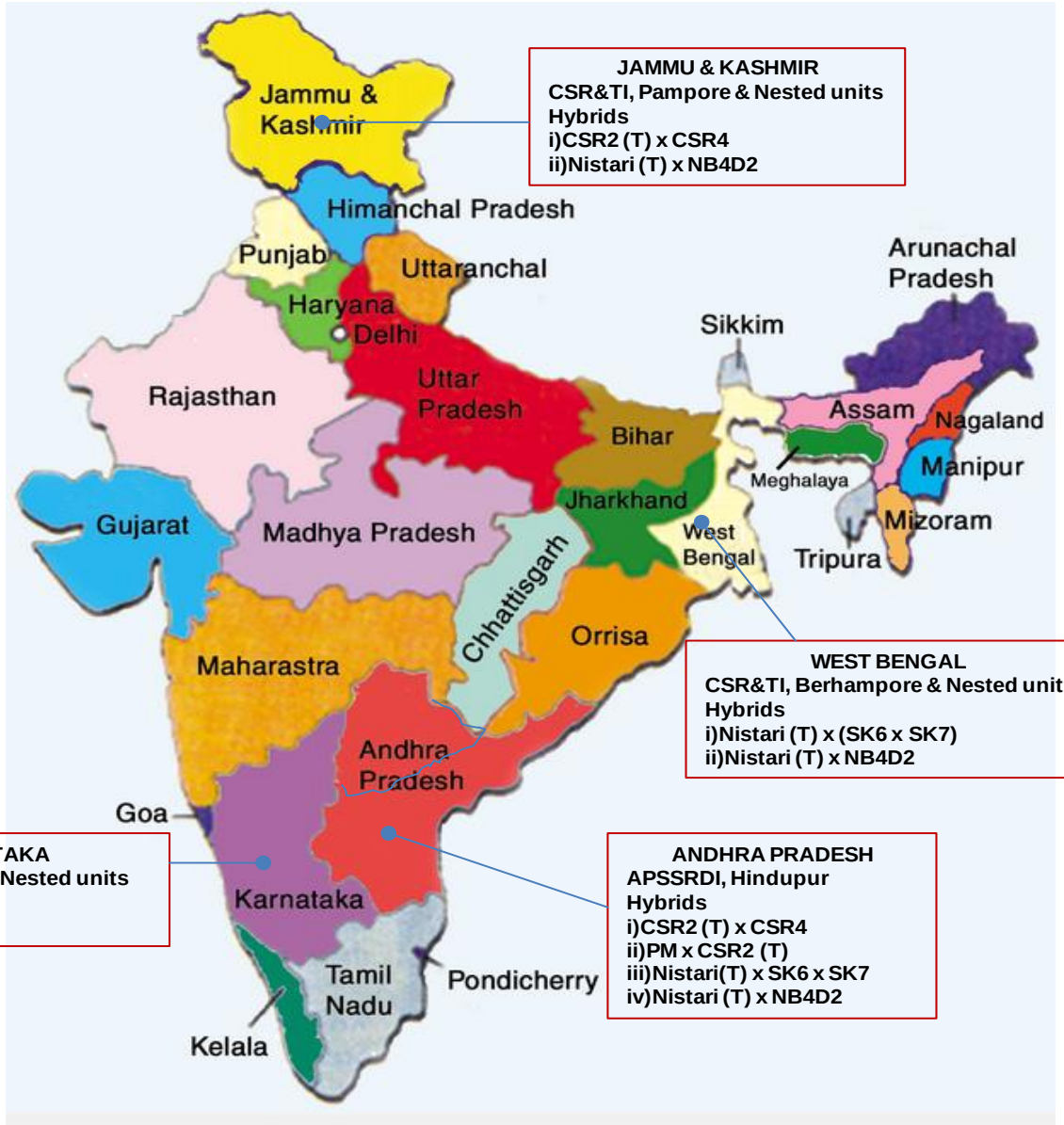
Multilocational contained trial locations



JAMMU & KASHMIR
CSR&TI, Pampore & Nested units
Hybrids
i)CSR2 (T) x CSR4
ii)Nistari (T) x NB4D2

WEST BENGAL
CSR&TI, Berhampore & Nested units
Hybrids
i)Nistari (T) x (SK6 x SK7)
ii)Nistari (T) x NB4D2

KARNATAKA
CSR&TI, Mysore & Nested units
Hybrids
i)CSR2 (T) x CSR4

ANDHRA PRADESH
APSSRDI, Hindupur
Hybrids
i)CSR2 (T) x CSR4
ii)PM x CSR2 (T)
iii)Nistari(T) x SK6 x SK7
iv)Nistari (T) x NB4D2

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Central Compliance Committee

- For monitoring field trials, a Central Compliance Committee was formed by Biosafety Support Unit of DBT

- The composition of CCC is as follows:

1. Dr. S.R. Rao, DBT, New Delhi
2. Dr. V. Siva Reddy, BSU, New Delhi
3. Dr. Gururaj Katti, IIRC, Hyderabad

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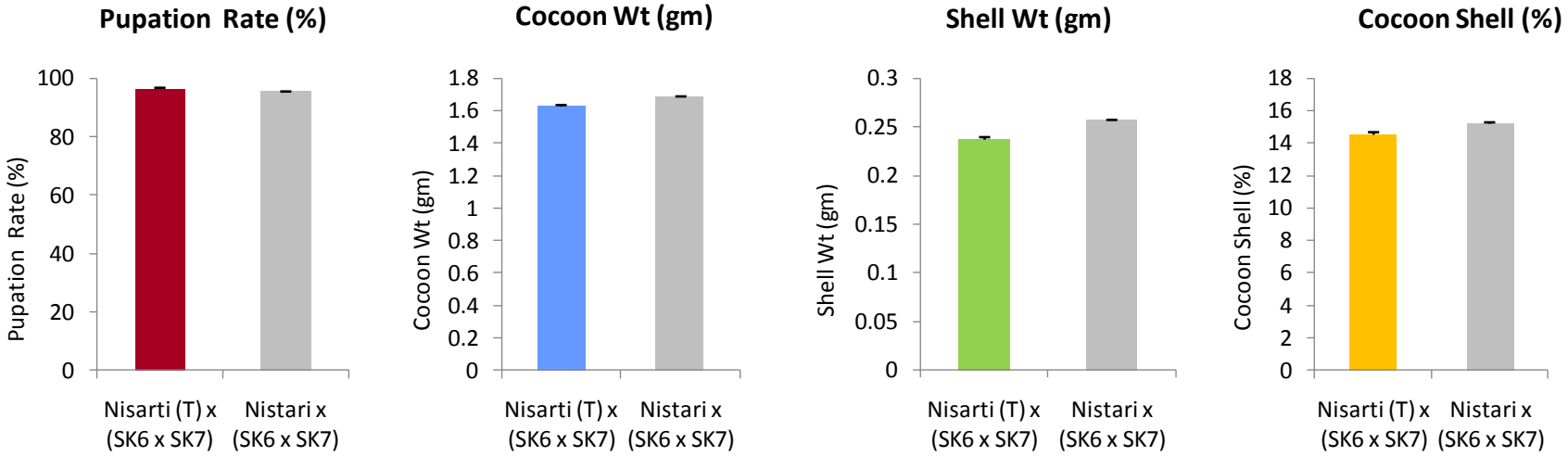
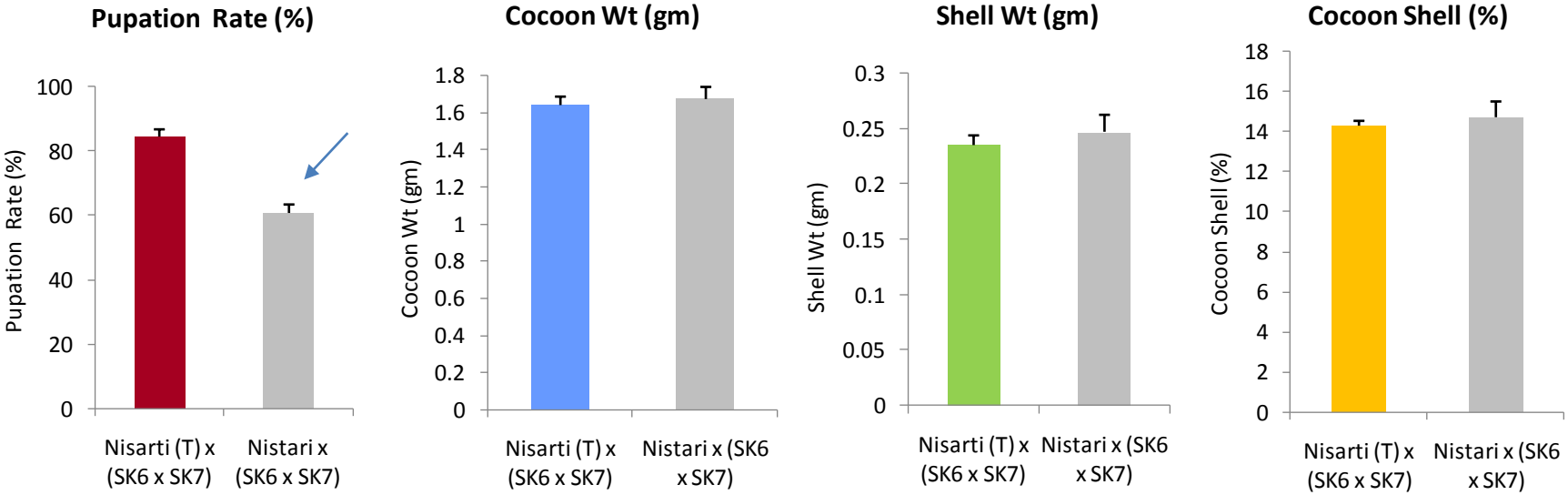
Performance of Nistari transgenic hybrids upon BmNPV challenge (Polyvoltine x Bivoltine)

Control



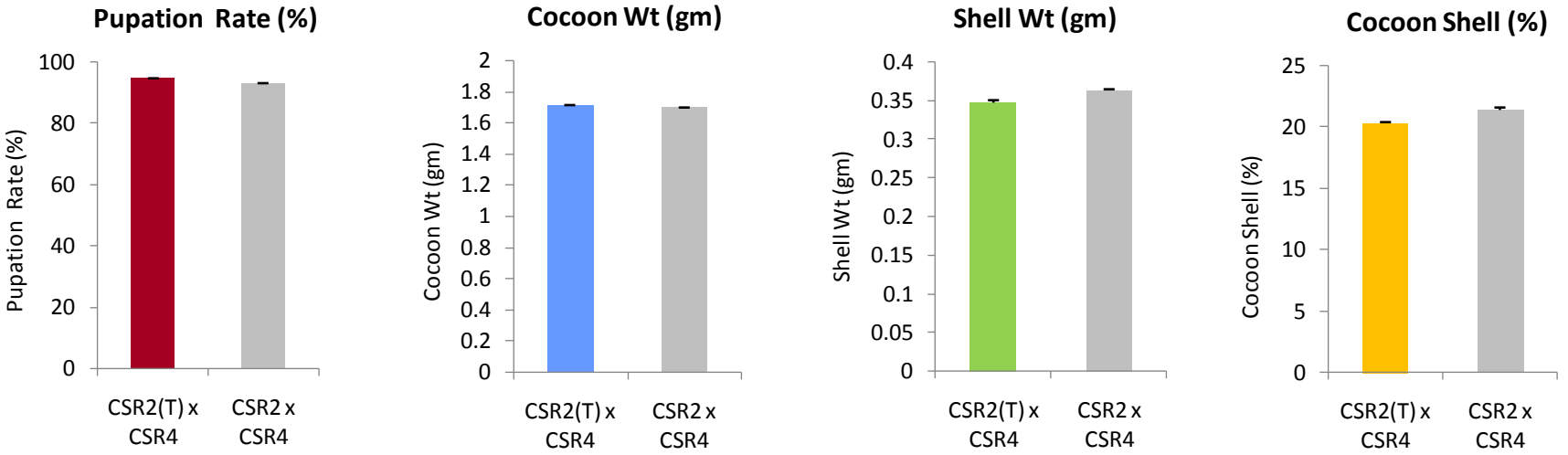
Infected



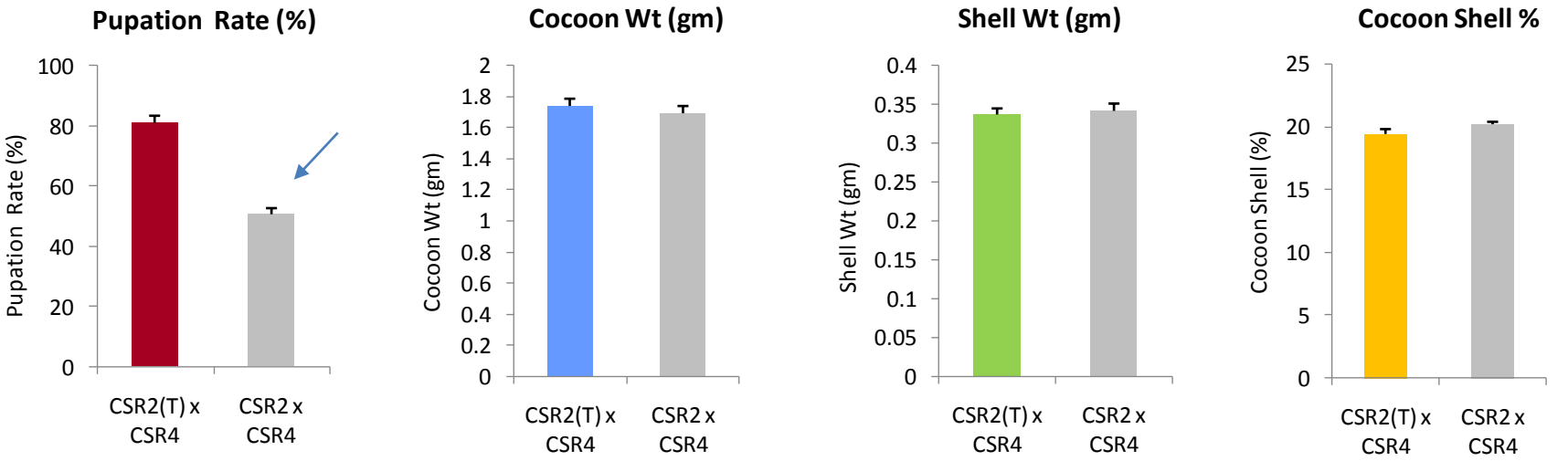
Control - grey; Transgenic – coloured

Performance of CSR2 transgenic hybrids upon BmNPV challenge (Bivoltine x Bivoltine)

Control



Infected



Control - grey; Transgenic - coloured

No environmental and biosafety issues

- Silkworms are always reared under contained conditions - can't survive outdoor
- Silkmoth can't fly out and it is a non-feeding stage
- Large scale rearing is done using only F1 hybrids. The F1 hybrids are not reproduced further
- GE silkworms can be identified with eye marker which enables easy monitoring.
- There is no functional gene incorporated in the present case

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ACKNOWLEDGEMENTS

DNA Fingerprinting and Diagnostics

Director, CDFD
Dr. C.V.E. Rajendra
Ms. B. SandhyaRani

Central Compliance Committee

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Dr. Gururaj Katti, IIRC, Hyderabad

Andhra Pradesh State Sericulture Research & Development Institute

Dr. P.J. Raju, Director
Dr. I. Basha
Mr. Ravi
Mr. Subba Rao
Ms. Nageena

Central Silk Board & its Centres

Dr. K.Vijayan, Director (Technical)
Dr. V. Sivaprasad, Director, CSR&TI, Mysore
Dr. M. Moorthy
Dr. K.Trivedy, Director, CSR&TI, Berhampore
Dr. M.K. Ghosh, Director, CSR&TI, Pampore

Biosafety Support Unit

Biotech Consortium India Limited

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Department of Biotechnology, Govt of India

- Centre of Excellence for Genetics and Genomics of Silkmoths (CoE-I)
- Biotechnology Industry Research Assistance Council - Contract Research Scheme (BIRAC CRS)

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