Virus-Resistant \((BmNPV)\) Transgenic Silkworm \(Bombyx\) mori L.  

Development, Efficacy and Biosafety Considerations  
for Commercial scale production

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*Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, India

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Laboratory of Molecular Genetics
Team Leader, DBT Centre of Excellence for Genetics and Genomics of Silkmoths
Centre for DNA Finger Printing and Diagnostics (CDFD), Hyderabad, India
LEARNING BASICS OF BIOSAFETY ISSUES....

SOUTH ASIA BIOSAFETY CONFERENCE AND WORKSHOPS
SEPTEMBER 18-20, 2013. NEW DELHI, INDIA
### SERICULTURE SCENARIO IN INDIA (2013-14)

<table>
<thead>
<tr>
<th>Mulberry Plantation (Lakh ha.)</th>
<th>2.03</th>
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<tbody>
<tr>
<td><strong>Raw Silk Production ( MT)</strong></td>
<td></td>
</tr>
<tr>
<td>Mulberry (Bivoltine)</td>
<td>2,559</td>
</tr>
<tr>
<td>Mulberry (Cross Breed)</td>
<td>16,917</td>
</tr>
<tr>
<td><strong>Sub Total (Mulberry)</strong></td>
<td>19,476</td>
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<tr>
<td><strong>Vanya (MT)</strong></td>
<td></td>
</tr>
<tr>
<td>Tasar</td>
<td>2619</td>
</tr>
<tr>
<td>Eri</td>
<td>4237</td>
</tr>
<tr>
<td>Muga</td>
<td>148</td>
</tr>
<tr>
<td><strong>Sub Total (Vanya)</strong></td>
<td>7004</td>
</tr>
<tr>
<td><strong>GRAND TOTAL</strong></td>
<td>26480</td>
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</table>
TYPES OF SILK AVAILABLE IN INDIA

Mulberry
(*Bombyx Mori* L.)

Tasar
(*Antheraea mylitta*)

Eri
(*Philosamia ricini*)

Muga
(*Antheraea assamensis*)
CSR2

• Fecundity: 500 – 550
• Cocoon Shell Percentage: 23 – 25
• Oval shape white cocoons
• Larval duration: 24 – 25 days
• Cocoon Weight: 1.7 ~ 1.9 g
• Larval colour: Bluish white without any marking on the body.
• Pupation Rate: 85 – 90%
• Raw Silk Percentage: 19 – 20
• Filament length: 1000 – 1100 m
• Neatness: 85 – 90% (points)
• Fecundity : 470 – 525
• Cocoon shell percentage: 22 – 24
• Peanut shape white cocoons
• Larval duration : 24 – 25 days
• Cocoon Weight : 1.6 ~ 1.8g
• Larval colour : Bluish white without any marking on the body.
• Pupation rate : 85 – 90%
• Raw silk percentage : 17 – 18
• Filament length : 900 – 950 m
• Neatness : 85 – 90 (points)
Productive Silkworm hybrid
CSR2 x CSR4

- Productive hybrids and easy to handle by farmers under hygienic conditions
- Hybrids with high cocoon shell ratio (24-25%) and raw silk recovery (19-20 %)
- Better fibre quality (2A –3A)
- Rearing during favourable months in southern states and spring in Jammu and Kashmir province
- Susceptible to disease

*Bm NPV*
Development of RNA Interference (RNAi) based Nuclear Polyhedrosis Virus (NPV) Resistant Transgenic Silkmoths
### Transgenic Nistari lines

<table>
<thead>
<tr>
<th>Category</th>
<th>Silkworm Stocks</th>
<th>Marker</th>
<th>Gene Insert</th>
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</thead>
<tbody>
<tr>
<td>Single Gene</td>
<td>126A, 126B, 54E</td>
<td>Green</td>
<td>ie-1</td>
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<tr>
<td>AS</td>
<td>126C, 239A</td>
<td>Red</td>
<td>PiggyBac Antisense</td>
</tr>
<tr>
<td>ABS</td>
<td>2G, 244E, 58L</td>
<td>Green</td>
<td>PiggyBac Sense</td>
</tr>
<tr>
<td>ABS x AS</td>
<td>244E.126C 58L.239A S2G.126C</td>
<td>Red/Green</td>
<td>PiggyBac Sense x PiggyBac Antisense</td>
</tr>
<tr>
<td>RNAi Control</td>
<td>Ta Fib 9</td>
<td>Green</td>
<td>Fibroin</td>
</tr>
<tr>
<td>Wild Type</td>
<td>Nm Inde</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>
Transfer of Transgenes for *BmNPV* Resistance

Plain larvae and white cocoon red eye males were backcrossed to CSR2 from BC1 onwards.

Red eye individuals sib-mated for further breeding.

Selection criteria:
- Strong RFP expression
- DNA samples for RFP amplification and CSR2 specific markers
- Per oral inoculation of *BmNPV* @ 40,000 PIB/ Larva@ 1ml per 100 larvae
- High Pupation rate
- Traits of CSR2
Baculovirus resistant transgenic silkworm hybrids

*Polyvoltine x bivoltine*
- PM x CSR2 (T)
- Nistari (T) x NB4D2

*Bivoltine x bivoltine*
- CSR2 (T) x CSR4
PM x CSR2 (Tg) and PM X CSR2 under normal and BmNPV inoculated conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inoculated</th>
<th>Control</th>
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<tbody>
<tr>
<td>Pupation Rate</td>
<td>80.0</td>
<td>97.2</td>
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<tr>
<td>Cocoon Wt.(gm)</td>
<td>1.823</td>
<td>1.886</td>
</tr>
<tr>
<td>Shell Wt.(gm)</td>
<td>0.329</td>
<td>0.365</td>
</tr>
<tr>
<td>Shell Ratio</td>
<td>18.05</td>
<td>19.35</td>
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</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inoculated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupation Rate</td>
<td>52.2</td>
<td>96.4</td>
</tr>
<tr>
<td>Cocoon Wt.(gm)</td>
<td>1.821</td>
<td>1.880</td>
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<tr>
<td>Shell Wt.(gm)</td>
<td>0.341</td>
<td>0.373</td>
</tr>
<tr>
<td>Shell Ratio</td>
<td>18.73</td>
<td>19.84</td>
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</table>

PM x CSR2 (Tg) and PM X CSR2 under normal and BmNPV inoculated conditions

PM x CSR2
Nistari (Tg) x NB4D2 and Nistari X NB4D2 under normal and \textit{BmNPV} inoculated conditions

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Control</th>
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</thead>
<tbody>
<tr>
<td>Pupation Rate</td>
<td>84.8</td>
<td>99.6</td>
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<tr>
<td>Cocoon Wt.(gm)</td>
<td>1.657</td>
<td>1.687</td>
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<tr>
<td>Shell Wt.(gm)</td>
<td>0.262</td>
<td>0.288</td>
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<td>Shell Ratio</td>
<td>15.81</td>
<td>17.07</td>
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</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inoculated</th>
<th>Control</th>
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<tbody>
<tr>
<td>Pupation Rate</td>
<td>58.0</td>
<td>95.6</td>
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<tr>
<td>Cocoon Wt.(gm)</td>
<td>1.635</td>
<td>1.706</td>
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<tr>
<td>Shell Wt.(gm)</td>
<td>0.255</td>
<td>0.291</td>
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<td>Shell Ratio</td>
<td>15.60</td>
<td>17.06</td>
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</table>
CSR2(Tg) x CSR4 and CSR2 X CSR4 under normal and BmNPV inoculated conditions

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Control</th>
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<tr>
<td>Pupation Rate</td>
<td>76.8</td>
<td>96.0</td>
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<td>Cocoon Wt.(gm)</td>
<td>1.764</td>
<td>1.856</td>
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<td>Shell Wt.(gm)</td>
<td>0.376</td>
<td>0.412</td>
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<td>Shell Ratio</td>
<td>21.32</td>
<td>22.20</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Control</th>
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<tbody>
<tr>
<td>Pupation Rate</td>
<td>44.4</td>
<td>94.4</td>
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<tr>
<td>Cocoon Wt.(gm)</td>
<td>1.688</td>
<td>1.858</td>
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<td>Shell Wt.(gm)</td>
<td>0.350</td>
<td>0.415</td>
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<td>Shell Ratio</td>
<td>20.73</td>
<td>22.34</td>
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## Reeling performance

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Particulars</th>
<th>717 X CSR4</th>
<th>CSR2 X CSR4</th>
<th>PM X 727</th>
<th>PM X CSR2</th>
<th>170B X (SK6 X SK7)</th>
<th>Nistari X (SK6 X SK7)</th>
<th>170B X NB4D2</th>
<th>Nistari X NB4D2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Cocoon Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>Single Cocoon Wt.</td>
<td>1.781</td>
<td>1.829</td>
<td>1.602</td>
<td>1.578</td>
<td>1.349</td>
<td>1.401</td>
<td>1.552</td>
<td>1.616</td>
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<tr>
<td>2</td>
<td>Single Shell Wt.</td>
<td>0.37</td>
<td>0.335</td>
<td>0.26</td>
<td>0.25</td>
<td>0.195</td>
<td>0.2</td>
<td>0.255</td>
<td>0.25</td>
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<tr>
<td>3</td>
<td>Shell Ratio%</td>
<td>20.77</td>
<td>18.31</td>
<td>16.22</td>
<td>15.83</td>
<td>14.45</td>
<td>14.27</td>
<td>16.43</td>
<td>15.46</td>
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<tr>
<td>4</td>
<td>No. Of Cocoons/Kg</td>
<td>565</td>
<td>551</td>
<td>625</td>
<td>634</td>
<td>743</td>
<td>734</td>
<td>645</td>
<td>624</td>
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<td>5</td>
<td>Defective Cocoons(%)</td>
<td>4.3</td>
<td>3.78</td>
<td>4.57</td>
<td>2.6</td>
<td>6.26</td>
<td>8.79</td>
<td>2.26</td>
<td>3.47</td>
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<tr>
<td>6</td>
<td>Avg. Filament Length(m)</td>
<td>968</td>
<td>965</td>
<td>646</td>
<td>647</td>
<td>454</td>
<td>501</td>
<td>670</td>
<td>592</td>
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<tr>
<td>7</td>
<td>Non Broken Filament Length(m)</td>
<td>762</td>
<td>778</td>
<td>422</td>
<td>526</td>
<td>316</td>
<td>445</td>
<td>447</td>
<td>501</td>
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<tr>
<td>8</td>
<td>Single cocoon filament denier</td>
<td>3.04</td>
<td>2.93</td>
<td>2.87</td>
<td>2.89</td>
<td>2.81</td>
<td>2.82</td>
<td>3.1</td>
<td>3.07</td>
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<tr>
<td></td>
<td><strong>Reeling characteristics</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>Reelability(%)</td>
<td>79.18</td>
<td>82.7</td>
<td>66.67</td>
<td>83.12</td>
<td>73.91</td>
<td>90.7</td>
<td>67.81</td>
<td>86.04</td>
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<tr>
<td>2</td>
<td>Raw Silk%(on green cocoons)</td>
<td>17.17</td>
<td>15.02</td>
<td>12.06</td>
<td>11.82</td>
<td>8.14</td>
<td>9.32</td>
<td>11.43</td>
<td>10.36</td>
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<tr>
<td>3</td>
<td>Renditta</td>
<td>5.8</td>
<td>6.6</td>
<td>8.3</td>
<td>8.4</td>
<td>12.3</td>
<td>10.7</td>
<td>8.7</td>
<td>9.6</td>
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<tr>
<td>4</td>
<td>Raw silk recovery%</td>
<td>82.66</td>
<td>82</td>
<td>74.35</td>
<td>74.66</td>
<td>56.3</td>
<td>65.28</td>
<td>69.56</td>
<td>67.02</td>
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<tr>
<td>5</td>
<td>Silk waste(%) on silk weight</td>
<td>14.34</td>
<td>17.4</td>
<td>14.39</td>
<td>21.79</td>
<td>29.17</td>
<td>22.65</td>
<td>26.11</td>
<td>21.72</td>
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Reeling test conducted at CSRTI, Bangalore
### Quality characteristics of raw silk reeled

<table>
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<tr>
<th>Sl. No.</th>
<th>Particulars</th>
<th>717 X CSR4</th>
<th>CSR2 X CSR4</th>
<th>PM X 727</th>
<th>PM X CSR2</th>
<th>170B X (SK6 X SK7)</th>
<th>Nistari X (SK6 X SK7)</th>
<th>170B X NB4D2</th>
<th>Nistari x NB4D2</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>value</td>
<td>Grad value</td>
<td>value</td>
<td>Grad value</td>
<td>value</td>
<td>Grade</td>
<td>value</td>
<td>Grad value</td>
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<tr>
<td>I</td>
<td>Average Size (d)</td>
<td>21.99</td>
<td>21.8</td>
<td>20.81</td>
<td>22</td>
<td>21.96</td>
<td>26.57</td>
<td>25.68</td>
<td>26.7</td>
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<tr>
<td>II</td>
<td>Major Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1</td>
<td>Standard Size deviation(d)</td>
<td>1.28</td>
<td>3A</td>
<td>1.2</td>
<td>3A</td>
<td>1.57</td>
<td>2A</td>
<td>1.57</td>
<td>2A</td>
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<td>2</td>
<td>Evenness variation - I (Stripes)</td>
<td>20</td>
<td>4A</td>
<td>30</td>
<td>4A</td>
<td>28</td>
<td>4A</td>
<td>22</td>
<td>4A</td>
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<tr>
<td>3</td>
<td>Evenness variation - II (Stripes)</td>
<td>0</td>
<td>4A</td>
<td>0</td>
<td>4A</td>
<td>0</td>
<td>4A</td>
<td>4</td>
<td>2A</td>
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<tr>
<td>4</td>
<td>Cleanness(%)</td>
<td>95</td>
<td>3A</td>
<td>97</td>
<td>4A</td>
<td>94</td>
<td>2A</td>
<td>97</td>
<td>4A</td>
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<tr>
<td>5</td>
<td>Neatness(%)</td>
<td>92</td>
<td>3A</td>
<td>92</td>
<td>3A</td>
<td>91</td>
<td>2A</td>
<td>91</td>
<td>A</td>
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<tr>
<td>6</td>
<td>Low Neatness(%)</td>
<td>90</td>
<td>3A</td>
<td>90</td>
<td>4A</td>
<td>85</td>
<td>2A</td>
<td>90</td>
<td>4A</td>
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<tr>
<td>III</td>
<td>Auxiliary Tests</td>
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<tr>
<td>1</td>
<td>Maximum Deviation(d)</td>
<td>2.29</td>
<td>1</td>
<td>1.68</td>
<td>1</td>
<td>2.35</td>
<td>1</td>
<td>2.34</td>
<td>1</td>
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<tr>
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<td>Evenness variation - III (Stripes)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>3</td>
<td>Winding breaks/10 skeins/hrs</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
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<tr>
<td>4</td>
<td>Tenacity(g/d)</td>
<td>3.9</td>
<td>1</td>
<td>3.9</td>
<td>1</td>
<td>3.9</td>
<td>1</td>
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<tr>
<td>5</td>
<td>Elongation(%)</td>
<td>18</td>
<td>1</td>
<td>18</td>
<td>1</td>
<td>18</td>
<td>1</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Cohesion(Strokes)</td>
<td>62</td>
<td>1</td>
<td>61</td>
<td>1</td>
<td>54</td>
<td>2</td>
<td>51</td>
<td>2</td>
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<tr>
<td>Overall Grade</td>
<td></td>
<td>3A</td>
<td>3A</td>
<td>A</td>
<td>3A</td>
<td>A</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

Note: Raw silk testing has been done as per the BIS standards
Limited multilocational trials to be conducted in the following centre

**CENTRAL SILK BOARD INSTITUTES**

<table>
<thead>
<tr>
<th><strong>CSR&amp;TI, Mysore, Karnataka</strong></th>
<th><strong>CSR&amp;TI, Berhampore, WB</strong></th>
<th><strong>CSR&amp;TI, Pampore, J&amp;K State</strong></th>
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<tbody>
<tr>
<td>1. TVDC, CSR&amp;TI, Mysore</td>
<td>1. CSR&amp;TI, Berhampore</td>
<td>1. RSRS, Jammu,</td>
</tr>
<tr>
<td>2. RSRS, Kodathi, Karnataka</td>
<td>2. REC, Nabagram (Farmer level)</td>
<td>2. Farmers under RSRS, Jammu</td>
</tr>
<tr>
<td>3. RSRS, Salem, Tamilnadu</td>
<td>3. REC Bagmara (Farm level)</td>
<td>3. RSRS, Sahaspur, Uttaranchal</td>
</tr>
<tr>
<td>4. RSRS, Anantapur, Andhra Pradesh</td>
<td></td>
<td>4. Farmers under RSRS, Sahaspur</td>
</tr>
<tr>
<td>5. RSRS, Chamrajnagar, Karnataka</td>
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<td></td>
</tr>
</tbody>
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**At Farmers Level**

5 identified farmers under nested units of CSR&TI, Mysore

1. APSSRDI, Hindupur, Andhra Pradesh
2. Anantapur and Chittoor districts
Proposed limited field trials

JAMMU & KASHMIR
1) Joint Director, RSSS, Jammu (CSIR)
2) Farmers under RSSS, Jammu (CSIR)

UTTARANCHAL
1) Joint Director, RSSS, Sahaspur (CSIR)
2) Farmers under RSSS, Sahaspur (CSIR)

WEST BENGAL
1) Director, CSR&TI, Berhampore (CSIR)
2) Joint Director, RFC, Nabagram (CSIR)
   Farmers level: two progressive farmers
3) Joint Director, REC, Bagmara (CSIR) – Farm level

KARNATAKA
1) Director, TVDC, CSR&TI, Mysore (CSIR)
2) Joint Director, RSSS, Kodathi (CSIR)
3) Joint Director, RSSS, Chamarajanagar (CSIR)
4) At Farmers level: 5 identified Farmers under nested units of CSR&TI, Mysore

ANDHRA PRADESH
1) Joint Director, RSSS, Ananthapar (CSIR)
2) Director, APSSRD, Hidrapur (AP State Sericulture Dept.)
3) Two farmers in Ananthapur District (AP State Sericulture Dept.)
4) Two farmers in Chittoor District (AP State Sericulture Dept.)

TAMIL NADU
Joint Director, RSSS, Salem (CSIR)
Visit of RCGM expert committee to APSSRDI on 07th Sep. 2012

1. Dr. Raj Bhatnagar
   Group leader,
   International center for Genetic Engineering and Biotechnology, New Delhi.

2. Dr. S.J. Rahman
   Principal Scientist & Head
   AICRP on Biological control of crop pests & weeds, ANGRAU, Hyderabad.

3. Dr. K. K. Tripathi
   Member Secretary, RCGM / Scientist – G, DBT, New Delhi.

4. Dr. S.K. Jalali
   Principal Scientist
   NBAI, Bangalore.

5. Dr. Vibha Ahuja
   General Manager
   Biotech Consortium India Ltd.
   New Delhi.

Clearance for conducting limited field trials of Transgenic silkworm hybrids
RCGM Expert committee
Project implementing lab
Transgenic Rearing House
Control of Uzi Fly

Uzi net for Doors

Uzi nets for Trays
Shoot Rearing
Multiple Mist System in rearing house
Cold Storage Facility

Cold storage

Anti chamber

Egg preservation chamber

Incubation chamber
Bed Waste pits for Transgenic & Non-Transgenic silkworms.
ISOLATED REARING HOUSE FOR INOCULATION
DBT-CoE Mulberry Garden

<table>
<thead>
<tr>
<th>PLOT NO</th>
<th>EXTENT OF AREA (Acres)</th>
<th>SPACING</th>
<th>VARIETY</th>
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<tr>
<td>15</td>
<td>0.65</td>
<td>2'x2'x4'</td>
<td>V1</td>
</tr>
<tr>
<td>16</td>
<td>0.60</td>
<td>2'x2'x4'</td>
<td>V1</td>
</tr>
<tr>
<td>17</td>
<td>0.60</td>
<td>2'x2'x4'</td>
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</tr>
<tr>
<td>18</td>
<td>0.50</td>
<td>6'x5'</td>
<td>V1</td>
</tr>
</tbody>
</table>
IBSC COMPOSITION

**APSSRDI, HINDUPUR**

- Prof. K. Muniyappa, IISc, Bangalore
- Dr. S. Raje Urs, DBT Nominee
- Dr. E.T. Ramamurthy, Biosafety Officer
- Dr. V. Siva Prasad, External Expert

**CSR&TI, MYSORE**

- Dr. V.Siva Prasad, Director
- Dr. N. Nataraja Karaba, DBT Nominee
- Dr. K. Nagendra Prasad, Biosafety Officer
- Prof. H. B. Manjunatha, External Expert

**CSR&TI, BERHAMPORE**

- Dr. S. Nirmal Kumar, Director
- Dr. Somnath Bhattacharya, DBT Nominee
- Dr. Rajib Kumar Sanyal, Biosafety Officer
- Mr. Rupak Kumar Bhadra, External Expert

**CSR&TI, PAMPORE**

- Dr. K.A.Sahaf, Director
- Dr. Raies Ahmad, DBT Nominee
- Dr. Surjeet Singh, Biosafety Officer
- Dr. Qazi Parveez Hassan, External Expert
Site visit of Expert team for funding by BIRAC, New Delhi to APSSRDI on 21st June, 2012.

Prof. K. Gopinathan  
IISc, Bangalore

Dr. Mohd. Aslam  
Director, DBT, New Delhi

Dr. J. Nagaraju  
Scientist-H, CDFD, Hyderabad

Dr. Vinita Jindal  
Programme Manager, BIRAC New Delhi

Mrs. Alpana Saxena  
Chartered Accountant New Delhi

Smt. C. Aruna Kumari  
(Rep. of COS, Govt. of AP.)

Dr. K. Vijayan  
(Rep. of The Member Secretary, CSB, Bangalore)

Provisionally sanctioned the budget for Rs. 209.2 lakhs
CDFD and APSSRDI are jointly initiating field trials of the transgenic silkworm hybrids at National level in association with Central Silk Board (CSB) for further exploitation with the assistance of Biotechnology Industrial Research Assistance Council (BIRAC), New Delhi in association with Biotech Consortium India Ltd (BCIL), New Delhi.

TRANSGENIC SILKWORM HYBRIDS
(For National level field testing)

- CSR2 (Tg) x CSR4 (South and North India)
- PM x CSR2 (Tg) (South India)
- Nistari (Tg) x NB4D2 (West Bengal)
- Nistari (Tg) x (SK6 x SK7) (West Bengal)

<table>
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<tr>
<th>INSTITUTIONS</th>
<th>BUDGET in Rs. (Lakh)</th>
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<tbody>
<tr>
<td>Academia - CDFD</td>
<td>Rs. 58.16</td>
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<tr>
<td>Collaborator 1- APSSRDI</td>
<td>Rs. 209.20</td>
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<tr>
<td>Collaborator 2 – CSB</td>
<td>Rs. 90.60</td>
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<tr>
<td>TOTAL PROJECT COST</td>
<td>Rs. 357.96</td>
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</tbody>
</table>
APSSRDI and CDFD have presented the details of action plan to conduct limited field trials of Transgenic hybrids
Appraisal meeting at CDFD, Hyderabad on 09.05.2013

1. Dr. J. Gowrishankar
   Director and Group leader,
   CDFD, Hyderabad

2. Dr. Vibha Ahuja
   General Manager
   BCIL, New Delhi.

3. Dr. P.J. Raju
   Director, APSSRDI,
   Hindupur

4. Dr. H.K. Basavaraja
   Breeding Consultant

5. Dr. C.G.P. Rao
   Director I/C, SBRL,
   Bangalore

6. Dr. K. Ibraheem Basha
   Scientist-B (FAC)
   APSSRDI, Hindupur

7. Dr. V.V. Satyavathi
   CDFD, Hyderabad
Dr. Toshiki Tamura & Dr. Kazuei Mita
National Institute of Agrobiological Sciences, JAPAN
(Visit to APSSRDI)

2nd SABC. Colombo, Srilanka 14th - 15th September, 2014
Suggestions of Institutional Biosafety Committee

3rd IBSC of APSSRDI, Hindupur held on 18.06.2013

- Manual on Do's and Don'ts involved in handling transgenic silkworm hybrids during field trials.
- Need to study medical report pertaining to the personnel involved in handling silkworm hybrids.
- Verification and certification of facilities at all the test centres.
- Toxicity to the farm animals such as cow, sheep, goat and hen due to the left overs of transgenic silkworm rearing disposals.
- Need to study toxicity studies and to keep the records for phase II trials.
- Environmental concerns.
Suggestions of Institutional Biosafety Committee

5th IBSC of CSR&TI, Mysore held on 03.06.2013
• Need to follow procedures as per the DBT guidelines (along with material transfer agreement, details of transgenic lines etc.,)
• Possible health hazards associated with transgenics

2nd IBSC of CSR&TI, Pampore held on 03.03.2014
• Need for checking NPV load during the trials, if required

2nd IBSC of CSR&TI, Berhampore held on 14.03.2014
• Need for definite SOPs
Co-ordinating Committee

Director, APSSRDI, Hindupur - Chairman
Director (Tech.), Central Silk Board, Bangalore - Special Invitee
Director, CSR&TI, Mysore - Member
Director, CSR&TI, Berhampore - Member
Director, CSR&TI, Pampore - Member
Dr. H.K. Basavaraja, Breeding Consultant – Member
Dr. V.V. Satyavathi, TO-IV, CDFD - Member
Dr. K. Ibrahim Basha, Scientist - Member
Dr. S.V. Seshagiri, Scientist - Member
Meeting on the status of Biosafety Regulatory Approvals on 5.09.2014

1. Dr. J. Gowrishankar  
   Director and Group leader,  
   CDFD, Hyderabad
2. Dr. B. Sesikeran  
   Former Director, NIN  
   Chairman, RCGM
3. Dr. B. S. Angadi  
   Director (Tech.)  
   CSB, Bangalore.
4. Dr. Vibha Ahuja  
   General Manager  
   BCIL, New Delhi.
5. Dr. P. J. Raju  
   Director, APSSRDI,  
   Hindupur
6. Dr. H. K. Basavaraja  
   Breeding Consultant
7. Dr. V. V. Satyavathi  
   CDFD, Hyderabad
8. Dr. K. Ibraheem Basha  
   Scientist-B(FAC)  
   APSSRDI, Hindupur
ACTION PLAN FOR
TESTING OF THE TRANSGENIC SILKWORM HYBRIDS

Parameters to be recorded in the field trials
- Compare the efficiency and any other changes in the GE silkworm vs Controls
- Fecundity
- Cocoon yield/10 KL (Kg)
- ERR (%)
- Pupation Rate (%)
- Cocoon Weight (g)
- Cocoon Shell Weight (g)
- Cocoon Shell Ratio (%)
- Moth Emergence Rage (%)
- Filament Rate (m)
- NB Filament Lenght (m)
- Filament size (d)
- Reelability (%)
- Raw Silk (%)
CHECK LIST BEFORE START OF THE TRIAL

- Provide information related to rearing facility to RCGM
- Trial-in-Charge’s name and contact details
- Permit No. From the regulatory authority
- Trial initiation date
- Duration of the trial
- Hybrids under evaluation
- No. of rearings and No. of cycles
- Trial Protocol in place

Compliance Records
- Experimental facilities
- Sub-contracts
- Cleaning of equipment
- Transportation
- Rearing of the GE silkworm
- How the surplus material is rendered non-viable

Contd......
Mandatory information

- Initiation of the trial information to RCGM
- Record of start of experiment
- Permit Number
- Material to be used
- Starting Date
- Transportation of GE material, if so detial
- Cleaning of any equipment
- Disposition of any surplus GE material

Photographs for experiment

- Start of the experiment
- Photograph covering stage and experimental details
CHECK LIST DURING TRIAL PERIOD

Monitoring of trial sites
• Trial-in-charge or designate monitor trial site once everyday during business hours
• Records made available for inspection
• Conduct of trial related records/originals to be retained by the Trial-in-charge

Unauthorized person entering the site
• Name
• Designation
• Purpose of visit

Photographs for experiment
• During mid of the experiment
• Indicate stage and experimental details

Chemical Treatment
• Chemical treatment time
• Post sign indicating date and time of spraying

Accidental release information
• Trial-in-charge should notify RCGM within 24 hours of any untoward incident by facsimile, emails or other means
• Trial Report to be sent to RCGM once in every 6 months with all the available data
CHECK LIST AT THE END OF THE TRIAL

Harvesting information submission
• Record of harvest for each trial site
• Date and method of harvest
• Amount of harvested material
• Disposition of any harvested material
• Cleaning of any equipment
• Method of destruction of any GE material on trial site
• Verified and signed by member of monitoring agency or any RCGM nominee during trial site inspection, harvest or within 15 days of harvest
• Records to be maintained up to 5 years

Photographs for experiment
• During mid of the experiment
• Indicate stage and experiment details
Standard Operating Procedures (SOPs) for contained field trials of regulated, Genetically Engineered (GE) silkworm, *Bombyx mori* L.

**ANDHRA PRADESH STATE SERICULTURE RESEARCH AND DEVELOPMENT INSTITUTE**
KIRIKERA – 515 211, HINDUPUR, ANANTAPUR Dt. (A.P)

In association with
Biotech Consortium India Ltd., New Delhi
## Trial locations and Trial In-charges

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Trial site</th>
<th>Trial In-charge</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>APSSRDI, Hindupur</td>
<td>Director, APSSRDI, Hindupur, Andhra Pradesh</td>
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<tr>
<td>2</td>
<td>CSR&amp;TI, Mysore</td>
<td>Director, CSR&amp;TI, Mysore</td>
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<tr>
<td>3</td>
<td>CSR&amp;TI, Berhampore</td>
<td>Director, CSR&amp;TI, Berhampore</td>
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<td>4</td>
<td>CSR&amp;TI, Pampore</td>
<td>Director, CSR&amp;TI, Pampore</td>
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</tbody>
</table>
Details of transgenic hybrids, rearing seasons, duration, institutions and number of rearings/cycles

<table>
<thead>
<tr>
<th>Institute</th>
<th>Transgenic Hybrids</th>
<th>No. Of Rearings/Cycles</th>
<th>Season</th>
<th>Duration</th>
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<tbody>
<tr>
<td>CSR&amp;TI, Mysore &amp; Nested units</td>
<td>CSR2 (T) x CSR4</td>
<td>10</td>
<td>Sept. 2014 – Aug.201</td>
<td>1 year</td>
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<tr>
<td>CSR&amp;TI, Berhampore &amp; Nested units</td>
<td>Nistari (T) x (SK6 x SK7) Nistari (T) x NB4D2</td>
<td>9</td>
<td>Sept. 2014 – Aug. 2015</td>
<td>1 year</td>
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<tr>
<td></td>
<td></td>
<td>9</td>
<td></td>
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<tr>
<td>CSR&amp;TI, Pampore &amp; Nested units</td>
<td>CSR2 (T) x CSR4</td>
<td>2</td>
<td>Sept. 2014 – Aug. 2015</td>
<td>1 year</td>
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<tr>
<td></td>
<td>CSR2 (T) x NB4D2</td>
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<tr>
<td>APSSRDI, Hindupur</td>
<td>CSR2 (T) x CSR4</td>
<td>5</td>
<td>Sept. 2014 – Aug. 2015</td>
<td>1 year</td>
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<td></td>
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<tr>
<td></td>
<td>PM x CSR2 (T)</td>
<td>5</td>
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<td></td>
<td>Nistari (T) x (SK6 xSK7) Nistari (T) x NB4D2</td>
<td>5</td>
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</table>
Declaration

I hereby declare that the transgenic silkworm stocks / hybrids issued to this centre will be used only for the purpose of evaluating their performance in the designated area under the supervision of the undersigned. These transgenic stocks / hybrids –

a) Will not be used for mating with any other transgenic / non-transgenic stocks

b) will not be distributed to non-designated areas for rearing

c) will not be given to any unauthorized persons

d) will not put to any used other than those specified

e) will not be put to commercial cultivation
Thank you ..........