AIT-3450: LONG-TERM CONSERVATION OF EGGS/ EMBRYOS OF SILKWORM GENETIC RESOURCES (*BOMBYX MORI* L) THROUGH CRYOPRESERVATION (DBT, New Delhi funded projects)

Period: November, 2010 – October, 2013

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Introduction:

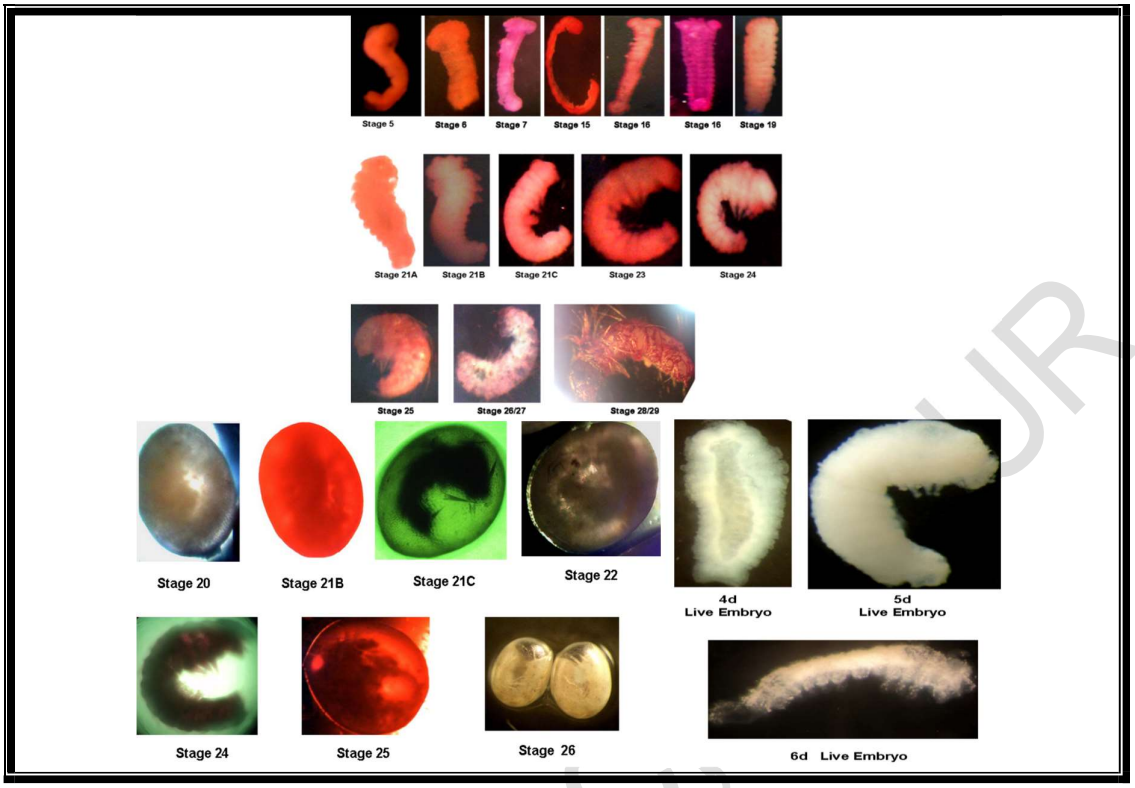
Conservation of the invaluable Silkworm genetic resources is of prime importance with respect to their utilization and improvement for wider exploitation. The newer techniques such as cryopreservation are available from which the genetic resources can be conserved *ex situ* for a longer period. This project was undertaken with an objective to develop suitable protocol for dechorionation of silkworm eggs, extraction of embryos, *in vitro* culture of silkworm embryo and cryopreservation of egg/embryos to establish cryo gene bank of embryos of silkworm genetic resources for commercial/future exploitation.

Objectives:

- To develop suitable protocol for dechorionation of silkworm eggs, extraction of embryos, in vitro culture of silkworm embryo and assessment of survival rates.
- To develop suitable protocols for cryopreservation of egg/embryos of silkworm genetic resources.
- To establish cryo gene bank of embryos of silkworm genetic resources for commercial exploitation.

Outcome:

- Technique for dechorionating silkworm eggs was developed. Treating silkworm eggs with 2% NaOH for two minutes followed by 5 % NaOCl for 10 minutes showed complete dechorionation of eggs.
- The eggs upon treatment with different chemicals at known concentrations have shown varied responses. It was found that silkworm of embryonic age upto 36 h were cold tolerant and embryo of 48 h was cold sensitive.
- *In vitro* culture of Silkworm embryos of early stages (20 to 36 h), incubated with yolk in insect media (TNM-FH) for development. The embryos survived for one day and further development of the embryo was not observed.
- Equilibration time for the CPA tolerance of different cryoprotectants on silkworm eggs after dechorionation, for 10 multivoltine races, was determined for use in cryopreservation of egg/embryos at room temperature. However, CPA tolerance is required to be standardized with respect to cryo-temperature of Liquid Nitrogen (LN) at -196° C.



Recommendations/Utilization

- ✓ Cryopreservation of embryos involves a series of complex and dynamic physio-chemical processes of temperature and water transport between embryo and the surrounding medium. At this stage there is no conspicuous evidence to explain the exact chill tolerant embryonic age.
- ✓ Silkworm eggs can tolerate cryoprotectants with 60% survival and embryos of 36hrs age are chill-tolerant and 48hrs embryos are chill-sensitive.
- ✓ Survival of eggs at cryo temperature could not be established as *in vitro* culture of silkworm embryos was not successful and requires further investigations taking leads from the observations obtained in the present study.
- ✓ Based on this outcome, one research project "Cryopreservation of tropical tasar silkworm *Antheraea mylitta* D semen and its artificial insemination" was proposed.