



INTERNATIONAL
SERICULTURAL
COMMISSION

UNITED NATIONS REG. NO. 10418

ISSN 0250-3980

Volume 54 | Number 3 | 2014

SERICOLOGIA

Journal of Silkworms
A Quarterly Publication of
International Sericultural Commission





INTERNATIONAL
SERICULTURAL
COMMISSION

UNITED NATIONS REG. NO. 10418

SERICOLOGIA

Journal of Silkworms

MANAGING DIRECTOR

Ms. Ishita Roy

CHIEF EDITOR

Dr. Julia S. Nair

EDITOR - AT - LARGE

Dr. G. Chavancy

ADMINISTRATIVE COUNCIL

Mr. K. K. Shetty

Mr. Dileep Kumar R.

Ms. Kalaiarasi R.

EDITORIAL BOARD

Dr. Basavaraja H. K., India

Dr. Cappellozza S., Italy

Dr. Chen Y., China

Dr. Couble P., France

Dr. Dandin S. B., India

Dr. Daniel Dezmirean, Romania

Dr. Das B. C., India

Dr. Fonseca T. C., Brazil

Dr. Freddi G., Italy

Dr. Gapuz C. F., Philippines

Dr. Goldsmith M. R., USA

Dr. He K., China

Dr. Homidy H., Uzbekistan

Dr. Iatrou K., Greece

Dr. Jolly M. S., India

Dr. Kobayashi M., Japan

Dr. Kusakabe T., Japan

Dr. Lea H. Z., Korea

Dr. Machii H., Japan

Dr. Madyarov S., Uzbekistan

Dr. Mase K., Japan

Dr. Mauchamp B., France

Dr. Miranda J. E., Brazil

Dr. Rajkhowa R., Australia

Dr. Shimada T., Japan

Dr. Sivaprasad V., India

Dr. Somashekhar T. H., India

Dr. Sonwalkar T. N., India

Dr. Trenczek T., Germany

Dr. Tzenov P., Bulgaria

Dr. Xia Q., China

Dr. Yamashita O., Japan

Dr. Yungen M., China

Published by International Sericultural Commission (ISC), Central Silk Board Complex, BTM Layout, Madiwala, Bengaluru - 560 068, INDIA. Tel: +91 80 26282191, 26680162, 26681663
Fax: +91 80 26681663, E-mail: sericologia@gmail.com, www.inserco.org

Printed at Ramya Reprographic Pvt. Ltd, No. 16/2B, Singasandra Village, Behind Dakshin Honda Show Room, Hosur Road, Bengaluru - 560 068, INDIA. Tel: +91 98440 36357, E-mail: ramyareprographic@gmail.com

Contents

Volume 54 | Number 3 | 2014

Review Papers

- Impact of major biotic factors on tropical silkworm rearing in India and monitoring of unfavourable elements A review
Impact des principaux facteurs biotiques sur l'élevage du ver à soie tropical en Inde et surveillance des éléments défavorables Revue
R. T. Gahukar 150

- QTL and their utilization in silkworm breeding programmes
Les QTL et leur utilisation dans les programmes de reproduction des vers à soie
Ritwika Sur Chaudhuri and G. Subramanya 171

Research Papers

- Reproductive efficiency of *Nesolynx thymus* (Girault) as influenced by host species – *Exorista bombycis* (Louis) and *Musca domestica* L.
La capacité de reproduction chez *Nesolynx thymus* (Girault) est influencée par l'espèce de l'hôte – *Exorista bombycis* (Louis) et *Musca domestica* L.
A. S. Aruna and D. Manjunath 181

- Evaluation of newly-evolved open-pollinated mulberry varieties
Evaluation de nouvelles variétés de mûriers obtenues par pollinisation ouverte
N. Damasco Crestilyn, A. Dacayanan Merlita, J. Tabafunda Minerva,
A. Ancheta Lilia and D. Supsup Roel 188

- Quantification of vitamin C in mulberry leaves
Quantification de la vitamine C des feuilles de mûrier
Aabid Khaliq Tantray and Kanika Trivedy 198

- Impact of mulberry red rust caused by *Aecidium mori* Linn. on the yield and quality of mulberry (*Morus alba* Linn.)
Impact de la rouille du mûrier causée par *Aecidium mori* L. sur le rendement et la qualité du mûrier (*Morus alba* L.)
Angelina T. Gonzales and Oscar S. Opina 202

Comparative account of morphological and karyotypic studies in three cultivars of mulberry (*Morus* spp.)

Compte-rendu des études morphologiques et caryotypiques comparatives chez trois cultivars de mûrier (*Morus* spp.)

K. H. Venkatesh, S. Shivaswamy and Munirajappa 208

Evaluation of promising mulberry genetic resources

Evaluation des ressources génétiques prometteuses du mûrier

N. Balachandran, S. R. Ramesh, Kiran B. Malali, M. Muchulakshmi and

M. M. Borpuzari 213





IMPACT OF MAJOR BIOTIC FACTORS ON TROPICAL SILKWORM REARING IN INDIA AND MONITORING OF UNFAVOURABLE ELEMENTS: A REVIEW

R. T. Gahukar

Arag Biotech Pvt. Ltd., Plot 220, Reshimbag, Nagpur 440009, India.

E-mail: rtgahukar@gmail.com

ABSTRACT

In India, the different genres of silkworms are reared on mulberry, castor, som, soalu and arjun leaves. The quality of leaves, number of broods/year, host plants and their varieties and environmental factors contribute largely towards their growth and development. Silkworm mortality has often been caused by feeding on foliage treated with chemical pesticides and due to attack of predators, parasitoids and entomopathogens. On the contrary, application of phytoecdysteroids, vermiwash or plant-derived products has shown favourable effects on larva leading to improvements in cocoon weight, shell percentage, cocoon yield and reeling parameters (filament length, denier value etc.). Both bivoltine and multivoltine races have extensively been exploited for better quality and quantity of silk production. In this review, the impact of biotic factors on silkworm rearing is critically discussed and measures for enhancing commercial production of raw silk are suggested.

Key words: Chemical pesticides, commercial silk production, natural enemies, plant-derived products, plant varieties, silkworm rearing.

INTRODUCTION

Silkworms used for commercial production of the raw silk in tropical countries include mulberry silkworm, *Bombyx mori* L. (Bombycidae), eri silkworm, *Philosamia ricini* Hutton (= *Samia cynthia ricini* Boisduval) (Saturniidae), muga silkworm, *Antheraea assamensis* Helfer (= *Antheraea assama* Westwood) and tasar silkworm, *Antheraea mylitta* Drury (Saturniidae). Mulberry silkworm being monophagous feeds on white mulberry (*Morus alba* L., *Morus indica* L.), whereas, red and black mulberry plants are rare host plants. Other primary host plants used for insect rearing are castor (*Ricinus communis* L.) for eri silkworm, som [*Persea bombycina* (King ex Hook) Kosterm] and soalu [*Litsaea monopetala* (Roxb.) Perb.] for muga silkworm, and arjun [*Terminalia arjuna* (Roxb.)] and asan [*Terminalia tomentosa* (Roxb.)] for tasar silkworm.

In silkworm rearing, the thumb rule works very well, i.e., healthy silkworms produce quality silk fibre.

Therefore, ideal conditions for the growth and development of silkworms have to be provided during rearing. Apart from ambient environment (relative humidity, temperature, photoperiod), and physical infrastructure (farm house, farmer's hut, commercial rooms) and personnel (skilled labour or trained technicians), a few biotic factors are responsible for silkworm growth and development and ultimately for the production of raw silk. These factors include firstly, food of silkworms and secondly, attack of natural enemies during rearing process. Except food, indoor rearing technology is same for all domesticated silkworms for which significant progress has been made during recent years through research and development in South Asia, South-East Asia and Africa. Most of the research was however concentrated on mulberry silkworm due to easy and simple rearing and the rising global demand for silk. Recently, the governments in some tropical countries have created a separate division for initiating and encouraging farmers to take up sericulture and to make this activity profitable for poor farmers by providing financial subsidy

and technical guidance (Sathe and Jadhav, 2001; Chakravorty *et al.*, 2004 a; Debaraj *et al.*, 2013). Currently, a compilation of research findings on all domesticated silkworms is not available at one source. This paper presents and discusses an account of recent information from various sources of literature on how biotic factors can affect silkworm rearing and how unfavourable conditions can be modified/controlled to improve the rearing process.

HOST PLANTS AND THEIR VARIETIES

Apart from primary host plants, alternative food plants have been assessed for silkworm rearing because availability, accessibility, quantity, crop season, plant growth and suitability are to be considered to procure sufficient foliage. Also, the leaf quality is an important factor which depends upon variety, plant spacing and soil fertilization (Krishnaswami *et al.*, 1971; Malik and Reddy, 2007).

1. Mulberry silkworm

Evaluation of mulberry varieties has been the theme of quite a lot of studies. Leaf quality in mulberry varieties influences the degree and uniformity in moulting and differences in leaf moisture, nutrition and biochemical constituents is reflected in cocoon parameters (Patil *et al.*, 2001; Malik and Reddy, 2007, 2010). Generally, nutritious foliage favours higher moulting ratio, ensures better larval growth rate and higher larval weight (Seidavi *et al.*, 2005). As such, silkworms fed on leaves containing high protein (main source for biosynthesis of silk) and low sugar are comparatively heavy (Chaluvachari and Bongale, 1996). Among genotypes evaluated in India, cv. V-1 was found significantly superior to local varieties for rearing bivoltine races of *B. mori* (Bhede *et al.*, 2013), and resulted in high larval weight (4.3 g), cocoon weight (1.93 g), shell weight (0.412 g), shell percentage (21.37 %) and cocoon yield (18.96 kg/10,000 larvae brushed) (Kamate *et al.*, 2011). Other suitable varieties include Kanva-2, S-36 and M-5 (Malik and Reddy, 2010), Mizosava and LMP (Kamate *et al.*, 2011) and S-41, S-54 and cv. Vishwa (Rayan, 2011). Also, the Mulberry Variety Authorization

Committee, an apex committee of the Central Silk Board, recommended a few varieties for each eco-region, e.g., cvs. V-1, S-13, S-36, G-4, RC-1, RC-2 and Anantha for central and southern regions; cvs. C-776, C-1730, C-2028, C-2038, S-1, S-799, S-1635, TR-10 and BC-59 for eastern and north-eastern regions, and cvs. S-140 and S-146 for north-western region. Further screening tests may reveal some more suitable genetic resources.

2. Eri silkworm

Host plants with difference in foliar constituents/nutrients can affect economic parameters in eri silkworm as demonstrated by Jayaramaiah and Sannappa (2000 a, b), Biswas and Dass (2001), Govindan *et al.* (2002 a, b) and Sarmah *et al.* (2002) who evaluated suitability of host plants in relation to the growth, development and reproductive potential of *P. ricini*. Further, Naik (2008) evaluated 23 plants other than major hosts and reported carrot (*Daucus carota* Linn.) and fountain tree (*Spathodea campanulata* Pal.) as suitable hosts. In other attempts, Hazarika *et al.* (2003) and Ramakrishna *et al.* (2003) recommended tapioca/cassava, *Manihot esculenta* Crantz (= *Manihot utilissima*), payam, *Evodia flaxinifolia* Hook and Kessuru, *Heteopanax fragrans* (Roxb.) for rearing. However, maximum volumetric attributes were noticed on castor followed by tapioca, ailanthus (*Ailanthus excelsa* Roxb.), plumeria/frangipani (*Plumeria acutifolia* Poir) and papaya (*Carica papaya* Linn.) (Neelu *et al.*, 2000; Hazarika *et al.*, 2003). Conclusively, castor is the most suitable plant for eri silkworm rearing because the leaves contain higher amino acids and nitrogen than other host plants (Chandrappa *et al.*, 2005) and the life cycle of silkworm was completed on castor, tapioca, *P. rubra* and *A. excelsa* in 46, 63, 62 and 72 days, respectively (Reddy *et al.*, 1989). Moreover, economic parameters were better in castor-reared silkworms than those reared on tapioca and plumeria (Sannappa and Jayaramaiah, 1999). Host plants also affected the amylase activity in digestive juice and haemolymph in fifth instar larvae of *P. ricini*; though activity increased steeply from first to fourth day and

decreased towards the onset of spinning, irrespective of silkworm breeds, host plants and their interactions. Larvae fed on *A. excelsa* showed activity at par with those fed on castor indicating that this plant could be a good substitute of castor (Sarithakumari *et al.*, 2009). This finding is pertinent to areas where rearing can be done on this plant particularly when castor foliage cannot be made available in plenty.

Among castor varieties cultivated in India, cvs. Aruna, RC-8 and NBR-1 were found suitable for larval development and survival of *P. ricini* (Sannappa and Jayaramaiah, 1999; Sannappa *et al.*, 2007). However, cvs. PCH-11 and GCH-4 were better in cocoon weight (2.32 g), shell weight (0.34 g), shell percentage (15.2 %), pupal weight (1.87 g) and egg hatching (99.2 %) compared to cvs. Kiran, Haritha and Kranthi (Lakshmi Narayanamma *et al.*, 2013). Among castor genotypes (Sannappa *et al.*, 2002), cv. PCH-11 showed significantly higher food consumption and digestion and mean consumption index (Lakshmi Narayanamma *et al.*, 2014). Murthy *et al.* (2013) reported cv. S-1708 as promising castor variety among 10 genotypes field tested in India. Feeding on this variety resulted in higher larval weight (943.21 mg/10 larvae), cocoon weight (2.15 g), shell weight (0.64 g), shell percentage (20.96%) and filament length (1142.7 m) and effective rate of rearing (ERR) of 79.9 %. It also gave lower values of renditta (5.07) and denier (2.08) which are preferred quality parameters. Fecundity was increased due to feeding on cv. DCS-85 (340 eggs/female) than cv. GCH-4 (275 eggs/female) (Chandrashekhar and Govindan, 2010). Two cvs. AC-03 and AC-04 were found superior in terms of agronomical and yield attributing traits viz., leaf biomass (334-345 g/plant), shell weight (0.5 g), shell percentage (15.5 %) and ERR (85.7 %) (Sarmah *et al.*, 2011). These authors reported a negative relationship of larval weight with leaf protein content but positive relationship with crude protein and sugar content. Therefore, these genotypes may be used in breeding programmes.

3. Muga silkworm

Host plants can affect the status of feeding and

moulting behaviour of muga silkworm and ultimately cocoon characteristics (Chakravorty *et al.*, 2004b). From field studies, Chowdhury *et al.* (2000), Rath (2000) and Singh and Goswami (2012) reported that the leaf quality of various genotypes/varieties of som, soalu and other natural host trees can affect growth and development of muga silkworm. Generally, increased cocoon weight and better grainage parameters in soalu-fed larvae and higher shell percentage in som-fed larvae have been reported. Similarly, among three plants (som, soalu, *Litsea citrata* Roxb.), the better quality silk (fibre length and width, sericin and amino acid content) was obtained from som leaves (Hazarika *et al.*, 1998). From these results, it was concluded that both plants can be considered suitable for rearing. Similarly, silkworms fed on primary host plants had higher pupal weight and oviposition rate than those fed on secondary host plants (Barah and Sengupta, 1991) because the leaves have high content of polysaturated fatty acids and total lipids (Unni *et al.*, 1996). In the case of first and second instars of *A. assamensis* larvae, feeding on soalu leaves resulted in a higher larval weight than those fed on som or *Litsea salicifolia* Roxb. ex Nees leaves whilst high moulting percentage was observed in larvae fed on som leaves. In som, contents of β -sitosterol, total phenol, and tannin are significantly higher in young leaves than in mature leaves, whereas contents of chlorogenic acid, and phytic acid and fibres are higher in mature leaves (Neog *et al.*, 2011 a). Young leaves of som are also better in respect of total minerals, crude protein, reducing sugar, total phenol, ascorbic acid, chlorogenic acid, lignin, crude fibre, tannins and chlorophyll (Neog *et al.*, 2013). Thus, nutritional superiority of young leaves over mature leaves was confirmed (Neog *et al.*, 2011 b).

It was observed that a mixture of flavonoids with β -sitosterol elicited the most biting behaviour in *A. assamensis* whereas linalyl acetate alone attracted larvae to host plants and azaindole deterred larvae from host plants (Neog *et al.*, 2011 b). Further, Chakravorty *et al.* (2004 b) recommended silkworm rearing on soalu due to shorter larval duration and high cocoon weight. It would therefore be necessary to evaluate available indigenous genotypes and only those with potential of high yield of

quality cocoons and better adaptation to local weather should be selected for rearing.

4. Tasar silkworm

Feeding on nutritious leaf can enhance larval growth, ERR and cocoon yield in tasar silkworm, and ultimately, other commercial traits of raw silk (Yadav and Mahobiam, 2010). In a comparison of host plants for physiological properties (leaf moisture content, net photosynthetic rate), biochemical parameters (chlorophyll, protein, carbohydrates, crude fibre) and food intake, digestion and assimilation, *Terminalia belerica* Roxb., *T. arujna*, *T. tomentosa* and *Lagerstroemia speciosa* (L.) Pers. performed better than *T. catappa* L., *T. chebula* Retz., *L. parviflora* Roxb. (Rath, 2000; Rai *et al.*, 2006; Deka and Kumari, 2013), *Shroea robusta* Gaertn. and *Ziziphus jujuba* Gaertn. (Dash *et al.*, 1992). From the point of important traits viz., larval weight, cocoon weight, shell weight, ERR, cocoon yield, silk percentage, fecundity and egg fertility, cocoons raised from *L. parviflora* were better than those from *T. tomentosa* and therefore, the leaves of this secondary host can be fed to silkworms during final larval stage (Reddy *et al.*, 2010). Currently, only the indigenous cultivars of *T. arjuna* and *T. tomentosa* are being used for rearing in tribal areas since breeding for improvement of genotypes has not yet been initiated.

Pest infested or diseased foliage

Infestation by thrips, mites and leaf roller results in undesirable biochemical changes in mulberry leaves that affect ultimately the silkworm growth and development (Narayanaswamy *et al.*, 1999; Narayanaswamy, 2003; Etebari and Bizhannia, 2006; Mahadeva, 2011). An increase in succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) indicated enhanced anaerobic glycolysis and oxidative metabolism in the leucocytes of silkworm (Naik *et al.*, 2011). Similarly, biochemical constituents of mulberry leaves (free amino acids, total soluble proteins, total reducing sugars, total soluble sugars, total carbohydrates, total proteins, starch and total phenols) and photosynthetic pigments (total chlorophyll,

chlorophyll a, b, a/b ratio and carotenoids) can create imbalance in nutritional status of leaves and adversely influence the growth and development of silkworms as well as quality of silk produced (Mahadeva, 2011). Ramesh *et al.* (2009) reported that the mealy bug infested or diseased (infected with *tukra*) mulberry leaves did not affect weight of larva, pupa and shell, pupation rate and reeling parameters. On the contrary, infestation of thrips (Sengupta *et al.*, 1999) or mites on leaves (Dar *et al.*, 2011; Ramegowda *et al.*, 2012) and nematodes on roots (Paul *et al.*, 1995) made mulberry leaves less nutritive. Consequently, silkworms reared on these leaves had low cocoon weight and shell weight, reduced shell percentage, decreased filament length and other commercial parameters. Apparently, adverse effects on silkworm physiology were not observed even after feeding on mealy bug infested leaves. This fact prompted for provision of infested leaves in acute shortage of healthy foliage (Ramesh *et al.*, 2009; Naik *et al.*, 2011). In this case, foliage must be checked for presence of other insect pests.

Among plant diseases, leaf rust significantly decreased the content of moisture, ash, lipid, crude fibre, carbohydrate, vitamins (β -carotene, B1, B2 and C) and minerals (P, Ca, Fe) in mulberry leaves but the content of protein and phenol was increased (Tang *et al.*, 2006).

Foliage treated with plant-derived products

Mulberry silkworm rearing

Plant-derived products include water and chemical (solvent) extracts, crude oil, commercial biopesticides and pure allelochemicals. Water extract of plant parts has been extensively used due to its low toxicity to silkworms, and easy and cheap preparation. The new plant-derived products (phytosteroids, allelochemicals, biopesticides) are not yet popular in rural areas particularly in small land holdings where farmers undertake silkworm rearing in the fields. These products were found beneficial to improve commercial parameters of mulberry silkworm through increased weight of cocoon and shell, higher food

utilization efficiency and digestive enzyme activity (Rajasekaragouda *et al.*, 1997). When silkworms (bivoltine hybrid) were fed on leaves sprayed with water extract (5 %, w/v) of neem, *Ocimum sanctum* L. or *Parthenium hysterophorus* L., activity of SDH and LDH gradually increased but that of glutamate dehydrogenase (GDH) decreased in haemolymph and fat bodies indicating that generally extracts did not affect enzyme activity (Mude *et al.*, 2012). A conventional mixture (1:1 proportion) of cow urine + *Melia azedarach* L. extract at 25 ml/l, Agrineem® at 4ml/l, and Nimbecidine® at 4ml/l killed only 1.39 %, 2.64 % and 5.9 % of silkworms, respectively, in the laboratory compared to >50 % mortality due to chemicals (Singh *et al.*, 2010). Strong feeding stimulant activity was recorded in 21-day old *B. mori* larvae under laboratory condition after feeding on leaves treated with 5 % (w/v) water extract of *Vitex negundo* L. or *Centella asiatica* (L.) Urban, 2 % (w/v) extract of *Pelargonium graveolens* L'Herit or *Murraya koenigii* (L.) Spreng. Growth promoting effects resulting in increased egg and silk production were noticed for the water or ether extract (10 %, v/v) of *Tribulus terrestris* L., *Phyllanthus niruri* Hook, *Boerhavia diffusa* Linn., *Psoralea corylifolia*, *Caesalpinia coriaria* (Jacq.) Willd., *P. hysterophorus* (Rajasekaragouda *et al.*, 1997), *Coffea arabica* L. (Murugan *et al.*, 1998), or extract of leaf, flower or pod of *Moringa oleifera* Lamk. (Rajeswari and Isaiaarasu, 2004). Spraying water extract of *V. negundo* (Sujatha and Rao, 2003) or other medicinal plants (Datta and Prasad, 1999; Bohidar *et al.*, 2004; Pandey and Srivastava, 2013) on mulberry crop significantly improved rearing standard particularly, the silk percentage and filament length. Among plants evaluated, *P. hysterophorus*, a common invasive weed in the tropics is known to cause adverse effects in humans and cattle. How it improves the silkworm physiology and development is rather controversial but can be known/confirmed by further experimentation.

Contrary to common belief on safety of plant-derived products, there are reports on the adverse effects on silkworms. For example, 1% aqueous extract of *Coleus aromaticus* Benth. or *Mentha arvensis* L. exerted strong

feeding deterrent effect on *B. mori* but without significant effect on larval period, pupal weight and survival of larvae and pupae (Pandey and Srivastava, 2013). In the laboratory, when grown-up larvae of *B. mori* were administered with extracts of 18 plants separately (1.5 ml/larva), all plants adversely affected larval weight, cocoon weight and shell weight (Bohidar and Choubey, 2005). Water extract (10 %) of *Andrographis paniculata* (Burm.) Wall. ex Nees (whole plant without roots) or *Plumbago zeylanica* L. (roots) reduced weight of cocoon, shell, filament and pupa, shell percentage, filament length and denier value (Takhliq, 2011). When leaves were sprayed with a NSKE-based product Vijay Neem® (0.005%) and fed to *B. mori* larvae, moths emerged from pupae of these larvae lived for 3.9 days compared to 5.7. days in control, laid fewer number of eggs (350 versus 547 eggs in control) and suffered a less egg hatching rate (73.1 % versus 93.4 % in control) (Kumtha *et al.*, 2013 b). Therefore, only those products which proved safe to silkworms should be recommended.

Nowadays, biofortification has been recommended for rearing and have shown beneficial effects on silkworms. Several biological diet supplements in solid or liquid form are readily available in the market, e.g., herbal tonic containing extract (2 %, v/v) of *Aloe barbadensis* Mill. (=Aloe vera) (Marimuthu and Isaiaarasu, 2010), ayurvedic medicines (Datta and Gupta, 2002), homeopathic drug containing *Strychnos nux-vomica* L. (Hiware, 2006), products containing multivitamins (Etebari and Matindoost, 2005; Radjabin *et al.*, 2006; Kanafi *et al.*, 2007), alpha-glycine (3 %) (Paital and Bohidar, 2011), amino acid alanine (Radjabin *et al.*, 2009) and alfalfa tonic containing essential nutrients (Laskar and Datta, 1998) or hydrolyzed soy protein (Krishnan *et al.*, 1995). All these products when fed to fifth instar larvae, significantly increased weight of silk gland, larva, pupa and cocoon, fibroin content of cocoon shell, relative growth rate and enhanced larval growth. Apart from these products, fortification of mulberry leaves with jaggery solution (10%, w/v), soybean powder (5 g/100 g leaves) (Sundarraaj *et al.*, 2000) or commercial protein powder Nutrilite® (Rani *et al.*, 2011) exhibited significant improvement in

filament length, cocoon yield, shell yield, silk percentage, denier and fibroin content because of the content of essential and non-essential amino acids and protein in the larval haemolymph that aid to protein synthesis. In Egypt, lettuce seed oil (0.1 %) was used as a food additive to fourth and fifth instar larvae which significantly enhanced silk productivity and moth fecundity (Shahin *et al.*, 2013). In Nigeria, Nguku *et al.* (2007) tested royal jelly obtained from local honey bee, *Apis mellifera scutellata* (Lepeletier) colonies and recorded considerable improvement in weight of larva, cocoon and pupa though no significant difference was noticed in shell weight and denier value. This can be tried in India by using honey from local bees.

Use of phytoecdysteroids (hormone-anabolic steroids of plant origin) showed favourable effects on larval maturity, spinning, cocoon and reeling parameters in *B. mori* (Shivakumar *et al.*, 1995, 1996). For example, a juvenile hormone analogue backuchiol extracted from *Psoralea corylifolia* L. leaves enhanced the cocoon parameters without a significant prolongation in the larval duration (Nair *et al.*, 2003) and a moulting hormone extracted from *Ipomoea hederacea* Jacq. seeds (Ghosh *et al.*, 2004) helped in the synchronization, hastening of larval maturation, reducing time for moulting and spinning, increased silk production and saved labour cost incurred in providing mulberry leaves. Considering the positive effect of new ready-made formulations and plant-derived products, intensive studies are needed on local plant material, extraction and isolation of phytochemicals and comparison with market products for cost reduction and easy availability in villages.

Eri silkworm rearing

Strong feeding stimulating activity in *S. ricini* was elicited by an aqueous extract (25-50 % conc. on fresh weight basis) of *A. paniculata*, *Aloe barbadensis* Miller, *Bacopa monnieri* (L.) Pennell and *Withania somnifera* (L.) Duval. The extract also increased larval weight, cocoon weight and shell percentage but did not affect larval duration (Chilana and Srivastava, 2010). Fortification of tapioca leaves with jaggery solution (10%,

w/v) and soybean powder (5 g/100 g leaves) exhibited significant improvement in larval period, larval weight, cocoon yield, shell yield, silk percentage and moth fecundity (Sakthivel and Qadri, 2013).

Foliage treated with chemical pesticides

Application of monocrotophos 36SL at 1.5 ml/l or endosulfan 35EC at 2ml/l on mulberry plants killed >80 % larvae, reduced number of harvested cocoons, moth emergence and fecundity in *B. mori* (Singh *et al.*, 2010), caused changes in levels of different bioconstituents and metabolic processes in the silkworm and degraded economic characteristics of cocoon (Bora and Handique, 1996; Nath *et al.*, 1997). Reduced rate of food intake and its assimilation, efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) have been reported in the late instar larvae of *B. mori* fed on leaves sprayed with 0.01 % fenvalerate 20EC (Wijayanthi and Subramanyam, 2002). Application of 0.05 % fenitrothion 50EC or 0.05 % ethion 40EC significantly decreased the activity of LDH, SDH and MDH and subsequently, induced toxicity by disturbing carbohydrate metabolism (Nath *et al.*, 1997). Also, levels of acetylcholine in the brain, fat body and silk gland were lowered, activity of acetyl cholinesterase was reduced and associated behavioural changes were observed (Nath and Kumar, 1999).

In the laboratory, when mulberry leaves treated with dichlorvos (0.0005 %) were fed to *B. mori* larvae, moths emerged from pupae were few in number (55 % versus 79% in control), lived for 4.3 days compared to 5.7 days in control, laid less number of eggs (485 versus 547 eggs in control) and egg hatching was affected (79.7 % versus 93.4 % in control) (Kumtha *et al.*, 2013 b). Greater insect growth regulatory effects, particularly larval-pupal intermediates, were also produced in *B. mori* with high doses of dichlorvos sprayed on mulberry crop (Kumtha *et al.*, 2013 a). Similarly, a dose of 2 g/l of buprofezin 25WP (Applaud®) applied to leaves and fed to *B. mori* larvae on first day of each instar affected most of the biological

characters, cocoon sericin and fibroin content but did not affect their mortality rate (Vassarmidaki *et al.*, 2000).

Silkworm breeds

Among mulberry silkworms currently exploited in the field, bivoltine broods (including double hybrids) give high cocoon shell percentage, better raw silk recovery and higher yield of 65-75 kg/100 disease free layings (dfls) (equivalent to about 40,000 larvae) with international silk grade of 3A-4A. Sericulturists in China nowadays prefer bivoltine races. However, over 95% of commercial raw silk in India is produced from a hybrid (multivoltine female x bivoltine male) that produces high quality silk with commercial grade of A-2A. These findings reveal considerable superiority of new hybrid strains over old hybrids (Ramesh *et al.*, 2009). Therefore, Naseema Begum *et al.* (2014) recommended a new robust bivoltine double hybrid (CSR 50 x CSR 52) x (CSR 51 x CSR 53) which is significantly superior over single hybrids in respect of egg yield by 34.2 % and dfls recovery by 18.5%. In the case of non-mulberry silkworms, multivoltine races of tasar silkworm have been bred and would be available in future. Thus, sericulturists would be able to select hybrids which produce better quality silk fibre in the 'vanya' sector too.

Natural enemies of silkworms

Predators

The dermestid beetle, *Dermestes ater* DeGeer (Coleoptera: Dermestidae) feeds on *B. mori* eggs and moths, besides boring into cocoons to feed on pupae within the cocoons. The common earwig, *Labia (Forficula) arachidis* Burr (Dermaptera: Forficulidae) attacks gravid females placed on the sheet for egg laying and cut open their abdomen. The injured moths die. The pentatomid bug, *Canthecona* sp., preying mantid, *Hierodulla bipapilla* (Serville), ants and wasps are common predators of *A. mylitta* (Shivakumar and Shamitha, 2013).

Management: Doors and windows provided with wire

mesh is ideal for rearing room. Another method is to rear silkworms in enclosures where predators can be ward off easily. Predators collected by vacuum cleaner can be destroyed by burning. The commercial product Raksharekha® containing insecticidal chalk is effective against ants and cockroaches (Anonymous, 2014). Grainage premises and places meant for cocoon storage should be kept clean. To kill harbouring predators, dusting of bleaching powder (36 % chlorine) or spraying of deltamethrin (0.028 %) on the floor, near the walls of storage rooms, on stands and in rearing room, and dipping wooden trays in dichlorvos emulsion (0.076 %) for 2-3 minutes followed by thorough washing with clean water after 10 days are effective preventive measures (Anonymous, 2014). In the case of pierced cocoons, they should be thoroughly sun-dried and treated with malathion dust (5 %) before storing in airtight bags which are previously dipped in deltamethrin solution (0.028 %) followed by shade drying (Anonymous, 2014). The bags can then be stored in a place away from the grainage.

Parasitoids

The major larval endoparasitoid of mulberry silkworm and tasar silkworm is an uzi fly, *Exorista sorbillans* (Lewis) (= *Tricholiga (Tachinia) bombycis*, *Tricholiga sorbillans*) (Diptera: Tachinidae). Its attack may incur economic loss to the extent of 20-80 % in seed crop (Sahu *et al.*, 2008). The incidence of the uzi fly in muga silkworm is higher on the crop reared during February-March than in larvae reared during December-January (Eswara Reddy and Rajan, 2011). This finding implies that precautionary measures should be adopted in advance to avoid or minimize fly attack. Other two fly species *Exorista japonica* Townsend and *Blepharipa zebina* Walker can occasionally cause significant mortality in silkworms. Early instar silkworms are killed and late instar larvae (up to early fifth instar, before spinning stage) spin weak and bad quality cocoons. Maggot entry is confirmed by the presence of black scar on the larval skin. Fly emerges by piercing cocoon which becomes unfit for commercial reeling (Anonymous, 2014).

Cocoons of the tasar silkworms are parasitized by a

larval-pupal endoparasitoid, *Xanthopimpla pedator* Fb. (Hymenoptera: Ichneumonidae) (Velide and Bhagavanulu, 2012) and larvae by a nematode, *Hexameris* sp. (Nematoda: Mermithidae) (Shivakumar and Shamitha, 2013); the former may bring loss of 9-15 % in cocoon production (Velide and Bhagavanulu, 2012). The ichneumonid causes more mortality (14.5 %) in second rearing season than in the first season (2.9 %) and prefers male pupae (85.6 %) (Bhatia and Yousuf, 2013). Barman (2010) reported a polyphagous braconid parasitoid, *Apanteles* sp. attacking second and third instar larvae of muga silkworm but data on the seasonal incidence and silkworm mortality are not available.

Management: Rearing rooms should be free from cracks and crevices. The access of female flies is restrained if doors and windows of rearing room are covered with wire mesh. Maintenance of sanitary and hygienic conditions in the rearing room, and collection and destruction of parasitized silkworms, maggots and pupae are quite efficient. Spraying of benzoic acid (1 %) against fly eggs laid on the mulberry silkworm and dusting of diflubenzuron (2.5 %) diluted with China clay against maggots and pupae of the uzi fly reduced infestation in muga silkworm (Anonymous, 2014). A water trap with chemical attractants dissolved in it can be placed near the windows. Flies attracted to the trap get drowned in the solution and killed. With this trap, fly population was reduced by up to 84 % (Anonymous, 2014). Certain chemical uzicides readily available in the market can also be used. For example, uzi powder® dusting on silkworms on the second day during third instar, second and fourth day during fourth instar, and on the third and fourth day during fifth instar has proven beneficial.

Water extract (4-5 %, w/v) of leaves or seeds of china berry (*Melia azedarach* L.) acts as a strong repellent to uzi fly, thereby reducing fly egg hatchability and increasing mortality in maggots and pupae in a dose-dependent manner (Narayanaswamy *et al.*, 1997). Oil (0.05 %) of *Ageratum conyzoides* L. or *Ocimum sanctum* L., alcoholic extract (10 %) of *A. conyzoides* or *Cantharanthus roseus* (L.) Don caused 78-100 % mortality in fly populations

(Bora and Khanikor, 2011). A neem-based product, Achook® (0.02-4 %) acting in a dose-dependent manner, reduced larval weight, prolonged pupation, and the flies emerged were small with abnormal proboscis and small abdomen. Major insect growth regulatory effect was exhibited by larval-pupal intermediates in *B. zebina* (Singh and Thangavelu, 1996). At least, eight hyperparasitoids of gregarious behaviour have been recorded on maggots or pupae of uzi fly (Kumar *et al.*, 1989, 1994; Kumar, 2003, 2004; Kaiser *et al.*, 1997; Sahu *et al.*, 2008). Among them, an ecto-pupal parasitoid, *Nesolynx thymus* Girault (Hymenoptera: Eulophidae) is efficient when released in three doses (8,000, 16,000 and 76,000 adults/100 dfls) corresponding to fourth and fifth instar larvae and within 1-2 days after cocoon harvest (Anonymous, 2014). Wasps should be released immediately after sunset in the rearing room, places of mountage and racks storage, near mountages with spinning worms and near the manure pits.

Insect diseases

Flacherie disease is caused due to infection of bacteria such as, *Bacillus* sp. (Bacillales: Bacillaceae) and *Pseudomonas aeruginosa* (Schroter) Migula (Pseudomonadales: Pseudomonadaceae). Sometimes, bacteria and viruses together can infect larvae when they feed on infected or contaminated leaves (Selvakumar *et al.*, 2005). Infected larva becomes soft, flaccid and pale in colour, stops feeding and suffer retarded growth. It fails to moult and starts vomiting releasing contaminated body fluid.

Management: As a precaution, utmost care is needed to maintain sanitation and hygiene in the rearing rooms and feeding beds. Appliances and rearing rooms need to be disinfected with bleaching powder before commencing rearing. In order to prevent further spread of diseases, early diagnosis of the disease is important (Anonymous, 2014). Likewise, separation of diseased larvae and discarding them, disinfection of silkworms, feeding on healthy and nutritious foliage and provision of good ventilation in rearing room can lower disease incidence

(Anonymous, 2014). Injury to the larvae, overcrowding of trays and accumulation of faeces in the rearing beds should be avoided. By spraying water extract (5 %, w/v) of *Curcuma longa* L., *Phyllanthus emblica* L., *Asparagus racemosus* Willd., *Aegle marmelos* (L.) Correa, *Boerhavia diffusa* L., *Allium sativum* L. or *Ocimum basilicum* L., Pachiappan *et al.* (2009) obtained larvae, cocoons and shells with higher weight and better economic parameters than non-treated larvae. A commercial product Muga-heal® prepared from fruits of *Terminalia chebula* Retz. proved effective against *P. aeruginosa* (strain AC3) in muga silkworm (Unni *et al.*, 2004, 2011). This product is easy to apply, improves cocooning and reeling due to uniform size of quality silk with unbreakable long filament, and can be recommended for rearing of other silkworms (Unni *et al.*, 2004, 2011). After collecting and burning infected larvae, faecal matter and bed refuses, spraying antibiotics such as, streptocycline at 1g/10 litres is useful (Anonymous, 2014). Also, dipping eggs of muga silkworm in formalin (2 %) and spraying the foliage with gallic acid (0.5 %) have been recommended by Kakati (2002).

White and green muscardine are caused in the mulberry silkworm by *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Clavicipitaceae) and *Spicaria prasina* (Maubl.) Sawada (Eurotiales: Trichocomaceae), respectively (Chandrasekharan and Nataraju, 2013). Infected larva has soft and flaccid body. After death, the fungal conidia grow on the body surface and larva becomes dry, mummified and hard with body fully covered with white or green powdery spores. Larvae fed on mite infested tender/young mulberry leaves particularly of cv. V-1 (Ramegowda *et al.*, 2012) and those fed on unhealthy and non-nutritious foliage (Chandrasekharan and Nataraju, 2013) are susceptible to muscardine disease. Another fungal disease, aspergillosis, is common in young larvae. Infected larva becomes lustrous and dies in a few days. The mycelial cluster of dark green fungus (*Aspergillus flavus* Link) or rusty brown fungus (*Aspergillus tamarii* Kita) (Eurotiales: Trichocomaceae) is seen on the dead body. In tasar silkworm, white muscardine is caused by *Penicillium*

citrinum Thom. (Eurotiales: Trichocomaceae) and yellow muscardine by *Isaria farinosa* (Holmsk.) Fr. (Kiran Kumar *et al.*, 2011).

Management: Sun-drying of rearing appliances, disinfecting the rearing room and trays/utensils with 2 % bleaching powder, early diagnosis of the disease and rejection of the infected lots can prevent further spread of the disease (Anonymous, 2014). Low temperature combined with high humidity in the room favours growth and multiplication of pathogens, rearing room should therefore be maintained with proper conditions and rearing beds should be kept thin and dry. Spraying Dithane M-45 at 3kg/100 dfls (Anonymous, 2014) and disinfecting the rearing room and trays with 4 % pentachlorophenol can control aspergillosis. Disinfection of eggs of muga silkworm with 2 % formaldehyde solution reduced the disease incidence (Das and Das, 2010). Against all fungal diseases, alcoholic extract of *Tridax procumbens* L., *O. sanctum* or *Acalypha indica* L. was more effective than aqueous extract (Isaiarasu *et al.*, 2011). Since *B. bassiana* is unable to invade the larva during its moulting period, plant extracts should be applied at this stage to prevent further spread (Chandrasekharan and Nataraju, 2011). In the laboratory, complete control of bacterial and fungal diseases of mulberry silkworm and eri silkworm was achieved by methanolic extract (16 %) of two sea grasses, *Rhizophora mucronata* Poir, *Syringodium isoetifolium* (Ash.) Dandy (Ravikumar *et al.*, 2009). The *Terminalia arjuna* leaves were sprayed with 1 % and 2 % solution of four commercial systemic fungicides (Bavistin®, Bayleton®, Thiram®, Dithane M-45®) and fed continuously for two days to fifth instar larvae of tasar silkworm. Also, larvae were topically inoculated with *Penicillium citratum* (4×10^6 conidia/ml) and then fed on fresh leaves. These treatments were comparatively less effective than Bavistin and Dithane (sprayed at 2 %) realising a complete control (Kiran Kumar *et al.*, 2011).

Silkworms are often infected by the Nuclear polyhedrosis virus (NPV) (jaundice or grasserie disease) and Cytoplasmic polyhedrosis virus (CPV) (Bhaskar *et al.*, 1999). The NPV parasitizes cell nucleus resulting in

swelling between segments, shiny body wall, milky white colour and incessant crawling. Larvae infected in the later period of fifth instar may form dark brown cocoons that rupture easily and turbid white body fluid containing hexagonal crystals oozes out. Larvae hang upside down with the anal claspers after dying. Larvae infected with CPV show symptoms of slow growth, stunted and atrophied body, dull white colour, transparent thorax, reduced food consumption, vomiting and diarrhoea. As the disease progresses, white faeces appear.

Management: Hygienic conditions are important to avoid spread of diseases. For this, firstly, sun drying of rearing appliances for 1-2 days and disinfection with 2 % bleaching powder of the rearing room, appliances and trays should be strictly followed. Secondly, proper ventilation and air circulation should be maintained. Thirdly, by keeping proper bed spacing, injury to the silkworms, overcrowding and accumulation of faeces in the rearing bed can easily be avoided. Moreover, this system helps to feed larvae with healthy nutritious leaves. Fourthly, in the case of diseased larvae, early diagnosis and rejection of infected lots is compulsory. This can be done by collecting and burning the infected larvae, faecal matter and bed refuses (Anonymous, 2014). Spray containing 800 ppm of hexane extract or aqueous extract of leaves of *P. coryleifolia* or *Plectranthus indicus* (Roth) Spreng, not only reduced the disease incidence in *B. mori* by up to 20-60 % but also increased larval weight, shell weight and cocoon weight, probably due to flavonoids (bakuchicin, bavacoumestan) present in the extract (Padma and Manimegalai, 2007; Manimegalai *et al.*, 2010). To control CPV in *A. mylitta* larvae, 13 medicinal plants were tested in the laboratory, aqueous extract of only three plants (*Aloe barbadensis*, *Psoralea coryefolia*, *Bougainvillea spectabilis* Willd.) reduced mortality in diseased larvae by 66.2 %, 64.5 % and 57.2 %, respectively (Kumar *et al.*, 2012 a). In the laboratory experiments, the ethanolic plant extract augmented activity of enzymes acted as antiviral against NPV. The plant species assayed included *Aegle marmelos* (L.) Corr., *Momordica charantia* L., *Argemone mexicana* L., *Curcuma longa* L., *Syzygium cumini* (L.) Skeels and

Euphorbia geniculata L. (Bhawane *et al.*, 2013).

As a general practice, dust the disinfectant Vijetha® (3kg/100 dfls) on the larvae after each moult and ½ h before starting of feeding, and spray 1 % water extract of *P. coryleifolia* on mulberry leaves, dry them in shade and feed silkworms once during third instar (Anonymous, 2014). A plant-based product Amruth® against flacherie and grasserie has been suggested (Anonymous, 2014). To prepare the solution, mix 20 g powder in a litre of water, spray 70 ml solution on a kilogramme of mulberry leaves. Other commercial products (Ankush® /Vijetha® /Sanitech® /Resham Jyothi®) can also be used as disinfectant at 2 l/m² of bed area. Additional measures including bed disinfection with lime powder (2 %) have been recommended by Reddy and Rao (2009).

Pebrine or microsporidiosis disease caused by a microsporidian, *Nosema bombycis* Nageli (Microsporidia: Nosematidae) is well studied in *B. mori* and documented (Singh and Saratchandra, 2003; Qadri and Khatri, 2005; Singh *et al.*, 2012). The disease is complex with its chronic and highly virulent nature because the microsporidian attacks all life stages from embryo to adult of *A. mylitta* but incidence is high in larvae (20-28 %) and pupae (6-8 %) (Velide and Rao, 2011). Larvae infected during the first and second instars show a normal growth up to third instar. If the contamination takes place in the third instar, larvae show symptoms of the disease in the late fifth instar. Late stage infected larvae lose appetite, retard in growth and moult irregularly. These yellowish green larvae discharge spores through faecal matter during the fourth and fifth instar. In advanced stage of infection, small brown/black spots cover the body and larvae are either unable to spin cocoons or else spin them loosely and finally die without pupating. Secondary infection to fourth instar larvae leads to formation of flimsy cocoons. Infected larvae have extended development period, small body and low larval weight (Rath *et al.*, 2003). Diseased larvae extrude faecal matter, gut juice and vomit containing pathogens. Infection also affects food consumption and digestion of the fifth instar *A. mylitta* larvae and declines significantly the silk gland

weight, shell weight and growth rate values (Rath *et al.*, 2003), moth emergence, fecundity, hatching, filament length, silk reeling and weight of fibre reeled (Velide and Rao, 2011).

Management: This disease is not common but control measures need to be studied for eventual disease infection in silkworms. For this purpose, the 'Fujiwara' method suggested by Kumar *et al.* (2012 b) can be used for early diagnosis. Other preventive measures include production of healthy silkworm eggs, disinfection of rearing room and appliances, maintenance of strict hygiene during rearing and surface disinfection of eggs in 2% formalin for 10 minutes before incubation. Whenever infected eggs, larvae, pupae and moths, bed refuses, faecal pellets *etc.* are noticed, that particular lot is rejected and destroyed through recommended methods (Anonymous, 2014).

CURRENT CHALLENGES AND OPPORTUNITIES

1. Organic farming

Of late, organic farming has been gaining popularity in view of maintaining an environment and human friendly agro-ecosystem. Nutritional status of mulberry leaves is improved in crops under organic farming and can positively influence cocoon production and economic characters (Rao *et al.*, 2007; Babu *et al.*, 2013). Green mulching of sunnhemp (*Crotalaria juncea* L.) in *Terminalia tomentosa* improved soil water holding capacity (56.9%), moisture content (88.5% and 37.9% at 30 and 45 cm depth, respectively), electrical conductivity (0.30 mhos/cm), pore space (50.4%), organic carbon (0.61%), nitrogen (109 kg/ha) and potassium (254 kg/ha). Ultimately, these properties led to better leaf yield (24.8t/ha) for rearing tasar silkworm (Manohar Reddy *et al.*, 2013). Improvement in soil physical, chemical and biological properties was also possible with compost prepared from the left over shoots of mulberry by using fungi (*Pleurotus* spp.) as decomposing agent. Application of compost, vermicompost (Das *et al.*, 2012; Nishitha Naik *et al.*, 2013) and biofertilizers containing bacteria or vesicular-arbuscular mycorrhizal fungi (Rao *et al.*, 2007) resulted in significant improvement in productivity and

quality of mulberry foliage.

2. Use of bio-principles

Application of plant growth promoting rhizobacteria improved quality and quantity of cocoons in muga silkworm reared on some plants. This was possible due to higher content of enzymes (trehalase, transaminase, phosphorylase) in the silk gland, haemolymph and fat body (Unni *et al.*, 2008). The large scale ground spraying or aerial spraying of two biological control agents (*Beauveria bassiana* Vuill. and *Bacillus thuringiensis* Berl.) has been recommended by Das *et al.* (2014) who cautioned about non-target effects on wild silkworms. Therefore, an appropriate control strategy needs to be developed in the near future. When vermiwash (10, 25 and 50%) was sprayed on mulberry leaves and fed to fifth instar of *B. mori* larvae, there was significant increase on dose-dependent basis, in the content of carbohydrates, protein and lipid of fat bodies, acid phosphatase and alkaline phosphatase that subsequently increased larval weight (3.42 g versus 2.16 g for untreated leaves) and shell weight (0.90 g versus 0.31 g for untreated leaves) in *B. mori* (Purushothaman *et al.*, 2012).

Readily available indigenous plant material comes from neem tree which has been extensively studied for plant protection in field crops (Gahukar, 2014). Most of the neem-derived products including pure azadirachtin are safe to silkworms due to low inherent contact toxic action and minimum residual toxicity (Sharma *et al.*, 2005). Other plants have been used on limited scale and further experimentation in the laboratory and field would reveal safer insecticides for producing chemical-free foliage needed for quality silk production.

3. Reduced use of chemicals

Chemical pesticides of different categories have been used in mulberry plantations but some of them adversely affected health of silkworms and cocoon quality, and even caused poisoning in field workers (Philip *et al.*, 1998; Gangwar *et al.*, 2000; Jeyapaul *et al.*, 2003; Tanki *et al.*, 2005). As a result, annual mulberry silk production has

decreased by up to 30 % in China (Li *et al.*, 2010). Therefore, testing of chemical pesticides on sericultural plants should be made mandatory so that those with proved bioefficacy against pests and diseases of host plants but not harmful to silkworms can be recommended. For indoor rearing, foliage should be tested for chemical residue level before feeding to silkworms. Unfortunately, this facility is either lacking or limited in all zones/regions of cultivation of sericultural plants. Sericulturists should be informed and trained for proper use of recommended chemicals. In the plantations, minimum use of chemical fertilizers and pesticides should be advocated while delivering package of practices to farmers.

4. Conservation of biodiversity

In the strategy of biodiversity conservation, heterozygosity plays an important role for which estimation of genetic diversity should be preferred over morphological traits. In India, 1120 host plants of *B. mori*, 13 species of this silkworm and an equal number of tasar silkworm have been recorded. Similarly, 14 morphotypes of som and 10 of soalu and 41 accessions of castor have been preserved (Tikader and Kamble, 2010). In reality, knowledge of genetic variation among populations, climatic adaptation, resistance to pests and diseases, geographic distribution, host plants, breeding *etc.* is needed for better conservation (Tikader *et al.*, 2013). In the case of *B. mori*, both markers and microsatellite sequences proved helpful in conducting genetic diversity studies on the morphotypes (Li *et al.*, 2005). In Manipur state (India) alone, 15 species (wild, semi-domesticated and domesticated) of silkworms in seven genera and two families have been reported through survey (Ibsorani Devi *et al.*, 2011). Out of 14 silkworm species, only four species are commercially exploited. Probably, the lack of knowledge of genetics and host plants is currently a bottleneck to improve rearing of domesticated and wild species.

New food plants of silkworms should not be neglected and surveys should be continued in a systematic way because in the period of scarcity, silkworms can survive on these plants. For example, *Litsea glutinosa* Lour. is a new

host plant recorded in India (Bindroo *et al.*, 2009). Further, wild species have to be conserved as there would be socio-economic perspective of rearing at least for the tribal communities (Kakati and Chutia, 2009). With conservation of natural resources and building community participation, establishment of the germplasm banks both for the host plants and silkworms can be envisaged. If possible, sericulture may be linked with local and global marketing so that farmers can earn tangible benefits even from their marginal land holdings.

Conservation is often affected by climatic changes in nature whereas the physical factors can be manipulated for maximum success in rearing rooms. With optimum temperature (20-31°C) and relative humidity (65-95 %), the moth emergence, fecundity and egg hatching are higher in the seed crops of muga silkworm (Zamal *et al.*, 2010). As per climatic conditions prevailing in the region, the cocoon production and quality differ but in general, the biological parameters of muga silkworm decline with the rise in temperature over the years. For example, Chaudhary (2003) reported decline in the silk production by 0.8 % during 2007-08 compared to previous year due to unfavourable climate. Nowadays, the natural balance in agro-ecosystems is getting disturbed. Therefore, efforts should be intensified to restore or at least to maintain the ecological balance in zones where host plants are cultivated or are found in the wild.

5. Silkworm rearing

Generally, farmers are habituated to rear silkworms on detached mulberry leaves. However, it has recently been proved that insect rearing on shoot is advantageous in many ways, *viz.*, considerable saving (up to 50 %) in labour cost, nearly 15 % less foliage requirement, reduced leaf drying in rearing bed, requirement of small bed area, avoidance or less contamination of pathogens and better aeration in rearing beds (Anonymous, 2014). Further, instead of several irregular feedings, two feedings / day at equal intervals are sufficient. Tender, succulent and nutritious leaves result in better growth and development of young larvae whereas progressively mature leaves with

less moisture content are required for later larval instars. For evaluating the quality of mulberry leaves, Himantharaj and Vindhya (2013) recommended a 'Keburui' test which was introduced for the first time in Japan.

In the laboratory rearing, there is scope for feeding silkworms on artificial diets. Artificial diet containing green gram [*Vigna radiata* (L.) Wilczek] was the most suitable medium for development of *S. ricini* larvae that produced cocoons and shells with higher weight and shell percentage than diet containing barnyard millet, soybean, corn, rice or French bean (Chaudhary and Gaur, 2013). This diet can easily be prepared with available local food grains. When *A. mylitta* was reared on fresh leaves of *T. arjuna* and semi-synthetic diet, the diet showed better survival of young larvae. Also, the ERR value, body weight, cocoon weight, shell percentage and protein profile were near to that of outdoor reared larvae (Kumar *et al.*, 2013). Therefore, artificial diets containing commercial product Nutrid® may be recommended for rearing of *B. mori*, particularly during early instars to provide them with balanced nutrients and to minimize human intervention by way of leaf feeding.

6. Integrated pest management

For an integrated management of parasitoids and diseases of silkworms, several measures have to be studied and the economic and effective strategy has to be formulated as discussed by Dutta *et al.* (2010) for muga silkworm. Use of bleaching powder as ovicide combined with release of a wasp (*N. thymus*) could prevent parasitization of uzi fly in tasar silkworm by up to 75.3 % (Ram Kishore *et al.*, 2009). Plant products if combined with safe and effective organic products, can be more economical. For example, mixing cow urine with plant products has shown significant advantages in pest control (Gahukar, 2013). Intensive research on development of effective IPM comprising of all suitable components in a compatible manner particularly, pest and disease resistant/tolerant cultivars, natural enemies, and sanitation and hygiene in the field/rearing room should receive

attention. This strategy would prove ideal to make plant protection effective and economical for the small and marginal farmers.

CONCLUSION

The favourable biotic factors as discussed above should be identified and appropriately applied. Unfavourable elements particularly larval parasitoids and diseases should be prevented from the beginning and utmost care is needed even during rearing. Since silk industry is gaining importance in retail marketing and export, rearing facilities, financial support / subsidy and technical assistance should be extended by the government.

ACKNOWLEDGEMENT

The author sincerely thanks Dr. V. Lakshmi Narayanamma, Sericulture Scientist, Agricultural Research Institute, Hyderabad (Andhra Pradesh), India, for critically reviewing the manuscript and suggesting certain changes.

REFERENCES

- Anonymous (2014) *AgriTech Portal*, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.
- Babu C. M., Dandin S. B., Thippeswamy T. and Renukeswarappa J. P. (2013) Nutritional status of mulberry leaf produced through organic farming and its impact on cocoon production. *Indian J. Seric.*, **52(1)**: 14-18.
- Barah A. and Sengupta A. K. (1991) Correlation and regression studies between pupal weight and fecundity of muga silkworm, *Antheraea assama* Westwood (Lepidoptera: Saturniidae) on four different food plants. *Acta Physiol. Hung.*, **78(3)**: 261-264.
- Barman H. (2010) *Apanteles* problem in muga silkworm crop. *Munis Entomol. Zool.*, **5(Suppl.)**: 1189-1190.
- Bhaskar R. N., Govindan R., Devaiah M. C. and Radhakrishna D. (1999) Influence of mulberry leaf nutrition and BMCPV infection on disease development and some cocoon traits in *Bombyx mori*. *Sericologia*, **39**: 25-29.
- Bhatia N. K. and Yousuf M. (2013) Parasitic behaviour of *Xanthopimpla pedator* Fabricius on tropical tasar silkworm,

- Antheraea mylitta* Drury (Lepidoptera: Saturniidae) reared on seven forestry host plants in Uttarakhand, India. *Int. J. Ind. Entomol.*, **27**: 243-264.
- Bhawane G. P., Chougale A. K., Patil S. J., Gaikwad Y. B. and Chavan J. A. (2013) Effects of ethanolic plant extracts on BMNPV inoculated silkworm, *Bombyx mori* in relation with midgut antioxidant enzymes, acid phosphatase and non-specific esterase. *Sericologia*, **53**:182-192.
- Bhede B. V., Lande U. L. and Pathrikar D. T. (2013) Evaluation of different mulberry varieties for commercial rearing of silkworm hybrid in Maharashtra. *J. Entomol. Res.*, **37**: 17-24.
- Bindroo B. B., Singh N. T. and Sahu A. K. (2009) *Litsea glutinosa* Lour.: A new host plant of muga silkworm (*Antheraea assamensis* Helfer). *Sericologia*, **49**: 231-237.
- Biswas N. and Dass P. K. (2001) Effect of food plant species on rearing performance of eri silkworm, *Samia cynthia ricini* (Boisduval). *Bull. Indian Acad. Seric.*, **5**(1): 36-39.
- Bohidar K. and Choubey M. (2005) Effect of indigenous plant extracts on the economic characters of mulberry silkworm, *Bombyx mori* L. *Indian J. Entomol.*, **67**: 238-240.
- Bohidar K., Choubey M. and Sahoo B. S. (2004) Preliminary studies on the effect of medicinal plant extracts on eri silkworm, *Samia cynthia ricini* (Boisd.). *Insect Environ.*, **10**: 33-34.
- Bora D. S. and Handique R. (1996) Insecticide induced biochemical changes in *Antheraea assama* Westwood. *Proc. Acad. Environ. Biol.*, **5**: 221-226.
- Bora D. S. and Khanikor B. (2011) Selective toxicity of *Ageratum conyzoides* and *Ocimum sanctum* against *Exorista sorbillans* (Diptera: Tachinidae) and *Antheraea assama* (Lepidoptera: Saturniidae). *Nat. Acad. Sci. Lett.*, **34**: 9-14.
- Chakravorty R., Barah A., Neog K., Rahman S. A. S. and Ghose J. (2004 a) *Package of practices of muga, eri and mulberry sericulture for North Eastern Region of India*. Central Muga and Eri Research and Training Institute, Jorhat, Assam, India, p. 23.
- Chakravorty R., Neog K., Suryanarayana N. and Hazarika L. K. (2004 b) Feeding and moulting behaviour of muga silkworm (*Antheraea assama*) on different food plants. *Sericologia*, **44**: 145-152.
- Chaluvachari A. and Bongale U. D. (1996) Bioassay moulting response of silkworm, *Bombyx mori* L. in relation to nutritive constituents in mulberry (*Morus* spp.) genotypes. *Indian J. Seric.*, **35**: 160-162.
- Chandrapa D., Govindan R., Sannappa B. and Venkataramana P. (2005) Correlation between foliar constituents of castor genotypes and economic traits of eri silkworm, *Samia cynthia ricini* Boisduval. *Environ. Ecol.*, **23**: 356-359.
- Chandrasekhar S. and Govindan R. (2010) Studies on the performance of castor genotypes on rearing cocoon of eri silkworm. *Int. J. Plant Prot.*, **3**: 219-224.
- Chandrasekharan K. and Nataraju B. (2011) *Beauveria bassiana* (Hyphomycetes: Moniliales) infection during ecdysis of silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). *Munis Entomol. Zool.*, **6**: 312-316.
- Chandrasekharan K. and Nataraju B. (2013) Effect of different nutritional conditions of silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) on its susceptibility to *Beauveria bassiana* (Hyphomycetes: Moniliales). *Munis Entomol. Zool.*, **8**: 613-618.
- Chaudhary M. (2013) Evaluation of impact of weather on cocoon shell weight of muga silkworm (*Antheraea assama* Ww.). *Sericologia*, **48**: 315-322.
- Chaudhary P. and Gaur N. (2003) Artificial diet for rearing of *Samia cynthia ricini*. *J. Insect Sci.*, **26**: 49-51.
- Chilara P. and Srivastava R. P. (2010) Impact of some medicinal plant extracts on cocoon production of eri silkworm, *Philosamia ricini* Hutton. *J. Insect Sci.*, **23**: 381-386.
- Chowdhury S. A., Ahmed R., Bhattacharya P. R., Dutta S., Das A. M. and Rajkhowa A. (2000) Performance of muga silkworm (*Antheraea assama* Ww.) on different som (*Persea bombycina* King ex Hook (F.) Kost.) plant collections from Assam, India. *Int. J. Wild Silk Moths Silk*, **5**: 66-69.
- Dar M. Y., Illahi I., Agrawal O. P., Mittal V. and Ramegowda G. K. (2011) Impact of mite infestation on mulberry leaf and silkworm, *Bombyx mori* L. *Indian J. Entomol.*, **73**: 378-381.
- Das R. and Das K. (2010) Muscardine in pre-seed cocoon muga crop and its management. *Indian Silk*, **48**(1): 16-17.
- Das R., Das K. and Giridhar K. (2014) Constraints in management for conservation of muga silkworm (*Antheraea assamensis* Helfer). *Munis Entomol. Zool.*, **9**: 879-883.
- Das R., Neog K., Pamehgama M. and Borua U. C. (2012) Effect of vermicompost application on leaf yield of mulberry and cocoon characters of silkworm, *Bombyx mori* in the North-eastern region of India. *J. Indian Acad. Seric.*, **15**(1): 49-52.

- Dash A. K., Nayak B. K. and Dash M. C. (1992) The effect of different food plants on cocoon crop performance in the Indian tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *J. Res. Lepidoptera*, **31**(1-2): 127-131.
- Datta M. and Gupta J. (2002) Application of plant extracts based ayurvedic medicine with feed to the silkworm, *Bombyx mori* L. Nistari race to assess its effect on cocoon production. *Environ. Ecol.*, **20**: 505-510.
- Datta M. and Prasad R. (1999) Effect of phytoextract-based medicines on the development of silkworm, *Bombyx mori* L. *Environ. Ecol.*, **16**: 875-877.
- Debaraj Y., Singh R., Biswas T. K. and Bindroo B. B. (2013) Sericulture with special reference to rearing and seed technologies. *Sericologia*, **53**: 1-9.
- Deka M. and Kumari M. (2013) Comparative study of the effect of different food plant species on cocoon crop performance of tropical tasar silkworm (*Antheraea mylitta* Drury). *Int. J. Res. Chem. Environ.*, **3**(1): 99-104.
- Dutta P., Neog K., Das R., Das K., Handique P. K. and Chakravorty R. (2010) Evaluation of some botanicals, antibiotics, carbon source and carrier against the bacterial disease of muga silkworm, *Antheraea assamensis*. *Sericologia*, **50**: 1-9.
- Eswara Reddy S. G. and Rajan R. K. (2011) Present status of uzi fly, *Exorista bombycis* (Louis) (Diptera: Tachinidae) incidence on muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae) in Upper Assam. *Munis Entomol. Zool.*, **6**: 856-858.
- Etebari K. and Bizhannia A. R. (2006) Effect of thrips (*Pseudodendrothrips mori* Niwa) infested mulberry leaves on silkworm growth and commercial cocoon parameters. *Caspian J. Environ. Sci.*, **4**(1): 31-37.
- Etebari K. and Matindoost L. (2005) Application of multivitamins as supplementary nutrients on biological and economical characteristics of silkworm, *Bombyx mori* L. *J. Asia-Pac. Entomol.*, **8**: 107-112.
- Gahukar R. T. (2013) Cow urine: A potential biopesticide. *Indian J. Entomol.*, **75**: 212-216.
- Gahukar R. T. (2014) Potential and utilization of plant products in pest control. In: *Integrated Pest Management: Current Concepts and Perspective*, D. P. Abrol (Ed.), Elsevier Inc., New York, USA, pp. 125-139.
- Gangwar S. K., Qadri S. M. H., Maji M. D., Pratheesh Kumar P. M. and Saratchandra B. (2000) Evaluation of fresh plant extracts for the control of mulberry powdery mildew. *Indian J. Seric.*, **39**: 76-78.
- Ghosh D., Tandon B. and Laddha K. S. (2004) *Ipomoea hederacea*: A useful insect moulting hormone in sericulture. *J. Entomol. Res.*, **28**: 211-223.
- Govindan, Sannappa B., Bharthi V. P., Singh M. P. and Hegde D. M. (2002 a) Influence of feeding leaves of some castor varieties on rearing performance of eri silkworm, *Samia cynthia ricini* (Boisduval). *Indian J. Environ. Ecol.*, **6**: 485-488.
- Govindan, Sannappa B., Bharthi V. P. and Ramakrishna Naik A. (2002 b) Influence of castor genotypes on economic traits of eri silkworm. *Insect Environ. Ecol.*, **8**: 72-73.
- Hazarika L. K., Saikia C. N., Katakya A., Bordoloi S. and Hazarika J. (1998) Evaluation of physico-chemical characteristics of silk fibres of *Antheraea assama* reared on different host plants. *Bioresource Technol.*, **64**: 67-70.
- Hazarika U., Barah A., Phukon J. D. and Benchamin K. V. (2003) Studies on the effect of different food plants and season on the larval development and cocoon characters of silkworm, *Samia cynthia ricini* Boisduval. *Bull. Indian Acad. Seric.*, **7**(1): 77-85.
- Himantharaj M. T. and Vindhya G. S. (2013) Keburui test: a new method for evaluating the quality of mulberry leaves. *Indian J. Seric.*, **52**: 79-81.
- Hiware C. J. (2006) Effect of fortification of mulberry with homeopathic drug *nux vomica* on *Bombyx mori* L. *Homeopathy*, **95**(3): 148-150.
- Ibsorani Devi K., Singh L. S., Singh N. I., Dutta K. and Chaoba Singh K. (2011) Biodiversity of sericigenous insects and their food plants in Manipur. *Ecospan*, **5**(1-2): 65-68.
- Isaiarasu L., Sakthivel N., Ravikumar J. and Samuthiravelu P. (2011) Effect of herbal extracts on the microbial pathogens causing flacherie and muscardine disease in the mulberry silkworm, *Bombyx mori* L. *J. Biopesticides*, **4**(2): 150-155.
- Jayaramaiah M. and Sannappa B. (2000 a) Correlation coefficient between foliar constituents of castor genotypes and economic parameters of the eri silkworm, *Samia cynthia ricini* Boisduval (Lepidoptera: Saturniidae). *Int. J. Wild Silk Moths Silk*, **5**: 162-164.
- Jayaramaiah M. and Sannappa B. (2000 b) Influence of castor genotypes on rearing performance of different silkworm breeds. *Int. J. Wild Silk Moths Silk*, **5**: 29-31.

- Jeyapaul C., Padmalatha C., Ranjitsingh A. J. A., Murugesan A. G. and Dhasarathan P. (2003) Effect of plant extracts on nutritional efficiency in mulberry silkworm, *Bombyx mori*. *Indian J. Seric.*, **42**: 128-131.
- Kaiser J., Jyothi K. N., Prasuna A. L., Murthy U. S. N. and Persoons C. J. (1997) *Trichopria khandulus* (Hymenoptera: Diapriidae): a new biocontrol agent of the uzi fly, *Exorista bombycis* (Dip.: Tachinidae). *J. Appl. Entomol.*, **121**: 99-102.
- Kakati L. N. and Chutia B. C. (2009) Diversity and ecology of wild sericigenous insects in Nagaland, India. *Trop. Ecol.*, **50**(1): 137-146.
- Kakati P. K. (2002) Adoption of prophylactic measures to control muga silkworm diseases. *Indian Silk*, **41**(1): 19-21.
- Kamate G. S., Lande U. L., Bhede B. V. and Mupade R. V. (2011) Evaluation of mulberry varieties for rearing performance of mulberry silkworm, *Bombyx mori* in Maharashtra. *J. Entomol. Res.*, **35**: 111-113.
- Kanafi R. R., Edabi R., Mirhosseini S. Z., Seidavi A. R., Zolfaghari M. and Etebari K. (2007) A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm, *Bombyx mori* L. *Indian J. Seric.*, **46**: 86-91.
- Kiran Kumar K. P., Sinha A. K., Singh G. P. and Madhusudan K. N. (2011) Efficacy of systemic fungicides for control of white muscardine in tasar silkworm, *Antheraea mylitta* D. *Res. J. Microbiol.*, **6**: 805-812.
- Krishnan M., Subbarathinam K. M. and Janarthanan N. (1995) Effects of hydrolysed soy protein (P-soyatox) on haemolymph protein profile, larval and pupal characters of silkworm, *B. mori* L. (Lepidoptera: Bombycidae). *Sericologia*, **32**: 227-235.
- Krishnaswami S., Kumararaj S., Vijayaraghavan N. and Kasivishwanathan K. (1971) Silkworm feeding trials for evaluating the quality of mulberry leaves as influenced by variety, spacing and nitrogen fertilization. *Indian J. Seric.*, **10**: 79-89.
- Kumar A. (2003) Parasites of uzi fly, *Exorista sorbillans* Wiedermann (Diptera: Tachinidae). V. Taxonomy of *Spilomicrus karnatakensis* Sharma (Hymenoptera: Diapriidae). *J. Exp. Zool.*, **6**: 105-107.
- Kumar A. (2004) Parasites of uzi fly, *Exorista sorbillans* Wiedermann (Diptera: Tachinidae). III. Biology of *Nesolynx thymus* (Hymenoptera: Eulophidae). *J. Ecobiol.*, **16**: 373-378.
- Kumar D., Pandey J. P., Sinha A. K., Mishra P. K. and Prasad B. C. (2013) Evaluation of novel tasar silkworm feed for *Antheraea mylitta*: Its impact on rearing cocoon trait and biomolecular profile. *American J. Biochem. Mol. Biol.*, **3**: 167-174.
- Kumar K. P. K., Singh G. P., Sinha A. K., Madhusudan K. N. and Prasad B. C. (2012 a) Antiviral action of certain medicinal plants against AmCPV and their effect on cellular and biochemical changes in tasar silkworm, *Antheraea mylitta* D. *Res. J. Med. Plants*, **6**: 92-97.
- Kumar K. P. K., Sinha A. K., Singh G. P. and Madhusudan K. N. (2012 b) Fujiwara method for pebrine detection in tasar silkworm - improvisation for better efficiency. *Indian Silk*, **52**(12): 18-19.
- Kumar P., Kishore R., Manjunath D. and Datta R. K. (1994) A preliminary study on the biology of *Brachymeria* sp. on *Exorista bombycis*, a new host record. *Indian J. Seric.*, **33**: 74-75.
- Kumar P., Ramadevi O. K., Singh B. D. and Jolly M. S. (1989) A new record of pupal endoparasite, *Exorista philippinensis* Ashmead (Hymenoptera: Encyrtidae) of the uzi fly, a serious parasite of silkworm. *Curr. Sci.*, **58**: 212-213.
- Kumtha P., Padmalatha C., Chairman K. and Ranjit Singh A. J. A. (2013 a) Effect of pesticides on the metamorphosis of silkworm, *Bombyx mori*. *Int. J. Mol. Zool.*, **3**(3): 10-13.
- Kumtha P., Padmalatha C., Chairman K. and Ranjit Singh A. J. A. (2013 b) Effects of pesticides on the reproductive performance and longevity of *Bombyx mori*. *Int. J. Curr. Microbiol. Appl. Sci.*, **2**(9): 74-78.
- Lakshmi Narayanamma V., Dharma Reddy K. and Vishnuvardhan Reddy V. (2013) Effects of castor genotypes on rearing and cocoon parameters of eri silkworm, *Samia cynthia ricini*. *Indian J. Plant Prot.*, **41**: 127-131.
- Lakshmi Narayanamma V., Dharma Reddy K. and Vishnuvardhan Reddy A. (2014) identification of promising castor genotypes for rearing eri silkworm, *Samia cynthia ricini*. *Indian J. Plant Prot.*, **42**: 135-140.
- Laskar N. and Datta M. (1998) Effect of alfalfa tonic and its inorganic ingredient on growth and development of silkworm, *Bombyx mori* L. *Environ. Ecol.*, **18**: 591-596.
- Li B., Wang Y., Liu H., Xu Y., Wei Z., Chen Y. and Shen W. (2010) Resistance comparison of domesticated silkworm (*Bombyx mori* L.) and wild silkworm (*Bombyx mandarina*

- M.) to phoxim insecticide. *African J. Biotechnol.*, **9**: 1771-1775.
- Li M., Shen L., Xu A., Miao X., Hou C., Sun P., Zhang Y. and Huang Y. (2005) Genetic diversity among silkworm (*Bombyx mori* L., Lepidoptera: Bombycidae) germplasms revealed by microsatellites. *Genome*, **48**: 802-810.
- Mahadeva A. (2011) Influence of thrips (*Pseudodendrothrips mori*) infestation on the biochemical constituents and photosynthetic pigments of mulberry (*Morus* spp.) leaves. *Int. J. Plant Anim. Environ. Sci.*, **1**(3): 57-63.
- Malik F. A. and Reddy Y. S. (2007) Role of mulberry nutrition on manifestation of post cocoon characters of selected races of the silkworm, *Bombyx mori* L. *Sericologia*, **47**: 63-76.
- Malik F. A. and Reddy Y. S. (2010) Effect of mulberry nutrition on biochemical constituents of haemolymph of selected races of the silkworm, *Bombyx mori* L. *Indian J. Entomol.*, **72**: 23-28.
- Manimegalai S., Rajeswari T., Shanmugam R. and Rajalakshmi G. (2010) Botanicals against nuclear polyhedrosis virus infecting three breeds of mulberry silkworm, *Bombyx mori* L. *J. Biopesticides*, **3**(1): 242-245.
- Manohar Reddy R., Kumar R., Rai S., Angadi B. S. and Sivaprasad V. (2013) Impact of legume green mulching on soil physico-chemical condition of rainfed tropical tasar silkworm food plants fields. *Sericologia*, **53**: 43-48.
- Marimuthu M. and Isaiarasu L. (2010) Influence of herbal tonic *Aloe* on the overall performance of the mulberry silkworm, *Bombyx mori* L. *J. Biopesticides*, **3**: 567-572.
- Mude S., Naik J. and Pala I. (2012) Effect of botanical extracts against thrips (*Pseudodendrothrips mori*) infested mulberry on enzyme activity in silkworm, *Bombyx mori* L. *J. Entomol. Res.*, **36**: 295-299.
- Murthy V. N. Y., Ramesh H. L., Munirajappa and Yadav B. R. D. (2013) Nutritional quality assessment of ten mulberry (*Morus*) germplasm varieties through moulting test, silkworm rearing technique and economic characters of bivoltine silkworm (*Bombyx mori* L.) for commercial exploitation. *Int. Res. J. Nat. Sci.*, **1**(2): 11-22.
- Murugan K., Jayabalan D., Senthilkumar N., Senthilnathan S. and Sivaprakasam N. (1998) Growth promoting effect of plant products on silkworm: A biotechnological approach. *J. Sci. Ind. Res.*, **57**: 740-745.
- Naik M. C. (2008) Growth, Development and Economic Cocoon Parameters of Eri Silkworm, *Samia cynthia ricini* Boisduval on New Hosts. *Dissertation*, University of Agricultural Sciences, Dharwar, Karnataka, India.
- Naik M. J., Schinde R. and Indira P. (2011) Role of SDH and IDH activity in silkworm, *Bombyx mori* L. with mealy bug infested mulberry leaves. *J. Entomol. Res.*, **35**: 63-67.
- Nair K. S., Nair J. S., Trivedy K. and Vijayan V. A. (2003) Influence of backuchiol, a JH analogue from bemchi (*Psoralea corylifolia*) on silk production in silkworm, *Bombyx mori* L. (Bombycidae: Lepidoptera). *J. Appl. Sci. Environ. Manage.*, **7**(2): 31-38.
- Narayanaswamy K. C. (2003) Biochemical composition of leaf roller infested mulberry leaf. *Insect Environ.*, **8**(4): 166-167.
- Narayanaswamy K. C., Geethabai M. and Raghuraman R. (1997) Effect of chinaberry leaf and seed extracts on egg hatchability and mortality of maggot and pupa of uzi fly, *Exorista bombycis* (Louis) (Diptera: Tachinidae). *Pestology*, **21**(3): 32-35.
- Narayanaswamy K. C., Ramegowda T., Raghuraman R. and Manjunath M. S. (1999) Biochemical changes in spiralling whitefly (*Aleurodicus dispersus* Russell) infested mulberry leaves and their influence on some economic parameters of silkworm (*Bombyx mori* L.). *Entomon*, **24**: 215-220.
- Naseema Begum A., Mal Reddy N., Manthira Moorthy S. and Bindroo B. B. (2014) A new robust bivoltine hybrid (CSR 50 x CSR 52) x (CSR 51 x CSR 53) of *Bombyx mori* L. for higher egg and cocoon yield. *Sericologia*, **54**: 66-72.
- Nath B. S. and Kumar R. P. (1999) Toxic impact of organophosphorous insecticides on acetyl cholinesterase activity in the silkworm, *Bombyx mori* L. *Ecotoxic. Environ. Safety*, **42**: 157-162.
- Nath B. S., Suresh A., Verma B. M. and Kumar R. P. (1997) Changes in protein metabolism in haemolymph and fat body of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) in response to organophosphorous insecticide toxicity. *Ecotoxic. Environ. Safety*, **36**: 169-173.
- Neelu N., Jagadish P. S. and Nagesh Chandra B. K. (2000) Evaluation of the volumetric attributes of the eri silkworm reared on various host plants. *Int. J. Wild Silk Moths Silk*, **5**: 36-38.
- Neog K., Ahmed G. U., Unni B. G., Dutta P., Gogoi D. K. and Giridhar K. (2013) Variation in biochemical composition of different host plants of muga silkworm, *Antheraea assamensis* Helfer. *Sericologia*, **53**(3-4): 207-218.
- Neog K., Das A., Unni B. G., Ahmed G. U. and Rajan R. K.

- (2011 a) Studies on secondary metabolites of som (*Persea bombycina* Kost): A primary host plant of muga silkworm (*Antheraea assamensis* Helfer). *Int. J. Pharm. Tech. Res.*, **3**(3): 1441-1447.
- Neog K., Unni B. G. and Ahmed G. U. (2011 b) Studies on the influence of host plants and effect of chemical stimulants on the feeding behaviour in the muga silkworm, *Antheraea assamensis*. *J. Insect Sci.*, **11**. Available on line: insectscience.org/11.133.
- Nguku E. K., Muli E. M. and Raina S. K. (2007) Larval, cocoon and post-cocoon characteristics of *Bombyx mori* L. (Lepidoptera: Bombycidae) fed on mulberry leaves fortified with Kenyan royal jelly. *J. Appl. Sci. Environ. Manage.*, **11**(4): 85-89.
- Nishitha Naik V., Pratheesh Kumar P. M., Sharma D. D., Dayakar Yadav B. R. and Qadri S. M. H. (2013) Production of compost from mulberry shoots using lingo-cellulolytic fungi and its impact on growth and yield of mulberry. *Sericologia*, **53**: 63-67.
- Pachiappan P., Aruchamy M. C. and Ramanna S. K. (2009) Evaluation of antibacterial efficacy of certain botanicals against bacterial pathogen *Bacillus* sp. of silkworm, *Bombyx mori* L. *Int. J. Ind. Entomol.*, **18**: 49-52.
- Padma S. and Manimegalai S. (2007) Efficacy of aqueous extract of some botanicals against grasserie disease of silkworm, *Bombyx mori* L. *Madras Agric. J.*, **94**(1): 144-147.
- Paital B. and Bohidar K. (2011) Effect of dietary alpha glycine on cocoon production in the silkworm, *Bombyx mori*. *Curr. Trends Biotechnol. Pharm.*, **5**(2): 1157-1162.
- Pandey R. and Srivastava R. P. (2013) Effect of some medicinal plants on development and economic parameters of mulberry silkworm. *Indian J. Entomol.*, **75**: 285-289.
- Patil S. V., Mallikarjunappa R. S., Dandin S. B. and Eswara Rao M. S. (2001) Evaluation of improved genotypes of mulberry through leaf chemical analysis and bioassay with silkworm race NB4D2. *Sericologia*, **41**: 219-226.
- Paul A., Sinhababu S. P. and Sukul N. C. (1995) Effect of nematode infected mulberry plants on the growth and silk production of *Bombyx mori* L. *Indian J. Seric.*, **34**: 18-21.
- Philip T., Gupta V. P., Govindaiah, Bajpai A. K. and Datta R. K. (1998) Diseases of mulberry in India: Research priorities and management strategies. *Int. J. Trop. Plant Dis.*, **12**: 1-21.
- Purushothaman S., Muthuvelu S., Balasubramanian U. and Murugesan P. (2012) Biochemical analysis of mulberry leaves (*Morus alba* L.) and silkworm, *Bombyx mori* enriched with vermiwash. *J. Entomol.*, **9**: 289-292.
- Qadri S. M. H. and Khatri R. K. (2005) Strategic management of pebrine in silkworm seed production. *Asian Tex. J.*, **14**(1-2): 72-75.
- Radjab R., Ebodi R., Fazilati M. and Mirhoseini S. Z. (2006) Nutritive effects of mulberry leaves enrichment with riboflavin vitamin on bio-economic characters of silkworm, *Bombyx mori* L. In: *Proceedings of 9th Arab Congress of Plant Protection*, 19-23 November 2006, Damascus, Syria.
- Radjab R., Ebodi R., Mirhoseini S. Z. and Nair S. (2009) Effects of feeding alanine-enriched mulberry leaves on the economic characters of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). *Formosan Entomol.*, **23**: 73-78.
- Rai S., Aggarwal K. K. and Babu C. R. (2006) Influence of host plant (*Terminalia arjuna*) defences on the evolution of feeding behaviour in the tasar silkworm. *Curr. Sci.*, **91**: 68-72.
- Rajasekaragouda R., Gopalan M., Jeyaraj N. and Natarajan M. (1997) Field performance of plant extracts on mulberry silkworm, *Bombyx mori* L. *Entomon*, **22**: 235-238.
- Rajeswari K. and Isaiarasu L. (2004) Influence of the leaf, flower and pod extracts of *Moringa oleifera* on the growth and reproductive parameters of *Bombyx mori* L. *Entomon*, **29**: 331-338.
- Ramakrishna N., Sannappa B. and Govindan R. (2003) Influence of castor varieties on rearing and grainage performance of different breeds of eri silkworm, *Samia cynthia ricini*. *J. Ecol.*, **15**: 279-285.
- Ramegowda G. K., Dar M. Y., Mittal V., Ahmed S. N., Guruswamy D., Illahi I., Agrawal O. P. and Dhar A. (2012) Effect of mite-infested mulberry leaves on rearing performance and reeling parameters of silkworm, *Bombyx mori* L. *Munis Entomol. Zool.*, **7**: 926-930.
- Ramesh C., Seshagiri S. V., Lakshmi H., Kumari S. S. and Rao C. G. L. (2009) Effect of tukra (mealy bug) infected mulberry leaves on the qualitative traits of new polyvoltine strain of silkworm, *Bombyx mori* L. *J. Entomol.*, **6**: 207-214.
- Ram Kishore A., Debnath K. and Suryanarayana N. (2009) Integrated management to control uzi fly, *Blepharidopterus zebina* Walker, an endoparasitoid of tropical and temperate tasar

- silkworms. *Sericologia*, **49**: 525-530.
- Rani A. G., Padmalatha C., Raj R. S. and Ranjit Singh A. J. (2011) Impact of supplementation of Amway protein on the economic characters and energy budget of silkworm, *Bombyx mori* L. *Asian J. Anim. Sci.*, **10**: 1-6.
- Rao D. M. R., Kodandaramaiah J., Reddy M. P., Katiyar R. S. and Rahmathulla V. K. (2007) Effect of VAM fungi and bacterial biofertilizers on mulberry leaf quality and silkworm cocoon characters under semi-arid conditions. *Caspian J. Environ. Sci.*, **5**: 111-117.
- Rath S. S. (2000) Studies on growth and development of *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) on different natural host plants. *Int. J. Wild Silk Moths Silk*, **5**: 99-103.
- Rath S. S., Prasad B. C. and Sinha B. R. (2003) Food utilization efficiency in fifth instar larvae of *Antheraea mylitta* (Lepidoptera: Saturniidae) infected with *Nosema* sp. and its effect on reproductive potential and silk production. *J. Invertebr. Pathol.*, **83**: 1-9.
- Ravikumar S., Ramanathan G., Sudhakaran M. and Ibaneson S. J. (2009) Antimicrobial compounds from marine halophytes for silkworm disease treatment. *Int. J. Medicine Med. Sci.*, **1**: 184-191.
- Rayan S. G. (2011) Performance of new bivoltine breeds of silkworm, *Bombyx mori* L. on improved mulberry varieties during rainy season. *J. Entomol. Res.*, **35**: 69-74.
- Reddy B. K. and Rao J. V. K. (2009) Seasonal occurrence and control of silkworm diseases: grasserie, flacherie and muscardine, and insect parasitoid, uzi fly in Andhra Pradesh, India. *Int. J. Ind. Entomol.*, **18**: 57-61.
- Reddy D. N. R., Kotikal Y. K. and Vijayendra M. (1989) Development and silk yield of eri silkworm, *Samia cynthia ricini* (Lepidoptera: Saturniidae) as influenced by the food plants. *Mysore J. Agric. Sci.*, **23**: 506-508.
- Reddy R. M., Chavan R., Prasad B. C., Shiva Reddy C., Manjula A. and Sivaprasad V. (2010) Rearing and grainage performance of Indian tropical tasar silkworm, *Antheraea mylitta* Drury fed on *Terminalia tomentosa* (W. & A.) and *Lagerstroemia parviflora* (Roxb.) food plants. *Acad. J. Entomol.*, **3**(3): 69-74.
- Sahu M., Sahu A. K. and Bindroo B. B. (2008) Biological control of uzi fly infestation in Muga. *Indian Silk*, **46**(10): 18-19.
- Sakthivel N. and Qadri S. M. H. (2013) Effect of fortification on senescent tapioca leaves on economic traits of eri silkworm. *Indian J. Seric.*, **52**: 44-47.
- Sannappa B. and Jayaramaiah M. (1999) Interaction of castor genotypes and breeds of silkworm in relation to cocoon and grainage parameters. *Mysore J. Agric. Sci.*, **33**: 214-223.
- Sannappa B., Jayaramaiah M. and Chandrappa D. (2002) Influence of castor genotypes on consumption indices of eri silkworm, *Samia cynthia ricini* Boisduval. *Environ. Ecol.*, **20**: 960-964.
- Sannappa B., Ramakrishna N., Govindan R. and Subramanya G. (2007) Influence of some castor genotypes on larval, cocoon and grainage traits of eri silkworm (*Samia cynthia ricini* Boisduval). *Int. J. Agric. Sci.*, **3**(1): 139-141.
- Sarithakumari S., Narayanaswamy K. C. and Gowda M. (2009) Amylase activity of eri silkworm, *Samia cynthia ricini* Boisduval as influenced by host plants. *Indian J. Ecol.*, **36**: 71-74.
- Sarmah M. C., Chutia M., Neog K. Das R., Rajkhowa G. and Gogoi S. N. (2011) Evaluation of promising castor genotypes in terms of agronomical and yield attributing traits, biochemical properties and rearing performance of eri silkworm, *Samia ricini* (Donovan). *Ind. Crops Prod.*, **34**: 1439-1446.
- Sarmah M. C., Datta R. N., Das P. K. and Benchamin K. V. (2002) Evaluation of certain castor genotypes for improving sericulture. *Indian J. Seric.*, **41**: 62-63.
- Sathe T. V. and Jadhav A. D. (2001) *Sericulture and Pest Management*. Daya Publishing House, New Delhi, India.
- Seidavi A. R., Bizhannia A. R., Sourati R. and Mavvajpour M. (2005) The nutritional effects of different mulberry varieties on biological characters of silkworm. *Asia Pac. J. Clin. Nutr.*, **14**: 122-124.
- Selvakumar T., Nagaraja B., Balavenkatasubbaiah M., Sharma S. D., Chandrasekharan K. and Sudhakara Rao P. (2005) Influence of environmental and nutritional factors on the development of flacherie disease in silkworm, *Bombyx mori* L. In: *Proceedings of 20th Congress of the International Sericultural Commission*, Bangalore, India, Vol. 1, pp. 207-211.
- Sengupta T., Misra A. K., Das C., Das K. K., Sen S. K. and Saratchandra B. (1999) Physiochemical changes in thrips infected mulberry leaves. *Sericologia*, **39**: 417-421.
- Shahin R., Gadelhak G., Mahmoud S. M., El-Agamy E. I. and Idriss M. (2013) The effects of lettuce seed oil on some

- economical parameters of the mulberry silkworm, *Bombyx mori* L. *J. App. Sci. Res.*, **9**: 2702-2707.
- Sharma S. P., Kishori R., Gupta S., Sinhadeo S. N. and Sinha B. R. R. P. (2005) Evaluation of azadirachtin for its visible residual toxicity to tasar silk moth, *Antheraea mylitta* D. (Lepidoptera: Saturniidae). *Sericologia*, **44**: 331-336.
- Shivakumar G. R., Raman K. V. A., Magadam S. B., Datta R. K., Hussain S. S., Banerji A. and Chowdhury S. K. (1996) Effect of phytoecdysteroids on the spinning, cocoon and reeling parameters of the silkworm, *Bombyx mori* L. *Allelopathy J.*, **3**: 71-73.
- Shivakumar G. R., Raman K. V. A., Venkata Ramireddy S. B., Magadam S. B., Datta R. K., Hussain S. S., Banerji A. and Chowdhury S. K. (1995) Effect of phytoecdysteroids on larval maturation and economic parameters of the silkworm, *Bombyx mori*. *Indian J. Seric.*, **34**: 46-48.
- Shivakumar G. R. and Shamitha G. (2013) Studies on larval mortality: Diseases, pest and predator menace in outdoor and indoor reared tasar silkworm, *Antheraea mylitta* Drury (Daba TV ecorace). *Res. J. Anim. Vet. Fishery Sci.*, **1**(4): 1-7.
- Singh R. N. and Thangavelu K. (1996) Influence of neem compound on the growth and development of immature forms of uzi fly, *Blepharipa zebina* Walk. (Diptera: Tachinidae). *Pestology*, **20**(3): 16-21.
- Singh K. I., Devi A. R., Singh M. P. and Gupta M. K. (2010) Residue toxicity of insecticides to mulberry silkworm, *Bombyx mori* Linnaeus. *Indian J. Entomol.*, **72**: 355-360.
- Singh N. I. and Goswami D. (2012) Food plant varietal effects on the rearing and grainage of muga silkworm, *Antheraea assamensis* Helfer, 1837. *Munis Entomol. Zool.*, **7**: 1023-1027.
- Singh T., Bhat M. M. and Khan M. A. (2012) Microsporidiosis in the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Pertanika J. Trop. Agric. Sci.*, **35**(3): 387-406.
- Singh T. and Saratchandra B. (2003) Microsporidian disease of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Int. J. Ind. Entomol.*, **6**: 1-9.
- Sujatha K. and Rao A. P. (2003) Effect of fortification of mulberry leaf with phytochemicals on the commercial traits of silkworm, *Bombyx mori* Linn. *J. Exp. Zool. India*, **6**: 321-323.
- Sundarraj S., Nangia N., Chinnaswamy K. P. and Sannappa B. (2000) Influence of protein supplement on performance of PMxNB4D2 silkworm breed. *Mysore J. Agric. Sci.*, **34**: 302-307.
- Takhliq M. (2011) Medicinal plant extracts: A great determinant to economic potency of silkworm, *Bombyx mori* L. *J. Entomol. Res.*, **35**: 231-233.
- Tang K., Abul Samed M. A., Akand H. A., Azhar S. B. and Absar N. (2006) Nutritional changes in four varieties of mulberry leaves infected with fungus (*Phyllactinia corylea*). *Pakistan J. Biol. Sci.*, **9**: 355-359.
- Tanki T. N., Munshi N. A., Zargar M. A., Sahaf K. A., Dar H. U. and Khan M. A. (2005) Comparative efficacy of triazoles and conventional fungitoxicants in the management of leaf spot disease (*Phloeospora maculans*) of mulberry. *Indian J. Seric.*, **44**: 171-174.
- Tikader A. and Kamble C. K. (2010) Seri-biodiversity with reference to host plants in India. *Asian Australasian J. Plant Sci. Biotech.*, **4**(1): 1-11.
- Tikader A., Vijayan K. and Saratchandra B. (2013) Muga silkworm, *Antheraea assamensis* (Lepidoptera: Saturniidae): An overview of distribution, biology and breeding. *European J. Entomol.*, **110**: 293-300.
- Unni B. G., Bora U., Singh H. R., Dileep Kumar B. S., Devi B., Wann S. B., Bhau B. S., Bora A., Neog I. K. and Chakravorty R. (2008) High yield and quality silk fibre production by muga silkworm, *Antheraea assama* through the application of plant growth promoting rhizobacteria. *Curr. Sci.*, **94**: 768-774.
- Unni B. G., Dowarah P., Wann S. B. and Gangadharrao P. (2011) Muga heal: *Terminalia chebula* based bioformulation as an anti-flacherie agent and a silk fiber enhancer. *Sci. Cult.*, **77**: 456-458.
- Unni B. G., Kakoty A. C., Khanikar D., Bhattacharya P. R., Pathak M. G., Pillai K., Chaudhary A., Saikia P. C. and Ghosh A. C. (1996) Lipid and fatty acid composition of muga silk worm, *Antheraea assama* host plants in relation to silkworm growth. *J. Lipid Mediators Cell Signalling*, **13**(3): 295-300.
- Unni B. G., Yadav A., Chaudhary A., Kumar J., Wann S. B. and Sarma R. (2004) Chebulic myrobalan for controlling bacterial disease on muga silkworm, *Antheraea assama*: A preliminary report. *Nat. Prod. Radiance*, **3**: 347-348.
- Vassarmidaki M. E., Harizanis P. C. and Katsikis S. (2000) Effect of Applaud on the growth of silkworm (Lepidoptera: Bombycidae). *J. Econ. Entomol.*, **93**: 290-292.
- Velide L. and Bhagavanulu M. V. K. (2012) Study on infestation

- of *Xanthopimpla pedator* on the cocoons of tropical tasar silkworm, *Antheraea mylitta* Drury. *Int. J. Plant Anim. Environ. Sci.*, **2**: 141-142.
- Velide L. and Rao A. P. (2011) Studies on the impact of microsporidiosis on tropical tasar silkworm, *Antheraea mylitta* Drury. *J. Appl. Biosci.*, **44**: 2994-2999.
- Wijayanthi N. and Subramanyam M. V. (2012) Effect of fenvalerate 20EC on sericigenous insects. 1. Food utilization in the late-age larva of the silkworm, *Bombyx mori* L. *Ecotoxicol. Environ. Safety*, **53**: 206-211.
- Yadav G. S. and Mahobiam G. P. (2010) Effect of different food leaves on rearing performance in Indian tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *UPJ. Zool.*, **30**: 145-152.
- Zamal T., Sarmah B., Hemchandra O. and Kalita J. (2010) Global warming and its impact on the productivity of muga silkworm (*Antheraea assamensis* Helfer). *Bioscan*, **1**(special issue): 199-209.

Revue

IMPACT DES PRINCIPAUX FACTEURS BIOTIQUES SUR L'ÉLEVAGE DU VER À SOIE TROPICAL EN INDE ET SURVEILLANCE DES ÉLÉMENTS DÉFAVORABLES: REVUE

R. T. Gahukar

Arag Biotech Pvt. Ltd., Plot 220, Reshimbag, Nagpur 440009, India.
E - mail: rtgahukar@gmail.com

RÉSUMÉ

En Inde, les différents genres de vers à soie sont nourris avec des feuilles de mûrier, de ricin, de som, soalu et arjun. La qualité des feuilles, le nombre d'élevages par an, les plantes hôtes et leurs variétés ainsi que les facteurs environnementaux contribuent grandement à leur croissance et à leur développement. La mortalité des vers à soie est souvent due à un nourrissage par des feuilles traitées avec des pesticides chimiques et aux attaques de prédateurs tels que des parasitoïdes et des entomopathogènes. En revanche, l'application de phytoecdystéroïdes, de vermi-compost ou de produits dérivés de plantes a donné des effets favorables sur les larves entraînant une amélioration du poids des cocons, de la richesse soyeuse, du rendement en cocons et des paramètres de filature (longueur du filament, titre *etc.*). Aussi bien les races bivoltines que polyvoltines ont été exploitées pour une meilleure qualité et une meilleure quantité de soie produite. Dans cette revue, nous discutons de manière critique l'impact des facteurs biotiques sur l'élevage des vers à soie et nous suggérons des mesures pour augmenter la production commerciale de soie.

Mots-clés: Pesticides chimiques, production de soie commerciale, ennemis naturels, produits dérivés de plantes, variétés de plantes, élevage de ver à soie.



QTL AND THEIR UTILIZATION IN SILKWORM BREEDING PROGRAMMES

Ritwika Sur Chaudhuri and G. Subramanya

Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysore - 570006, India.

E-mail: ritwika87@gmail.com

ABSTRACT

With the rising demand of silk in the global scenario, several silkworm breeding strategies are being developed to improve the quality and quantity of silk. The present aim of silkworm breeding in India is to evolve disease resistant and highly productive silkworm strains. Disease resistance breeding has gained momentum in recent years and is important in this context, since a major percentage of crop loss in sericulture is due to incidences of silkworm diseases. Molecular approaches viz., QTL and marker assisted breeding have added a new dimension to the conventional breeding principles, with respect to reduced cost and time. Since the expression of disease resistance and several quantitative traits in silkworm is the result of a complex interplay of several genes and environment, QTL analysis would enable the identification of the genes in question and their effects. Different QTL mapping approaches are employed for that purpose. However, the integrated approaches discussed herein promise a deeper understanding of the genetic basis of the traits and will provide a right direction towards sustainable development of sericulture.

Key words: *Bombyx mori*, QTL, marker assisted selection, silkworm breeding.

INTRODUCTION

Sericulture is an important agrobased industry and has occupied a prominent place in agriculture in many of the tropical and temperate countries for the production of silk. Silk, being a natural fibre, has an ever-increasing demand in the global textile industries. It is estimated that the global raw silk production had crossed 1.52 lakh MT during the year 2012, compared to 0.81 lakh MT in the year 2000. The silkworm is geographically demarcated into univoltines, bivoltines and multivoltines. The univoltines and bivoltines produce superior quality of silk but the production of these breeds and their hybrids was limited to temperate regions as they exhibit low survivability and disease susceptibility in the tropical climatic conditions. Multivoltines, on the other hand, are the hardy races that are well-acclimatized to tropical climatic conditions, but exhibit low productivity. As a result, silkworm breeders of the tropics have always strived to improve the productivity of the multivoltines, in addition to introducing the quasi inbred strains of the temperate region to the tropics. In the conventional

breeding methodology, the promising parents are selected and the F_1 progenies are inbred for many generations to isolate the best lines with desired traits. The breeders opt for highly heritable qualitative traits in the early generations and phenotypic selection for quantitative traits through long processes of pedigree breeding strategies. Consequently, the breeding procedures become laborious with respect to cost and time. Though silkworm breeders have tried to evolve superior silkworm breeds through several combinations of multivoltines and bivoltines, yet succeeded in obtaining only a handful of breeds with desired traits. In most of the cases, the traits did not stabilize, rather got lost during the subsequent generations because of continuous inbreeding. In India, hybridization programmes were initiated in the 1920s (Basavaraja *et al.*, 1994) and presently, the most sought after methodology to produce gradable quality of raw silk by exploiting the multi-bi hybrids, yet fails to meet the international standards of raw silk. Since phenotypic selection was the only basis of choice of breeds, even with the knowledge of gene combination and segregation

through Mendelian principles, desired results were hardly achieved, for alleles of a particular gene may be beneficial for one trait, but have negative effect on the other (Williams, 2005).

Economic traits of silkworm, *viz.*, fecundity, hatching percentage, cocoon weight, shell weight, filament length, renditta, denier, larval duration as well as disease resistance are all polygenic in nature, *i.e.*, they are controlled by several genes, thus making it difficult to understand their complex pattern of inheritance, since the contribution of each of the genes towards the expression of the quantitative trait in question remains unknown. To add to the problem, one cannot undermine gene-gene and gene-environment interactions. Such intricate problems can only be solved if the molecular information on the traits in question is made available. Thus, molecular and genetic approach to silkworm breeding can considerably overcome the limitations of conventional breeding methods. Developments in the field of quantitative genetics, such as the concepts of Quantitative Trait Loci (QTL) and marker-assisted selection (MAS) have added a new dimension to the conventional breeding principles. Knowledge of the genes controlling the trait/s in question and their corresponding location (locus) within the genome enables a breeder to gain precision and accuracy in the selection process and evolve breeds with desired traits rapidly. In recent years, molecular markers have been employed in the detection and mapping of the complex quantitative traits among several plant and animal species. Disease resistance, which is another important trait in this context, needs to be fully exploited for sustainable production. The present review discusses the basics of QTL and its application in silkworms to enable improvement of economic traits and enhance disease resistance in silkworm breeds.

Dissecting the complex QTL

Advances in quantitative genetics have enabled us to understand that every trait which is expressed in an individual is a result of expression of one or several genes. Discontinuous traits are those wherein the phenotypes are

'fixed' and therefore predictable if the genotype is known. On the other hand, continuous traits are those wherein phenotypic expression is continuously varying and therefore not predictable solely through the genotype. Because such traits are measured quantitatively, they are called quantitative traits. Quantitative traits are controlled by several genes and their expression is a complex interplay of gene effects (additive, dominance and interaction) and environment. The genes that control such traits are called quantitative trait loci (QTL). QTL may be defined as the stretch of DNA or a region in the chromosome that is associated with a particular phenotypic trait. Often, many QTLs add up to manifest a particular trait and are located on different chromosomes. These genes have small contributions and thus make up the genetic architecture of that trait.

QTL analysis encompasses the study of genetic variation, exploration of QTL, their precise location, their interaction and their effects (Kearsey, 1998). It is a statistical method that links two types of information-phenotypic data (trait measurements) and genotypic data (usually molecular markers) in an attempt to explain the genetic basis of variation in complex traits (Falconer and Mackay, 1996). A major objective of QTL analysis is to find out genetic markers that can be implemented in breeding programmes via marker assisted selection. The QTL mapping experiments quantify the co-inheritance of genetic markers with variance of traits of interest and thereby are able to localize the sources of significant amounts of genetic variability to a certain region of a chromosome (QTL). This information can be used to identify a major gene by a common approach called positional cloning. After the initial mapping, fine mapping could be performed to improve the accuracy of the localization of the genes. QTL mapping or assigning precise locations of these genes to a particular region in the chromosome has been facilitated by the application of molecular markers. Molecular markers are usually the non-coding regions of a chromosome closely linked to the QTL of interest. These markers discern genetic differences of a segregating population and thus allow the study of genetic variation even among individuals of a species.

While genetic markers for QTL that are linked to the trait gene could be used to choose animals for selective breeding programmes, the most effective markers are the functional mutations within the trait genes caused by single nucleotide polymorphisms.

QTL mapping approaches

a) ANOVA

Broman (2001) reviewed that the simplest method for QTL mapping is Analysis of Variance (ANOVA) sometimes called 'marker regression' at marker loci. In this method, in a backcross, one may calculate t-statistics to compare the average of the two marker genotype groups. For other types of crosses (such as the intercross), where there are more than two possible genotypes, F-statistics is used. The advantages of analysis of variance at the marker loci are that the method is simple, does not require genetic map for the markers and may be easily extended to account for multiple loci. A further advantage is the easy inclusion of covariates, such as, sex, treatment, or an environment effect. However, the ANOVA approach for QTL mapping has its limitations. First, separate estimates of QTL location and QTL effect are not obtained. QTL location is indicated only by looking at which markers give the greatest differences between genotype group averages, and the apparent QTL effect at a marker will be smaller than the true QTL effect as a result of recombination between the marker and the QTL. Second, individuals whose genotypes are missing at the marker must be discarded. Third, when the markers are widely spaced, the QTL may be quite far from all markers, and so the power for QTL detection will decrease.

b) Interval mapping

Currently, this is the most popular approach for QTL mapping in experimental crosses. The method makes use of a genetic map of the typed markers and like ANOVA, assumes the presence of a single QTL. It uses information

on values of two flanking markers to estimate the QTL position. The probability that the data could be obtained assuming a QTL at several positions between the markers is calculated and QTL is declared where the probability of obtaining the observed data is the highest (Broman and Speed, 2002).

c) Composite interval mapping

Composite interval mapping evaluates the probability that a marker or an interval between two markers is associated with a QTL affecting the trait, while simultaneously controlling for the effects of other markers on the trait. This method combines interval mapping with linear regression and includes additional genetic markers in the statistical model in addition to an adjacent pair of linked markers for interval mapping (Zeng, 1994).

DNA markers can be used to map useful genes using recombinant frequencies of linked genes. The markers near QTL co-segregate with them. The markers that are tightly linked to QTL are detected by ANOVA. Parents that differ for a trait are selected. Then the two parents are screened for polymorphic marker loci. Recombinant inbred lines are generated and screened in field for the desired phenotype. The mean of dominant and recessive marker genotypes, MM and mm lines at every marker locus is drawn and QTL is declared where MM-mm is greatest (in the case of multiple marker analysis) or a separate ANOVA is done on the effect of each marker and QTL is declared where F-test is significant (in the case of single marker analysis). Markers do give more information if we learn how to evaluate it and use it correctly (Haley, 1995). However, results from QTL studies cannot be directly implemented into breeding schemes through MAS before finding out whether the QTL identified are real and whether the identified QTL are segregating in the breeding population. Hence, one strategy is to combine P-values from many QTLs and another is to establish a confirmation study (Spelman and Bovenhuis, 1998).

Wide arrays of QTL mapping softwares are available, viz., QTL Mapper (Wang *et al.*, 1999), QTL express (Seaton *et al.*, 2002), QGA station (Chen *et al.*, 2012) that allow rapid identification and mapping of QTLs.

Taking clues from plant and animal breeding strategies

The application of QTL in economically important plants, agriculture, livestock, fisheries, *etc.* has become increasingly indispensable for the past three decades. A common aim for both plant and animal breeders is to enhance the process of improvement of quantitative traits. With the knowledge of QTL, a breeder can quickly identify the individuals containing the QTL that contribute to the superior trait under selection. QTL mapping was carried out in *Arabidopsis* species for heavy metal tolerance genes that has important implications on human health (Roosens *et al.*, 2008). QTLs have been utilized to predict heterosis for yield traits in rapeseed using molecular markers (Shen *et al.*, 2006 a). In addition, disease resistance breeding has extensively been carried out in plants. In maize, QTL were identified for resistance to common smut disease (Ding *et al.*, 2008), root-knot nematode resistance in *Gossypium hirsutum* (Shen *et al.*, 2006 b), and late blight disease resistance in potato (Leonards-Schippers *et al.*, 1994). Through genome wide association mapping, disease resistance QTLs were identified for barley (Gutierrez *et al.*, 2013). Among animals, QTLs affecting body weight and condition, resistance to pancreatic necrosis, in Atlantic Salmon (Reid *et al.*, 2004; Houston *et al.*, 2008; Moen *et al.*, 2009), QTLs affecting temperature tolerance in Tilapia hybrids (Cnaani *et al.*, 2003) and common carp (Sun and Liang, 2004) were identified. QTLs for production traits were detected in chicken (Hansen *et al.*, 2005) and QTL mapping was done in pigs (Rothschild *et al.*, 2007) and QTLs affecting muscle mass (Jeon *et al.*, 1999) and fat (Perez-Enciso *et al.*, 2000; Ovilo *et al.*, 2000) were identified. In cattle, detection of QTLs influencing milk production and health traits were carried out by Hendleder *et al.* (2003) and Boichard *et al.* (2003). In sheep, QTLs have been identified for disease resistance (Crawford *et al.*, 1997), wool quality (Bicinos *et al.*, 2008), milk production rates, growth rate and body weight (Raadsma *et al.*, 2009). Such studies in the sericulture scenario are rather obscure and needs to be taken up by the silkworm breeders, not only to improve the raw silk production, but also to understand the genetic architecture of the desired traits.

QTL: Current scenario in silkworm

The concept of QTL and marker studies transfused into the field of science and technology in the 1980s and saw its application in sericulture in recent years. Silkworm, *Bombyx mori*, has 28 linkage groups and the initial linkage maps of silkworm were constructed on the basis of morphological markers (Doira, 1983). The classical linkage maps in silkworms that were based on morphological, biochemical and behavioral markers were gradually replaced by molecular linkage maps including RFLP (Goldsmith, 1991; Shi *et al.*, 1995; Nguu *et al.*, 2005), RAPD (Promboon *et al.*, 1995; Yasukochi, 1998), AFLP (Tan *et al.*, 2001; Lu *et al.*, 2004; Yang-Hu *et al.*, 2006), microsatellites (Miao *et al.*, 2005;) and SNP (Yamamoto *et al.*, 2006). Preliminary attempts were made for QTL mapping of a few economic traits using molecular markers viz., RAPD (Chatterjee and Pradeep, 2003), ISSR (Chatterjee and Mohandas, 2003), AFLP (Lu *et al.*, 2004; Li *et al.*, 2006; Mirhosseini, 2010; Nguyen and Olsen, 2012) and SNPs (Sreekumar *et al.*, 2011; Sreekumar *et al.*, 2012). Several attempts were made to establish an accurate and rapid method for identifying and mapping QTLs. Yuande *et al.* (1998) proposed the DAF (Distribution Area of Frequencies) method using morphomarkers for mapping QTLs. More recently, Xu and his co-workers (2011) came up with a mixed model for mapping QTL that could accurately estimate additive, dominance and epistatic effects. Application of QTL is still at a nascent stage and needs to be imbibed in silkworm breeding programmes. Knowing the positions of the genes that control the economic traits would enable a breeder to gain accuracy on effective selection gain. To locate their positions on chromosome and estimate their contribution to the variation of trait is a key step for positional cloning and follow-up utilization of those genes. Nevertheless, determining the genetic basis of traits under the control of several genes with small effects and their variation in the manifestation of phenotypes with changing environment may prove difficult (Goldsmith, 2010). But it is important that production of many offsprings from a single brood opens up a new avenue for QTL mapping in silkworm, *Bombyx mori*.

Disease resistance breeding in silkworm

Infectious diseases are ubiquitous and are amongst the most challenging selective evolutionary forces encountered by species and individuals (Seal, 1991). Silkworms are affected with various diseases caused by virus, bacteria, fungi and protozoans. Viral diseases caused by BmNPV, BmCPV and BmDNV are serious and cause a great extent of crop damage. Fungal diseases, like muscardine, pose a major threat to silk cocoon production during rainy and winter seasons. Pebrine disease outbreak due to protozoan infection can also result in severe damage since it is maternally transmitted to the offsprings. There are strong theoretical bases to speculate that inbreeding and loss of genetic diversity will reduce the ability of populations to cope with diseases (Frankham *et al.*, 2002). Because of long period of domestication and continuous inbreeding, the silkworms have developed the tendency to succumb to diseases. Therefore, breeders have been careful in evading the incidences of such diseases to certain extent in silkworm by providing optimized rearing conditions and nutritious leaves. Yet, management of the symptoms alone, rather than the underlying cause of disease, may mask problems and lead to greater problems in future. It has been observed that the manifestation of disease resistance traits is predominantly expressed in the multivoltine and their hybrids, whereas in temperate bivoltines, the productive traits are distinctly superior.

Since the occurrence of a disease is a multi-factorial process and depends on the presence of disease-resistance/susceptibility gene(s) present in an individual, response of the host to the pathogen and gene-environment interaction work towards/against the manifestation of a disease. Host responses vary according to the mode of infection and pathophysiology, including behavioral avoidance of pathogens, barriers to infection and innate infection (Lewontin, 1974; Burdon, 1987). Resistance and tolerance are often quantitative phenomena. Continuous variation is seen when resistance or tolerance is due to the combined effects of several genes. Disease resistance seems to be specific to

particular diseases rather than general to all diseases. This is because genetic resistance to different diseases is controlled at least to a major degree by different mechanisms. Thus, different genes will generally influence resistance to unrelated diseases. Thus, similar genes may influence resistance to closely related infections. The greater the number of genes governing resistance, the greater the difficulty the parasite will have in overcoming the barriers.

Using genetics to assist in the management of the symptoms of disease, *i.e.*, genetically increasing the tolerance of a population to infection will be effective in reducing the incidence or the effect of disease in the target population. Clearly, actual disease resistance genes or QTL have the benefit of enabling the selection of animals without exposing them to infection. For many diseases, molecular techniques may be the only way of enabling effective selection for disease resistance. While considering variations in the immune response or resistance to disease challenge, one possible candidate locus could be the major histocompatibility complex locus that includes genes coding for cell surface proteins functioning in binding peptides from pathogens and presenting them to the cells of the immune system for initiation of a proper immune response. Detection of the genes controlling the disease-resistance trait and their locations within the genome will further help in selection of the individuals that contain the particular gene and evolving breeds with higher disease-resistant traits (Bishop *et al.*, 2010). The incorporation of disease resistance genes into the bivoltine breed would be brought about initially by crossing the multivoltine and bivoltine breeds. The F_1 generation population would then be backcrossed to the bivoltine breed and the individuals obtained then genotyped for the genes involved in disease resistance with the help of markers. Individuals carrying the favorable alleles would be selected for breeding and then backcrossed again to the bivoltine breed. Thus, repeated backcrossing and selective breeding from the individuals carrying the favorable disease resistance alleles, will possibly retrieve majority of the genome from

the bivoltine breed while maintaining the disease resistance that originated from the multivoltine breed (Figure 1).

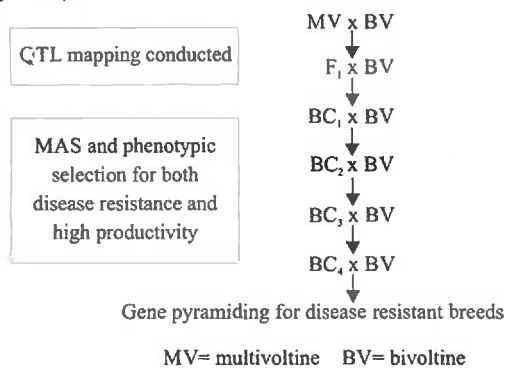


Figure 1: Schematic representation of development of disease resistant progenies based on QTL

The cross involves the utilization of multivoltine breed as a donor parent for disease resistance (DR) and bivoltine breed as a donor of superior productivity traits. Multi-generation QTL analysis included four backcross populations- BC₁, BC₂, BC₃ and BC₄.

Future prospective

Early attempts to identify genes controlling quantitative traits in silkworm were hampered by the lack of informative marker loci. There have been very few successful experiments to identify a major gene through QTL and their molecular basis have been studied (Van Laere *et al.*, 2003; Roff, 2007). The downside is that QTL studies require very large sample size and they can help map only those differences that are captured between the initial parental strains. Because these strains are unlikely to contain segregating alleles of large effect at every locus contributing to variation in natural populations, some loci will remain undetected (Miles and Wayne, 2008). QTLs themselves do not indicate the presence of one or more genes on a marker position in the genome. QTL mapping rarely pinpoints the exact functional genetic variants because QTLs are often wide and cover hundreds of genes. In this context, silkworm offers an ideal model system where it can produce a large population size in a short span of time. In addition, the molecular linkage maps constructed of this animal makes it ideal for undertaking QTL studies. Nowadays, genome-wide association studies are becoming increasingly popular in genetic research and they are an excellent complement to QTL mapping (Miles and Wayne, 2003). Large sets of

ESTs that have been generated through cDNA libraries and automated sequencing methods and map based on SNP-EST covering the majority of the genome and the combined whole genome sequence (International Silkworm Genome Consortium, 2008) will be of great benefit in locating and characterizing the genes of our interest. Knowledge of the genes underlying the expression of qualitative and quantitative traits will help researchers to search for novel combinations of alleles to make further improvement in the desired traits. Studies should not be limited to major genes and their effects but also on the interactions between the genes, which could be obtained from gene expression studies. If the expressions of the genes under a QTL can be monitored, then further clues to the identity of the genetic variants responsible for that trait could be identified. Microarrays can be used to monitor the expression of genes which help to narrow down the candidate genes for a QTL (Jansen and Nap, 2001). Information on QTL may therefore be used in 'candidate gene' approach where prior and external information is used to hypothesize that the candidate gene may be responsible for a known major gene effect. Research can then focus on identifying that gene through prior information on its sequence (from other species), then identifying that sequence variation in that gene, and finally associating the various alleles with phenotypic variation of the trait in question (Albers *et al.*, 2006). To develop a sustainable industry, it will be necessary to address the potential problems associated with conventional approaches and full exploitation of the new technologies available for selection of genetically superior animals as well. Thus, the integrated approaches will become more and more common, and they promise a deeper understanding of the genetic basis of complex traits, including disease resistance (Hubner *et al.*, 2006).

ACKNOWLEDGEMENT

The first author wishes to thank Department of Science and Technology, New Delhi for providing financial assistance. The authors wish to thank University of Mysore and Department of Studies in Sericulture Science, Manasagangotri, Mysore for the facilities provided.

REFERENCES

- Albers G. A. A., Rattink A. P. and Vereijken A. L. J. (2006) The future of molecular genetics in poultry breeding. In: *XII European Poultry Conference*, Verona, Italy.
- Anonymous (2008) The genome of a lepidopteran model insect, the silkworm *Bombyx mori*, International Silkworm Genome Consortium *Insect Biochem. Mol. Biol.*, **38**(12):1036-1045.
- Basavaraja H. K., Suresh Kumar N., Malreddy N. and Datta R. K. (1994) New approaches to bivoltine silkworm breeding. *Proceedings of National Workshop on "Silkworm breeding"*, Oxford & IBH, New Delhi, India, pp. 137-140.
- Bidinost F., Roldan D. L., Dodero A. M., Cano E. M., Taddeo H. R., Mueller J. P. and Poli M. A. (2008) Wool quantitative trait loci in Merino sheep. *Small Ruminant Research*, **74**(1): 113-118.
- Bishop S. C., Axford R. F. E., Nicholas F. W. and Owen J. B. (2010) *Breeding for Disease Resistance in Farm Animals*, 3rd Edition, CABI.
- Boichard D., Grohs C., Bourgeois F., Cerqueira F., Faugeras R., Neau A. and Levéziel H. (2003) Detection of genes influencing economic traits in three French dairy cattle breeds. *Genet. Sel. Evol.*, **35**(1): 77-102.
- Broman K. W. and Speed T. P. (2002) A model selection approach for the identification of quantitative trait loci in experimental crosses. *J. Royal Stat. Society: Series B (Statistical Methodology)*, **64**(4): 641-656.
- Broman K. W. (2001) Review of statistical methods for QTL mapping in experimental crosses. *Lab Anim.*, **30**(7): 44-52.
- Burdon J. J. (1987) *Diseases and plant population biology*. Cambridge University Press, Cambridge, UK.
- Chatterjee S. N. and Mohandas T. P. (2003) Identification of ISSR markers associated with productivity traits in silkworm, *Bombyx mori* L. *Genome*, **46**(3): 438-447.
- Chatterjee S. N. and Pradeep A. R. (2003) Molecular markers (RAPD) associated with growth, yield and origin of the silkworm, *Bombyx mori* L. in India. *Genetika*, **39**: 1612-1624.
- Chen G., Zhu Z., Zhang F. and Zhu J. (2012) Quantitative genetic analysis station for the genetic analysis of complex traits. *Chinese Sci. Bull.*, **57**(21): 2721-2726.
- Cnaani A., Hallerman E. M., Ron M., Weller J. I., Indelman M., Kashi Y. and Hulata G. (2003) Detection of a chromosomal region with two quantitative trait loci, affecting cold tolerance and fish size, in an F2 tilapia hybrid. *Aquaculture*, **223**(1): 117-128.
- Crawford A. M., Phua S. H., McEwan J. C., Dodds K. G., Wright C. C., Morris C. A. and Green R. S. (1997) Finding disease resistance QTL in sheep. *Anim. Biotech.*, **8**(1): 13-22.
- Ding J. Q., Wang X. M., Chander S. and Li J. S. (2008) Identification of QTL for maize resistance to common smut by using recombinant inbred lines developed from the Chinese hybrid Yuyu22. *J. Appl. Genet.*, **49**(2): 147-154.
- Doira H. (1983) Linkage maps of *Bombyx mori*-status quo in 1983. *Sericologia*, **23**: 245-269.
- Falconers D. S. and Mackay T. F. C. (1996) *Introduction to Quantitative Genetics* (4th Edn), Harlow, Longman.
- Frankham R., Briscoe D. A. and Ballou J. D. (2002) *Introduction to conservation genetics*. Cambridge University Press, Cambridge, UK.
- Goldsmith M. R. (1991) The *Bombyx mori* genome-mapping project. *Sericologia*, **31**: 145-155.
- Goldsmith M. R. (2010) Recent progress in silkworm genetics and genomics. In: *Molecular Biology and Genetics of the Lepidoptera*, Goldsmith M. R. and Marec F. (Eds.), Boca Raton, CRC press, pp.25-47.
- Gutiérrez L., Berberian N., Capettini F., Falcioni E., Fros D., Germán S. and Castro A. (2013) Genome-Wide Association Mapping Identifies Disease-Resistance QTLs in Barley Germplasm from Latin America. In: *Adv. Barley Sci.*, Springer Netherlands, pp. 209-215.
- Haley C. S. (1995) Livestock QTLs—bringing home the bacon?. *Trends in Genet.*, **11**(12): 488-492.
- Hansen C., Yi N., Zhang Y. M., Xu S., Gavora J., and Cheng H. H. (2005) Identification of QTL for production traits in chickens. *Anim. Biotech.*, **16**(1): 67-79.
- Hiendleder S., Thomsen H., Reinsch N., Bennewitz J., Leyhe-Horn B., Looft C. and Erhardt G. (2003) Mapping of QTL for body conformation and behavior in cattle. *J. Heredity*, **94**(6): 496-506.
- Houston R. D., Hale, C. S., Hamilton A., Guy D. R., Tinch A. E., Taggart J. B. and Bishop S. C. (2008) Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmosalar*). *Genet.*, **178**(2): 1109-1115.
- Hubner N., Yagil C. and Yagil Y. (2006) Novel integrative approaches to the identification of candidate genes in hypertension. *Hypertension*, **47**(1): 1-5.
- Jansen R. C. and Nap J. P. (2001) Genetical genomics: the added

- value from segregation. *Trends in Genet.*, **17**(7): 388-391.
- Jeon J. T., Carlborg Ö., Törnsten A., Giuffra E., Amarger V., Charden P. and Andersson L. (1999) A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. *Nat. Genet.*, **21**(2): 157-158.
- Kearsey M. J. (1998) The principles of QTL analysis (a minimal mathematics approach). *J. Exp. Botany*, **49**(327): 1619-1623.
- Leonards-Schippers C., Gieffers W., Schäfer-Pregl R., Ritter E., Knapp S. J., Salamini F. and Gebhardt C. (1994) Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an allogamous plant species. *Genet.*, **137**(1): 67-77.
- Lewontin R. C. (1974) The genetic basis of evolutionary change. Columbia University Press, New York.
- Li B., Lu C., Zhao A. C. and Xiang Z. H. (2006) Multiple Interval Mapping for Whole Cocoon Weight and Related Economically Important Traits QTL in Silkworm (*Bombyx mori*). *Agric. Sci. China*, **5**(10): 798-804.
- Lu C., Li B., Zhao A. and Xiang Z. (2004) QTL mapping of economically important traits in silkworm (*Bombyx mori*). *Sci. China Series C: Life Sci.*, **47**(5): 477-484.
- Miao X. X., Xub S. J., Li M. H., Li M. W., Huang J. H., Dai F. Y. and Huang Y. P. (2005) Simple sequence repeat-based consensus linkage map of *Bombyx mori*. *Proc. Nat. Acad. Sci. United States Amer.*, **102**(45): 16303-16308.
- Miles C. and Wayne M. (2008) Quantitative trait locus (QTL) analysis. *Nat. Educ.*, **1**(1): 208.
- Mirhosseini S. Z., Bizhannia A. R., Rabiei B., Taeb M. and Siedavi A. R. (2010) Identification of AFLP markers linked with cocoon weight genes in silkworm (*Bombyx mori* L.). *Afri. J. Biotech.*, **9**(10): 1427-1433.
- Moen T., Baranski M., Sonesson A. and Kjølglum S. (2009) Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*): population-level associations between markers and trait. *BMC Genomics*, **10**(1): 358.
- Nguu K. E., Kadono-Okuda K., Mase K., Kosegawa E. and Hara W. (2005) Molecular linkage map for the silkworm, *Bombyx mori*, based on restriction fragment length polymorphism of cDNA clones. *J. Insect Biotech. Sericology*, **74**(1): 5-13.
- Nguyen K. and Olsen W. (2012) Importance of QTL mapping on economical traits in Silkworm. *Eur. J. Life Sci. Tech.*, **8**: 10-22.
- Ovilo C., Pérez-Enciso M., Barragán C., Clap A., Rodríguez C., Oliver M. A. and Noguera J. L. (2000) A QTL for intramuscular fat and back fat thickness is located on porcine chromosome 6. *Mammalian Genome*, **11**(4): 344-346.
- Pérez-Enciso M., Clap A., Noguera J. L., Ovilo C., Coll A., Folch J. M. and Sanchez A. (2000) A QTL on pig chromosome 4 affects fatty acid metabolism: evidence from an Iberian by Landrace intercross. *J. Anim. Sci.*, **78**(10): 2525-2531.
- Promboon A., Shimada T., Fujiwara H. and Kobayashi M. (1995) Linkage map of random amplified polymorphic DNAs (RAPDs) in the silkworm, *Bombyx mori*. *Genet. Res.*, **66**(1): 1-8.
- Raadsma H. W., Thomson P. C., Zenger K. R., Cavanagh C., Lam M. K., Jonas E. and Nicholas F. W. (2009) Mapping quantitative trait loci (QTL) in sheep. I. A new male framework linkage map and QTL for growth rate and body weight. *Genet. Sel. Evol.*, **41**(1): 34.
- Reid D. P., Szanto A., Glebe B., Danzmann R. G. and Ferguson M. M. (2004) QTL for body weight and condition factor in Atlantic salmon (*Salmo salar*): comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*). *Heredity*, **94**(2): 166-172.
- Roff D. A. (2007) A centennial celebration for quantitative genetics. *Evol.*, **61**(5): 1017-1032.
- Roosens N. H., Willems G. and Saumitou-Laprade P. (2008) Using Arabidopsis to explore zinc tolerance and hyper accumulation. *Trends Plant Sci.*, **13**(5): 208-215.
- Rothschild M. F., Hu Z. L. and Jiang Z. (2007) Advances in QTL mapping in pigs. *Int. J. Biol. Sci.*, **3**(3): 192.
- Seal U. S. (1991) Disease and captive conservation of threatened species. *SSC Working Group meeting*, 28-29 May, 1991, National Zoo, Washington D.C.
- Seaton G., Haley C. S., Knott S. A., Kearsey M. and Visscher P. M. (2002) QTL Express: mapping quantitative trait loci in simple and complex pedigrees. *Bioinformatics*, **18**(2): 339-340.
- Shen J. X., Fu T. D., Yang G. S., Tu J. X. and Ma C. Z. (2006 a) Prediction of heterosis using QTLs for yield traits in rapeseed (*Brassica napus* L.). *Euphytica*, **151**(2): 165-171.
- Shen X., Zhang T., Guo W., Zhu X. and Zhang X. (2006 b) Mapping fiber and yield QTLs with main, epistatic, and QTL × environment interaction effects in recombinant inbred lines of upland cotton. *Crop Sci.*, **46**(1): 61-66.
- Shi J., Heckel D. G. and Goldsmith M. R. (1995) A genetic linkage map for the domesticated silkworm, *Bombyx mori*, based on restriction fragment length polymorphisms. *Genet.*

- Res.*, **66(02)**: 109-126.
- Spelman R. J. and Bovenhuis H. (1998) Moving from QTL experimental results to the utilization of QTL in breeding programmes. *Anim. Genet.*, **29(2)**: 77-84.
- Sreekumar S., Ashwath S. K., Saha A. K., Bindroo B. B. and Kadono-Okuda K. (2012) Identification of SNP markers linked to genes controlling cocoon and post-cocoon traits in the mulberry silkworm, *Bombyx mori*. *Current Biotech.*, **13(15)**: 65.
- Sreekumar S., Ashwath S. K., Slathia M., Kumar S. N. and Qadri S. M. H. (2011) Detection of a single nucleotide polymorphism (SNP) DNA marker linked to cocoon traits in the mulberry silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) *Eur. J. Entomol.*, **108**: 347-354.
- Sun X. and Liang L. (2004) A genetic linkage map of common carp (*Cyprinus carpio* L.) and mapping of a locus associated with cold tolerance. *Aquaculture*, **238(1)**: 165-172.
- Tan Y. D., Wan C., Zhu Y., Lu C., Xiang Z. and Deng H. W. (2001) An amplified fragment length polymorphism map of the silkworm. *Genet.*, **157(3)**: 1277-1284.
- Van Laere A. S., Nguyen M., Braunschweig M., Nezer C., Collette C., Moreau L. and Andersson L. (2003) A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. *Nature*, **425(6960)**: 832-836.
- Wang D. L., Zhu J., Li Z. K. L. and Paterson A. H. (1999) Mapping QTLs with epistatic effects and QTL × environment interactions by mixed linear model approaches. *Theor. Appl. Genet.*, **99(7-8)**: 1255-1264.
- Williams J. L. (2005) The use of marker-assisted selection in animal breeding and biotechnology. *Revue Scientifique et Technique-Office International des Epizooties*, **24.1**: 379.
- Xu H., Wei C., Tang Y., Zhu Z., Sima Y. and Lou X. (2011) A new mapping method for quantitative trait loci of silkworm. *BMC Genet.*, **12**: 19.
- Yamamoto K., Narukawa J., Kadono-Okuda K., Nohata J., Sasanuma M., Suetsugu Y. and Mita K. (2006) Construction of a single nucleotide polymorphism linkage map for the silkworm, *Bombyx mori*, based on bacterial artificial chromosome end sequences. *Genet.*, **173(1)**: 151-161.
- Yang-Hu S., Bin L., Da-Xia C., De-Bin S., Ai-Chun Z., Lie Z. and Zhong-Huai X. (2006) Construction and analysis of an AFLP molecular linkage map of the silkworm (*Bombyx mori*). *Chinese J. Agric. Biotech.*, **3(01)**: 25-31.
- Yasukochi Y. (1998) A dense genetic map of the silkworm, *Bombyx mori*, covering all chromosomes based on 1018 molecular markers. *Genet.*, **150(4)**: 1513-1525.
- Yuande T., Fade L., Cheng L. and Zhonghuai X. (1998) Mapping QTLs in Silkworm by Distribution Area of Frequencies [J]. *J. Biomathematics*, **1**: 018.
- Zeng Z. B. (1994) Precision mapping of quantitative trait loci. *Genet.*, **136(4)**: 1457-1468.

Revue

**LES QTL ET LEUR UTILISATION DANS LES PROGRAMMES DE
REPRODUCTION DES VERS A SOIE**

Ritwika Sur Chaudhuri and G. Subramanya

Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysore - 570006, India.

E-mail: ritwika87@gmail.com

R E S U M E

Avec la demande croissante de soie au niveau global, plusieurs stratégies de reproduction sont développées pour améliorer la qualité et la quantité de soie. Le but actuel du lignage des vers à soie en Inde est d'obtenir des souches de vers à soie résistantes aux maladies et hautement productives. La résistance aux maladies s'est accélérée ces dernières années et est importante dans ce contexte, puisque la plus grande partie des pertes en sériciculture est due à l'incidence des maladies. Des approches moléculaires telles que les QTL et le breeding assisté par des marqueurs ont ajouté une nouvelle dimension aux principes de la reproduction conventionnelle, eu égard à la réduction du coût et du temps. Puisque l'expression de la résistance aux maladies et plusieurs caractères quantitatifs chez le ver à soie sont le résultat d'un jeu complexe entre plusieurs gènes et l'environnement, l'analyse des QTL peut permettre l'identification des gènes en question et de leurs effets. Différentes approches cartographiques des QTL sont utilisées dans ce but. Cependant, les approches intégrées discutées ici promettent une compréhension approfondie des bases génétiques des caractères et fourniront une voie correcte vers le développement durable de la sériciculture.

Mots-clés: *Bombyx mori*, QTL, sélection assistée par marqueurs, lignage du ver à soie.



REPRODUCTIVE EFFICIENCY OF *NESOLYNX THYMUS* (GIRAULT) AS INFLUENCED BY HOST SPECIES – *EXORISTA BOMBYCIS* (LOUIS) AND *MUSCA DOMESTICA* L.

A. S. Aruna¹ and D. Manjunath²

¹Central Tasar Research and Training Institute, Central Silk Board, Ranchi, Jharkhand, India.

²Department of Sericulture, University of Mysore, Mysore, Karnataka, India.

E-mail: arun_9639@rediffmail.com

ABSTRACT

A host of factors viz., age and size of host, age and size of parasitoid, availability and quality of host and temperature are reported to influence the reproductive efficiency of parasitoids. *Nesolynx thymus* Girault (Hymenoptera: Eulophidae) is one among 20 hymenopteran parasitoids reported to attack the uzi fly, *Exorista bombycis* (Louis) (Diptera: Tachinidae), which in turn infests the silkworm, *Bombyx mori* L. accounting a cocoon yield loss of 10-20 %. *N. thymus* is the recommended biocontrol agent for the management of uzi fly. The parasitoid can be mass produced in the insectary for field release on the puparia of house fly and uzi fly. In the present study, an attempt was made to record the influence of parasitoid age on its reproductive performance by providing puparia of 3 day-old *E. bombycis* and 2 day old *Musca domestica*, separately at the parasitoid : host ratio of 1:4 and 1:20, respectively to different age *N. thymus* female (0-10 day-old). The observations on number of puparia parasitized, developmental duration, parasitoid recovery per pupa, progeny production and sex ratio were recorded/calculated. No significant difference was observed among the parameters when *E. bombycis* puparia were offered. However, parasitization and progeny production decreased significantly with age of *N. thymus* when developed on puparia of *M. domestica*. Sex ratio was influenced by parasitoid age when it was multiplied on primary host (uzi fly puparia). However, the reproductive performance decreased with its age when multiplied on secondary host (house fly puparia).

Key words: *Exorista bombycis*, *Musca domestica*, *Nesolynx thymus*, parasitoid age, reproductive performance.

INTRODUCTION

Parasitoid-related factors chiefly comprise parasitoid size with which the other related factors viz., longevity, mating ability, fecundity and progeny production are associated. The information on the influence of parasitoid age on biological parameters of parasitoids is rather scanty and is limited to only sex allocation. Honda and Kainoh (1998) have observed higher response of parasitoid females to host odour when they were young (2 – 5 days) which decreased thereafter, leading to diminished parasitisation in *Ascogaster reticulatus*. With regard to progeny production, an enhanced fecundity has been realized in younger parasitoid females than older ones (Guang and Oloo, 1990). An inverse relationship between parasitoid age and progeny allocation and production and percentage of females has been noticed in

Glyptapanteles flavicoxis (Hu *et al.*, 1986). Insofar as the parasitoid sex ratio is concerned, male-biased sex ratio is reported in older parasitoids and female-dominant sex ratio in younger counterparts (Simser and Coppel, 1980; Laetemia *et al.*, 1995).

Nesolynx thymus Girault (Hymenoptera: Eulophidae) is an ecto-pupal gregarious parasitoid of the tachinid (uzi) fly, *Exorista bombycis* (Louis) (Diptera: Tachinidae), which is an endo-larval parasitoid of the mulberry silkworm, *Bombyx mori* L. Incidentally, *E. bombycis* inflicts a cocoon yield loss of 10 – 20 % in silkworm rearings in the southern sericultural belt comprising Karnataka, Andhra Pradesh and Tamil Nadu in India. Owing to the possession of a number of desirable attributes of a biocontrol agent, *N. thymus* has been

recommended for biological control of *E. bombycis*. The puparia of *Musca domestica* (Linnaeus) (Diptera: Muscidae) serve as an alternate host for mass production of *N. thymus*. In view of the above, in the present investigation, an attempt was made to study the influence of age of *N. thymus* on its reproductive performance on *E. bombycis* (Louis) and *M. domestica*.

MATERIALS AND METHODS

Culturing of parasitoid

The cultures of *N. thymus* (Girault) were maintained on the puparia of *E. bombycis* (Louis) in laboratory at 23 – 28°C and 60 – 85 % RH (Kumar *et al.*, 1996).

Host puparia

The cultures of *M. domestica* (Linnaeus) were maintained at 23 – 28°C and 60 – 85 % RH on a culture medium prepared from cow dung cake (40 g), wheat bran (30 g), saw dust (25 g), yeast (5 g) and water. The puparia were obtained by allowing the grown up maggots in the cultured medium to pupate in a tray containing saw dust. The uzi fly maggots were collected from Govt. Cocoon

Market, Kollegal, Karnataka and were allowed to pupate in a tray containing saw dust. The age of the puparia were counted from the time of pupation of maggots.

To study the effect of parasitoid age, a pair of *N. thymus* adults of different ages (0 – 10 day-old) were allowed to parasitize 3 day-old uzi fly puparia and 2 day-old house fly puparia separately in a test tube (12.5 cm length and 1.5 cm dia) at parasitoid - host ratios of 1:4 and 1:20, respectively. The parasitoid adults were fed with 50 % aqueous honey solution and were allowed to parasitize the host puparia for a period of 3 days at 23 – 28°C and 60 – 85 % RH. Thereafter, they were separated from the host pupae and the latter were kept for parasitoid emergence. After emergence of the parasitoid, observations on number of puparia parasitized, parasitoid development duration, progeny production per pupa and per female and sex ratio in first generation were recorded. Each of the age group comprised of 10 replications. The data were subjected to one-way ANOVA followed by DMRT for test of significance and correlation analysis to understand the relationship between parasitoid age and its reproductive performance.

Table 1: Effect of *N. thymus* age on its reproductive performance on puparia of *E. bombycis*

Parasitoid age (days)	No. of puparia parasitized [@]	Developmental duration (days)	Parasitoid recovery per puparia (No.)			Progeny production per female (No.)			Sex ratio (♂/♀)
			Male	Female	Total	Male	Female	Total	
0	2.77 ± 0.14 (69.25)	15.11 ± 0.11	2.77 ± 0.41	51.98 ± 3.46	54.75 ± 3.70	7.77 ± 1.22	145.22 ± 12.58	153.00 ± 13.28	1:22.04 ± 3.15
1	2.66 ± 0.16 (66.5)	15.44 ± 0.17	2.46 ± 0.20	46.33 ± 3.83	48.79 ± 3.96	6.55 ± 0.70	126.55 ± 15.85	133.11 ± 16.43	1:19.27 ± 1.49
2	2.88 ± 0.26 (72.0)	15.22 ± 0.14	3.16 ± 0.21	46.02 ± 2.24	49.19 ± 2.40	9.44 ± 1.13	136.66 ± 14.52	146.11 ± 15.57	1:14.76 ± 0.64
3	3.12 ± 0.12 (78.0)	15.25 ± 0.16	2.67 ± 0.10	45.50 ± 0.78	48.17 ± 0.84	8.37 ± 0.49	142.37 ± 6.90	150.75 ± 7.31	1:17.17 ± 0.65
4	2.70 ± 0.21 (67.5)	15.10 ± 0.10	2.86 ± 0.29	52.69 ± 3.50	55.55 ± 3.71	7.60 ± 0.90	141.40 ± 12.76	149.00 ± 13.46	1:19.40 ± 1.55
5	2.44 ± 0.37 (61.0)	15.22 ± 0.14	4.47 ± 1.14	45.73 ± 1.74	50.20 ± 2.04	7.66 ± 0.50	111.77 ± 17.89	119.44 ± 17.64	1:15.71 ± 2.86
6	3.12 ± 0.22 (78.0)	15.25 ± 0.16	3.09 ± 0.31	49.62 ± 2.34	52.71 ± 2.59	9.25 ± 0.64	152.37 ± 7.87	161.62 ± 7.92	1:17.19 ± 1.93
7	3.33 ± 0.16 (83.25)	15.00 ± 0.00	2.90 ± 0.17	44.21 ± 1.04	47.12 ± 1.04	9.77 ± 0.87	146.33 ± 5.23	156.11 ± 5.95	1:15.72 ± 1.17
8	3.00 ± 0.23 (75.0)	15.22 ± 0.14	2.93 ± 0.13	44.15 ± 2.27	47.09 ± 2.32	8.88 ± 0.90	130.55 ± 8.91	139.44 ± 9.72	1:15.19 ± 0.82
9	2.80 ± 0.29 (70.0)	15.10 ± 0.10	3.09 ± 0.32	47.22 ± 2.02	50.31 ± 2.26	8.90 ± 1.39	135.00 ± 15.90	143.90 ± 17.02	1:16.67 ± 1.72
10	2.66 ± 0.28 (66.5)	15.22 ± 0.14	3.02 ± 0.19	45.48 ± 2.26	48.50 ± 2.32	8.11 ± 1.05	124.44 ± 16.36	132.55 ± 17.26	1:15.42 ± 1.14
F test	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values are means of 10 replications (mean ± SE)

@ - Number of puparia parasitized by parent female out of 4 puparia provided

NS - Non-significant; Figures in parentheses are the per cent parasitization values.

Table 2: Influence of age of *N. thymus* on its reproductive performance on puparia of *M. domestica*

Parasitoid age (days)	No. of puparia parasitized*	Developmental duration (days)	Parasitoid recovery per puparia (No.)			Progeny production per female (No.)			Sex ratio (♂/♀)
			Male	Female	Total	Male	Female	Total	
0	9.10±0.48 ^a (45.5)	15.10±0.28	0.46±0.04 ^{cd}	5.37±0.49	5.83±0.53	4.10±0.35	47.10±2.20 ^{ab}	51.20±2.41 ^{ab}	1:11.96±0.79 ^{ab}
1	8.80±0.49 ^a (44.0)	15.10±0.28	0.44±0.05 ^{cd}	5.74±0.56	6.18±0.59	3.80±0.44	48.50±2.60 ^a	52.30±2.87 ^a	1:14.04±1.45 ^a
2	9.00±0.58 ^a (45.0)	16.00±0.15	0.39±0.06 ^d	5.13±0.38	5.52±0.43	3.50±0.58	46.50±4.85 ^{ab}	50.00±5.30 ^{ab}	1:14.33±1.12 ^a
3	7.10±0.50 ^b (35.5)	14.90±0.28	0.76±0.06 ^{ab}	5.38±0.35	6.14±0.37	5.20±0.25	37.90±3.44 ^{bc}	43.10±3.44 ^{abc}	1:7.46±0.79 ^c
4	7.10±0.46 ^b (35.5)	15.40±0.22	0.64±0.13 ^{bcd}	4.99±0.47	5.63±0.53	4.50±0.89	35.40±3.70 ^{ac}	39.90±4.16 ^{bc}	1:9.25±1.43 ^{bcd}
5	6.40±0.54 ^{bc} (32.0)	15.20±0.20	0.70±0.11 ^{bc}	6.77±0.74	7.47±0.82	4.30±0.58	41.40±3.31 ^{abc}	45.70±3.77 ^{abc}	1:10.68±1.07 ^{bc}
6	6.20±0.42 ^{bc} (31.0)	15.20±0.20	0.59±0.07 ^{bcd}	6.42±0.63	7.01±0.68	3.60±0.43	38.60±2.91 ^{abc}	42.20±3.21 ^{abc}	1:11.56±1.04 ^{bc}
7	6.40±0.54 ^{bc} (32.0)	15.20±0.20	0.65±0.07 ^{bcd}	6.10±0.59	6.75±0.65	4.00±0.39	37.00±2.22 ^{bc}	41.00±2.50 ^{bc}	1:9.83±0.86 ^{bcd}
8	6.10±0.53 ^{bc} (30.5)	15.30±0.26	0.86±0.14 ^{ab}	6.33±0.85	7.19±0.99	4.70±0.50	35.50±2.97 ^c	40.20±3.38 ^{bc}	1:7.83±0.57 ^{cd}
9	6.00±0.33 ^{bc} (30.0)	15.40±0.27	0.87±0.09 ^{ab}	5.94±0.58	6.81±0.64	5.00±0.37	35.10±3.04 ^c	40.10±3.17 ^{bc}	1:7.32±0.80 ^c
10	5.00±0.39 ^c (25.0)	15.30±0.26	1.03±0.10 ^a	6.90±0.87	7.92±0.95	4.90±0.38	32.50±3.51 ^c	37.40±3.73 ^c	1:6.74±0.69 ^c
F test	**	NS	**	NS	NS	NS	**	*	**

Values are means of 10 replications (mean ± SE); @ - Number of puparia parasitized out of 20 puparia provided

* - Significant at 5% ; ** - Significant at 1% ; NS - Non-significant; Figures in parentheses are the per cent parasitization values.

Figures followed by the same superscript in columns are statistically not significant.

RESULTS

Influence of *N. thymus* age on its reproductive effectiveness on the puparia of *E. bombycis*

The number of puparia parasitized by 0–10 day-old *N. thymus* females ranged from 2.66±0.16 (1 and 10 day-old) to 3.33±0.16 (7 day-old) among the treatments. The developmental duration of the progenies of 0–10 day-old *N. thymus* varied from 15.00 (7 day-old) to 15.44±0.17 days (1 day-old) among the treatments (Table 1).

The recovery of male progeny per pupa was the highest and the lowest for 1 (2.46±0.20) and 2 day-old (3.16±0.21) *N. thymus*, respectively. The female progeny recovered per pupa fluctuated from 52.69±3.50 (4 day-old) to 44.15±2.27 (8 day-old) among the treatments. The total progeny recovery was maximum and minimum for 4 (55.55±3.71) and 8 day-old (47.09±2.32) parasitoid, respectively.

Among the age groups, the production of male progeny by 0–10 day old *N. thymus* adults fluctuated between 9.77±0.87 (7 day-old) and 6.55±0.70 (1 day-old). Maximum and minimum values for the female progeny production per female were 152.37±7.87 (6 day-old) and 111.77±17.80 (5 day-old), respectively among the treatments. The values for total parasitoid progeny

produced per female varied between 156.11±5.95 (7 day-old) and 119.44±17.64 (5 day-old). The sex ratio of *N. thymus* among the age groups ranged from 1:22.04±3.15 (0 day-old) to 1:14.76±0.64 (2 day-old).

Statistical analysis of the results for the above mentioned parameters did not reveal significant variation. Similarly, correlation analysis of the data exhibited no significant relationship between the parameters and the age of *N. thymus*, except the parasitoid sex ratio where it was significant and negative ($r = -0.252$) (Table 1). Correlation coefficients are provided in Table 3.

Effect of *N. thymus* age (0–10 days at an interval of 1 day) on its reproductive efficiency on *M. domestica* puparia

Higher ($P \leq 0.01$) number of puparia were parasitized by *N. thymus* females up to the age of 4 days (7.10±0.50) maximizing being in the case of 0 day-old parasitoid (9.10±0.48) in comparison to the rest of the age groups revealing statistically significant variations (6.40±0.54–5.00±0.39). However, no significant correlation was obtained between the number of puparia parasitized and the age of *N. thymus* (Table 2).

The developmental duration varied from 16.00±0.15 (2 day-old) to 14.90±0.28 days (3 day-old) among different

age groups, but statistically *on par* with each other. However, negative correlation ($r = -0.628$) was established between developmental duration and the parasitoid age.

The parasitoid male progeny enhanced with advancement in age of *N. thymus* and such an increase was less consistent. The male parasitoid recovery varied from 1.03 ± 0.10 (10 day-old) to 0.39 ± 0.06 (2 day-old) among different age groups. The male progeny recovery at 8 (0.86 ± 0.14) – 10 day-old treatments was *on par* and significantly higher ($P \leq 0.01$) than those for the rest of the age groups with most values being statistically not significant. Further, significant positive correlation ($r = 0.498$) was recorded between the parameter and the parasitoid age. The variations in the mean values for the parameter among the treatments were non-significant. Further, the female parasitoid recovery showed an increasing trend with increase in age of the parasitoid with the values fluctuating between 6.90 ± 0.87 (10 day-old) and 4.99 ± 0.47 (4 day-old). However, the female parasitoid recovery established significant positive correlation ($r = 0.220$) with age of *N. thymus*. The total progeny of *N. thymus* recovered per pupa among the different age groups ranged between 7.92 ± 0.95 (10 day-old) and 5.52 ± 0.43 (2 day-old), which did not differ significantly. The results for the parameter did not vary significantly when compared. However, significant positive correlation ($r = 0.272$) was obtained between total progeny recovery and the parasitoid age.

Maximum and minimum parasitoid male progeny production was realized for 3 day (5.20 ± 0.25) and 2 day-old (3.50 ± 0.58) group, respectively. Further, comparison of the mean values among the age groups of

N. thymus showed no significant variation. Also, correlation analysis of male progeny production per female established no significant relationship with the parasitoid age. More or less consistent reduction in the parasitoid female progeny production was observed as the age of *N. thymus* increased. The progeny production among the treatments ranged between 48.50 ± 2.60 (1 day-old) and 32.50 ± 3.51 (10 day-old). Furthermore, the female recovery realized among different age groups up to 6 day were *on par* and significantly maximum ($P \leq 0.01$) as against the remaining age groups with the highest value for 7 day-old *N. thymus* (37.00 ± 2.22). Likewise, the female progeny production per female indicated significant negative correlation ($r = -0.412$) with the parasitoid age. The total progeny realized among the age groups ranged from 52.30 ± 2.87 (1 day-old) to 37.40 ± 3.73 (10 day-old). It decreased almost consistently as the age of the parasitoid advanced. The values scored for the parameter for majority of the age groups up to 7 day (41.00 ± 2.50) were significantly higher ($P \leq 0.05$) as compared to the remaining age groups that recorded the maximum value at 8 day-old *N. thymus* (40.20 ± 3.38). In the same manner, a significant negative correlation ($r = -0.367$) was noticed between the parameter and *N. thymus* age.

The age groups up to 2 day produced comparable and significantly maximum ($P \leq 0.01$) sex ratios (1: 14.33 ± 1.12) for *N. thymus* in sharp contrast to most of the remaining age groups where the sex ratio fluctuated between 1: 11.56 ± 1.04 (6 day-old) and 1: 6.74 ± 0.69 (10 day-old). The sex ratio obtained in most age groups from 3 day (1: 7.46 ± 0.79) to 10 day were statistically non-significant. Similarly, the correlation between the

Table 3: Correlation between the age of *N. thymus* and its reproductive parameters on the puparia of *E. bombycis* and *M. domestica*

Host puparia	No. of puparia parasitized	Developmental duration	Parasitoid recovery per puparium			Progeny production per female			Sex ratio
			Male	Female	Total	Male	Female	Total	
<i>E. bombycis</i>	0.046 ^{NS}	0.087 ^{NS}	0.081 ^{NS}	-0.153 ^{NS}	-0.132 ^{NS}	0.140 ^{NS}	-0.052 ^{NS}	0.040 ^{NS}	-0.252*
<i>M. domestica</i>	0.019 ^{NS}	-0.628**	0.498**	0.220*	0.272**	0.165 ^{NS}	-0.412**	-0.367**	-0.477**

* - Significant at 5% ; ** - Significant at 1% ; NS - Non-significant

parasitoid sex ratio and the parasitoid age was significant and negative ($r = -0.477$) (Table 2). Correlation coefficients are provided in Table 3.

Reproductive performance of *N. thymus* (0 - 10 day-old) on puparia of *E. bombycis* and *M. domestica*

The production of *N. thymus* male progeny by 0 - 10 day-old females of *N. thymus* on *E. bombycis* and *M. domestica* puparia varied from 9.77 ± 0.87 to 6.55 ± 0.70 and 5.20 ± 0.25 to 3.50 ± 0.58 , respectively. When the values for male progeny on both the hosts were compared in respective age groups, significantly higher number of parasitoid males was observed on *E. bombycis* puparia. Similarly, production of parasitoid female progeny by *N. thymus* females on *E. bombycis* and *M. domestica* pupae

varied from 1: 22.04 ± 3.15 to 1: 14.76 ± 0.64 when developed on *E. bombycis* and 1: 14.33 ± 1.12 to 1: 6.74 ± 0.69 when emerged from *M. domestica* puparia. Comparison of mean values from respective age groups revealed significantly higher parasitoid sex ratio on *E. bombycis* puparia (Table 4).

DISCUSSION

In majority of parasitic species, females oviposit a high proportion of fertilized eggs immediately after mating (Brodeur and McNeil, 1994). A number of factors are observed to influence a parasitoid's decision to oviposit in a patch, viz., host searching efficiency, host

Table 4: Effect of age of *N. thymus* on its reproductive performance on puparia of *E. bombycis* and *M. domestica*

Parasitoid age (days)	Progeny production per female (No.)									Sex ratio (♀/♂)		
	Male			Female			Total			E.b.	M.d.	F test
	E.b.	M.d.	F test	E.b.	M.d.	F test	E.b.	M.d.	F test			
0	7.77±1.22	4.10±0.35	**	145.22±12.58	47.10±2.20	**	153.00±13.28	51.20±2.41	**	1:22.04 ± 3.15	1:11.96±0.79	**
1	6.55±0.70	3.80±0.44	**	126.55±15.85	48.50±2.60	**	133.11±16.43	52.30±2.87	**	1:19.27 ± 1.49	1:14.04±1.45	**
2	9.44±1.13	3.50±0.58	**	136.66±14.52	46.50±4.85	**	146.11±15.57	50.00±5.30	**	1:14.76 ± 0.64	1:14.33±1.12	**
3	8.37±0.49	5.20±0.25	**	142.37±6.90	37.90±3.44	**	150.75±7.31	43.10±3.44	**	1:17.17 ± 0.65	1:7.46±0.79	**
4	7.60±0.90	4.50±0.89	**	141.40±12.76	35.40±3.70	**	149.00±13.46	39.90±4.16	**	1:19.40 ± 1.55	1:9.25±1.43	**
5	7.66±0.50	4.30±0.58	**	111.77±17.89	41.40±3.31	**	119.44±17.64	45.70±3.77	**	1:15.71 ± 2.86	1:10.68±1.07	**
6	9.25±0.64	3.60±0.43	**	152.37±7.87	38.60±2.91	**	161.62±7.92	42.20±3.21	**	1:17.19 ± 1.93	1:11.56±1.04	**
7	9.77±0.87	4.00±0.39	**	146.33±5.23	37.00±2.22	**	156.11±5.95	41.00±2.50	**	1:15.72 ± 1.17	1:9.83±0.86	**
8	8.88±0.90	4.70±0.50	**	130.55±8.91	35.50±2.97	**	139.44±9.72	40.20±3.38	**	1:15.19 ± 0.82	1:7.83±0.57	**
9	8.90±1.39	5.00±0.37	**	135.00±15.90	35.10±3.04	**	143.90±17.02	40.10±3.17	**	1:16.67 ± 1.72	1:7.32±0.80	**
10	8.11±1.05	4.90±0.38	**	124.44±16.36	32.50±3.51	**	132.55±17.26	37.40±3.73	**	1:15.42 ± 1.14	1:6.74±0.69	**

Values are means of 10 replications (mean ± SE); * - Significant at 5%; ** - Significant at 1%; NS - Non-significant
E.b. - *Exorista bombycis*; M.d. - *Musca domestica*

was significantly more at all treatments on *E. bombycis*. The total parasitoid progeny produced by *N. thymus* fluctuated from 161.62 ± 7.92 to 119.44 ± 17.64 and 52.30 ± 2.87 to 37.40 ± 3.73 when parasitized on *E. bombycis* and *M. domestica*, respectively. Statistical analysis of the data revealed significantly maximum total progeny production on *E. bombycis* at all age groups. The sex ratio of the progenies of 0 - 10 day-old parasitoid

quality, female longevity, egg load, etc. (Iwasa *et al.*, 1984; Parker and Courtney, 1984; Skinner, 1985; Waage, 1986; Charnov and Skinner, 1988; Minkenberg *et al.*, 1992; Heimpel *et al.*, 1997; Mackauer, 1996). Hu *et al.* (1986) observed decrease in daily percentage parasitization in *Glyptapanteles flavicoxis* (Marsh) with parasitoid age. In *A. reticulatus* (Watanabe), Honda and Kainoh (1998)

reported that the parasitoid females exhibited more response to host odour up to 2–5 days after their eclosion and thereafter the response decreased resulting in diminished parasitisation. With reference to progeny production, Guang and Oloo (1990) observed an elevated fecundity in younger parasitoid females than in older ones in *Trichogramma* sp. Nr. (Mwanzai Schulten & Feijn) *chilonis*. In an effort to record the day-wise progeny allocation by a parasitoid, production and percentage of female was found decreased with parasitoid age in *G. flavicoxis* (Hu *et al.*, 1986). As far as the parasitoid sex ratio was concerned, Barbosa and Frongillo (1979) recorded a male biased sex ratio in older females of the parasitoid, *Brachymeria intermedia* (Nees). Similarly, older females of *B. lasus* (Simser and Coppel, 1980) and *T. minutum* Riley (Leatemia *et al.*, 1995) produced male dominant progeny compared to their younger counterparts.

The present efforts related to the impact of *N. thymus* age on the per cent parasitization and progeny production (male, female and sex ratio) of the parasitoid when developed on *E. bombycis* revealed non-significant correlation with all the parameters, except the sex ratio where the correlation was significant and negative. In contrast, when the parasitoid was developed on *M. domestica*, significant negative correlation was obtained for all the parameters. The results indicating positive trend for male progeny and negative relationship for female and total progeny and sex ratio substantiate the observations of Guang and Oloo (1990), and Honda and Kainoh (1998). However, the reduction in sex ratio of *N. thymus* with the parasitoid age can be a reflection of declined female progeny in relation to more or less constant population of the parasitoid male.

A superior progeny production (male and female) has been recorded for *N. thymus* when the parasitoid adults parasitized *E. bombycis* compared to *M. domestica*. Irrespective of age, *N. thymus* performed better when it developed on *E. bombycis* which categorically proved that *E. bombycis* was preferred to *M. domestica* for parasitization, perhaps based on quality criterion.

REFERENCES

- Barbosa P. and Frongillo E. (1979) Host parasitoid interactions affecting reproduction and oviposition by *Brachymeria intermedia*. *Entomophaga*, **24**: 139-143.
- Brodeur J. and McNeil J. N. (1994) Seasonal ecology of *Aphidius nigripes* (Hymenoptera: Aphidiidae), a parasitoid of *Macrosiphum euphorbiae* (Homoptera: Aphididae). *Environ. Entomol.*, **23**: 292-298.
- Charnov E. L. and Skinner S. W. (1988) Clutch size in parasitoids: the egg production rate as a constraint. *Evolution Ecol.*, **2**: 167-174.
- Guang L. Q. and Oloo G. W. (1990) Host preference studies on *Trichogramma* sp. nr. Mwanzai Schulten & Feijen (Hymenoptera : Trichogrammatidae) in Kenya. *Insect Sci. Appl.*, **11**: 757–763.
- Heimpel G. E., Rosenheim J. A. and Kattari D. (1997) Adult feeding and lifetime reproductive success in the parasitoid *Aphytis melinus*. *Entomol. Exp. Appl.*, **83**: 305–315.
- Honda T. and Kainoh Y. (1998) Age-related fecundity and learning ability of the egg-larval parasitoid *Ascogaster reticulatus* Watanabe (Hymenoptera : Braconidae). *Biol. Control*, **13**: 177–181.
- Hu Cui, Barbosa P. and Martinat P. J. (1986) Influence of age of female *Glyptapanteles flavicoxis* (Marsh) (Hymenoptera: Braconidae) and its periodicity of oviposition activity on levels of parasitism and reproductive output. *Ann. Entomol. Soc. Am.*, **79**: 280–282.
- Iwasa Y., Suzuki Y. and Matzuda H. (1984) Theory of oviposition strategy of parasitoids. I. Effect of mortality and limited egg number. *Theor. Popul. Biol.*, **26**: 205–227.
- Kumar P., Manjunath D., Vinod Kumar, Ahsan M. M. and Datta R. K. (1996) Industrial production of biocontrol agents for the key pests of mulberry and silkworm – prospects and challenges, *Proceedings of the International Conference on Sericulture – Global Silk Scenario – 2001*, Oxford & IBH Publishers, pp. 189–199.
- Leatemia J. A., Laing J. E. and Corrigan J. E. (1995) Effect of adult nutrition on longevity, fecundity, and offspring sex ratio of *Trichogramma minutum* Riley (Hymenoptera : Trichogrammatidae). *Can. Entomol.*, **127**: 245–254.

- Mackauer M. (1996) Sexual size dimorphism in solitary parasitoid wasps: influence of host quality. *Oikos*, **76**: 265 – 272.
- Minkenberg O. P. J. M., Tatar M. and Rosenheim J. A. (1992) Egg load as a major source of variability in insect oviposition behaviour. *Oikos*, **65**: 134 – 142.
- Parker G. A. and Courtney S. P. (1984) Models of clutch size in insect oviposition. *Theor. Popul. Biol.*, **26**: 27 – 48.
- Simser D. H. and Coppel H. C. (1980) Courtship and mating behaviour of *Brachymeria lasus* (Hymenoptera : Chalcididae), an imported gypsy moth parasitoid. *Entomophaga*, **25**: 349 – 356.
- Skinner S. W. (1985) A third extrachromosomal factor affecting the sex ratio in the parasitoid wasp, *Nasonia vitripennis*. *Genetics*, **109**: 745 – 759.
- Waage J. K. (1986) Family planning in parasitoids : Adaptive pattern of progeny and sex allocation. In: *Insect Parasitoids*, Wang J. and Greathead D. (Eds.), Academic Press, London, pp. 63-95.

Article scientifique

LA CAPACITE DE REPRODUCTION CHEZ *NESOLYNX THYMUS* (GIRAULT) EST INFLUENCEE PAR L'ESPECE DE L'HÔTE – *EXORISTA BOMBYCIS* (LOUIS) ET *MUSCA DOMESTICA* L.

A.S. Aruna¹ and D. Manjunath²

¹Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles, Ranchi, Jharkhand, India.

²Department of Sericulture, University of Mysore, Mysore, Karnataka, India.

E-mail: arun_9639@rediffmail.com

R E S U M E

L'âge et la taille de l'hôte, l'âge et la taille du parasitoïde, la disponibilité et la qualité de l'hôte ainsi que la température influencent l'efficacité de reproduction des parasitoïdes. *Nesolynx thymus* Girault (Hymenoptera: Eulophidae) est l'un des 20 hyménoptères parasitoïdes connus pour attaquer la mouche uzy, *Exorista bombycis* (Louis) (Diptera: Tachinidae), qui elle-même infeste *Bombyx mori* L. entraînant une perte de 10-20% de cocons. *N. thymus* est recommandé comme agent de contrôle biologique pour gérer la mouche uzy. Le parasitoïde peut être produit en masse dans l'insectarium sur des pupariums de mouches domestiques ou de mouches uzy avant le lâchage sur le terrain. Dans notre étude, nous avons tenté d'enregistrer l'influence de l'âge du parasitoïde sur ses performances de reproduction en fournissant des pupariums de *E. bombycis* de 3 jours et des pupariums de *Musca domestica* de 2 jours séparément avec un ratio parasitoïde/hôte de 1/4 et 1/20 respectivement pour des âges différents (0-10 jours) des femelles de *N. thymus*. Les observations sur le nombre de pupariums parasités, la durée de développement, le nombre de parasitoïdes récupérés par pupes, la production de progénitures et le sex-ratio ont été enregistrées. Aucune différence significative n'a été observée parmi les paramètres lorsque les pupariums de *E. bombycis* sont utilisés. Toutefois, le parasitisme et la progéniture diminuent significativement avec l'âge de *N. thymus* lorsqu'elle est développée sur des pupariums de *M. domestica*. Le sex-ratio est influencé par l'âge du parasitoïde lorsqu'il est multiplié sur l'hôte primaire (pupariums de la mouche uzy). Les performances de reproduction diminuent avec l'âge lorsque la multiplication est réalisée sur l'hôte secondaire (pupariums de la mouche domestique).

Mots-clés: *Exorista bombycis*, *Musca domestica*, *Nesolynx thymus*, âge du parasitoïde, performance de reproduction.



EVALUATION OF NEWLY-EVOLVED OPEN-POLLINATED MULBERRY VARIETIES

N. Damasco Crestilyn, A. Dacayanan Merlita, J. Tabafunda Minerva,
A. Ancheta Lilia and D. Supsup Roel

Don Mariano Marcos Memorial State University-Sericulture Research and Development Institute, Bacnotan,
La Union, Philippines.

E-mail:crestilyndamasco@yahoo.com

ABSTRACT

The performance evaluation and selection of open-pollinated mulberry varieties (OPVs) was made based on growth, leaf yield and quality parameters covering five years that included eleven seasons. Alf-004 was observed the best in terms of leaf yield, longest shoot and plant height whereas for leaf quality with the highest moisture content (MC) and moisture retention capacity (MRC), it was Alf-025. Significant seasonal variation on growth, leaf yield and quality were observed. The highest leaf yield of 625.39 g/plant was obtained in July 2008. However, the quality of leaves was superior in November 2007 in terms of moisture content and in August and October 2011 for moisture retention capacity. Regression Analysis revealed that temperature did not affect the growth, yield and quality characters but rainfall and relative humidity affected the plant height, MC and MRC of the OPVs. A significant negative correlation could be established between leaf yield and number of branches. Highly significant positive correlations between plant height and length of shoots; plant height with moisture content and moisture retention capacity and moisture content and moisture retention capacity were also revealed. Considering the over-all evaluation on growth, yield and quality characters, the top three promising OPVs selected were Alf-004, S61-019 and S54-019.

Key words: Growth, leaf quality, leaf yield, open-pollinated mulberry varieties.

INTRODUCTION

The present scenario of sericulture industry demands new varieties suitable for various agronomic conditions. Evaluation of crop performance is the basic requirement to evolve new varieties suitable for specific zones for commercial utilization. Suitable parent material needs to be identified from the different accessions for the purpose.

Open-pollinated mulberry varieties (OPVs) are known to be hardier and have more flexibility than hybrid varieties as these seeds are dynamic, that is they mutate and adapt to the local ecosystem, as opposed to hybrids, which are static. Yet, the suitability of these OPV's has to be considered from two aspects *i.e.*, agronomic and silkworm rearing. Out of a few OPVs developed, seven promising strains were identified which recorded 30-70 % increase in yield per hectare over the widely used variety, Batac (Almojuela *et al.*, 2001).

Agronomic characters such as good rooting, fast growth, high yield, adaptability, pest/disease resistance and drought tolerance are factors to be considered in the selection of cultivars. However, the final aim of plant selection is to achieve better yield and a more nutritious product with high moisture content to realize higher cocoon yield and quality. Thus, the present study was conducted to evaluate the performance of newly evolved OPVs on growth, yield and quality characters for effective utilization in cocoon production.

MATERIALS AND METHODS

1. Treatments, field lay-out and maintenance of the OPVs

Seven-year old plantation of OPVs named as Alf-004, Alf-018, Alf-025, Alf-028, S61-011, S61-019, S54-019 and the local variety Batac were used in the study. These varieties were selected from the short listed OPVs

considered as promising strains in terms of yield potential with desirable leaf characteristics, moisture content, moisture retention capacity and bioassay as to cocoon yield/box as shown in Table 1. The parental varieties of these OPVs are S54, S61 and Alfonso. The plants were laid out in Randomized Complete Block Design with three replications with 30 plants per variety per block. The spacing was 1m between treatments; 0.5 m between hills and 1.75 m between blocks.

Recommended cultural management practices were employed uniformly to all treatments. The mulberry plants were pruned at a height of 0.5 m. Two weeks after pruning, fertilizer was applied through drill method using mixed urea (45-0-0) and complete (14-14-14) fertilizer at the rate of 300-120-120 kg /ha/year. Hence, each application comprised of a mixture of 10 g urea and 32.14g complete / plant followed by irrigation. Weeding was done twice within 45 days. Data were gathered sixty days after pruning.

Table 1: Characteristics of the parental varieties

Parent Character	Alfonso	S54	S61
Leaf shape	Elliptic	Cordate	Ovate
Leaf apex shape	Sharp	Caudate	Ovate
Leaf base shape	Linear	Cordate	Cordate
Lamina lobation	Entire	Entire	Entire
Leaf nature	Heterophyllus	Uniform	Uniform
Leaf margin	Crenate	Crenate	Crenate
Stem color	Light brown	Light green	Light green
Branching nature	Erect	Drooping	Erect
Leaf texture	Glossy	Glossy	Glossy
Leaf yield (g/plant)	1783.19	2620.77	1931.84
Moisture content (%)	72.60	77.10	73.61
Moisture retention capacity (%)	45.43	42.01	43.51
Cocoon yield/box (kg/box)	35.51	33.14	31.86

Source: Mulberry Breeding and Improvement Unit, DMMMSU-SRDI.

2. Data Collection

Four plants were randomly sampled for 11 harvests from 2007-2011 for the following traits:

Leaf yield

All harvestable leaves / plant were gathered and put in polyethylene bags. Fresh weight was taken immediately using 10 kg capacity weighing scale. Leaf yield was determined using the equation:

$$\text{Leaf yield (g plant}^{-1}\text{)} = \frac{S_1 + S_2 + S_3 + S_4}{4}$$

Number of branches

All the branches were counted and recorded.

Length of the longest shoot (cm)

Measured from the base of the shoot or branch to the tip of the apical leaf.

Plant height (cm)

Measured from the base of the plant to the tip of the largest glossy leaf of the longest branch.

Moisture content of leaves

All harvestable leaves / plant were harvested, put in polyethylene bags and sealed. Fresh weight was taken immediately using electronic weighing scale. After three days of air drying, the leaves were further oven-dried at 70°C for 48-72 hours till the constant weight was attained. Moisture content was calculated using the formula:

$$\text{MC (\%)} = \frac{\text{Fresh weight} - \text{oven dry weight}}{\text{Fresh weight}} \times 100$$

Moisture retention capacity (MRC)

All harvestable leaves / plant were harvested, put in polyethylene and sealed. Fresh weight was recorded. Leaves were kept in open condition at room temperature and then weighed after 6 hours of harvest. Leaves were further dried thoroughly at 70°C for 72 hours in the oven and then weighed. The moisture retention capacity was computed using the formula:

$$\text{MRC} = \frac{\text{mg} - \text{md}}{\text{mg}} \times 100$$

Where: MRC – moisture retention capacity
mg – leaf weight after 6 hours
md – oven dry weight of leaves

Other observations

Data on rainfall, average temperature and relative humidity were recorded at the DMMMSU-Agromet Station, NLUC during the research implementation period.

3. Data analysis

Data were subjected to Combined Analysis of Variance (ANOVA) across season. Duncan Multiple Range Test (DMRT) was used as test of significance. The degree of association between yield and related characters was determined using the Simple Linear Correlation Analysis. Regression analysis was used to determine the effects of climatic factors on the different growth, yield and quality characters of the different OPVs.

varieties were then ranked for all the data gathered with rank 1 for the highest value and rank 8 for the lowest value. Then the ranks for all the data of each variety were added. The top three varieties having the lowest ranks were considered promising.

RESULTS AND DISCUSSION

Performance of the newly evolved open-pollinated varieties on growth, yield and quality characters (2007-2011)

Mulberry is the only food for raising silkworm (*Bombyx mori* L.) crop for commercial exploitation. The recommended mulberry genotype should provide a large quantity of high quality foliage in unit space and time. Selection of improved genotypes of mulberry is based

Table 2: Performance of the newly evolved open-pollinated mulberry varieties and Batac in terms of growth, yield and quality characters (2007-2011)

Character Genotype	Leaf yield (g plant ⁻¹)	Number of branches	Length of the longest shoot (cm)	Plant height (cm)	Moisture content (%)	Moisture retention capacity (%)
Alf-004	433.03 a	12.44 b	183.02 a	251.12 a	78.32 b	62.48 ab
Alf-018	326.58 cd	13.48 c	126.29 c	176.22 cd	78.71 ab	61.66 bc
Alf-025	335.58 cd	8.69 d	126.65 c	173.36 d	79.17 a	63.80 a
Alf-028	251.21 e	10.92 c	113.72 d	157.83 e	76.99 c	59.78 cd
S61-011	323.68 cd	13.33 ab	128.11 c	186.73 c	77.04 c	59.99 cd
S61-019	386.75 b	10.97 c	154.21 b	216.97 b	78.02 b	59.56 d
S54-019	364.80 bc	13.73 a	137.89 c	205.97 b	77.21 c	58.74 de
Batac	294.95 d	8.77d	149.82 b	207.51 b	75.80 d	57.43 e
SE	68.67	1.89	18.53	18.69	1.19	3.07
CV (%)	24.77	20.14	16.21	11.62	1.88	6.22

In a column, means followed by a common letter are not significantly different at 5 % level by DMRT.

4. Identification and selection of promising OPVs

Promising open- pollinated mulberry varieties were identified based on growth, leaf yield and quality parameters. The criterion for selection was arbitrarily devised in which each parameter was given weight expressed in percentage. Leaf yield was given the highest weight of 30 % and leaf quality, 15 %. The mulberry

particularly on characters such as number of branches, length of shoot, plant height, moisture content and moisture retention capacity to improve the target traits in the breeding program. The general trend in breeding is to maintain the balance between yield and quality of the leaves.

The analysis of variance (Table 2) revealed highly

significant differences among the genotypes for growth, yield and quality characters.

Leaf yield (g plant⁻¹): Among the eight genotypes, Alf-004 significantly produced the highest leaf yield of 433.03 g plant⁻¹ which is 11.97-72.38 % higher than the other varieties. Use of this variety may serve as an effective way to increase yield in cocoon per unit area of mulberry leaves due to higher leaf yield than the other varieties. The next highest in yield but significantly lower than Alf-004 were S61-19 and S54-19 with 386.75 and 364.80 g plant⁻¹. The lowest yield was produced by Alf-028 at 251.21 g plant⁻¹.

Number of branches: The number of branches produced by a genotype has been considered as one of the growth indices in mulberry (Dandin and Kumar, 1989). S54-019 and S61-011 have the most number of branches with 13.73 and 13.33 branches (average), respectively, while Alf-025 (8.69) and Batac (8.77) produced the least number of branches that differed significantly from the rest of the OPVs.

Length of the longest shoot (cm): Significant variations were also observed among OPVs in terms of the longest shoot. Alf-004 exhibited the longest shoot at 183.02 cm which differed significantly from all other OPVs. This indicated that among the varieties, Alf-004 manifested more vigorous and faster growth. Alf-028 by far had the shortest shoot. Batac ranked third among the varieties for the longest shoot but at par with S61-019 (154.21 cm).

Plant height (cm): Plant height is a characteristic which can be considered an ideotype in the selection of superior plant (Sengupta and Dandin, 1989). The optimum value for total length of shoot for mulberry is 500 cm and the length of the tallest shoot is 150 cm. Alf-004 was markedly tall at 251.12 cm qualifying as the only promising OPV in terms of plant height. Further, it was significantly higher than all other OPVs with height ranging from 157.83 (Alf-028) – 216.97 cm (S61-019). This may in turn be an advantage for Alf-004 in terms of

leaf yield since taller plants have more leaf surface, resulting in more biomass production and greater photosynthetic activity (Masilamani *et al.*, 1996; Chaitanya *et al.*, 2002).

Moisture content (%): A major determinant of nutritive quality of mulberry is the moisture content of the leaves. Dacayanan *et al.* (2013), further stressed that higher moisture content in mulberry leaves is known to increase the ingestion and digestion ability of silkworm because moisture acts as olfactory and gustatory stimulant (Ito, 1963; Paul *et al.*, 1992), and thus serves as a criterion in estimating the leaf quality (Parpiev, 1968). Alf-025 recorded the highest MC with a mean of 79.17 % followed by Alf-018 at 78.71 %. These varieties proved to have higher palatability and digestibility qualifying more suitable for silkworm. On the other hand, among the OPVs, MC of Batac was the lowest at 75.80 %.

Moisture retention capacity (%): Due to the time gap between harvesting and feeding of leaves to silkworm, a considerable amount of moisture loss can occur from the harvested leaves. The optimum moisture retention capacity of mulberry leaves is 68 % after 6 hours of harvest. Since MRC has a direct relationship with feeding quality, it is an important consideration in the selection of variety. Significant differences were noted in the MRC of the different OPVs. This is in conformation to Kasiviswanathan *et al.* (1973) and Sastry *et al.* (1988) in their pronouncement that genotypic characteristics among mulberry play a major role in retention and loss of moisture. The highest mean MRC was obtained by Alf-025 (63.80 %) which performed comparably well with Alf-004 at 62.48 %. Batac recorded the lowest MRC of leaves of 57.43 % but did not differ significantly from S54-019 at 58.74 %. However, harvested leaves of OPVs should be provided with effective preservation method to maintain their quality as feed for silkworm.

Seasonal performance of the newly evolved open-pollinated mulberry varieties (2007-2011)

The knowledge of complex characters in mulberry *viz.*, leaf yield and its component characters which are sensitive

to environmental conditions, can obviously be of considerable use for a rational approach for the improvement of leaf yield. As presented in Table 3, there are significant seasonal variations in the performance of OPVs in growth, yield and quality.

The highest leaf yield of 652.39 g plant⁻¹ was recorded in July 2008 which was significantly higher than the rest of the seasons. Yield was consistently low in March (157.18 and 176.34 g plant⁻¹) in two years (2008 and 2009, respectively) as well as in October 2011 (230.35 g plant⁻¹).

The most number of branches was produced in March 2008 (18.98). In July 2008, November 2007, August 2007 and August 2011, branches produced markedly dropped to 7.62-9.90. Number of branches in July 2008 was the least at 7.62.

Mulberries registered significantly longest shoot at 216.60 cm in October 2011 which was at par with mulberry shoots harvested during August 2007 (205.25 cm). The shortest was noticed in March 2009 with 73.48 cm.

Plants were significantly taller at a height of 282.29 cm in October 2011 than in all other seasons while plants were found to have grown only to a height of 136.52 cm in March 2009 which is the lowest. No significant

differences were seen between the seasons July 2010, August 2009 and March 2008 with the height of plants at a mean of 157.90, 168.77 and 160.21 cm, respectively.

Leaf quality also varied with season. November 2007 registered the highest MC with a mean of 84.67 % which significantly differed from the rest of the seasons. October 2011 exhibited the second highest MC at 82.49 % which was on par with that in August 2011 (80.91 %). The lowest MC was recorded in March 2009 at 71.68 %.

Moisture retention capacity of OPVs during different seasons varied significantly. Leaves produced in August 2011 retained the highest moisture content with a mean of 74.34 % which was on par with those produced in October 2011 (73.68 %). Leaves tended to lose moisture fast in March 2009 with 44.59 %, hence, preservation methods during this season should be implemented vigilantly to maintain the freshness or the quality of leaves.

In addition, the very low moisture retention capacity is not only due to genetic reasons but could be attributed to the prevailing climatic conditions coupled with high maximum temperature at 33.05 °C and lower relative humidity of 85.09 % which undoubtedly hastened the leaf moisture retention capacity of newly evolved OPVs and Batac.

Table 3: Seasonal performance of the newly evolved open-pollinated mulberry varieties on growth, yield and quality characters (2007-2011)

Season Parameter	Aug 2007	Nov 2007	March 2008	July 2008	Oct 2008	March 2009	Aug 2009	Oct 2009	July 2010	Aug 2011	Oct 2011
Leaf yield (g plant ⁻¹)	548.32 b	424.82 c	157.18 g	652.39 a	364.01 cd	176.34 fg	301.97 de	288.13 de	262.24 df	329.56 ce	230.35 eg
Number of branches	9.73cd	7.65 d	18.98 a	7.62 d	10.75 cd	11.40 c	12.77 bc	12.33 bc	10.54 bc	9.90 cd	14.63 b
Length of the longest shoot (cm)	205.25 a	149.59 cd	109.42 e	166.22 bc	104.33e	73.48f	104.67e	170.44b	95.90e	143.73d	216.60a
Plant height (cm)	209.96 bc	206.31 bc	160.21 d	206.73 bc	201.85 c	136.52 e	168.77 d	227.15 b	157.90 d	208.92 bc	282.29 a
Moisture content (%)	77.27 c	84.67 a	72.07 e	75.28 d	77.34 c	71.68 e	77.19 c	78.53 c	76.84 cd	80.91 b	82.49 b
Moisture retention capacity (%)	63.25 cd	64.86 bc	50.06 f	49.66 f	67.43 b	44.59 g	57.56 e	61.42 d	57.90 e	74.34 a	73.68 a

In a row, means followed by a common letter are not significantly different at 5% level by DMRT.

Leaf yield of the newly evolved open-pollinated mulberry varieties as affected by genotype and season

There was a significant variation in leaf yield of the newly evolved open-pollinated mulberry varieties as affected by genotypes x season (Table 4). As yield and yield contributing characters are quantitative in nature, the observed variation includes variation due to genotype and environment and their interaction. Knowledge of the available variability within the species for the component characters enables the breeder to determine superior genotypes.

evolved OPVs and different climatic factors (average temperature, rainfall and relative humidity). Results revealed that temperature made no significant effect on the different characters. This may be an indication that the OPVs are adapted to the local ecosystem. During the nine seasons of evaluation, the minimum temperature ranged from 21.45 (March 2008) - 24.31°C (July 2010). The maximum temperature ranging from 30.77- 33.60 °C was recorded in the months of March 2008 and July 2010 when faster evaporation of the ground moisture was observed leading to drought condition that was aggravated by low

Table 4: Leaf yield (g plant⁻¹) of the newly evolved open-pollinated mulberry varieties as affected by genotype and season

Season Genotype	Aug 2007	Nov 2007	March 2008	July 2008	Oct 2008	March 2009	Aug 2009	Oct 2009	July 2010	Aug 2011	Oct 2011
Alf-004	700.00 bc	600.00c-h	142.73 D-G	882.33 a	499.30 e-m	242.38 r-G	456.05 f-o	254.45 q-G	373.37 j-z	404.67 j-t	208.07 v-G
Alf-018	606.58c-g	360.33 k-z	132.77 D-G	591.73 c-i	292.20 o-F	183.95 z-G	279.08 o-G	334.08 l-C	234.57 s-G	347.73k-B	229.38 t-g
Alf-015	526.39 c-k	308.33 n-E	122.18E-G	512.75 d-l	396.78 j-v	188.65 v-G	280.15 o-G	462.68 f-u	228.65 t-G	360.50 k-z	304.26 n-F
Alf-028	450.00 f-p	421.67 h-s	213.20 G	415.98 i-t	241.57 s-G	113.95 FG	168.80 A-G	97.20 G	199.67 x-G	247.49 r-G	193.82 x-G
S61-011	442.44 g-q	391.67 j-w	128.75D-G	683.66 b-d	553.47 c-j	155.18 B-G	291.80 o-F	201.40 w-G	206.15 w-G	291.92 o-F	214.10u-G
S61-019	587.22 c-i	426.58 g-r	185.22x-G	703.20 bc	315.86 m-D	189.47 x-G	377.92 j-Y	415.82 i-t	379.05 j-x	401.75 j-u	272.15o-G
S54-019	625.00 c-f	530.00 c-k	180.23 z-G	785.74 ab	344.08 k-B	170.02 z-G	295.12 o-F	335.18 l-C	207.16 o-G	319.06 m-D	158.16A-G
Batac	448.89 f-o	360.00 k-z	152.37 C-G	643.71 c-e	268.82O-G	167.12 A-G	266.85 o-G	204.20 w-G	206.32 w-G	263.35 p-G	262.86p-G

Means followed by a common letter within a row and within a column are not significantly different at 5% level by DMRT.

Alf-004 and S54-019 produced higher yield of 882.30 and 785.74 g plant⁻¹, respectively in July 2008 that differed significantly from the rest of the varieties. S54-019 performed comparably well with S61-019 in July 2008, Alf-004 in Aug 2007 and S61-011 in July 2008 with a respective mean of 703.20, 700.00 and 683.66 g plant⁻¹. These varieties could be exploited commercially for silkworm rearing in the respective seasons.

The lowest yield among the OPVs was recorded by Alf-028 at 97.20 g plant⁻¹ harvested during October 2009 season.

Regression analysis between climatic factors and growth, yield and quality characters of the newly evolved OPVs

Table 5 shows the regression coefficients between the different growth, yield and quality characters of the newly

humidity. High volumes of water is needed during this season of evaluation (Table 6). In a study made by Choudhury and Giridhar (1987), the most suitable range of temperature recorded for good growth of mulberry was from 20-30 °C. The mean temperature should not exceed 37°C and above 40°C, plant growth suffers due to high evapo-transpiration rate. They were able to refix the range as 23.9–26.6 °C as optimum for the best growth.

On the other hand, rainfall significantly affected the plant height (5.35*) and moisture retention capacity (1.50**) of the newly evolved OPVs with coefficients of determination (r^2) of 45.2 and 60.6 %, respectively. Result implies that the stated percentages of the variation on plant height and moisture retention capacity are due to rainfall.

Rainfall was the heaviest during October 2008 with a total of 1430 mm while only 11 mm of rainfall was

recorded in March 2008, the most critical month of irrigating the plants (Table 6). Mulberry can adapt to a wide rainfall range of 635 to 2500 mm (Anonymous, 1987).

Results also revealed that plant height (11.61*), moisture content (1.23*) and moisture retention capacity (3.08*) of the OPVs were significantly affected by relative humidity. There was a coefficient of determination in plant height (44 %), moisture content

(49.9 %) and moisture retention capacity (96.6 %) indicating that the extent of variation in stated characters of the OPVs was contributed by relative humidity. The highest relative humidity was recorded during October 2011 (91.57 %) while the lowest was experienced during July 2008 at 85.09 %.

An increase in the level of RH would have a corresponding increase in the plant height, moisture content and moisture retention capacity (Table 6).

Table 5: Regression coefficients between the climatic factors and the different growth, yield and quality characters of the newly evolved OPVs

Climatic Factor Character	Rainfall (mm)		Average temperature		Relative humidity (%)	
	b	r ²	b	r ²	b	r ²
Leaf yield	7.184E-02 ns	0.056	68.71 ns	0.199	-12.09 ns	0.033
Number of branches	-1.07E-03 ns	0.028	-1.352 ns	0.170	8.857E-02ns	0.044
Length of the longest shoot	4.579E-02ns	0.245	2.329 ns	0.002	6.735 ns	0.109
Plant height	5.352E-02*	0.452	-5.213 ns	0.017	11.612 *	0.440
Moisture content	4.717E-03ns	0.357	-0.451 ns	0.013	1.226 *	0.499
Moisture retention capacity	1.497E-02 **	0.606	-2.771ns	0.080	3.082 *	0.966

Table 6: Rainfall, temperature (min. and max.) and relative humidity during the growing period of the different OPVs and Batac

Season	Total rainfall (mm)	Temperature (°C)		Relative humidity (%)
		Min.	Max	
Aug 2007	1170	23.99	32.53	86.44
Nov 2007	652	22.88	31.36	89.58
Mar 2008	11	21.45	30.77	85.58
July 2008	512	23.41	33.05	85.09
Oct 2008	1432	23.15	31.16	90.30
Mar 2009	26	22.22	31.58	86.06
Aug 2009	1240	23.69	31.87	89.29
Oct 2009	1050	23.49	31.88	89.85
July 2010	586.8	24.31	33.60	85.53
Aug 2011	951.6	24.28	33.15	91.19
Oct 2011	1392.1	22.08	31.31	91.57

Correlation between yield related characters

Leaf yield in mulberry is a complex entity and is observed to be associated with a number of component traits (Susheelamma *et al.*, 1988). The correlation analysis revealed the negative correlation between leaf yield and number of branches; positive correlation was disclosed between plant height x length of the longest shoots x moisture content and moisture retention capacity; and moisture content and moisture retention capacity (Table 7).

Leaf yield x Number of branches

Significant negative association was disclosed between leaf yield and number of branches which connotes that the increase in the number of branches resulted to a decrease in leaf yield. Further, it was found

that 53.88 % variation in yield per plant is due to the number of branches as indicated by $r = -0.734$. This is contrary to the findings of Sarkar *et al.* (1987) who reported positive correlation between branch number with leaf yield and hence, suggested to consider this trait for selection of mulberry. Earlier to this, Das and Krishnaswamy (1969) reported that plant height and branches have positive correlation with leaf yield.

Table 7: Correlation matrix of the different growth, yield and quality characters

Character	LY	NoB	LLS	PH	MC	MRC
LY	-					
NOB	-0.734*	-				
LLS	0.466	-0.128	-			
PH	0.268	-0.073	0.879**	-		
MC	0.205	-0.367	0.552	0.708*	-	
MRC	0.062	-0.136	0.527	0.736**	0.832**	-

Legend:

LY- Leaf yield

NoB- Number of branches

LLS- Length of the longest shoot

PH- Plant height

MC- Moisture content

MRC- Moisture retention capacity

Plant height x Length of the longest shoot x Moisture content and moisture retention capacity

Plant height was significantly correlated with the length of the longest shoot as indicated by the r value of 0.879. A variation of 77.26 % in plant height was due to length of the longest shoot. This connotes that an increase in plant height significantly increases length of the longest shoot. This conforms to the findings of Gapuz (1998) and Damasco (1999).

Plant height of the different OPVs were observed to be significantly correlated with moisture content (0.708*) and moisture retention capacity (0.736**). This connotes that an increase in plant height significantly increases the moisture content and moisture retention capacity of harvested leaves of the OPVs.

Moisture content and moisture retention capacity

Highly significant positive correlation was disclosed between moisture content and moisture retention capacity. This proposes that increases in moisture content of the leaves significantly increases the moisture retention capacity of mulberry leaves 6 hours after harvesting.

This conforms to reports that mulberry leaves contain

Table 8: Values and ranks on growth, yield and quality parameters of the different open-pollinated varieties and Batac

OPV	LY	Rank	NoB	Rank	LLS	Rank	PH	Rank	MC	Rank	MRC	Rank	Total
AIf-004	433.03	1	12.00	3	183.02	1	251.12	1	78.32	3	62.48	2	11
AIf-018	326.58	5	10.00	6	126.29	7	176.22	6	78.71	2	61.66	3	29
AIf-025	335.58	4	8.69	8	126.65	6	173.36	7	79.17	1	63.80	1	29
AIf-028	251.21	8	11.00	5	113.72	8	157.83	8	76.99	7	59.78	5	41
S61-011	323.68	6	13.00	2	128.11	5	186.73	5	77.04	6	59.99	4	28
S61-019	386.75	2	10.97	4	154.21	2	216.97	2	78.02	4	59.56	6	20
S54-019	364.80	3	14.00	1	137.89	4	205.97	4	77.21	5	58.74 d	7	24
Batac	294.95	7	9.00	7	149.82	3	207.51	3	75.80	8	57.43	8	36

Legend:

LY- Leaf yield

NoB- Number of branches

LLS- Length of the longest shoot

PH- Plant height

MC- Moisture content

MRC- Moisture retention capacity

64-83 % water by weight (JOCV, 1975). The correlation was detected between moisture content and moisture retention capacity with r value of 0.832.

The above relationship could be explained by the fact that water comprises the major weight of the leaves as has been stated earlier and that it contributes to the weight of the total harvestable leaves. Moreover, Dandin and Kumar (1989) stated that the moisture content of leaves is a determinant of its nutritive quality and hence plays an important role in cocoon yield and quality.

Identification and selection of promising OPVs

In the criteria for selection, the agronomic characters were given a total weight of 55 % and the remaining 45 % was allotted for the biochemical characters and the resulting cocoon yield and quality. Leaf yield and yield related parameters were given a weight of 30 %, and 15 % for leaf quality. The varieties under study were ranked based on the values of traits computed. The top three varieties were identified as promising.

Considering the over-all performance on growth, yield and quality, OPVs viz., Alf-004, S61-019, and S54-019 were identified as top three, high performing varieties (Table 8).

REFERENCES

- Almojuela M. N. and Damasco C. N. (2001) *Mulberry variety for quality leaf yield production*. DMMMSU-SRDI, pp. 2-3.
- Anonymous (1987) Mulberry cultivation. In: *Manual on sericulture, Agricultural Services Bulletin*, No. 15/1, Bangalore, India, p. 42.
- Bose P. C. and Bindroo B. B. (2001) A comparative biochemical study of seven promising (*Morus alba* L.) varieties under rainfed condition of sub-tropical region. *Indian J. Seric.*, **40**(2): 171-173.
- Chaudhary P. C. and Giridhar K. (1987) Mulberry cultivation. In: *Appropriate Sericulture Techniques*, Jolly M. S. (Ed.), ICTRETS, Mysore, India, p. 18.
- Dacayanan M. N., Damasco C. N. and Supsup R. D. (2013) *Growth and yield of newly evolved 15 mulberry hybrids under La Union conditions*. DMMMSU-SRDI, p. 9.
- Damasco C. N. (1999) Preliminary evaluation of open-pollinated mulberry lines. *Unpublished Thesis*, Don Mariano Marcos Memorial State University, NLUC, p. 60.
- Damasco C. N. (2012) Growth, yield and quality of newly evolved open-pollinated mulberry varieties. *Terminal report*, DMMMSU-SRDI, p. 20.
- Datta R. K. (2000) Mulberry cultivation and utilization in India. Retrieved from <http://www.fao.org> on July 11.
- Gapuz C. F. (1998) Preliminary evaluation of open-pollinated mulberry hybrids and identification of mulberry ideotypes. *Unpublished Thesis*, Don Mariano Marcos Memorial State University, NLUC, p. 53.
- Kumar R. and Dandin S. B. (1989) Evaluation of mulberry genotypes for different growth and yield parameters. In: *Genetic Resources of Mulberry and Utilization*, Central Sericultural Research and Training Institute, Mysore, India, p. 146.
- Parpiev B. A. (1968) Water metabolism in silkworm with a different mulberry strain changing diets. *Shelk*, **39**: 15-17.
- Paul D. C. (1992) Impact of dietary moisture on nutritional indices of the growth of *Bombyx mori* and concomitant larval durations. *J. Insect Physiol.*, **38**(3): 229.
- Sengupta K. and Dandin S. B. (1989) *Genetic resources of mulberry and utilization*. First edition, Central Sericultural Research and Training Institute, Mysore, India.
- Susheelamma B. N., Jolly M. S. and Sengupta K. (1989) Evaluation of germplasm for stress resistance genetic resources of mulberry and utilization. Central Sericultural Research and Training Institute, Mysore, India, p. 143.
- Tikader A. and Kamble C. K. (2009) Performance of exotic mulberry (*Morus* spp.) germplasm on growth and yield traits in Indian conditions. *African J. Plant Sci.*, **3**(2): 30-36.
- Villamor C. C. (2008) Morphological Characterization and Nutritional Bioassay of Different mulberry leaves (*Morus alba* L.) with Silkworm (*Bombyx mori* L.). *Unpublished Dissertation*, Central Luzon State University, pp. 4-5, 6-8.

Article scientifique

**EVALUATION DE NOUVELLES VARIETES DE MURIERS OBTENUES PAR
POLLINISATION OUVERTE**

**N. Damasco Crestilyn, A. Dacayanan Merlita, J. Tabafunda Minerva,
A. Ancheta Lilia and D. Supsup Roel**

Don Mariano Marcos Memorial State University-Sericulture Research and
Development Institute (DMMMSU-SRDI), Bacnotan, La Union, Philippines.

E-mail:crestilyndamasco@yahoo.com

R E S U M E

L'évaluation de la performance et la sélection de variétés obtenues par pollinisation libre (OPVs) ont été effectuées sur la base de la croissance, du rendement en feuilles et des paramètres de qualité pendant cinq années représentant onze saisons. Alf-004 est la meilleure en termes de rendement foliaire, longueur de tiges et hauteur de plant alors que pour la qualité des feuilles avec la plus forte teneur en eau et de capacité de rétention d'eau, c'est Alf-025. Des variations saisonnières significatives ont été observées pour la croissance, le rendement en feuilles et la qualité. Le plus fort rendement en feuilles de 625.39g/plant a été observé en Juillet 2008. Toutefois la qualité des feuilles a été la meilleure en Novembre 2007 en terme de contenu en eau et en Août et Octobre 2011 en ce qui concerne la capacité de rétention de l'humidité. L'analyse de régression montre que la température n'affecte pas la croissance, le rendement et les caractères qualitatifs alors que la pluie et l'humidité relative influencent la hauteur du plant, la quantité d'eau et la capacité de rétention chez les OPVs. Une corrélation négative significative a été établie entre le rendement en feuilles et le nombre de branches. On observe des corrélations positives fortement significatives entre la hauteur du plant et la longueur des tiges, la hauteur du plant avec le contenu en eau ainsi que la capacité de rétention. Considérant l'ensemble des données, les trois OPVs les plus prometteurs sont Alf-004, S61-19 et S54-019.

Mots-clés: Croissance, qualité de la feuille, rendement en feuilles, variétés de mûriers en pollinisation ouverte.



QUANTIFICATION OF VITAMIN C IN MULBERRY LEAVES

Aabid Khaliq Tantray¹ and Kanika Trivedy

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

¹Department of Sericulture, Govt. Boys Degree College, Sopore-19320, Baramulla, Jammu & Kashmir, India.

E-mail: aabsimda@gmail.com

ABSTRACT

Vitamin C was quantified in the leaves of mulberry at three maturity stages viz., tender, medium and coarse from six different varieties viz., V1, S34, S36, S13, G4 and K2 following dinitrophenyl hydrazine (DNPH) method. The vitamin content varied according to the age of leaves. Tender leaves recorded the highest vitamin C content in terms of dry weight followed by medium and coarse leaves. However, opposite trend in vitamin C content was observed in terms of fresh weight of leaves, being the lowest in tender followed by medium and coarse leaves. Leaves of G4 variety recorded the highest content of vitamin C (5.694 mg/g dry wt. & 1.707 mg/g fresh wt.) followed by V1 (5.224 mg/g dry wt. & 1.603 mg/g fresh wt). S36 variety stood third in vitamin C quantum by dry and fresh weight basis, respectively as 4.838 and 1.565 mg/g followed by S34 (4.589 & 1.555 mg/g), S13 (4.568 & 1.473 mg/g) and K2 (4.517 & 1.349 mg/g). The present study envisages the correlation of the vitamin C content present in mulberry leaves with the quantification method followed and also attempts to distinguish the most reliable technique to quantitate the exact vitamin C content present in the mulberry leaves fed to silkworms.

Key words: *Bombyx mori* L., quantification, vitamin C.

INTRODUCTION

Vitamin C ($C_6H_8O_6$), a carbohydrate derivative closely related to glucose, is one of the first substances synthesized in the growing seed to nourish the plant embryo (Wheeler *et al.*, 1998). It plays an essential role in several biological processes including growth, differentiation and metabolism (Foyer *et al.*, 1993) of plants and can accumulate in millimolar concentrations in both photosynthetic and non-photosynthetic tissues. Leaves often contain more vitamin C (VC) than chlorophyll, with the VC pool representing over 10 % of the soluble carbohydrate (Noctor and Foyer, 1998). Except possibly for a few microorganisms, those species of animals including silkworm that cannot make their own VC (Ito and Arai 1965) require it in their food if they are to survive. The silkworm, *Bombyx mori* L. is a typical monophagous lepidopteran deriving its entire essential nutrients, the VC being one amongst them, from the leaves of mulberry for its normal growth and development. VC has been quantified in the mulberry leaves by a host of workers (Ali *et al.*, 1992; Babu *et al.*, 1992; Sakre *et al.*, 1992) using some titrimetric methods. It is in this direction, the present attempt has been made to quantitate the VC present in some popular cultivars of mulberry by a comparatively more reliable process.

MATERIALS AND METHODS

Extraction

Mulberry leaves of all the three maturity stages viz., tender, medium and coarse from the six varieties viz., V1, S34, S36, S13, G4 and K2, were dried in the hot air oven at below 40 °C and ground to fine powder. Moisture % of all the samples was calculated by the following formula.

$$\text{Moisture \%} = \frac{\text{Fresh weight-dry weight}}{\text{Fresh weight}} \times 100$$

The non-polar fractions from the leaf powder were removed by thorough agitation with chloroform (1:3 w/v) followed by centrifugation at 8000 rpm. The chloroform was made to evaporate from the pellet, to which ether was added (1:3 w/v), mixed thoroughly and again centrifuged. The ether was evaporated from the pellet and the de-etherized pellet was used as sample for extraction of VC. Further extraction of the sample was carried out by following m-Phosphoric acid (MPA): acetic acid (AA) extraction method of AOAC (Anonymous, 1995).

Quantification

All samples of the mulberry leaf extracts were first filtered through Whatman No. 41 filter paper to obtain particle free extract and then quantified for VC by the following Dinitrophenyl hydrazine (DNPH) method (Raghuramulu *et al.*, 2003).

Experimental procedure

Standard VC solution was prepared by dissolving 40mg of VC in 250 ml of 5% TCA and designated as stock solution. 10 ml of this solution was pipetted out into 100ml of standard flask and the volume was made up to the mark by adding 5 % TCA solution. This solution was kept as working standard solution. A series of 0-1 ml of working standard solution was pipetted out into 6 clean and dry test tubes. The solution in each test tube was made up to 1 ml by adding 5 % TCA. 1 ml of 2, 4-dinitrophenyl hydrazine (DNPH) reagent was added to each test tube and later were boiled for 8-10 minutes in water bath. The test tubes were cooled and 8 ml of 65 % H_2SO_4 was added. The solution in each of the test tubes was mixed well and optical density (OD) was read at 540 nm using Digital Spectrophotometer Model No. 301, Growel Instruments, Bangalore, India.

A known quantity from each of the leaf extracts was taken into clean and dry test tubes in three replications in a series of dilutions. The solution in each test tube was made up to 1 ml with 5 % TCA. 1 ml of DNPH reagent was added to each test tube and later boiled for 8-10 minutes in water bath. The test tubes were cooled and 8 ml of 65 % H_2SO_4 was added. The solution in each of the test tubes was mixed well and optical density (OD) was read at 540 nm.

Estimation

Amount of VC needed to be read by a unit optical density (OD) was calculated and kept as standard (constant) to measure the amount of VC in an unknown sample. The calculation was directly made using the

values of standard VC constant.

Amount of VC in an unknown sample was calculated as follows.

Amount of VC in an unknown sample = OD of sample x (amount of standard VC/ OD)

RESULTS AND DISCUSSION

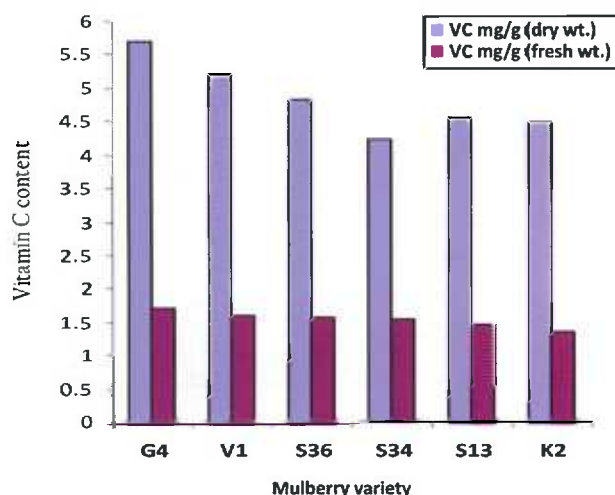
The quantum of VC (mg/g dry wt.) was found the highest in tender followed by medium and coarse leaves in all the mulberry varieties in general. Among the varieties, G4 recorded the significantly highest VC (mg/g dry wt.) of tender (5.766 mg), medium (5.692 mg) and coarse (5.622mg) leaves followed by V1 variety (5.600, 5.183 and 4.885 mg, respectively). S36 variety stood third in VC (mg/g dry weight) recovery as 5.530 mg/g in tender, 4.991 mg/g in medium and 3.990 mg/g in coarse leaves. S34 (5.720, 4.706 & 3.339 mg/g), S13 (5.481, 4.230 & 3.991 mg/g) and K2 (5.106, 4.251 & 4.191 mg/g) recorded comparatively lower values. However, vitamin C content per gram fresh weight was recorded in increasing order from tender to coarse in all the mulberry varieties. In G4 variety, significantly highest VC was recorded as 1.659, 1.691 & 1.770 mg/g, followed by V1 (1.512, 1.600 & 1.696 mg/g), S36 (1.497, 1.556 & 1.643 mg/g), S34 (1.487, 1.546 & 1.663 mg/g), S13 (1.425, 1.457 & 1.536 mg/g) and K2 (1.302, 1.332 & 1.414 mg/g), respectively (Table 1 and Figure 1).

In this study, VC in different mulberry leaves displayed a decreasing trend in terms of dry weight from tender to coarse whereas, quantum of VC by fresh weight had an opposite (increasing) trend from tender to coarse due to decrease in moisture % from 73-67 % (Table 1). Ali *et al.* (1992) determined 2.565 mg VC per gram fresh weight of mulberry leaf by 2, 6- dichlorophenolindophenol (DCIP) titration method. Sarkre *et al.* (1992) estimated the amount of VC present in tender and mature leaves of three mulberry varieties, S1, S799 and S76 by DCIP and reported that tender and the mature leaves contained 67.70 to 106.40 mg and 71.18 to 114.00 mg/100 g fresh leaf, respectively. Vitamin C content of mulberry leaves increased with the increase in maturity of leaves (Talyshinskii and Gasannov, 1965; Anonymous, 1975).

Table 1: Quantification of vitamin C in fresh and dry mulberry leaves

Mulberry variety	Leaf quality	VC mg/g (Dry wt.)	Moisture (%)	VC mg/g (Fresh wt.)
G4	T	5.766	71.97	1.659
	M	5.692	70.00	1.691
	C	5.622	67.03	1.770
V1	T	5.600	73.00	1.512
	M	5.183	70.00	1.600
	C	4.885	68.00	1.696
S36	T	5.530	73.00	1.497
	M	4.991	72.10	1.556
	C	3.990	70.00	1.643
S34	T	4.719	71.10	1.487
	M	4.706	69.00	1.546
	C	3.339	68.00	1.633
S13	T	5.481	71.10	1.425
	M	4.230	69.00	1.457
	C	3.991	67.00	1.536
K2	T	5.106	73.10	1.302
	M	4.251	71.10	1.332
	C	4.191	69.00	1.414
	SE $\pm$	0.104	1.924	0.020
	CD 5 %	0.147	2.737	0.028

T: Tender, M: Medium, C: Coarse

**Figure 2: Vitamin C content in different mulberry varieties**

Babu *et al.* (1992) measured the VC in M₁ mulberry leaves and found that the vitamin content varied according to age of leaves. The ascorbic acid content was 1.357, 1.907 and 1.706 g/100 g dry weight of tender, medium and coarse leaves, respectively. The values of VC quantification by these workers are at contradiction with the average values obtained in the present study. The variation is surmised to be due to the erratic method of VC estimation. Among the titrimetric methods, iodine and

DCIP are applied commonly. However, these methods do not allow the determination of dehydroascorbic acid (DHA), which is characterised by the same vitamin activity as the ascorbic acid (Moszczynski and Pyc, 1999). In addition, titration methods are not sufficiently selective as reduction substances in food products interfere with the process of VC determination and distort the obtained results (Azar and Ojani, 1997; Moszczynski and Pyc, 1999; Arya *et al.*, 2002). On the other hand, spectrophotometric method (DNPH), the one used in the present study, provides more selective possibilities of vitamin C determination of the sum of ascorbic acid and dehydroascorbic acid. This method allows a simultaneous determination of both ascorbic acid and dehydroascorbic acid and is therefore more reliable compared to DCIP and iodine methods. The present results agree with Chakrabarathi and Singhal (1996) who measured the VC content of ten promising genotypes of mulberry (*Morus* sp.) and concluded that S13 variety has the maximum quantum of VC (3.321 mg/g). Same variety was found to contain an average of 4.568 mg/g of VC in the present experiment. Higher values could be once again attributed to the type of quantification method used. Singhal and Roopa (1998) could estimate 3.089 mg/g of VC in S13 mulberry variety which was found to be drastically reduced under water stress of only six days. In leaves of a number of plant species, there is a strong relationship between light and VC concentration. Acclimation to high light increases the VC pool whereas transfer to low light or darkness causes its decrease (Smirnoff *et al.*, 2001).

REFERENCES

- Ali R., Alam S., Qaiyyum M. A., Alam N. and Alam M. S. (1992) Estimation of vitamin C (ascorbic acid) content in mulberry leaf during pathogenesis with *Cercospora moricola*, Cooke. *Bull. Seric. Res.*, **3**: 79.
- Anonymous (1975) *Text book of Tropical Sericulture*, Japan Overseas Cooperation Volunteers, Tokyo, Japan, p. 391.
- Anonymous (1995) *Official Methods of Analysis*, AOAC 16th Ed., Ascorbic acid in vitamin preparations and juices. Arlington, Virginia, Method 967.21.
- Arya S., Mahajan P. M. and Jain P. (2002) Non-spectrophotometric methods for the determination of Vitamin C. *Analytica Chimica Acta*, **417**(1): 1-14.

- Azar M. H. P. and Ojani R. (1997) A selective catalytic voltammetric determination of vitamin C in pharmaceutical preparations and complex matrices of fresh fruits juices. *Talanta*, **44**: 297-303.
- Babu M., Swamy M. T., Rao P. K. and Rao M. S. (1992) Effect of ascorbic acid- enriched mulberry leaves on rearing of *Bombyx mori* L. *Indian J. Seric.*, **31**: 111-114.
- Chakrabarthi S. and Singhal B. K. (1996) Seasonal variation in ascorbic acid of promising mulberry genotypes. *Indian J. Plant Physiol.*, **1**(4): 298-299.
- Foyer C., Rowell J. and Walker D. (1993) Measurement of the ascorbate content of spinach leaf protoplasts and chloroplasts during illumination. *Planta*, **157**: 239-244.
- Ito T. and Arai N. (1965) Nutrition of silkworm *Bombyx mori* L. IX. Further studies on the nutritive effects of ascorbic acid. *Bull. Seric. Expt. Stn. Jpn.*, **20**: 1-19.
- Moszczynski P. and Pyc R. (1999)* *Biochemia witamin*. Witaminy lipofilne I kwas askorbionowy. Czesc II. PWN Warszawa.
- Noctor G. and Foyer C. H. (1998) Ascorbate and glutathione: keeping active oxygen under control. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **49**: 249-279.
- Raghuramulu N., Nair K. M. and Kalyanasundaram S. (2003) Vitamin C analysis, In: *A Manual of Laboratory Techniques*, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, pp. 157-162.
- Sarkre A. A., Yasmin T., Quade M. A. and Absar N. (1992) Studies on vitamin and amino acid constituents of mulberry leaves. *Bull. Seric. Res.*, **2**: 103-106.
- Singhal B. K. and Roopa K. (1998) Changes in physiological and biochemical parameters of promising mulberry (*Morus indica* L.) under water stress condition. *Philippine J. Sci.*, **127**(4): 277-285.
- Smirnoff N., Conklin P. L. and Loewus F. A. (2001) Biosynthesis of ascorbic acid in plants: a renaissance. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **52**: 437-467.
- Talyshinskii G. M. and Gasannov A. S. (1965)* The content of carotene, rutin and vitamin C of fruit and leaves of mulberry trees. *Dokl. Akad. Nauk. Azer. S.S.R.*, **10**: 69-73.
- Wheeler G. L., Jones M. A. and Smirnoff N. (1998) The biosynthetic pathway of vitamin C in higher plants. *Nature*, **393**: 365-369.

* Original not seen

Article scientifique

QUANTIFICATION DE LA VITAMINE C DES LES FEUILLES DE MÛRIER

Aabid Khaliq Tantray¹ and Kanika Trivedy

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

¹Department of Sericulture, Govt. Boys Degree College, Sopore-19320, Baramulla, Jammu & Kashmir, India.

E-mail: aabsimda@gmail.com

R E S U M E

Nous avons mesuré la quantité de vitamine C dans des feuilles de mûriers à trois étapes de maturité différentes, c'est-à-dire tendre, moyenne et dure chez six variétés de mûrier, V1, S34, S36, S13, G4 et K2 en utilisant la méthode à la dinitrophényl-hydrazine (DNPH). Le contenu en vitamine C varie selon l'âge des feuilles. Le plus fort taux de vitamine par unité de poids sec est enregistré chez les feuilles tendres, il est inférieur chez les feuilles moyennes et le plus faible chez les feuilles dures. La tendance est inversée lorsque le taux est exprimé en fonction du poids frais. Les feuilles de la variété G4 présentent le plus fort taux de vitamine (5.694 mg/g de poids sec et 1.707 mg/g de poids frais) et celles de V1 (5.224 mg/g de poids sec et 1.603 mg/g de poids frais). La variété S36 arrive en troisième position avec 4.838 et 1.565 mg/g suivie par S34 (4.589 et 1.555 mg/g), S13 (4.568 et 1.473 mg/g) et K2 (4.517 et 1.349 mg/g). Cette étude analyse la corrélation entre le taux de vitamine C dans les feuilles de mûrier et la méthode utilisée pour le mesurer. Elle tente également de définir la meilleure technique pour connaître le taux exact de vitamine présent dans les feuilles fournies aux vers à soie.

Mots-clés: *Bombyx mori* L., quantification, vitamine C.



IMPACT OF MULBERRY RED RUST CAUSED BY *AECIDIUM MORI* LINN. ON THE YIELD AND QUALITY OF MULBERRY (*MORUS ALBA* LINN.)

Angelina T. Gonzales¹ and Oscar S. Opina²

¹ Sericulture Research and Development Institute, Don Mariano Marcos Memorial State University, Bacnotan, La Union, Philippines

² University of the Philippines, Los Banos, Laguna, Philippines
E-mail: angelinatadurangonzales@yahoo.com

ABSTRACT

The impact of mulberry red rust (MRR) infestation on the yield and quality of mulberry leaves (cv. Batac), and its management intervention were assessed. Yield loss assessment showed that an average severity of 17.15 % can cause 7.4 % reduction in yield from 9.305 to 10.05 t/ha in mulberry cv. Batac. Tissue analyses of MRR-infected leaves revealed significant reduction in moisture content, total protein and vitamins. Moisture and protein content were found the highest in leaves occupying the top position of the branches. Sugar, starch, carotenoids, b-carotene and ascorbic acid did not vary significantly regardless of leaf position.

Key words: *Aecidium mori*, quality loss, mulberry, red rust, yield loss.

INTRODUCTION

In the Philippines, mulberry red rust (MRR) caused by the fungus, *Aecidium mori* Linn. is becoming very alarming due to its adverse effect on the mulberry leaf biomass. It was once reported as the most destructive disease of mulberry (Biswas, 1992 ; Teotia and Sen, 1994). It causes a significant decrease in the amount of moisture, crude protein, reducing sugars and total sugars of the infected leaves (Sundareswaran *et al.*, 1988). While Biswas and Sengupta (1994) claimed that the disease is also known to cause 10-30 % leaf yield loss and also decreases the nutritive value of leaves, hastens yellowing, senescence, and defoliation; Philip *et al.* (1994) attributed the poor quality and quantity of mulberry leaves as the perennial problem hindering successful silkworm rearing and cocoon production.

Since no related study has been conducted so far to investigate the intensity and impact of mulberry rust infestation in Philippines, the present work was planned with an aim to assess the effect of MRR on the leaf biomass production and leaf quality of mulberry cv. Batac.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted in farmers' fields in Tinaan, Ilocos Sur Province and Bacnotan, La union during the period of high MRR incidence. Each farm (500 m²), serving as block was planted with mulberry cv. Batac and was divided into two equal parts. One half of the farm was sprayed with Thiobicarbamate fungicide immediately after sprouting to keep leaves free from MRR. The other half was exposed to rust infestation by growing them without any chemical spray. Recommended cultural practices were employed on both farms to avoid unwanted experimental error.

Disease Assessment

The disease intensity was assessed from 20 randomly selected and previously tagged sample plants per block. Observation from the non-sprayed farm were recorded at maximum leaf age (ready for rearing) of 55 days after pruning (DAP). The intensity of infection was graded as per the following rating scale.

Scale	Description
0	No Infection
1	less than 1% of the surface area covered with pinhead eruptive lesion
2	>1- 5 %
3	>5-10 %
4	>10-25 %
5	>25-50 %
6	>50-75 %
7	>75 %

Data on disease severity and yield were gathered. The severity of the disease in the non-sprayed plants was determined 55 days after pruning (at maximum leaf age, ready for silkworm rearing) using the rating scale (Figure 1). The yield was estimated based on the average weight of mulberry leaves harvested 55 DAP from 20 plants each of two treatments. The yield loss was determined using the following formula: $\text{Yield Loss} = \{(Y1-Y2)/Y1\} \times 100$, where: Y1 is the yield in healthy plants; Y2 is the yield in MRR infected plants.

The data were analyzed using the Analysis of Variance (ANOVA); and the means were separated using the T-test at 5 % level of significance.

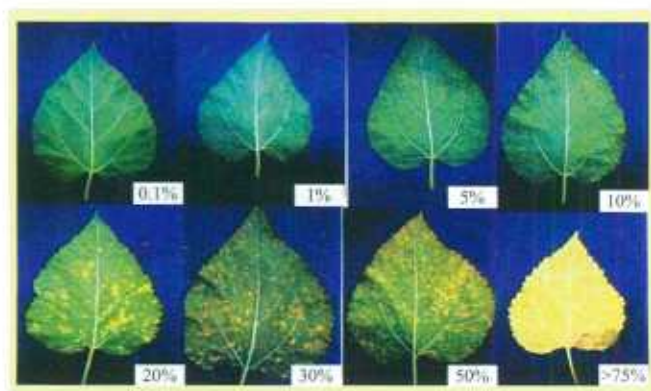


Figure 1: Leaves showing different percentages of mulberry red rust (MRR) infection based on the surface covered with pinhead eruptive lesions.

Leaf quality loss assessment

Mulberry leaf sampling and tissue analysis: Rust infected leaves from five randomly selected mulberry plants (in non-sprayed plot) per block were harvested (55 DAP). and grouped according to their maturity (young, medium and mature leaves) or relative leaf position (top, middle and bottom portion of the branch). The same groups of leaves were sampled in the other treatment (healthy leaves) and the harvested leaves were separately air-dried, pounded and analysed in the Chemistry Laboratory of the Institute of Plant Breeding (IBP).

Data on moisture content, protein, sugars and starch, vitamins viz., carotenoids, b-carotene and ascorbic acid were determined from the top, middle and bottom positioned mulberry leaves of healthy and infected categories. The data on different biochemical components of the mulberry leaves were subjected to ANOVA and the means were separated using the T-test and DMRT at 5 % level of significance.

RESULTS AND DISCUSSION

Yield loss assessment: Assessment of yield loss of mulberry cv. Batac due to MRR indicated that the amount of harvestable mulberry leaves was determined by the severity of the mulberry rust infection (Table 1). Leaf yield was consistently higher in rust-free plots compared to rust-infected plots. An average disease severity of 15.62 % resulted in 7.35 % reduction of harvestable leaf biomass.

The yield obtained from the rust-infected field in a sericulture model farm in Sapilang, Bacnotan, La union with 16.92 % disease severity, was 9.33 t.ha⁻¹. This was significantly lower compared with the yield of rust-free field in the same area equivalent to 10.33 t.ha⁻¹. In another location, i.e., in a sericulture farm in Barangay Tinaan, Sta. Maria, Ilocos Sur, a similar result was obtained under the same experimental set-up. Results confirmed that when mulberry cv. Batac was infected with MRR with an average disease severity of 14.33 %, a 5.01 % leaf biomass was reduced compared with the disease free situation (Table 1).

Table 1: Impact of MRR infection on yield of mulberry in Sta. Maria, Ilocos Sur and Bacnotan, La Union

Farm/Location	Treatment	Disease severity (%)	Yield ¹ (t/ha ¹)	Yield loss (%)
Ilocos Sur	Rust-Infected	14.33	9.28 b	5.01 b
	Rust-Free	-	9.77 a	-
La Union	Rust-Infected	16.92	9.33 b	9.68 a
	Rust-Free	-	10.33 a	-
Mean	Rust-Infected	15.62	9.305 a	7.41
	Rust-Free	-	10.05 b	-

¹ Means in a column in each location with the same letter are not significantly different at 5 % level, T-test

The present results on the yield-loss of mulberry cv. Batac due to MRR corroborate with those of Siddaramaiah *et al.* (1978) and Teotia and Sen (1994) who reported 5-10 % of reduction in yield. But Sikdar and Krishnaswami (1980) claimed a comparatively higher range of 17.6–22.2 % reduction in leaf yield in Kanva-2 cultivar in India.

Many diseases have been reported on mulberry causing 10-15 % leaf yield loss besides reducing its quality. Leaf spot causes 10-15 % yield loss during July-December. Powdery mildew causes 40 % leaf yield loss during November-December. Leaf rust is one of the major diseases of mulberry causing 10-30 % leaf yield loss during severe condition from November to January (Gunasekhar and Govindiah, 1993). Philip *et al.* (1994) cited that 10-15 % yield loss is observed in mulberry plants attacked by *Fusarium*. Rust was one of the major foliar diseases reported to cause great yield losses in a number of economic crops such as beans, wheat and other cereals (Agrios, 1997).

The reduction in yield due to MRR in this experiment could be explained by the fact that rust fungi invade host cells and actively transport nutrients from the host to the haustorial plasma membrane (Farrel, 1971).

Quality loss assessment: Results of chemical analyses of MRR-infected and rust-free mulberry leaves are summarized in Tables 2 and 3. Healthy mulberry leaves contained significantly higher amount of moisture, total protein, carotenoids and β -carotene compared with red rust-infected mulberry. Rust-free and MRR-infected mulberry leaves appeared to have the same amount of

total starch and ascorbic acid. Table 3 showed that the relative position of the mulberry leaves, *i.e.*, the top, middle and bottom portions of mulberry branch, did not significantly influence the amount or concentration of the identified chemical components. The percentage of moisture content was higher in the leaves taken from top portion compared with those of the middle and bottom levels. Protein content of the leaves from the top and middle portions did not significantly vary but the protein content of the leaves from top was significantly higher compared with the leaves from the bottom portion. It was also evident that the concentrations of sugars, starch, carotenoids, α -carotene and ascorbic acid from the leaves of mulberry did not vary with leaf position. In advanced condition, the disease has been found to reduce crude leaf protein content by as much as 33 %, although the reduction in protein varies according to degree of tolerance or susceptibility of mulberry cultivars.

Moisture, protein and ascorbic acid levels were found decreasing as the leaves mature while the total sugars, starch, carotenoids and β -carotene contents were observed the highest in the leaves from the middle portion of the mulberry branches. Nutrient composition in mulberry leaves differed according to age which corroborate to UN-ESCAP (Anonymous, 1990) report indicating that young/tender leaves have higher moisture content, protein and lower carbohydrates while mature leaves are rich in vitamin C.

The lower chemical components and moisture content as recorded from diseased mulberry leaves in this study is supported by the findings of Mendgen (1981) that nutrient was used by biotrophic pathogens *viz.*, rust fungi from susceptible host plants. Rust fungi derive nutrients from living host cells and therefore, stored nutrients are consumed by the fungus and become less available in rust-infected leaves. The rust pustules accumulate many metabolites. The fungus derives nutrients by alteration of the direction of the normal phloem transport. Since sporulation ruptures the host epidermis, increased water loss through the pustules makes more nutrients available for the fungus by increased transpiration. Sundareswaran *et al.* (1988) observed that rust infected leaves had reduced moisture, crude protein, and total sugars, which are

important bioconstituents for growth and development of silkworm. The increase in sugars and starch of MRR infected leaves in the middle part of the branch (Table 2) was believed to be due to massive increase of respiratory activity and accumulation of cytokinins in the infected area which indicate that the normal translocation of photosynthate metabolites in the host cells undergo

analyses were parts of fungal components.

The reduction in yield due to MRR in this experiment could be explained by the fact that rust fungi invade host cells and actively transport nutrients from the host to the haustorial plasma membrane (Farrel, 1971). Rust

Table 2: Biochemical components of a healthy and mulberry red rust infected leaves of top, middle and bottom portion of mulberry branch on 55 days after pruning

Leaf position	Leaf quality	Moisture content (%)	Protein (%)	Total sugars (%)	Starch (%)	Carotenoids (mg/100 g)	β -carotene (mg/100 g)	Ascorbic acid (mg/100 g)
Top	Healthy	81.19 a	24.92 a	4.07 a	13.38 a	5.73 a	3.96 a	0.25 a
	Diseased	73.5 b	21.28 b	4.53 a	12.68 b	5.35 b	3.69 b	0.22 b
Middle	Healthy	75.5 a	23.30 a	4.53 a	13.44 b	6.11 a	4.21 a	0.25 a
	Diseased	68.8 b	20.39 b	4.60 a	16.02 a	5.45 b	3.76 b	0.23 b
Bottom	Healthy	71.20 a	20.40 a	4.53 a	15.84 a	0.12 a	4.22 a	0.24 a
	Diseased	68.50 b	18.29 b	4.33 a	13.32 b	5.38 b	3.71 b	0.26 a
Average	Healthy	75.96 a	22.87 a	4.37 a	14.22 a	5.98 a	4.13 a	0.25 a
	Diseased	70.27 b	19.98 b	4.48 a	14.00 a	5.39 b	3.72 b	0.24 a

Each figure represents an average of three replications; means of different leaf position in a column with the same letter are not significantly different at 5% level, T-test; Top – from the largest light green and glossy leaf to 3rd leaf downwards (this could be identified in a fully grown shoot, by scooping the top leaves and making the largest leaf by its tip); Middle – from 4th to 6th leaf downwards, on the branch with brownish buds and dark green stem; Bottom – leaves in lower portion of the plant with dark green and brownish branches.

Table 3: Biochemical components of healthy and mulberry red rust infected leaves on 55 days after pruning

Component	Rust infected	Rust free
Moisture content (%)	70.27 b	75.96 a
Protein (%)	19.98 b	22.87 a
Total sugars (%)	4.48 a	4.37 a
Starch (%)	14.22 a	14.00 a
Carotenoids (%)	5.39 b	5.98 a
β -Carotene (mg/100 g)	3.72 b	4.13 a
Ascorbic Acid (mg/100 g)	0.24 a	0.25 a

Each figure represents an average of three replications. Means in a row with the same letter are not significantly different at 5% level, T-test.

dramatic alteration from host cells to rust fungus (Dekhuizen, 1976). As such, sugars (sucrose) accumulate in the rust lesion area during the synthesis of fungal tissue (Lewis, 1978) and the detected sugars and starch during

infection (*Uromyces phaseoli*) induced abnormal phloem transport of nutrients (mobilization of nutrients) to the infected tissue instead of uninfected leaves. These infected tissues become sinks for photo assimilates and centers of intense metabolic activity and considered as “field of dominance”. Carbohydrates and other metabolites accumulate strongly at the sites of infection.

CONCLUSION

The present study dealt with the assessment of the effect of MRR on the leaf biomass production and quality of mulberry leaves.

Yield loss assessment indicated that MRR can reduce the yield of mulberry cv. Batac from 10.05 to 9.305 t.ha⁻¹ i.e., 7.4 %. An average disease severity of 17.15 % can be translated into 7.35 % reduction on harvestable leaves. Tissue analyses of MRR-infected leaves revealed that MRR can significantly reduce moisture content, total protein and vitamins viz., carotenoids and β -carotene.

On the basis of above results, it is concluded that MRR can pose an alarming threat to the Philippine silk industry by seriously reducing mulberry production. The following measures are suggested to be implemented as a package to curtail the disease incidence: (a) elaborate assessment of yield loss of mulberry due to MRR to distinguish moderately resistant and resistant cultivars by

establishing damage function; (b) determination of the yield-loss in relation to the disease tolerance level of mulberry and (c) development and evaluation of integrated disease management of MRR using host resistance and cultural practices such as sanitary pruning and branch thinning as major interventions.

REFERENCES

- Agrios G. N. (1997) *Plant Pathology*, 4th Edn., Academic Press, Inc., New York, p. 635.
- Anonymous (1990) *Handbook on pest and disease control of mulberry and silkworm*, UN-ESCAP, pp. 5-7.
- Biswas S. (1992) Fungicides for Mulberry disease control – an appraisal. *Indian Silk*, (8): 26-29.
- Biswas S. and Sengupta K. (1994) Genetic control of disease resistance in mulberry. *Indian J. Seric.*, 28(1): 36-46.
- Dekhuizen H. M. (1976) Endogenous cytokinins in healthy and disease plants. In: *Physiological Plant Pathology*, R. Heitefuss and P. H. Williams (Eds.), Springer-Verlag, New York, pp. 526-559.
- Farrel G. M. (1971) Localization of photosynthetic products in potato leaves infected by *Phytophthora infestans*. *Physiol. Plant Pathol.*, 1: 457-467.
- Gunasekhar V. and Govindaiah D. D. (1993) Monitoring of mulberry diseases. *Indian Silk*, February, pp. 50-52.
- Lewis (1978) Influence of infection count, host vigour and culture filtrates on canker production by *Botryodiplodia theobromae* conidia in Sycamore. *Pl. Dis. Repr.*, 62: 934-937.
- Mendgen K. (1981) Nutrient uptake in rust fungi. *The Amer. Phytopathol. Soc.*, 71: 9.
- Philip T., Govindaiah D. D., Bajpai A. K. and Datta R. K. (1994) Chemical control of mulberry diseases in India – a review. *Indian J. Seric.*, 33: 1-2.
- Siddaramaiah A. K., Krishnaprasad K. S. and Hegde R. H. (1978) Epidemiological studies of mulberry leaf spot caused by *Cercospora moricola*. *Indian J. Seric.*, 17: 44-47.
- Sikdar A. K. and Krishnaswami (1980) Assessment of leaf yield loss of the two mulberry varieties due to leaf spot disease. *J. Seric.*, X(9): 9.
- Sundareswaran P., Govindaiah D. D., Srinivasan E. B. and Jolly M. S. (1988) Genetic control of disease resistance in mulberry.
- Teotia R. S. and Sen S. K. (1994) Mulberry diseases in India and their control. *Sericologia*, 34(1): 1-18.

Article scientifique

IMPACT DE LA ROUILLE DU MÛRIER CAUSEE PAR *AECIDIUM MORI* L. SUR LE RENDEMENT ET LA QUALITE DU MÛRIER (*MORUS ALBA* L.)

Angelina T. Gonzales¹ and OSCAR S. Opina²

¹Sericulture Research and Development Institute, Don Mariano Marcos Memorial State University, Bacnotan, La Union, Philippines

²University of the Philippines, Los Banos, Laguna, Philippines
E-mail: angelinatadurangonzales@yahoo.com

R E S U M E

Nous avons étudié l'impact de l'infestation par la rouille du mûrier (MRR) sur le rendement et la qualité des feuilles (cultivar Batac) ainsi que son contrôle. Les résultats montrent qu'une sévérité de 17.15% peut entraîner une perte de rendement de 7.4% qui passe de 10.05 t/ha à 9.305 t/ha chez le cultivar Batac. L'analyse des tissus des feuilles infectées montre une diminution significative du taux d'humidité en protéines et en vitamines. Le taux d'humidité et la quantité de protéines sont les plus élevés chez les feuilles du haut des branches. Les taux de sucres, d'amidon, de caroténoïdes, de b-carotène et d'acide ascorbique ne varient pas significativement entre les feuilles occupant différentes positions sur la branche.

Mots-clés: *Aecidium mori*, perte de qualité, mûrier, rouille, perte de rendement.



COMPARATIVE ACCOUNT OF MORPHOLOGICAL AND KARYOTYPIC STUDIES IN THREE CULTIVARS OF MULBERRY (*MORUS* spp.)

K. H. Venkatesh¹, S. Shivaswamy² and Munirajappa¹

¹Mulberry Breeding and Genetics Laboratory, Department of Sericulture,
Bangalore University, Bangalore – 560006, India.

²Department of Sericulture, Bharati College, BharatiNagara – 571422, Karnataka, India.
E-mail: cytogenetics1@gmail.com

ABSTRACT

Three mulberry genotypes namely, Kajali, S₃₄ and Thailand were analysed for morphological and karyotypic attributes. Kajali and S₃₄ are diploid with 2n=28 and Thailand is triploid with 2n=42 somatic chromosome numbers. Somatic chromosome length ranges from 1.46 to 3.06 µm whereas arm ratio ranges from 1.49 to 2.36 µm. Stomatal frequency is less in triploid variety when compared to diploid mulberry varieties. Three to four types of chromosomes have been observed irrespective of varieties. Chromosomes are small with a narrow range of variation in length.

Key words: Diploids, karyotype analysis, mitosis, morphology, mulberry (*Morus* spp.), triploid.

INTRODUCTION

Mulberry (*Morus* spp.) is a multipurpose, predominantly dioecious, heterozygous and out breeding tree. The foliage of the plant is used mainly as a unique source of feed for silkworm (*Bombyx mori* L.) and cultivated in over 40 countries (Machi and Katagiri, 1991). Most of the natural species of *Morus* is diploid (2n = 28), but triploids (2n = 42), developed by controlled crosses, are often superior to diploids with respect to foliage yield, quality and adaptability to environmental stimuli (Das and Prasad, 1974). The study of karyotype which deals with not only the number but also the shape and size of chromosomes is of great value in modern taxonomy. For a few Indian species, cytological investigations were carried out by Das *et al.* (1970) and Kundu and Sharma (1976). But the reports of Janaki Ammal (1948) and Venkatesh (2007) are confined only to the chromosome counts. Meiotic behaviour of a few cultivars has been reported by Basavaiah *et al.* (1990), Venkatesh *et al.* (2012) Venkatesh and Munirajappa (2012) and Venkatesh *et al.* (2013). For most of the species, there is no information on karyomorphology. In the present study, an attempt has been made to assess the

karyo-morphological and micro- morphological details in three mulberry cultivars.

MATERIALS AND METHODS

Three mulberry varieties, namely Kajali, S₃₄ and Thailand which are maintained in the mulberry germplasm bank of Department of Sericulture, Bangalore University, Bangalore, India, were selected for the present study. Cuttings of these varieties were planted in pots for experimental use. Somatic preparations were made from excised root tips. Root tips were pre-treated with aqueous solution of 0.002 molar 8 - hydroxyquinoline for 3 hours at 10°C and fixed in 1 : 3 acetic acid : alcohol for 24 hours. After washing in water, the root tips were hydrolysed in 1N HCl for seven minutes at 50°C and stained in 2 % aceto-orcein. Squash preparations were made in 45 % acetic acid. Photomicrographs and drawings of good and clear plates were made immediately, to ascertain the chromosome number and their morphology. Ideograms were drawn using suitable scale. Karyotype classification was made according to Levan *et al.*

(1964). Stomatal frequency was also determined by nail polish impression method. Stomatal frequency was calculated by using the formula and expressed as number of stomata/mm² (Sikdar *et al.*, 1986; Aneja, 2001).

$$\text{Stomatal frequency} = \frac{\text{Number of stomata}}{\text{Area of microscopic field}} \times \text{mm}^2$$

RESULTS AND DISCUSSION

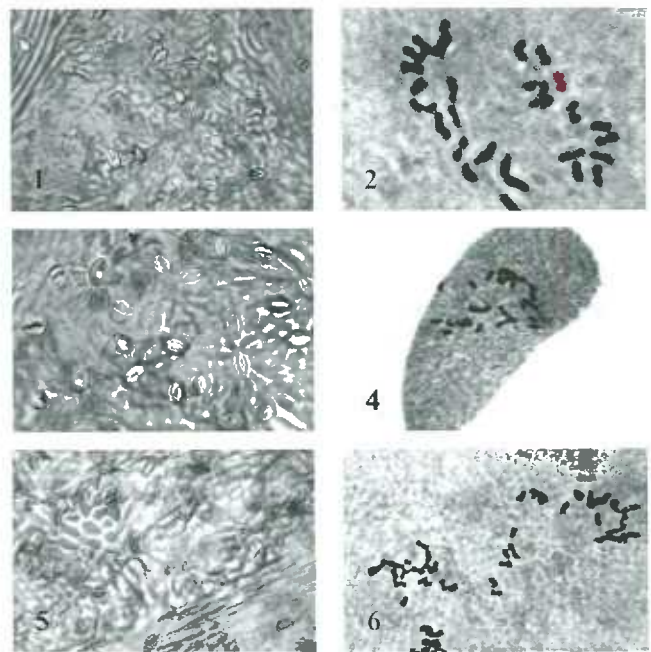
Details of the stomatal frequency, somatic chromosome number, ploidy level, range of chromosome length, karyotype formula, arm ratio and haploid chromatin length are presented in Table 1. Out of the following three cultivars studied, two are diploids ($2n=28$) and one is triploid ($2n=42$).

Kajali: It is a male variety, grown in tropical conditions. It is a good rooter and possesses wide acclimatization to different agro-climatic conditions. Stem is of light green to brown colour. This variety bears lobed light green leaves with long internodes. Stomatal frequency was found to be 177.27/mm² (Figure 1). This is a diploid cultivar with $2n=28$ somatic chromosomes (Figure 2). The karyotype formula of this variety is $2n=28=18B^m+10B^{sm}$ (Figure 7). Chromosome length varies from 2.00 to 3.06 μm with arm ratio ranging from 0.74 to 1.06 μm .

S₃₄: It is a female variety, grown in tropical region. Under ideal agro climatic conditions, this genotype yields 45 tonnes of leaf per/year/hectare. Stem is light green to brown in colour. Leaves are entire, unlobed, dark green with smooth texture. Stomatal frequency was found to be 156.06/mm² (Figure 3). This is also a diploid cultivar with $2n=28$ somatic chromosomes (Figure 4). The karyotype

formula of this variety is $2n=28=14B^m+10B^{sm}+2C^m+2C^{sm}$ (Figure 8). Chromosome length varies from 1.90 to 2.46 μm with arm ratio ranging from 0.68 to 1.00 μm .

Thailand: It is an exotic triploid female variety brought from Thailand to India. Triploid varieties are supposed to originate from the fertilization of diploidized gametes with normal haploid gametes (Osawa, 1920 a). Stem is green to brownish in colour. Leaves are simple, unlobed, dark green, serrate and ovate having long internodes. Stomatal frequency was found to be 150/mm² (Figure 5). This triploid cultivar possesses $2n=42$ somatic chromosomes (Figure 6). The karyotype formula of this genotype is $2n=28=6B^m+22B^{sm}+10C^m+4C^{sm}$ (Figure 9). Chromosome length varies from 1.46 to 2.86 μm with arm ratio ranging from 0.49 to 2.36 μm .



Figures 1-6: Stomatal frequency and somatic chromosomes of variety Kajali (1,2), S₃₄ (3,4) and Thailand (5,6).

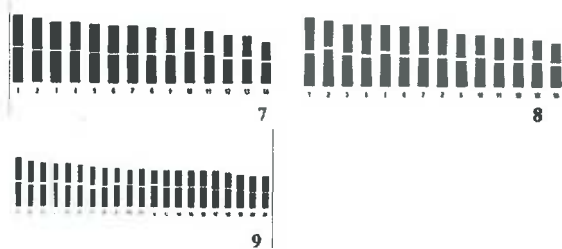
Table 1: Karyomorphological details of three mulberry varieties

Mulberry variety	Stomatal frequency/mm ²	2n chromosome number	Ploidy level	Karyotype formula	Range of chromosome length (μm)	Range of arm ratio (μm)	Haploid chromatin length (μm)
Kajali	177.27	28	Diploid	$2n=28=18B^m+10B^{sm}$	2.00-3.06	0.74-1.06	35.53
S ₃₄	156.06	28	Diploid	$2n=28=14B^m+10B^{sm}+2C^m+2C^{sm}$	1.90-2.46	0.68-1.00	31.09
Thailand	150.00	42	Triploid	$2n=28=6B^m+22B^{sm}+10C^m+4C^{sm}$	1.46-2.86	0.49-2.36	45.46

Basic chromosome number of the genus *Morus* L. as $x=14$ have been reported by Datta (1954) and Das (1961) for majority of the species. However, $2n=26$ (Janaki Ammal, 1948), $2n=30$ (Kundu and Sharma, 1976), for *Morus alba* L., $2n=30$ and 44 , (Kundu and Sharma, 1976) for *M. Nigra* reported will not fall in line of polyploidy series.

Koidzumi (1917) and Hotta (1953) have classified the Genus *Morus* based on the morphological characters of the female flower. Katsumata (1972) described some anatomical characters in addition to morphological features. The above systems are considered to be incomplete as data from cytology, embryology etc. are lacking. The other available reports are confined only to chromosome number (Tahara, 1909; Sinoto, 1929; Katsumata, 1979). Gill and Gupta (1978) while studying cytology of sex types of *Morus* species reported the presence of large sized chromosomes.

In the present investigation, Kajali, S_{54} and Thailand belonging to *Morus alba* are observed morphologically distinct with some similarities in their leaves viz., identical leaf margin and dissimilarities in their leaf lobation, stem colour, sexuality and stomatal frequency. Cytologically, Kajali and S_{54} revealed $2n=28$ chromosomes and Thailand, $2n=42$ chromosomes confirming the earlier reports and also exhibited similar karyotype with only two types of chromosomes, i.e., of equal chromatin length and arm ratio. Despite common origin, Kajali and S_{54} are diploids and Thailand is a triploid. These different chromosome numbers has reflected on their morphology and comparative micro propagation efficiency. Information on different ploidy level of same mulberry varieties are scanty (Khurana *et al.*, 2003). This type of chromosomes observed in each complement of all three varieties confirms the report of Kundu and Sharma (1976).



Figures 7-9: Ideograms of Kajali (7), S_{54} (8) and Thailand (9) mulberry varieties.

Cultivars Kajali and S_{54} have certain similarities in their adaptative parameters. However, triploid forms are better in rooting, grow more quickly and possess larger leaves when compared to diploids and tetraploids (Eswar Rao *et al.*, 2000). The frequencies of stomata are significantly less in triploids compared to diploids. Stomatal frequency is considered as an important parameter while selecting drought resistant genotypes as being correlated with drought and disease resistance (Hatalli *et al.*, 1993; Nautiyal *et al.*, 1994). Less frequency per unit area is advantageous for rain fed conditions.

Previous and present reports on chromosome number and cytology show the presence of $2n=28$ chromosomes in most of the cultivars in this genus. In all the above cases, the cytological identity of each cultivar has been represented in phenotypic variation. These karyomorphological and micro-morphological investigations can be made use of while selecting the parents for evolving progeny of different ploidy level both by hybridization and colchicine treatment. In addition, the information will be of much use in establishing a phylogenetic relationship and evolution of mulberry and will also help in selecting mother plants for hybridization based on chromosome number.

REFERENCES

- Aneja K. R. (2001) *Experiments in microbiology, plant pathology, tissue culture and mushroom production technology*, 3rd Edition, New Age International Publishers, New Delhi, India.
- Basavaiah Mala V. R., Dandin S. B., Suryanarayana N. and Sengupta K. (1990) Chromosomal association and meiotic behaviour of four triploid varieties of mulberry (*Morus* spp.). *Cytologia*, **55**: 327-333.
- Das B. C. (1961) Cytological studies on *Morus indica* L. and *M. laevigata* Wall. *Caryologia*, **14**: 159-162.
- Das B. C. and Prasad D. N. (1974) Evaluation of some tetraploid mulberry varieties through chemical analysis and feeding experiments. *Indian J. Seric.*, **13**: 17-22.
- Das B. C., Prasad D. N. and Sikdar A. K. (1970) Colchicine induced tetraploids of mulberry. *Caryologia*, **23** (3): 283-293.

- Datta M. (1954) Cytological studies in the species of *Morus*. *Cytologia*, **19**: 86-95.
- Eswar Rao M. S., Mllikarjunappa R. S. and Dandin S. B. (2000) Evaluation of tetraploids and triploids mulberry genotypes for propagation, growth and yield parameters. *Proceedings of Natl. Conf. Stra. Seri. Res. Devpt.*, November 16-18, CSR TI Mysore, India, p. 2.
- Gill B. S. and Gupta R. C. (1978) Cytological studies in the sex types of *Morus alba* L. Moraceae, *Curr. Sci.*, **48** (1): 35-36.
- Hatalli S. R., Chetty M. B. and Koti R. V. (1993) Interrelationship of stomatal frequency, interveinal distance and yield under depleting soil moisture regime in wheat genotypes. *Indian J. Plant Physiol.*, **36** (3): 187-188.
- Hotta T. (1953) Taxonomical study on the wild mulberry in Japan and near countries. *Bot. Mag. Tokyo*, **66**: 177-180.
- Janaki Ammal E. K. (1948) The origin of black mulberry *J. Roy. Hort. Soc.* **75**: 117-120.
- Katsumata F. (1972) Mulberry species in Java and their peculiarities *J. Seric. Sci. Jpn.*, **41**: 213-222.
- Katsumata F. (1979) Chromosome of *Morus nigra* L. from Java and hybridization affinity between this species and some mulberry species in Japan. *J. Seric. Sci. Jpn.*, **48** (3): 418-422.
- Khurana P., Bhatnagar S. and Kumari S. (2003) Tissue culture and morphogenetic studies in mulberry - an overview. *Indian J. Seric.*, **42**: 93-110.
- Koidzumi (1917) Kawazokushakubatsuko. *Bull. Imp. Seric. Expt.*, **III**: 1-PI-XI.
- Kundu D. and Sharma A. (1976) Chromosome studies in some Indian Moraceae. In: *Recent advances in Botany*, P. Kachroo (Ed.), Bishen Singh Mahendra Pal Singh, Dehradun, pp. 348-369.
- Levan A. K., Fredga and Sandberg A. A. (1964) Nomenclature for centromeric position on chromosome. *Hereditas*. **52**: 201-220.
- Nautiyal S., Badola H. K., Pal H. and Negi D. S. (1994) Plant responses to water stress changes in growth dry matter production Stomatal frequency and leaf anatomy. *Biol. Plant.*, **36** (1): 91-97.
- Osawa I. (1920) Cytological and experimental studies in *Morus* with special reference to triploid mutants. *Bull. Imp. Seric. Exp. Stn. Jpn.*, **1** (3): 318-369.
- Sikdar A. K., Jolly M. S., Susheelamma B. N. and Giridhar K. (1986) Stomatal chloroplast count technique as a tool to ascertain different ploidy level in mulberry. *Indian J. Seric.*, **25** (2): 88-90.
- Sinoto Y. (1929) Chromosomes studies in some dioecious plants with special reference to the allosomes. *Cytologia*, **1**: 109-144.
- Tahara M. (1909) On chromosomes of *Morus alba*. *Bot. Mag., Tokyo*, **23**: 343-353.
- Venkatesh K. H. and Munirajappa (2012) Cytogenetical studies in two triploid mulberry varieties. *J. Cytol. Gen.*, **13** (NS): 29-34.
- Venkatesh K. H., Munirajappa and Narayanaswamy V. (2012) Cytological studies in diploid varieties of mulberry. *J. Cytol. Gen.*, **13** (NS): 73-77.
- Venkatesh K. H. (2007) Cytogenetical investigation in the Genus *Morus* L. *Ph. D., Thesis*, Bangalore University, Bangalore, India.
- Venkatesh K. H., Nijagunaiah R. and Munirajappa (2013) Cytogenetical studies in some diploid mulberry varieties (Moraceae). *Cytologia*, **78** (1): 69-72.

Article scientifique

**COMPTE-RENDU DES ETUDES MORPHOLOGIQUES ET CARYOTYPIQUES
COMPARATIVES CHEZ TROIS CULTIVARS DE MÛRIER (*MORUS* SPP.)**

K. H. Venkatesh¹, S. Shivaswamy² and Munirajappa¹

¹Mulberry Breeding and Genetics Laboratory, Department of Sericulture, Bangalore University, Bangalore – 560006, India.

²Department of Sericulture, Bharati College, Bharati Nagara – 571422, Karnataka, India.

E-mail: cytogenetics1@gmail.com

R E S U M E

La morphologie et les caryotypes de trois génotypes de mûrier à savoir Kajali, S54 et Thailand ont été analysés. Kajali et S54 sont diploïdes avec $2n=28$ et Thailand est triploïde avec $2n=42$ (autosomes). La longueur de ces chromosomes varie entre 1.46 et 3.06 μm alors que le rapport des bras va de 1.49 à 2.36. La fréquence des stomates est plus faible chez la variété triploïde que chez les diploïdes. Trois à quatre types de chromosomes ont été observés chez toutes les variétés. Les chromosomes sont petits et diffèrent peu en longueur.

Mots-clés: Diploïdes, analyse de caryotype, mitose, morphologie, mûrier (*Morus* spp.), triploïde.



EVALUATION OF PROMISING MULBERRY GENETIC RESOURCES

N. Balachandran, S. R. Ramesh, Kiran B. Malali, M. Muthulakshmi and M. M. Borpuzari

Central Sericultural Germplasm Resources Centre, P. B. No.44, Thally Road, Hosur-635109, Tamil Nadu, India.

E-mail: balucsgrc@yahoo.co.in

ABSTRACT

Central Sericultural Germplasm Resources Centre (CSGRC), Hosur is a unique and exclusive station involved in conservation of different mulberry and silkworm genetic resources in India. CSGRC maintains 1254 mulberry accessions representing 26 countries (indigenous-984 and exotic-270) and 458 silkworm genetic resources from 14 countries. These valuable genetic resources are thoroughly evaluated during the conservation process for their qualitative and quantitative economic traits and potential accessions are identified for inclusion in the crop improvement programmes. In the present study, 189 (147 indigenous and 42 exotic) mulberry accessions were evaluated over a period of three years and data of thirteen crops in different seasons were analysed. The identified 20 better performing mulberry accessions along with 3 control accessions were subjected to bioassay to ascertain their suitability and preference by silkworm (*Bombyx mori* L.). The popular bivoltine hybrid CSR2 x CSR4 was used for bioassay in three different seasons. The pooled data of ten rearing traits and three post cocoon traits of economic importance were assessed using multiple trait evaluation index. The results based on the evaluation index value revealed accession MI-0669 the superior most for 9 traits followed by ME-0012 for 8 traits and ME-0165 for 7 traits. Three exotic accessions, ME-0089, ME-0139 and ME-0151 performed better for five traits and MI-0025 and MI-0653 for 4 traits. These promising accessions can serve as a potential breeding material in evolving superior mulberry varieties.

Key words: Bioassay, mulberry genetic resources, multiple trait evaluation index.

INTRODUCTION

Mulberry (*Morus* spp.) being the exclusive food plant for the silkworm, *Bombyx mori* is cultivated across different agroclimatic zones of the country where mulberry sericulture is being practiced as a means of revenue generation. The industry always demands improved mulberry varieties, which can produce quality leaves over and above the existing and popular mulberry varieties in the field. In this perspective, the breeders are always in search of genetic resources with wider variability and adaptability to be included in their parental selection. Further, the purpose of conserving such a large collection of mulberry genetic resources would not be serving any purpose if it is not utilized in the breeding programmes. Hurd (1976) stressed the importance of

diverse germplasm pool to improve stress resistance in crop plants since it probably involves many genes having small effects that are difficult to measure. Frankel and Bennet (1976) emphasized that evaluation is the process of correlating the genetic traits of the crop with the consumer needs and it is an essential preliminary step of utilization.

In this regard, Central Sericultural Germplasm Resources Centre (CSGRC), Hosur which is the nodal agency for conservation of mulberry and silkworm genetic resources in India can come in handy. Presently, CSGRC, Hosur has 1254 accessions with 984 of them being indigenous representing 29 states and the exotic accessions are 270 from 26 countries.

Since the growth of the silkworm, quality and quantity

of silk produced and reproductive parameters of silkworm *B. mori* are directly influenced by the quality of feed given and the nutritional contents of the mulberry variety (Sengupta *et al.*, 1973; Saratchandra *et al.*, 1992; Chaluvachari and Bongale, 1996; Sujathamma and Dandin, 1998), these factors indirectly decide the success of the crop, production of good quality cocoons and in turn higher returns to the sericulture farmers as well as the reelers. In view of the above, it is pertinent to evaluate, the existing mulberry germplasm accessions for growth and yield attributing parameters to know their genetic potential for crop improvement programme through bioassay. Such biological appraisal is the most direct and reliable method for assessing the quality of the mulberry leaf, which could be done both at early and late instars of silkworm growth to identify superior and promising genetic resources.

MATERIALS AND METHODS

In the present study, 189 mulberry germplasm accessions comprising of 147 indigenous and 42 exotic ones were evaluated during 2008-2012 for growth and yield parameters. The exotic accessions are from countries *viz.*, Afghanistan (2), China (1), Egypt (1), France (4), Italy (3), Japan (18), Pakistan (3), South Korea (1), Spain (2) and Turkey (2) with five of them of unknown exotic origin. The indigenous accessions are from the states of Andhra Pradesh (3), Himachal Pradesh (9), Jammu & Kashmir (5), Jharkhand (8), Karnataka (27), Kerala (15), Maharashtra (4), Punjab (3), Rajasthan (7), Tamil Nadu (27), Uttar Pradesh (19), Uttaranchal (1) and West Bengal (19). Species representation of the mulberry accessions considered for evaluation under the study included *Morus indica* (102) *M. alba* (60) *M. latifolia* (20) *M. laevigata* (3) and each of the other 4 species of *M. bombycis*, *M. rubra*, *M. serrata* and *M. tiliaefolia* represented by single accession.

The experiment was laid as per the Augmented Randomized Block Design (ARBD) with 12 plants per accession under 90 x 90 cm spacing with MR2, Kosen and V-1 varieties as checks. Recommended package of practices were adopted for maintenance of the plantation

in low bush form. The experimental plot was pruned after one year of establishment and data were recorded 60 - 75 days after pruning. The data on leaf yield and various growth traits of economic importance *viz.*, number of branches, average shoot length (cm), length of the longest shoot (cm), inter nodal distance (cm), shoot yield /plant (kg), leaf yield (kg), harvest index, lamina index, hundred leaf weight (g), moisture content (%) and moisture retention capacity (%) were recorded for four different seasons in a year for three consecutive years. The twelve crops' data were pooled and based on the average data, 20 better performing indigenous and exotic accessions were short-listed.

These accessions were subjected to bioassay using the popular bivoltine hybrid CSR2 x CSR4 under different seasons (summer, autumn and winter) with two replications of 300 larvae per replication. The larvae were fed with leaves from the selected 20 elite mulberry accessions and three control accessions after third moult under standard rearing conditions (Krishnaswami, 1978). The data on the ten important rearing traits *viz.*, larval weight (g), total larval duration (h), fifth age larval duration (h), ERR by number (No.), ERR by weight (kg), pupation rate (%), cocoon weight (g), shell weight (g), shell %, cocoon yield/100 Dfls (kg) and three reeling traits *viz.*, total filament length (m), non broken filament length (m) and denier (d) were also recorded (Table 3). Based on the average data over the seasons, the superior mulberry germplasm with better rearing traits were again assessed using the multiple trait evaluation indices (Mano *et al.*, 1993) for identifying the promising accessions.

RESULTS AND DISCUSSION

The short listed, 20 promising indigenous and exotic mulberry accessions along with their national accession number, name, scientific name and other parameters are listed in Table 1. The average data of twelve crops on 12 growth and yield traits of 20 better performing indigenous and exotic mulberry accessions and three control accessions are presented in Table 2. Out of these, indigenous accessions MI-0523, MI-0627, MI-0546, MI-

Table 1: List of mulberry accessions evaluated

Acc. No.	Nat. Acc. No.	Name	Species	State/Country	Leaf lobation	Sex
MI-0523	IC-314003	CUCKPILLA	<i>M. indica</i>	Karnataka	UL	F
MI-0627	IC-405775	BERHAMPORE-B	<i>M. indica</i>	West Bengal	UL	M
MI-0546	IC-313899	S-162	<i>M. alba</i>	West Bengal	UL	F
MI-0479	IC-313950	ERRC-12	<i>M. indica</i>	Kerala	SL	F
MI-0616	IC-405764	BENDORA	<i>M. laevigata</i>	Jharkhand	DL	F
MI-0652	IC-405800	Pouri-2	<i>M. indica</i>	Jammu and Kashmir	UL	M
MI-0550	IC-314090	YERCAUD-1	<i>M. indica</i>	Tamil Nadu	ML	F
MI-0594	IC-313960	KADAGANJI	<i>M. indica</i>	Karnataka	UL	F
MI-0653	IC-405801	Nomai	<i>M. latifolia</i>	Jammu and Kashmir	UL	F
MI-0669	IC-405817	Madhopur-3	<i>M. indica</i>	Punjab	UL	M
ME-0089	EC-493846	SUKAKUCHI	<i>M. alba</i>	Japan	ML	F
ME-0029	EC-493786	PKS 1-2	<i>M. alba</i>	Pakistan	SL	F
ME-0139	EC-493866	KAERAYONG-PPONG	<i>M. alba</i>	South Korea	SL	M
ME-0024	EC-493781	ACC.56	<i>M. alba</i>	Egypt	DL	MFBI
ME-0074	EC-493831	TOMESO	<i>M. alba</i>	Japan	SL	F
ME-0151	EC-493878	SOSUKE-II	<i>M. alba</i>	Japan	SL	F
ME-0012	EC-493769	PKS 1-11	<i>M. alba</i>	Pakistan	SL	F
ME-0165	EC-493892	CHINA BLACK-A	<i>M. alba</i>	China	UL	M
ME-0031	EC-493788	PKS 1-14	<i>M. alba</i>	Pakistan	UL	MLBI
ME-0126	EC-493853	M. TILIAEFOLIA	<i>M. tiliaefolia</i>	Japan	UL	F
*ME-0066	EC-493823	KOSEN	<i>M. latifolia</i>	Japan	UL	MFBI
*MI-0308	IC-313996	V-1	Hybrid	India	UL	MFBI
*MI-0025	IC-313974	MR-2	<i>M. sinensis</i>	India	UL	MFBI

UL – Un lobed, SL – Shallow lobed, DL – Deep lobed, ML – Medium lobed, F – Female, M – Male, MFBI – Male female bisexual, MLBI – Male bisexual, * – Control.

0479, MI-0616, MI-0652, MI-0550, MI-0594, MI-0653, MI-0669 represented *Morus indica*, *M. alba*, *M. laevigata* and *M. latifolia*. Exotic accessions ME-0089, ME-0029, ME-0139, ME-0024, ME-0074, ME-0151, ME-0012, ME-0165, ME-0031 belonged to *M. alba* and ME-0126 to *M. tiliaefolia*.

The growth and yield parameters of the 23 accessions selected for bioassay (Table 2) indicated higher number of branches (15.6) in two mulberry accessions ME-0012 and MI-0627. Higher shoot length (114.2 cm), length of the longest shoot (164 cm) and the internodal distance (7.5 cm) was observed in accession MI-0616. Accession MI-0523 showed higher shoot yield (2.3 kg) and leaf yield (1.3 kg). The harvest index was more in accession ME-0139 (0.8). Mulberry accession ME-0074 recorded

maximum lamina index (92.3) and hundred leaves weight (570.6 g). The moisture content was high in accession MI-0653 (80.4 %) and the moisture retention capacity was more in accession MI-0308 (69.9 %).

The rearing and post cocoon data of three seasonal trials were pooled and presented in Table 3. Among the 13 silkworm economic traits (10 rearing and 3 reeling) evaluated for bioassay, under the rearing traits, weight of 10 larvae was high (41.65 g) in accession MI-0669 and the low total larval duration (596 h) and fifth age larval duration (152 h) were observed in accession MI-0669. Accession MI-0669 also recorded the high effective rate of rearing (ERR) (by no.) of 9600 per 10000 larvae reared whereas, the ERR (by wt.) was high in accession ME-0012 with 16.7 kg. Pupation rate was high (91.0 %) in

Table 2: Growth and yield performance of experimental accessions

Accession No.	NBR (No.)	SL (cm)	LLS (cm)	INT NOD (cm)	Sh. Yield (kg)	Leaf yield (kg)	H I	Lam Ind	Hun wt (g)	MC (%)	MRC (%)
MI-0523	14	111.2	151	5.7	2.3	1.3	0.6	86.6	434	63.5	47.7
MI-0627	15.6	108.2	141	5.3	2.2	1.3	0.6	90.1	444.4	75.3	50.6
MI-0546	10	81.1	106	4.8	0.8	0.5	0.7	92	284.2	73.8	55.3
MI-0479	12.8	85.4	121	3.7	1	0.5	0.6	91.7	113.8	77.5	44
MI-0616	15	114.2	164	7.5	1.6	0.9	0.6	89.5	510.2	79.7	56.8
MI-C652	11.8	86.4	112	4.2	1	0.7	0.7	88	351.8	74.9	45.6
MI-C550	10.2	88.2	106	4.6	0.8	0.5	0.7	92	201.4	74.9	32.8
MI-0594	7.8	77.8	103	5.2	0.4	0.2	0.6	88.7	151	78.8	29.6
MI-0653	5.8	96.6	122	5.1	0.6	0.4	0.7	88.7	341.2	80.4	46.3
MI-0669	12.2	86.9	110	4.2	1.5	0.9	0.6	89.2	391.4	71.5	58.4
ME-C089	8.6	90.9	121	4.6	1.1	0.8	0.7	87.3	490.4	79.1	59
ME-C029	10.8	87	123	5	0.9	0.7	0.7	90	266.8	75.3	51.4
ME-C139	9.4	87.2	114	2.9	1.2	0.9	0.8	87.7	300	78.9	49.7
ME-0024	13.3	92.1	121.3	6.2	1.7	1.1	0.7	90.6	544.8	75.4	51.6
ME-0074	5	83	103	4.2	0.6	0.4	0.7	92.3	570.6	79	52.6
ME-0151	12.6	93	121	5.3	1.3	0.8	0.6	90.6	328.4	78.8	63.3
ME-0012	15.6	96.1	129	4.6	1.2	0.8	0.6	89	242.4	74.8	58.6
ME-0165	9	97.1	115	4.8	1.6	1.1	0.7	89.6	434.6	77.1	65.8
ME-0031	14	87.4	108.6	4.9	1.3	0.9	0.7	84.9	206.6	74.1	49
ME-0126	6.6	87.9	105.6	7.5	0.7	0.5	0.6	87.3	468	79.9	56.8
*ME-C066	7.9	67.9	96	4.8	0.8	0.5	0.7	89	463	75.4	68.4
*MI-0308	12.8	96.4	137.2	5.4	1.7	1.1	0.6	87.5	408.3	76.2	69.9
*MI-0025	14.1	79.4	106.9	5.1	1.6	0.9	0.6	89.1	357.7	76.4	60.7

NBR - No. of branches, SL - Shoot length, LLS - Length of the longest shoot, INT NOD - Internodal distance, Sh. yield - Shoot yield, H.I. - Harvest Index, Lam Ind - Laminar index, Hun wt - Hundred leaf weight, MC - Moisture content, MRC - Moisture Retention Capacity, * - Control.

Data are mean of 12 trials.

accession MI-0669. Accession ME-0012 showed higher cocoon weight (1.904 g) and shell weight (0.454 g) and the shell % was high (25.32 %) in accession ME-0126. Higher Cocoon yield per 100 dfls was recorded in accession ME-0012. Among the reeling parameters studied, higher filament length and non-broken filament length was recorded in accession ME-0012 with 1223.9 m and 1166.9 m, respectively. Accession MI-0627 showed the low denier of 2.22 d among the 23 accessions studied. Similar evaluation of germplasm genotypes through bioassay have already been reported by many workers for different purposes (Sarkar, 1993; Sikdar, 1993; Chaluvachari and Bongale, 1995; Bongale and Krishna, 1997; Muniraju *et al.*, 2000; Chakravorty *et al.*, 2003; Chakravorty and Neog, 2003).

The data were analysed with Mano's evaluation index method (Mano *et al.*, 1993) and cumulative evaluation index values worked out for 13 parameters, revealed

accession ME-0012 and MI-0669 and ME-0151 qualifying with 12 parameters with CEI values of 63.25, 57.62 and 56.84, respectively, followed by accessions ME-0165, ME-0029 with 11 parameters and accessions ME-0139 and MI-0616 with 10 parameters (Table 4).

Further, the data of these trials were subjected to multiple traits evaluation by CSGRC method (Table 5) and the results were in conformity with that of Mano's cumulative evaluation index method. Mulberry accessions MI-0669, ME-0012 and ME-0165 were found to be good in 9, 8 and 7 selected rearing and reeling parameters studied while ME-0089, ME-0139 and ME-0151 in five of the parameters. ME-0012, MI-0669, ME-0151, ME-0139 and ME-0151 are adjudicated as the top five accessions in both the evaluation methods. The study is on the lines of earlier one by Balachandran *et al.* (2011) in which 199 mulberry germplasm accessions were

Table 3: Crop performance of bioassay

Accession No.	Wt 10 (g)	TLD(h)	VLD(h)	ERR (No.)	ERR (Wt)(kg)	PR%	C Wt.(g)	S Wt.(g)	Shell %	Yld/100d fls(kg)	AFL(m)	NBFL(m)	Denier(d)
ME-0024	30.1	603	168	9009	13.8	70.3	1.527	0.355	23.33	55.01	1086.1	694.4	2.35
ME-0031	28.3	608	167	9114	12.9	50.8	1.445	0.358	24.86	51.38	1006.7	787.9	2.36
ME-0074	31.1	600	162	9020	13.5	73.5	1.51	0.344	23.12	54.07	1104.3	921.9	2.35
ME-0126	23.1	642	201	8844	11.8	76.3	1.183	0.294	25.32	47.25	792.4	667.4	2.27
ME-0151	33.3	603	162	9426	15.5	79.8	1.654	0.404	24.72	62.01	1196.3	956.3	2.51
MI-0025	27.0	600	163	9441	14.2	68.7	1.505	0.298	19.80	56.71	1164.5	1032.5	2.41
MI-0479	28.4	603	165	9258	11.6	52.6	1.516	0.347	22.95	46.26	1107.7	933.0	2.24
MI-0546	30.6	603	162	9018	13.9	74.7	1.506	0.354	23.76	55.40	1126.4	875.7	2.46
MI-0594	32.6	603	159	9324	14.2	73.2	1.633	0.328	20.06	56.86	1045.1	915.6	2.55
MI-0627	30.1	615	174	9235	13.5	74.0	1.555	0.347	22.37	54.02	1042.8	849.7	2.22
MI-0653	33.1	601	157	8716	11.4	62.1	1.725	0.394	22.89	45.37	1074.8	1024.5	2.54
ME-0012	38.8	605	166	9393	16.7	83.6	1.904	0.454	24.03	66.80	1223.9	1166.9	2.86
ME-0029	34.6	605	167	9293	14.8	82.7	1.588	0.376	23.83	59.23	1101.4	960.1	2.60
ME-0066	28.2	603	165	8484	13.5	73.1	1.326	0.281	21.00	53.77	941.5	941.5	2.55
ME-0139	37.1	642	201	9346	15.4	84.1	1.67	0.589	23.38	61.45	1152.5	1056.5	2.69
ME-0165	38.4	600	162	9448	15.8	89.9	1.712	0.394	23.04	63.29	1075.9	993.1	2.76
MI-0308	31.0	605	165	9212	13.1	87.7	1.496	0.332	22.47	52.20	956.3	910.6	2.61
MI-0523	31.3	601	160	9108	11.6	82.7	1.368	0.318	23.44	46.21	942.4	865.8	2.43
MI-0550	35.1	600	162	9270	13.8	87.0	1.467	0.338	23.25	55.04	1011.2	962.7	2.69
ME-0089	38.1	603	162	9449	13.6	75.0	1.684	0.412	24.70	54.37	1097.2	1009.6	2.75
MI-0616	34.5	615	174	9436	14.6	89.3	1.595	0.378	23.88	58.55	1085.5	1036.8	2.68
MI-0652	31.5	606	162	9251	13.2	80.7	1.528	0.346	22.77	52.85	996.8	911.7	2.67
MI-0669	41.6	596	152	9600	16.4	91.0	1.729	0.402	23.46	65.6	1083.8	985.3	2.65

Wt 10 - Weight of 10 grown up larvae, TLD -Total larval duration, VLD - Fifth age larval duration, ERR (No.) - Effective rate of rearing /10000 larvae, ERR (Wt.) Effective rate of rearing per 10000 larvae in wt., PR % - Pupation rate in %, C wt.- Cocoon weight, S wt - Shell weight, Yld/100 dfls- Cocoon yield /100 dfls, AFL - Average filament length, NBFL - Non broken filament length.

evaluated and two exotic and two indigenous accessions were found to be better than the national check (V-1), which can serve as potential breeding material.

The growth of the silkworm, quality and quantity of silk produced and reproductive parameters of silkworm *B. mori* are directly influenced by the quality of feed given and the nutritional contents of the mulberry variety (Sengupta *et al.*, 1973; Saratchandra *et al.*, 1992; Chaluvachari and Bongale, 1996; Sujathamma and Dandin, 1998). These factors indirectly decide the success of the crop, production of good quality cocoons and in turn higher returns to the sericulture farmers as well as the reelers. The superiority of certain varieties with reference to the larval growth, successful crop and better cocoon characters were studied (Abdullaev *et al.*, 1966; Das and Sikdar, 1970; Krishnaswami *et al.*, 1971; Narayanan *et al.*, 1971)

With the advancement in sericulture technologies, it has been possible to rear mulberry silkworms throughout the year in tropical conditions, because of the availability of mulberry leaf in all seasons. As sericulture is a highly productive enterprise particularly in southern states of India, to increase the leaf productivity per unit area and for repeated harvest throughout the year, evolving productive mulberry varieties to fit different cultivation practices in various agro-climatic zones is of prime importance. In view of the above, the mulberry germplasm accessions such as MI-0669, ME-0012, ME-0165, ME-0139 and ME-0151 identified as promising ones not only in terms of growth and yield attributing parameters but also through bioassay, such biological appraisal is the most direct and reliable method for assessing the quality of the mulberry leaf for silkworm growth to identify superior and promising genetic resources and for inclusion in the mulberry improvement programmes.

Table 4: Mano's evaluation index values and cumulative evaluation index of bioassay

Acc.No/ Traits	Wt 10 (g)	TLD(h)	VLD(h)	ERR (No.)	ERR (Wt)	PR%	C Wt.(g)	S Wt.(g)	Shell %	Coc.Yd	AFL(m)	NBFL (m)	Denier(d)	CEI	Para.
ME-0012	64.39	48.28	49.26	57.15	69.24	56.42	72.68	72.92	56.40	69.18	67.07	70.80	68.46	63.25	12
MI-0669	70.80	40.69	37.38	64.99	67.21	53.25	61.22	60.46	52.26	67.16	52.34	54.65	56.70	57.62	12
ME-0151	51.79	46.59	45.87	58.40	61.11	52.91	56.31	60.94	61.42	61.13	64.17	52.07	50.86	56.84	12
ME-0165	53.47	44.06	45.87	59.23	63.14	52.23	60.11	58.54	49.21	63.28	51.51	55.34	62.86	56.83	11
ME-0029	54.77	48.28	50.11	53.37	56.36	55.59	51.99	54.22	54.95	56.47	54.19	52.41	53.90	53.58	11
ME-0139	60.50	79.48	78.97	53.37	60.43	56.88	57.36	57.34	51.68	60.19	59.56	60.98	58.94	61.36	10
MI-0616	54.54	56.71	56.05	58.78	55.01	61.68	52.45	54.70	55.31	55.32	52.52	59.23	58.38	56.21	10
ME-0089	62.79	46.59	45.87	59.27	48.23	48.48	58.27	62.85	61.27	48.31	53.75	56.81	62.30	54.98	9
*MI-0025	37.36	44.06	46.71	58.97	52.30	42.67	46.55	35.52	25.66	52.24	60.83	58.85	43.25	46.54	8
MI-0550	55.92	44.06	45.87	52.49	49.59	59.56	44.06	45.11	50.73	49.43	44.71	52.64	58.94	50.24	7
MI-0546	45.61	46.59	45.87	42.95	50.27	48.21	46.62	48.95	54.44	50.04	56.82	44.90	46.05	48.25	7
MI-0594	50.19	46.59	43.32	54.54	52.30	46.82	54.93	42.71	27.55	52.49	48.27	48.45	51.10	47.64	7
MI-0653	51.33	44.90	41.62	31.52	33.33	36.58	60.96	58.54	48.12	33.20	51.39	58.14	50.54	46.17	7
MI-0479	40.57	46.59	48.41	52.04	34.68	27.81	47.27	47.27	48.55	34.69	54.85	50.00	33.73	43.58	5
MI-0523	47.21	44.90	44.17	46.36	34.68	55.59	37.58	40.32	52.11	34.61	37.47	44.02	44.37	43.34	5
MI-0652	47.67	49.12	45.87	51.77	45.52	53.74	48.06	47.03	47.24	45.76	43.19	48.10	57.82	48.53	4
*MI-0308	46.52	48.28	48.41	50.30	44.84	60.20	45.96	43.67	45.06	44.66	38.93	48.01	54.46	47.64	4
ME-0074	46.75	44.06	45.87	43.03	47.55	47.10	46.88	46.55	49.79	47.80	54.50	49.01	39.89	46.83	4
ME-0024	44.46	46.59	50.96	42.61	49.59	44.15	47.99	49.19	51.31	49.38	52.58	28.78	39.89	45.96	4
*ME-0066	40.11	46.59	48.41	22.73	47.55	46.73	34.83	31.44	34.38	47.30	37.38	50.75	51.10	41.49	3
MI-0627	44.46	56.71	56.05	51.17	47.55	47.56	49.83	47.27	44.34	47.72	48.03	42.59	32.61	47.38	2
ME-0031	40.34	50.81	50.11	46.59	43.49	26.15	42.62	49.91	62.43	43.29	44.23	37.09	40.45	44.42	2
ME-0126	28.43	79.48	78.97	36.36	36.04	49.68	25.46	34.56	65.78	36.35	21.70	26.37	35.41	42.66	2

Wt 10 - Weight of 10 grown up larvae, TLD - Total larval duration, VLD - Fifth age larval duration, ERR (No.) - Effective rate of rearing /10000 larvae, ERR (Wt.) Effective rate of rearing per 10000 larvae in wt., PR % - Pupation rate in %, C wt.- Cocoon weight, S wt - Shell weight, Yld/100 dfls- Cocoon yield /100 dfls, AFL - Average filament length, NBFL - Non broken filament length, CEI - Cumulative evaluation index, Para. - No. of parameters, * - Control Accession.

Table 5: Better performing accessions based on multiple traits in bioassay

Accession No.	No. of traits	*Trait No. (Value)
MI-0669	9	1(41.6), 2(596), 3(152), 4(9600), 5(164), 6(91), 7(1.7), 8(0.40), 10(65.6)
ME-0012	8	1(38.7), 5(16.7), 7(1.9), 8(0.45), 9(24.0), 10(66.7), 11(1223), 12(1166)
ME-0165	7	1(38.4), 2(600), 4(9448), 5(15.8), 6(89), 7(1.7), 10(63.2)
ME-0089	5	1(38.0), 4(9449), 7(1.6), 8(0.41), 9(24.6)
ME-0139	5	1(37.1), 5(15.3), 10(61.4), 11(1152), 12(1056)
ME-0151	5	5(15.535), 8(0.40), 9(24.7), 10(62.0), 11(1196)
MI-0025	4	2(599), 4(9441), 11(1164), 12(1032)
MI-0653	4	3(157), 7(1.7), 8(0.39), 12(1024)

*1 - Weight of 10 larvae (g), 2 - Total larval duration (h), 3 - Fifth age larval duration (h), 4 - ERR (by no.), 5 - ERR (by wt.), 6 - Pupation rate (%), 7 - Cocoon weight (g), 8 - Shell weight (g), 9 - Shell %, 10 - Cocoon yield/100 dfls (kg), 11 - Filament length (m), 12 - NBFL (m), 13 - Denier.

REFERENCES

- Abdullaev I. K., Alieve M. O., Gusejnova P. A. and Mahmudbekova N. I. (1966) Chemical analysis of the fodder qualities of the leaves of promising mulberry varieties in the conditions of Karabah area. *Azerb.SSR Elmer Akad. Heberler. Biol. Elmer. Ser.*, 1: 36-43.
- Balachandran N., Chandrasekar M., Muthulakshmi M., Saraswat R. P., Srinivasa Babu G. K. and Kamble C. K. (2011) Evaluation elite mulberry genetic resources through bioassay. *Bull. Indian Acad. Seric.*, 15(1): 57-66.
- Bongale U. D. and Krishna M. (1997) Leaf quality in four important varieties of mulberry (*Morus indica*) in relation to biochemical components and bioassay rearing (*Bombyx mori* L) in respect to soil organic matter status. In: *Abstracts of National Conference on Morphological, Physiological, Biochemical and Molecular aspects of stress tolerance*, Tiruchirappally, India, 22-23, February, 1997, pp. 29-30.
- Chakravorty R., Goswami B. C. and Hazarika L. K. (2003)

- Evaluation of thirteen mulberry varieties through bioassay feeding techniques. In: *Abstracts of National conference on tropical sericulture for Global Competitiveness*, Central Sericultural Research and Training Institute, Mysore, India, 5 - 7 November, 2003, p. 3.
- Chakravorty R. and Neog K. (2003) Bioassay moulting response of silkworm (*Bombyx mori* L.) on thirteen mulberry varieties. In: *Abstracts of National conference on tropical sericulture for Global Competitiveness*, Central Sericultural Research and Training Institute, Mysore, India, 5 - 7 November, 2003, p. 3.
- Chaluvachari and Bongale U. D. (1995) Evaluation of leaf quality of some germplasm genotypes of mulberry through chemical analysis and bioassay with silkworm *Bombyx mori* L. *Indian J. Seric.*, **34**(2): 127-132.
- Chaluvachari and Bongale, U. D. (1996) Bioassay moulting response of silkworm *Bombyx mori* L. in relation to leaf nutritive constituents in mulberry (*Morus* spp.) genotypes. *Indian J. Seric.*, **35**(2): 160-162.
- Das B. C. and Sikdar A. K. (1970) Evaluation of some improved strains of mulberry by feeding experiment. *Indian J. Seric.*, **9**: 26-30.
- Frankel C. H. and Bennet E. (1976) Genetic Resources in Plants: Their exploration and conservation. *IBH Handbook No.11*, Blackwell scientific publications, Oxford, pp. 157-159.
- Hurd E. A (1976) Plant breeding for drought resistance In: *Water Deficits and Plant Growth*, T. T. Kozlowski (Ed.), Vol -4, Academic press, New York and London, pp. 317-353.
- Krishnaswami S. (1978) New technology of silkworm rearing. *Bulletin No.2*, Central Sericultural Research and Training Institute, Mysore, India, pp. 1-23.
- Krishnaswami S., Kumararaja S., Vijayaraghavan K. and Kasiviswanathan K. (1971) Silkworm feeding trials for evaluating the quality of mulberry leaves as influenced by variety, spacings and nitrogen fertilization. *Indian J. Seric.*, **10**: 79-89.
- Mano Y., Nirmal Kumar S., Basavaraja H. K., Mal Reddy N. and Data R. K. (1993) A new method to select promising silkworm hybrid combination. *Indian Silk*, **30**: 53.
- Muniraju E., Sekarappa B. M. and Raghuraman R. (2000) Seasonal bioassay response of silkworm (*Bombyx mori* L.) to the mulberry (*Morus* spp.) leaf preservation methods. *Sericologia*, **40**(4): 623-631.
- Narayanan E. S., Kasiviswanathan K. and Iyengar M. N. S. (1971) Effect of varietal feeding, irrigation, levels of nitrogen fertilization on larval development and cocoon characteristics of *Bombyx mori* L. *Indian J. Seric.*, **10**: 13-17.
- Saratchandra B., Rajanna L., Philomena K. L., Paramesha C., Ramesh S. P., Jayappa T. and Sabitha M. G. (1992) An evaluation of elite mulberry varieties for yield and quality through bioassay. *Sericologia*, **32**: 127-134.
- Sarkar A (1993) Effects of feeding different races of silkworm (*Bombyx mori* L.) with different mulberry (*Morus indica* L.) leaves varying in ploidy level. *Sericologia*, **33**: 25-34.
- Sengupta K., Mukherjee S. K., Sikder A. K., Mustafi J. C. and Sengupta D. (1973) A coordinated study on the effects of variety, spacings in cultivation and level of nitrogen fertilization on the cocoon yield and cocoon character of multivoltine silkworm *Bombyx mori* L. *Indian J. Seric.*, **12**: 39-45.
- Sikdar A. K. (1993) Chemo and bioassay of high yielding triploids and diploids of mulberry (*Morus* spp.). *Indian J. Seric.*, **32**(2): 169-174.
- Sujathamra P. and Dandin S. B. (1998) Evaluation of mulberry (*Morus* spp.) genotypes for yield under Rayalaseema condition of Andhra pradesh. *Indian J. Seric.*, **37**(1): 13-16.

Article scientifique

EVALUATION DES RESSOURCES GENETIQUES PROMETTEUSES DU MÛRIER

N. Balachandran, S. R. Ramesh, Kiran B. Malali, M. Muthulakshmi and M. M. Borpuzari

Central Sericultural Germplasm Resources Centre, P. B. No.44, Thally Road, Hosur - 635109, Tamil Nadu, India.

E-mail:balucsgrc@yahoo.co.in

RESUME

Le Central Sericultural Germplasm Resources Centre (CSGRC) à Hosur est une station unique en Inde consacrée exclusivement à la conservation des ressources génétiques de mûriers et de vers à soie. Le CSGRC entretient 1254 lignées de mûriers provenant de 26 pays (984 indigènes et 270 exotiques) et 458 souches de vers à soie provenant de 14 pays. Ces ressources génétiques sont sérieusement évaluées pendant le processus de conservation pour leurs caractères économiques qualitatifs et quantitatifs et les lignées à potentiel sont incluses dans les programmes d'amélioration des récoltes. Dans notre étude, 189 (147 indigènes et 42 exotiques) lignées de mûriers ont été évaluées pendant trois ans et les données de treize récoltes à différentes saisons ont été analysées. Les 20 lignées les plus performantes ainsi que 3 témoins ont été soumises à un test biologique pour confirmer leur utilité et la préférence des vers à soie (*Bombyx mori* L.). L'hybride populaire CSR2xCSR4 a été utilisé dans un essai biologique à trois saisons différentes. Les données rassemblées de dix caractères d'élevage et de trois caractères post cocon ont été évaluées par indice d'évaluation de caractères multiples. Les résultats indiquent que la lignée MI-0669 est supérieure pour 9 caractères suivie par ME-0012 pour 8 caractères et ME-0165 pour 7 caractères. Trois lignées exotiques, ME-0089, ME-0139 et ME-0151 sont plus performantes pour cinq caractères alors que MI-0025 et MI-0653 sont meilleures pour 4 caractères. Ces lignées prometteuses peuvent servir de matériel de breeding pour obtenir des variétés de mûriers supérieures.

Mots-clés : Essai biologique, ressources génétiques de mûrier, indice d'évaluation de caractères multiples.

The 23rd International Congress of International Sericultural Commission on Sericulture & Silk Industry was held during 24th~27th November 2014 at Bangalore, India. Hotel Leela Palace, Bangalore, India could rightly provide an apt and serene ambience for the four day long scientific extravaganza.

The Congress witnessed the active participation of 315 delegates belonging to various fields of interests, across 17 countries.

Marking the commencement of the Congress, Ms. Ishita Roy, Secretary General, International Sericultural Commission extended a warm welcome to all the delegates and dignitaries. She hoped that the Bangalore Congress would script a refreshing new chapter in the 54 years long existence of ISC.

In the grand opening ceremony on the first day, the Governor of Karnataka, His Excellency Shri Vajubhai Vala inaugurated the event in the presence of Dr. Sanjay Kumar Panda, IAS, Secretary to the Govt. of India, Ministry of Textiles who delivered the keynote address. Shri Kumar Tuhin, IFS, Joint Secretary to the Govt. of India, Ministry of External Affairs and Shri Rajeev Chawla, IAS, Principal Secretary to the Govt. of Karnataka, Horticulture Department were present during the inaugural function and delivered brief addresses.



True to its tradition, the Congress was spanned out for four eventful days with a total of eight sessions as follows:

1. Mulberry
2. *Bombyx mori*
3. Non-Mulberry Silkworms
4. Ecology of Silkworms
5. Post-Cocoon Technology
6. Economy
7. Sericulture in Non-Textile Industries
8. Silk Processing, Trading & Marketing

The above sessions were chaired by eminent scientific personalities such as Dr. Souad Morsy MAHMOUD, Prof. Daniel Dezmirean, Dr. Alok Sahay, Dr. Kadono Okuda, Ms. Somying Chuprayoon, Mr. Farzam Pourramezan, Dr. Yasushi Tamada and Mr. M. K. Ghosh, respectively.

Each session commenced with the presentation of a lead paper by some sterling scientific personalities viz., Dr. Shankar Dandin, Dr. H. K. Basavaraja, Mr. Dharendra Kumar, Dr. Kadono Okuda, Ms. Somying Chuprayoon, Ms. Suwannee Cunvong, Prof. Subhas Kundu and Mr. Andrea Nembri.

A highly organized and functional scientific committee comprised by scientists of Central Silk Board, Govt. of India was very vital in conducting the scientific sessions, hassle free.



During the occasion, three eminent scientists *viz.*, Dr. Hiroaki Machii, Ms. Suwannee Cunvong and Dr. B. S. Angadi were conferred with the prestigious Louis Pasteur award.

Though the response to the Congress, both from home and abroad was unprecedented, 226 papers were shortlisted after examining the scientific content and value for presentations. These presentations were bifurcated into oral (129) and poster (97) categories for effective and meaningful interaction.

The Congress in general addressed the most relevant scientific and economic developments in the field of sericulture science and allied aspects. The presentations were of matter of facts and the interactions followed were meaningful.

The Commission was extremely fortunate to have Sri Santosh Kumar Gangwar, the Hon'ble Minister of State for Textiles (Independent charge), Govt. of India to inaugurate and chair the Valedictory Function.

The plenary session conducted on the fourth day was chaired by Padmabhushan Dr. M. Mahadevappa. The chairpersons of individual sessions presented the gist of each session along with the session's recommendations. Dr. Mahadevappa summed up the recommendations to mark the end of the Congress.





INTERNATIONAL

SERICULTURAL

COMMISSION

UNITED NATIONS REG. NO. 10416

INTERNATIONAL SERICULTURAL COMMISSION

An intergovernmental organization instituted in 1960 and registered with United Nations

APPLICATION FOR ASSOCIATE MEMBERSHIP

Name (Personal Associate Membership for Individuals)			
Person In-Charge (Collective Associate Membership for Institutions/Libraries etc)			
Address			
Country			
Tel		Fax	
E-mail			
Privileges Personal Associate Member : Receives Sericologia Collective Associate Member : Receives Sericologia Receives Scientific and Technical information as and when sought Subsidized Registration fee for participation in ISC Congress			

I wish to become a Personal Associate/Collective Associate Member of ISC

New Application

☐

Renewal

☐

Rates : Personal Associate Members
Collective Associate Members

: US\$ 175 per year
: US\$ 470 per year

Applicant's signature : _____ Date : _____

Membership fees can be remitted either by cheque or by Bank Transfer

Beneficiary : International Sericultural Commission

Account No. **3188283389**

Bank & Branch: Central Bank of India, CSB Branch, Bengaluru, India

Bank Code: IFSC – CBIN0283975, SWIFT – CBININBBMRB

For overseas transfer

THROUGH BANK OF AMERICA, NEW YORK CHIPS ABA 0959, FED ABA 026009593, SWIFT CODE – BOFAUS3N TO
A/C NO.6550991916 OF CENTRAL BANK OF INDIA, OVERSEAS BRANCH, MUMBAI, SWIFT: CBININBBOSB

This form along with your cheque or the references of your Bank Transfer must be mailed to the office of the International Sericultural Commission whose address is given below.

Ground Floor, Central Silk Board Complex, B.T.M. Layout, Madiwala, Bengaluru – 560 068. INDIA Tel: +91 80 26680162,
26282186 | Fax: +91 80 26681663 Email: iscbangalore@inserco.org

www.inserco.org

INFORMATION TO CONTRIBUTORS

SERICOLOGIA is a peer reviewed quarterly scientific journal dedicated to the science of sericulture, published by the International Sericultural Commission. Papers submitted to SERICOLOGIA should carry original contributions of scientific research (basic or applied) or reviews on any aspect of sericulture.

Submission of a paper to SERICOLOGIA implies that it has not previously been published, that it is not under consideration for publication elsewhere and that, if accepted in SERICOLOGIA, it will not be published elsewhere in the same form without the written consent of the Chief Editor.

Manuscripts and illustrations must be prepared in British English on a standard A4 size paper setting. It should be typed in 12 point Times New Roman or Arial font in double spacing and single column with well set margins on top, bottom, left and right. Page numbers should be given at the bottom centre of every page.

The typescript should contain the following features:

- **Title** of the paper should be in a 14 point font. It should be bold typed, centered and fully capitalized.
- **Authors'** full name, address, mobile/fixed line numbers, and email/alternate email address should be in 10 point font and centered underneath the title. The first address should be of the centre at which the study was conducted. In the case of multiple ownership, the authors may indicate the correspondence address.
- **Abstract:** must be the gist of the paper which should explain the background, aims, methods, results & conclusion in a single paragraph not exceeding 200 words.
- **Key words** should follow the abstract, subject to a maximum of five, arranged in alphabetic order.
- **Main text** should have serially arranged sections such as Introduction, Materials and Methods, Results and Discussion for an effective and systematic presentation of the contents. Acknowledgements may be included if relevant. Only standard abbreviations should be used. Whenever specialized abbreviations are used, it should firstly be given in full with the abbreviation indicated in parenthesis. Scientific names should be given for all species used in the investigation.
- **Tables** should be simple, centered, separately numbered & self explained, and titles must be above the tables. Sources of data if any, should be mentioned below the table. All tables and figures should be referred to in the text.
- **Illustrations:** All necessary illustrations should accompany the manuscript but should not be inserted in the text. Photographs, graphs and diagrams should be numbered consecutively in Arabic numerals in the order in which they are referred to in the text. Digital/electronic version of the graphs and photographs should be submitted as separate files. Graphs should be in excel format. Photographs should be of high resolution in jpeg format. Legends to figures should be typed on a separate sheet and not at the back of the original.
- **References:** In the text, the referencing pattern should be as Dupont (1964)/ (Dupont, 1964)/ Dupont and Durand (1964) or (Dupont and Durand, 1964). When a citation includes more than two authors, e.g., Dupont, Durand and Martin, the paper should be cited in the text as Dupont *et al.* (1964) or (Dupont *et al.*, 1964). If papers by the same author(s) in the same year are cited they should be distinguished by the letters a, b, etc. following year of publication.
- At the end of the text, the list of references prepared following Harvard Style of Referencing should be alphabetically arranged. References not cited in the text should not be included. References should be cited in the following pattern:

Dollo M., Samal P. K., Sundriyal R. C. and Kumar K. (2009) Environmentally sustainable traditional natural resource management and conservation in Ziro Valley, Arunachal Himalaya, India. *J. Am. Sci.*, 5:41–52.

Ceci S. J., Williams W. M. and Barnett S. M. (2009) Women's underrepresentation in science: Sociocultural and biological considerations. *Psychol. Bull.*, 135: 218–261.

Russell E. W. (1961) Soil conditions and plant growth. (9th edition), John Wiley and Sons, New York.

Power J. F. (1990) Fertility management and nutrient cycling. In: *Dry land agriculture, strategies for sustainability. Advances in soil science*, Singh R. P., Parr J. F. and Stewart B. A. (Eds.), Springer Verlag, New York.

Eggoonee I., Schmuterer R. and Ascher K. R. S. (1987) Use of neem in vegetable crop protection in Mauritius. *Proceedings of the 3rd International Neem Conference*, Nairobi, Kenya, 10-15 July, 1986, pp. 419-429.

Online Resources: Always indicate the date that the source was accessed, as online resources are frequently updated or removed.

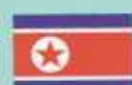
Printing

Print-ready proof is sent electronically. Corrections to proof should be restricted to printer's errors only. The author should take a printout of the proof, mark the corrections legibly in red ink and send by speed post within 3 days of receipt. Alternatively, scanned images of the corrected proof can also be sent via email. If corrections are minimum, it may be indicated page-wise, column-wise and line-wise and intimated to the Chief Editor via email. If there are no corrections, the same may also be intimated to the Chief Editor.

Reprints

Sericologia sends the reprints electronically in the form of PDF to the principal or corresponding author.

The articles should be submitted in duplicate along with the soft copy to the Chief Editor, Sericologia, International Sericultural Commission, I Floor, Central Silk Board Complex, BTM Layout, Madiwala, Bengaluru - 560068, INDIA. Tel: +91 80 26282191, Fax: +91 80 26681663, E-mail: sericologia@gmail.com, www.inserco.org



Sericologia

International Sericultural Commission

CSB Complex

BTM Layout, Madiwala, Bengaluru - 560068

Karnataka, India

Phone: +91-80-26282191

+91-80-26680162

+91-80-26681663

Fax: +91-80-26681663

Email: sericologia@gmail.com

Website: www.inserco.org