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SALT TOLERANCE MECHANISMS IN MULBERRY AND THEIR IMPLICATIONS

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Abstract

Soil salinization and alkalization are major threats to sericulture in arid and semi-arid regions, where water scarcity and inadequate drainage of irrigated lands severely reduce mulberry leaf yield and quality. The salt-affected soil leads to cascades of responses in mulberries at morpho-anatomical, physio-biochemical, and molecular levels due to salt-induced osmotic and ionic stress. Mulberry is a salt-sensitive crop, and understanding salt-stress responses and tolerance mechanisms is important for the development of salt-resilient mulberry for sustained leaf productivity in the future. This review focuses on the response of the mulberry plant to salt stress, particularly its morpho-anatomical and physio-biochemical changes and its adaptation to salt-affected soils through osmoregulation, ion homeostasis, synthesis of antioxidants, and hormonal regulation. Future research is needed on management strategies, such as breeding for salt-tolerant cultivars, application of molecular markers to select salt-tolerant germplasm, and exploration of the potential of genetic transformation for salt resistance.

Key words: Alkalinity, antioxidant enzymes, breeding, mulberry, salinity, salt-tolerant.

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Introduction

Soil salinization and alkalization are widespread environmental problems throughout the world. It has been estimated that about 932.2 million hectares of land are salt-affected worldwide (Shahid *et al.,* 2018). Around 6.727 million hectares of land area in India is salt-affected, of which 2.956 million hectares are saline and the rest, 3.771 million hectares are alkaline (Arora *et al.,* 2016; Arora and Sharma, 2017). Nearly 75 % of salt-affected soils in the country exists in the states of Gujarat (2.23 million ha), Maharashtra (0.61 million ha), West Bengal (0.44 million ha), and Rajasthan (0.38 million ha) (Mandal *et al.,* 2018). Salt-affected soils are classified into three types based on the nature and concentration of salt: (i) saline soils [pH <8.5; ESP <15; ECe (dS m^{-1}) >4; SAR<13], which contain mainly sodium chloride (NaCl)/sulphate ions (Na_2SO_4); (ii) alkaline soils [pH >8.5; ESP >15; ECe (dS m^{-1}) < 4; SAR>13], which contain high amounts of sodium carbonate (Na_2CO_3)/ sodium bicarbonate ions and (iii) saline-sodic soils [pH <8.5; ESP >15; ECe (dS m^{-1}) > 4; SAR>13] that are intermediate between saline and alkaline soils. Saline soils are white in color and mostly found in arid or semi-arid regions where less rainfall and high evaporation rates tend to concentrate the salts in soils, whereas alkaline soils are black in color and have poor permeability to water and air (Szabolcs, 1994). This adversely affects both the physical and nutritional properties of the soil and thus makes the soil very inhospitable for plant growth. Therefore, these kinds of soil conditions and the yield reduction can be overcome in different ways, *viz.,* by scrapping, surface flushing, and leaching, which is the best way of reclamation, or by the farming of improved salt-tolerant crops.

India is the world's second-largest silk producer. In India's rural and semi-urban areas, the sericulture sector employs around 8.8 million people. India is the only country that produces all the five commercial silks, namely mulberry, tropical tasar, oak tasar, eri, and muga, with mulberry silk accounting for roughly 74.03 % of total silk output. In India, sericulture has traditionally been practiced in tropical climates *i.e.,* in the states of Karnataka, Tamil Nadu, Andhra Pradesh, and West Bengal, as well as to a lesser extent, in the temperate area of Jammu and Kashmir (Anonymous, 2022). Mulberry (*Morus* spp.) is an important commercial crop grown extensively as a food plant for the silkworm, *Bombyx mori* L. and forms a prime host plant for the sericulture industry. It is a highly heterozygous, cross-pollinated, deep-rooted, perennial crop that continues to grow and produce its foliage throughout the year. Chiefly, most of the *Morus* species are diploid in nature with 2n=2x=28, but a few have 3x (42), 4x (56), 6x (84), 8x (112), and docosoploidy with 2n=22x=308 chromosomes (Kumara *et al.,* 2021). Mulberry is being exploited by the sericulture, food, beverage, pharmaceutical, and cosmetic industries. It has a broader geological distribution across the continents with the ability to be cultivated in different forms; multiple uses of leaf foliage; and also has positive impact in environmental safety approaches, such as eco-restoration of degraded lands, bioremediation of polluted sites, conservation of water, prevention of soil erosion, and improvement of air quality by carbon sequestering (Rohela *et al.,* 2020). Though, it can be grown efficiently in red loamy soil with a soil pH ranging from 6.5-7.2, increasing exposure to soil salinity (pH >7.5) causes cascades of responses at the morpho-anatomical, physio-biochemical, and molecular levels due to salt-induced osmotic and ionic stress, resulting in decreased growth and production. Salt problems are most pronounced in mulberry gardens in tropical and sub-tropical regions because of insufficient annual rainfall to flush accumulated salts from the mulberry root zone. It is also caused by the combination of high evaporative demand and shallow depth of groundwater, which causes significant salt to be moved to the soil surface and accumulate during evaporation. The main sources of salt in tropical and sub-tropical mulberry gardens are rainfall, mineral weathering, fossil salts, and various bodies of surface and groundwater that redistribute accumulated salts, often as a result of human activities (Bose and Sengupta, 1990).

Abbreviations

ABA = Abscisic acid; CAT = Catalase; CMS = Carboxymethyl starch; Cl- = Chloride ion; Ca2+ = Calcium ion; CO_2 = Carbon dioxide; ECe = Electrical conductivity: ESP = Exchangeable sodium percentage; LEA = Late embryogenesis abundant proteins; GR = Glutathione reductase; MI = *Morus* indigenous; MDA = Malondialdehyde; NR = Nitrate reductase; Na/K = Sodium/Potassium ratio; NO = Nitric oxide; Na+ = Sodium ion; N = Nitrogen; POD = Peroxidase; pH = potential of hydrogen ion; P = Phosphorus; K+ = Potassium ion; ROS = Reactive oxygen species; SOD = Superoxide dismutase; SAR = Sodium adsorption ratio.

The quality and quantity of mulberry leaves produced have a direct bearing on the silkworm cocoon harvest and, in turn, the sericulture farmer's economy. Almost 70 % of the mulberry leaf protein is converted into silk protein through biosynthesis in silkworms. Mulberry leaf protein is the quintessence for the synthesis of sericin and fibroin, the components of silk protein. Thus, mulberry leaf is the central dogma in sericulture, and the increased biomass (leaves) in mulberry varieties is the principal determining factor of higher cocoon yield (Bukhari and Kour, 2019). Thus, there is an immense need for improving mulberry varieties for salt tolerance in terms of nutritive value and increased biomass to ensure profitable cocoon production (Figure 1).

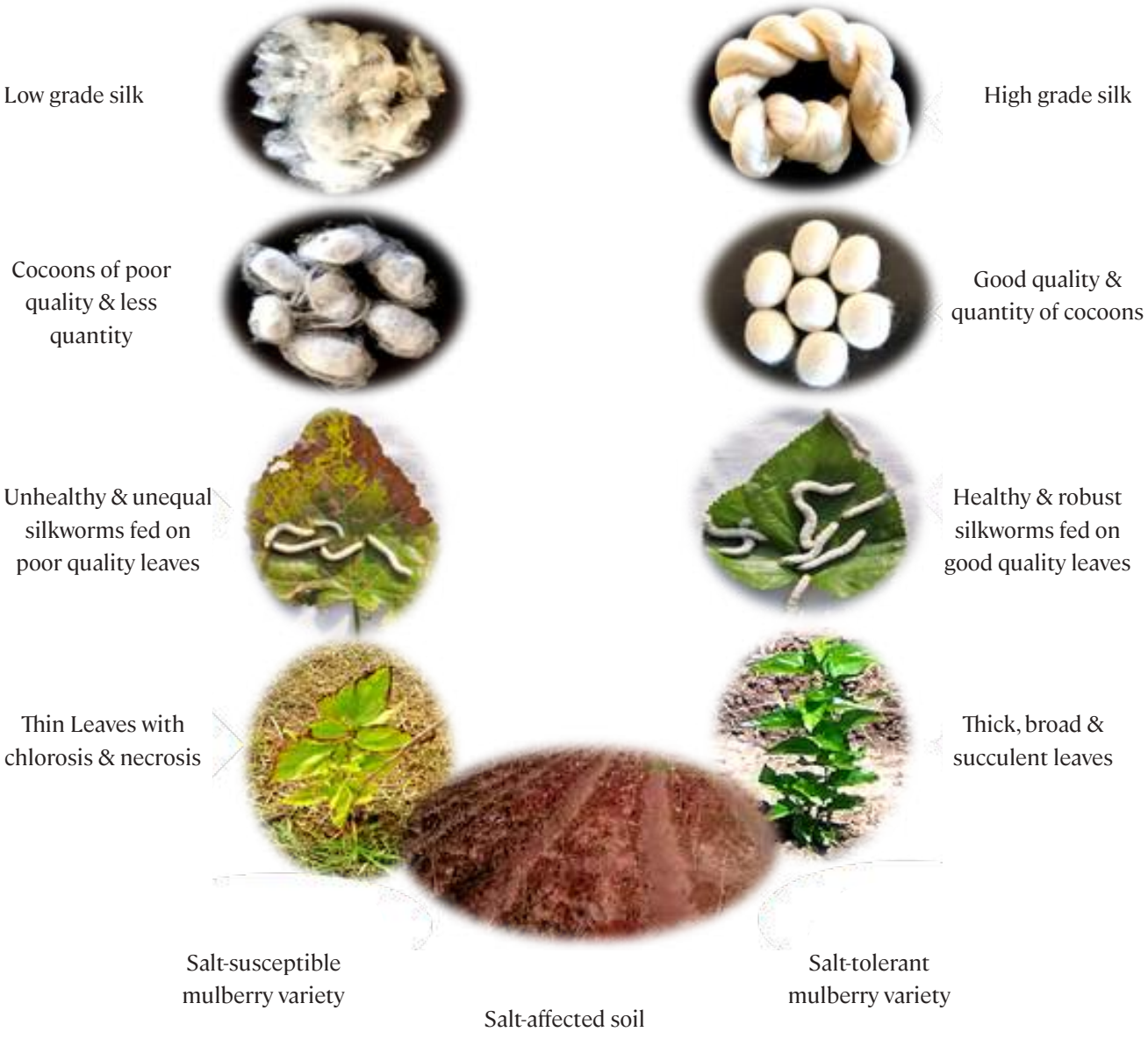

Figure 1: An illustration of the effects of salt stress on salt-tolerant and salt-susceptible mulberry varieties with respect to the sericulture industry

1. Effects of salt stress on mulberry

Salt stress inhibits the normal growth and development of mulberries by modifying their morphological, anatomical, and physiological functions. The adverse effect of salt stress on mulberry plants is generally divided into three categories. First, the presence of salt in the soil solution reduces the ability of the plant to take up water, and this leads to a reduction in the growth rate; this is referred to as the osmotic or water-deficit effects (Prakash *et al.,* 1998). Second, it reduces the nutrient uptake from the soil (Jhansilakshmi *et al.,* 2016), and third, if excessive amounts of salt enter the plant in the transpiration stream, there will be an injury to cells in the transpiring leaves, and this may cause further reduction in growth; this is called the salt-specific or ion-excess effects (Mogili *et al.,* 2002). Osmotic stress causes difficulty in the absorption of water and essential minerals for normal growth and development because the osmotic pressure of the soil solution is greater than that in plant cells. Mulberries are unable to obtain sufficient water and nutrients as a result of salt stress.

a) Morpho-anatomical changes

Morphological symptoms of salt toxicity and nutrient imbalance or deficiency are both present in salt-stressed mulberry plants (Mogili *et al.,* 1998; Jhansilakshmi *et al.,* 2016). Salt toxicity in mulberries occurs when concentrations of salts are imbalanced inside the cell and inhibit cellular metabolism and other processes (Tewary *et al.,* 2000). Toxicity due to increased uptake of Na^+ and Cl^-, may result in a range of symptoms, such as reduced growth, vigor; leaf necrosis; (initially yellow patches that later turn into black necrotic areas), top growth ceasing, and drying of the shoot tip. Furthermore, leaf senescence occurs during the growth stage, followed by defoliation, which eventually causes the mulberry plant to die (Mogili *et al.,* 2002; Jhansilakshmi *et al.,* 2016). Salt stress also induces anatomical changes in the roots, stems, and leaves of mulberry plants. As a result, as salt increases, leaf thickness and area decrease dramatically. A decrease in chlorophyllous palisade tissue and stomatal frequency occurs, all of which are directly related to a decrease in mulberry leaf yield (Vijayan *et al.,* 2008 a). The most important anatomical response to salinity is related to cell wall modification (Ceccoli *et al.,* 2011).

b) Growth and development

The higher concentration of salinity delays the seed germination rates and seedlings' growth in mulberry (Vijayan *et al.,* 2004 a; Vijayan *et al.,* 2004 b; Sun *et al.,* 2009). Most of the researchers opined that plants are the most susceptible to salt during the seedling and early vegetative growth stages (Ayers *et al.,* 1952; Maas and Poss, 1989). These kinds of effects are mainly due to the osmotic changes outside the root, which cause changes in cell-water relations under stress conditions. The severe effects of salt stress on mulberry indicate a direct effect on the meristematic tissue of the growing region of root tips and sprouting buds, which reduces rooting and sprouting abilities (Ramanjulu *et al.,* 1994; Ahmad *et al.,* 2007), via lower water uptake and higher Na^+ and Cl^- ion accumulation (Shaik and Vivekanandan, 1999). Further, shoot growth is reduced along with shoot length, inter-node distance, leaf area, and the number of branches per plant (Agastian and Vivekanandan, 1997; Urs *et al.,* 2011). The inadequate supply of calcium ions, reduce shoot growth, particularly leaf area, more than root growth in mulberry (Hossain *et al.,* 1991). The correlation coefficient between leaf yield and its component traits changes significantly under different salt stresses (Vijayan *et al.,* 2010).

c) Physiological and biochemical changes

Physiological parameters indicate a decrease in water potential, chlorophyll content, photosynthetic, and transpiration rates in mulberries due to salt stress. It induces the synthesis of abscisic acid (ABA), which closes the stomata when transported to guard cells, resulting in a decrease in stomatal conductance. Consequently, rates of transpiration (*i.e.,* water loss) and photosynthesis (CO_2 uptake) are reduced (Lakshmi *et al.,* 1996; Ahmad *et al.,* 2006; Ahmad *et al.,* 2007; Sun *et al.,* 2009). Higher Cl^- concentrations in leaf tissue

may impair photosynthetic function by inhibiting nitrate reductase activity (Xu *et al.,* 2001). Changes in Na_2CO_3 levels are associated with gradual changes in the biosynthesis of chlorophyll and total carotenoid pigments (Agastian *et al.,* 2000). The reduction in photosynthesis is associated with a decrease in total chlorophyll content and distortions in chlorophyll ultrastructures (Kader and Lindberg, 2010).

Salt stress affects membrane integrity, as evidenced by increases in solute leakage (Kumar *et al.,* 2000; Vijayan *et al.,* 2002). Mulberry leaf growth is gradually reduced as the water content in leaves decreases with increasing salinity (Harinasut *et al.,* 2000). Further, it enhances the senescence of old leaves and then transports salt into transpiring leaves, as a result of the very high concentration of Na^+ and Cl- in the tender leaves (Kumar *et al.,* 1999). The higher salt accumulation induces osmotic stress, which is responsible for the oxidative stress caused by reactive oxygen species (ROS). It is involved in a variety of negative reactions, including lipid peroxidation, pigment oxidation, membrane disruption, protein denaturation, and DNA mutation (Sudhakar *et al.,* 2001; Harinasut *et al.,* 2003; Ahmad *et al.,* 2006), and its activity of nitrate reductase (NR) decreases (Ramanjulu *et al.,* 1993). Proline dehydrogenase and proline oxidase enzymes are inhibited (Veeranjaneyulu and Kumar, 1989), and the ability of mulberries to synthesize enzymatic antioxidants may be impaired (Harinasut *et al.,* 2003; Reddy *et al.,* 2004). As a result, protein levels change quantitatively and qualitatively in the mulberry leaves (Liu *et al.,* 2019).

d) Leaf yield and quality

Mulberry leaf is the sole food plant for silkworm larvae; it is ingested and digested by the silkworm for metabolism and to supply all kinds of essential nutrients for its growth and development. Leaf yield in mulberry is a complex trait contributed by many components, such as plant height, the number of branches, leaf length, weight, and water retention capacity (Sahu *et al.,* 1995; Vijayan *et al.,* 1997; Chakraborti *et al.,* 2000). Due to salt stress, the leaf yield, total soluble sugar, protein, free amino acids, lipids, ascorbic acid, and minerals decrease (Agastian and Vivekanadan, 1997). Mulberry leaf quantity and quality have a direct influence on silkworm cocoon production (Hassanein *et al.,* 1972). Mulberry leaf protein can directly affect the growth and development of the silkworm and the combination of silk substances (Fukuda *et al.,* 1959). Carbohydrate is the most important resource for silkworm survival, and while total free amino acids and total sugars are also important components in silkworm nutrients, ascorbic acid acts as a phagostimulant as well as a nutrient for the silkworm (Shamsuddin, 2009). Hence, decreases in the nutritional as well as biochemical components of leaf directly affect the silkworm's growth, development, and cocoon yield.

e) Nutrient absorption

Salt stress reduces multiple mineral elements in the leaves of mulberry (Ramanjulu *et al.,* 1993). Salinity reduces plant nutrient uptake, resulting in nutrient imbalance and deficiency in mulberry (Jhansilakshmi *et al.,* 2016). The high concentration of NaCl acts antagonistically to the uptake and reduces the other nutrients, *viz.,* K^+, Ca^{2+}, N, and P (Jimenez *et al.,* 2003). Ion toxicity occurs mainly due to sodium's (Na^+) and chlorine's (Cl-) presence. Deficiencies of the elements, K and Ca appear to play an important role in the observed growth depression in many saline soils (Finck *et al.,* 1977). Sodium ions at the root surface interfere with potassium ion nutrition in the plant by inhibiting both potassium uptake and enzymatic activation within the cell (Lauchi *et al.,* 1990). Potassium regulates about 50 enzymes and is important for maintaining cell turgor pressure, creating membrane potential, and regulating enzymatic activities (Kadar and Lindberg, 2010). The plant senses salinity through trans-membrane proteins and enzymes, which are the messengers of cytosol and calcium (Zhu *et al.,* 2012).

2. Mechanisms of salt tolerance in mulberry

The basis of salt tolerance

Mulberry salt tolerance can be defined as the ability of mulberry plants to survive and yield leaf under adverse conditions caused by soil salinity and alkalinity. Salt tolerance of mulberry is typically expressed in terms of the decreased leaf yield associated with increased soil salinity or as the relative leaf yield on saline versus non-saline soils (Bose and Sengupta, 1990). Salt resistance is a genetically controlled physiological property of plant species. Resistance to salt is associated with various morpho-anatomical and physio-biochemical traits in mulberries (Vijayan *et al.,* 2008 a). Salt tolerant mulberry is resistant to water stress, osmotic stress, and ion toxicity. The best way to manage salinity problems is to evaluate promising varieties of mulberry for salt tolerance (Hossain *et al.,* 1991; Mogili *et al.,* 1998).

a) Morpho - anatomical basis

Morphological and anatomical characters associated with salt-tolerant mulberry genotypes include shape, size, frequency, and structure of the stomata; thickness and succulence of leaves; rooting pattern; plant growth habit, *etc*. Osmotic stress is the most important cause of the salt effect, and modified sunken, small sized, and fewer stomata are associated with reduced transpiration by the closure of their stomata in response to water deficit, while control of stomatal aperture is important in salt resistance. Furthermore, rapid stomatal closure during the development of water scarcity aids in maintaining a higher water potential in the tissue by reducing transpiration rate and thus avoiding water stress caused by soil salinity (Maksimovic *et al.,* 2010; Batool *et al.,* 2014; Yan *et al.,* 2019). Salt-tolerant mulberry genotypes show increased leaf thickness due to spongy parenchyma cell layers with increased succulence on the dorsal side of the leaf (Vijayan *et al.,* 2008 a). Anatomical changes could compromise the plant's ability to conduct water and nutrients (Ortega *et al.,* 2006) while increasing the mesophyll thickness associated with an increase in leaf thickness, succulence, and specific leaf mass. Increased leaf succulence is one of the most important and noticeable adaptive characteristics in response to increasing salinity (Waisel *et al.,* 1986). There is also evidence that succulence is crucial for the dumping of toxic ions in relatively inert areas like vacuoles (Mimura *et al.,* 2003). The relative increases in leaf succulence as a function of time of stress were higher in tender leaves than in older leaves, while increases in leaf succulence could be considered an important morpho-anatomical adaptation under salt stress (Lauchli and Epstein, 1990). The increase in chlorophyll concentration and leaf thickness under high salinity can be considered as preliminary selection parameters for salt tolerance in mulberry (Vijayan *et al.,* 2008 a). Root proliferation parameters, such as the number of roots per sapling, root length, and root volumes are reported to be associated with salt tolerance, while an increase in the depth, width, and branching of root systems leads to decreases in plant water stress to survive under salinity (Shaik *et al.,* 1999). High genotypic score, survival percentage, sprouting percentage, low leaf necrosis, and high biomass yield under salt stress are indicators of salt-tolerant genotypes (Mogili *et al.,* 1998; Sarkar *et al.,* 2000; Mogili *et al.,* 2002; Jhansilakshmi *et al.,* 2016).

b) Physio-biochemical basis

The plant develops various physiological and biochemical mechanisms in order to survive in soils with high salt concentrations. Principal mechanisms include, but are not limited to, (1) ion homeostasis and compartmentalization, (2) ion transport and uptake, (3) biosynthesis of osmoprotectants and compatible solutes, (4) antioxidant enzyme activation and synthesis of antioxidant compounds, (5) polyamine synthesis, (6) NO generation, and (7) hormone modulation (Agarwal *et al.,* 2013). Under stress conditions, plants need to maintain an internal water potential below that of the soil and maintain turgor and water uptake for growth (Tester and Davenport, 2003). Plant hormones are signal molecules produced in the plant and are found in very low concentrations. Abscisic acid plays a vital role in the response of plants to salt stress (Xu *et al.,* 2001).

Several studies have found that salt stress tolerance in mulberry plants is associated with increased accumulation of key osmoprotectants *viz.,* proline, glycine betaine, phenols, soluble sugars, soluble proteins, free amino acids, sucrose, and starch, as well as up-regulation of antioxidant enzymes, such as SOD, CAT, POD, and GR; these bio-molecules could act as potential biochemical selection criteria for mulberry improvement (Veeranjaneyulu and Kumar, 1989; Kumar *et al.,* 2000; Harinasut *et al.,* 2003; Ahmad *et al.,* 2006). The accumulation of compatible solutes is required for plant osmo-tolerance. Compatible solutes help to stabilize membranes and protect enzymes from denaturation (Ashraf and Foolad, 2007), whereas proline, glycine betaine, and abscisic acid accumulation are quantifiers in the control of salt-stressed mulberry leaves (Kumar *et al.,* 2003).

Salt-tolerant mulberry genotypes exhibit less electrolyte leakage (Kumar *et al.,* 2000; Vijayan *et al.,* 2002), less reduction in net CO_2 assimilation rate, and higher stomatal conductance, as well as high water use efficiency and high water potential (Reddy *et al.,* 2004; Kumari *et al.,* 2007) with a high photosynthetic rate, low transpiration rate, high relative water content (Chackraborti *et al.,* 2010; Ahmad *et al.,* 2014), and the expression (Vijayan *et al.,* 2002) of abundant late embryogenesis proteins (LEA; groups 1, 2, 3, and 4). Salt tolerance shows a comparatively lower Na/K ratio at the respective salinity level in all the plant parts. The lower ratios in the tolerant genotypes indicate selective uptake and transport and preferential portioning of minerals between the organs (Mogili *et al.,* 2002; Ghosh *et al.,* 2006; Vijayan *et al.,* 2009). Identification of promising mulberry genotypes for salt tolerance at the whole plant level using reliable traits, such as Na^+ exclusion and Na^+/K^+ ratios will be of immense value in breeding for salt tolerance (Mogili *et al.,* 2002).

Mogili *et al.* (2002) discovered that mulberry tolerant to salinity and alkalinity has less Na^+ and Cl^- accumulation and less electrolyte leakage, which has been linked to greater membrane integrity under stress conditions, and these are referred to as salt stress-tolerant genotypes. The CMS and MDA content may be considered simple physiological traits to assess the salt-tolerant potential of mulberry genotypes. The salt tolerance is positively correlated with the activity of the antioxidant enzyme and the increases in osmotic stress, which help the mulberry plant to withstand the environmental challenges (Tewary *et al.,* 2000; Harinasut *et al.,* 2003; Ahmad *et al.,* 2007). The hydrogen peroxide content and activities of guaiacol-specific peroxidase, superoxide dismutase, ascorbate peroxidase, and glutathione reductase increased with increasing salinity, while peroxidase activity appears to play an active role in scavenging reactive oxygen species in salt-tolerant mulberry genotypes (Vijayan *et al.,* 2002; Harinasut *et al.,* 2003; Vijayan, 2009).

3. Genetics of salt tolerance

A study on the inheritance of salt tolerance in plants was initiated by Lyon (1941). The genetic constitution of plants is determined by the mode of pollination. Generally, asexually propagated species are highly heterozygous and have a broad genetic base, wide adaptability, and more flexibility. Mulberry is propagated asexually by stem cuttings, while the dioecy and monoecy modes of reproduction are promoted by cross-pollination, which permits new gene combinations from different sources and also allows individuals to have a deleterious recessive gene that is concealed by masking the effect of the dominant gene. In a random mating population, each genotype has an equal chance of mating with all other genotypes. Mulberry has genetic variation for salinity resistance both within and between species. The ratio of general combining ability (GCA) to specific combining ability (SCA) indicated the predominance of non-additive genes in mulberry (Vijayan *et al.,* 2008 b).

Mulberry's phenotypic plasticity also contributed to its ability to grow and survive in harsh environmental conditions (Gray, 1990). Salt tolerance-determining genes can be categorized into two functional groups; the first includes genes that encode effectors, which are responsible for the process that is necessary for stress alleviation or adaptation. The second, consists

of regulatory genes that control the expression and activity of these effectors (Hasegawa *et al.,* 2003). Several studies indicate that salt tolerance is governed by both additive and dominant effects (Flowers and Yeo, 1995).

4. Genetic resources and screening techniques

Mulberry genetic resources represent the basis for sericultural development and a reservoir of genetic adaptability that acts as a buffer against environmental change. India has 1292 mulberry genotypes in its Central Silk Board-Central Sericultural Germplasm Resources Centre (CSGRC) at Hosur (Tamil Nadu State, India), of which 1007 are indigenous and the remaining 285 are exotic collections (CSGRC, 2018). CSGRC has developed screening techniques for salt stress while some genotypes resistant to this stress have been identified. Besides, a systematic programme for screening the available germplasm and breeding material to identify the sources of resistance and their use in a specific breeding programme to evolve high-yielding varieties for the salt-affected area has been well documented. The mulberry genotypes are screened by imposing salinity and alkalinity stresses in micro-plots or pots; the *in vitro* method is followed for screening seeds and cuttings on a small scale; and hotspots of salt-affected areas are used for screening genotypes on large scale, to identify the salt-tolerant ones.

a) *in vivo* (hotspot areas/micro-plots/pot culture) screening

Agastian and Vivekanandan (1997) evaluated twelve mulberry genotypes for saline tolerance under field conditions in the coastal saline area (Vedarannyam, Tamil Nadu). Of which, BC-259 and S-30 were found to have comparatively higher leaf productivity, and the economic characteristics of cocoons were also found to be superior. Das *et al.* (2009) screened fifteen mulberry germplasm accessions in alkaline soils (RSRS, Chamarajanagar, Karnataka). The ranking of germplasm showed that four accessions are superior to S-34 in four parameters, while five accessions are superior to AR-12 in six parameters. Vaijayanthi *et al.* (2019) evaluated forty alkaline stress contrast genotypes of mulberry in two alkaline hotspots (Koppal and Kinakahalli, Karnataka), and four genotypes (MI-0025, MI-0775, MI-0764, and MI-0017) exhibited relatively more tolerance for alkaline stress.

To provide potential parents for the development of salt-tolerant varieties, the diverse germplasm mulberry accessions were screened by CSGRC, Hosur (CSGRC, 2013, 2014) using field screening and the micro-plot technique. The accessions, *viz.,* MI-0476, MI-0242, MI-0129, MI-0245, MI-0161, MI-0763, MI-0716, MI-0310, MI-0145, MI-0497, MI-0499, MI-0027, MI-0139, MI-0764, MI-0437, MI-0376, MI-0327, MI-0670, MI-0657, and MI-0012, were identified as salinity tolerant (EC 8 dS/m) which showed better performance than check C-776. The tolerant accessions continued to grow with very little or no leaf necrosis, resulting in higher biomass and leaf yield/plant. The accessions, *viz.,* MI-0226, MI-0670, MI-0836, MI-0652, MI-0762, MI-0449, MI-0764, MI-0437, MI-0716, MI-0822, MI-0310, MI-0248, MI-0702, MI-0190, MI-0643, MI-0499, MI-0788, and MI-0466, were identified as tolerant to alkalinity at pH 9.0. These accessions out performed the check variety, AR-12.

b) *in vitro* screening

As screening a large number of genotypes in *ex vitro* conditions requires a significant investment and often complicates the assessment due to the interaction of other soil factors, screening *in vitro* is an appealing alternative that is easier, more efficient, and requires less space and time to screen a large number of seeds and genotypes for salt tolerance in mulberry (Vijayan *et al.,* 2004 b). The *in vitro* screening of mulberry through tissue culture and the use of axillary buds and shoot tips is found to be an efficient, rapid, and cost-effective method for the early detection of salt tolerance in mulberry. Sodium chloride, sodium carbonate, and bicarbonate are commonly used *in vitro* for screening salt tolerance (Dennis, 2002). Vijayan *et al.* (2004 b) screened 43 genotypes under NaCl conditions through the seed germination stage; among them, seeds of English Black, Rotundiloba, KPG-3, Mysore local, and Sujanpuri showed considerable tolerance to salinity. Tewary *et al.* (2000) selected the G-4 cultivar as a salt-tolerant genotype using *in vitro* selection, whereas

Jhansilakshmi *et al.* (2010) cultured (nodal explants) the cv. Local and Sujanpuri to investigate alkalinity tolerance.

5. Breeding methods

Most of the mulberry breeders' primary goals are increased leaf yield and resistance to biotic and abiotic stress. This has been accomplished by providing varieties that are basically more productive due to greater physiological efficiencies. Genetic improvement on salt tolerance in mulberry can be achieved by plant introduction, use of wild varieties from the germplasm collection, clonal selection, hybridization, changing the ploidy level, mutation, and biotechnological methods. Plant breeders have used genetic variation in mulberry at intra-specific, inter-specific, and inter-generic levels to produce stress-tolerant lines. This is due to the cross-pollination and the highly heterozygous nature of the parents, so that the progeny exhibits variability immediately on crossing. Such variability promises the success of conventional breeding. They mate freely, as there is no such crossing barrier among the species.

a) Plant (mulberry) introduction

Plant introduction is one of the oldest and the most effective methods of mulberry breeding. The main function of plant introduction is to make available the germplasm that can be utilized in mulberry breeding programmes. In this process, mulberry plants are transferred from their original habitat to a place with different climatic conditions. It may involve new varieties of mulberry or the wild relatives of mulberry species. The S-1 genotype was introduced from Burma (Myanmar), which is a salt-tolerant variety (Dandin and Giridhar, 2014) that is directly used as a variety in eastern and north-eastern India for cultivation under salt-affected soils (primary introduction), while *M. multicaulis* and English Black genotypes were introduced from China, and both of these genotypes were used as salt-tolerant parents in the hybridization programme (secondary introduction) (Tikader and Kamble, 2008).

b) Hybridization

When the desired traits cannot be found in any of the existing mulberry germplasm sources, the breeder creates variation by hybridization. Artificial hybridization is the most widely used method for the breeding of mulberries, and both intra-specific and inter-specific hybridization have yielded valuable results. Female and male parents are selected for crossing. Parental selection is important because it determines the range and nature of the variability in the first generation. The vegetative method is used to propagate desired seedlings after they have been selected (Sung, 2002). In mulberry, a hybrid variety is defined as an F_1 population used for commercial plantings. Miralimov (1979) had some success in developing salt-tolerant hybrids through controlled hybridization by crossing a local cultivar with a salt-resistant genotype. This further confirms the possibility of developing salt-resistant hybrids in mulberries through conventional breeding if appropriate parents are used. Further, the inter-specific transfer of salinity resistance has been attempted in varieties of the Ber, C-776 cultivar (English Black x *M. multicaulis*) (Tikader and Kamble, 2008), while salt-tolerant lines, *viz.,* SR-1, SR-2, and SR-3 genotypes, were developed through a cross between English Black and Ber, C-776 parents (Vijayan *et al.,* 2009).

c) Polyploidy breeding

Mulberry exhibits a high degree of polyploidy, ranging from haploid (2n=14) to docosoploid (2n=22x=308). But most of the *Morus* species are diploid in nature, with 2n=2x=28 chromosomes. The most common method for developing induced polyploidy in mulberry is the use of colchicine (0.1 to 0.4 % aqueous solution) on the buds through a cotton swab or by injecting colchicine solution into the buds (Das *et al.,* 1970). The common effect of polyploidy is to increase the size of the vegetative portion of the plant, making it more vigorous than the corresponding diploids. However, with the increase in ploidy level, the leaf surface

becomes rougher, making it unsuitable for silkworm rearing (Laltanmawii and Roychowdhuri, 2010). Thus, the polyploidy breeding programmes of mulberry are aimed at the evolution of superior triploids, and the optimum level of ploidy is considered to be triploids (2n=3x=42). Generally, triploids are obtained by controlled hybridization between tetraploid and diploid parents. Many triploid varieties of mulberry are considered superior to diploids in yield and nutritive qualities of leaves, cold and disease resistance, and in producing quality cocoons compared to diploids and tetraploids (Vijayan and Chakraborti, 1998). A triploid alkalinity-resistant mulberry cultivar, AR-12, was evolved from cross-pollinated hybrids of S-41 (4x) and Ber. C-776 (2x) in the year 2000 by Central Sericultural Research and Training Institute, Mysore. It is a fast-growing mulberry variety with high rooting ability in alkaline soils. Bushes are slightly spread, medium-branching, and greyish with short internodes. Leaves are unlobed, large, cordate, thick, and dark green with a slightly rough surface. The variety is suitable for alkaline soils with a pH range of 8.0-9.4 and yield potential of about 25 MT/ha/year in alkaline soils under irrigated conditions with the recommended package of practices (Dandin and Giridhar, 2014).

d) Induction of somaclonal variation

Plant tissue culture may generate genetic variability, *i.e.,* somaclonal variations, as a result of gene mutation or changes in epigenetic marks. Somaclonal variation has provided a new and alternative tool to breeders for obtaining genetic variability relatively rapidly and without sophisticated technology in horticultural crops that are either difficult to breed or have a narrow genetic base (Krishna *et al.,* 2016). In mulberry, somaclonal variation has been obtained by culturing the inter-nodal callus of *Morus alba* cv. S-1 (SV-1) in MS medium. Under salt stress conditions, the variants demonstrated increased branching with improved leaf characteristics and yield (Narayan *et al.,* 1989). For the induction of somoclonal variation in mulberries, callus culture is the most reliable option (Dennis, 2002).

e) Genetic engineering

Plant breeding strategies for salt tolerance have not been very successful due to their polygenic nature, reproductive barriers, and the risk of other undesirable traits being transferred. A genetic engineering strategy is preferred because it only deals with the transferred gene(s). Genetic engineering for salt tolerance in mulberries has focused on genes that encode compatible organic solutes, antioxidants, ion transport, signal transduction, and transcription factors. Machii (1989) first isolated DNA from *Morus acidosa* leaves and developed genetically transformed plants by culturing mulberry leaf discs with *Agrobacterium tumefaciens* LBA 4404. Transgenic mulberry plants contain several desired genes and insertion of those genes into the plant genome via *Agrobacterium tumefaciens, A. rhizogenes,* particle bombardment-mediated, and electroporation methods (Lal *et al.,* 2007; Umate *et al.,* 2010) have been developed. Salinity tolerance genes include the AlaBlb gene and the barley HVA1 and group-3 LEA proteins (Shabir *et al.,* 2013; Wang *et al.,* 2018). However, bch1 (b-carotene hydroxylase-1), NHX, and tobacco osmotin genes (Das *et al.,* 2011) showed both salt and drought tolerance in mulberry. Physiological, biochemical, and molecular studies revealed that this transgenic mulberry plant performed much better than the non-transgenic plant when subjected to salinity (200 mMNaCl) and drought (2 % PEG, MW 6000)-induced stresses (Lal *et al.,* 2007).

Conclusion

This article provides a comprehensive review of major research advances in morphological, anatomical, physio-biochemical, genetic, and molecular mechanisms regulating mulberry plant adaptation and tolerance to salt stress. The genetic improvement process in mulberry for salt tolerance is hindered by its long juvenile phase, the dioecious nature of the plant, and the lack of knowledge of genetic linkage between the desirable and weak traits. It is difficult to overcome this situation through the conventional breeding

approach. Thus, taking advantage of the latest advancements in the field of genomics, transcriptomic, proteomic, and metabolomic techniques, mulberry breeders are focusing on the development of a complete profile of genes, proteins, and metabolites responsible for different mechanisms of salt tolerance in different mulberry species. Genetic engineering has been shown to be an effective method for developing salt tolerance in mulberry plants, and this method will become more powerful as more candidate genes associated with salinity tolerance are identified and widely used.

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DEVELOPMENT AND PRACTICAL USE OF A SEX-LIMITED HYBRID "MR" WITH THE NAKED PUPA TRAIT FOR THE RECOMBINANT PROTEIN PRODUCTION IN THE SILKWORM, *BOMBYX MORI*

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Abstract

In recent years, baculovirus-silkworm *(Bombyx mori)* expression system has been widely used for the production of recombinant proteins, such as pharmaceuticals and diagnostic agents. Based on the productivity of the target protein, either the pupae or 5th instar silkworm larvae will be used for production of the recombinant proteins. Nevertheless, it has been pointed out that recombinant proteins produced from female pupae are contaminated with host proteins derived from eggs. In addition, the process also involves a lot of labor to remove the cocoon layer. In order to overcome these challenges, we first bred three new parental varieties "JT", "CT" and "WJT" of silkworms with naked pupa traits *(Nd)*. Next, a practical silkworm Hybrid "MR", that has both traits of naked pupa and sex-limited, which can be differentiated by the larval spot, was created for recombinant protein production by crossing the newly bred variety "WJT" with the traits of both naked pupa and sex-limited and the normal Chinese silkworm variety "WR" with the sex-limited trait.

Key words: *Bombyx mori,* hybrid, naked pupae, recombinant protein, sex-limited.

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Introduction

Maeda *et al.* (1984) for the first time, developed the technique for the production of recombinant protein using the silkworm, *Bombyx mori*. In this process, a recombinant baculovirus is created by replacing the viral polyhedrin gene with 'the gene of the protein to be produced' using recombinant technology, and then this virus is inoculated into the silkworm. The technique facilitates large-scale production of the target protein in the body of the silkworm inoculated with this virus (Maeda *et al.,* 1985; Maeda, 1993; Fukushima *et al.,* 2011).

At the time of development, only a few companies were involved in the commercialization of the technology due to the huge hurdles in introducing the technology for silkworm rearing, *etc.* However, gradually the technology was introduced, mainly by biotechnology companies developing pharmaceuticals and *in vitro* diagnostics (Usami, 2014).

Currently, the 5th instar larvae or pupae of regular silkworm hybrids, such as "Kinshu × Shouwa", which are larger in size, are used in this method. The choice of larvae or pupae for recombinant virus inoculation is determined by the productivity of the recombinant protein intended to be produced. Although larvae aren't considered much of a problem when producing recombinant proteins, it has been suggested that pupae produce recombinant proteins with low purity. This could be due to the female ovaries that grow rapidly and contain large amounts of proteins serving as egg material, and these remain foreign to the recombinant protein recovered from the pupae after inoculation. It has been mentioned that the time required for the process of removing pupae from cocoons accounts for approximately 70 % of the total work, and labor-saving in this process is also an important issue leading to improved productivity of recombinant protein and lower production costs. Possible solutions to overcome these problems include ① using only male pupae, which contain fewer foreign proteins than females, and ② facilitating the pupal retrieval process by utilizing the trait of inability to spit out threads. The authors, therefore, considered that it is essential to develop practical silkworm varieties that have both the sex-limited trait, as well as naked pupa trait.

To develop practical silkworm hybrids with the sex-limited trait, it is necessary to make the cross combination, such as "♀ (a silkworm variety with the sex-limited trait) × ♂ (a silkworm variety with the sex-limited trait)" or "♀ (a silkworm variety with the sex-limited trait) × ♂ (a silkworm variety without the spot trait)". At present, one Japanese and five Chinese silkworm varieties with the sex-limited trait are owned by the Institute of Sericulture and Silk Science, Ibaraki, Japan.

In this study, the Japanese variety "JT" without the spot trait, the Chinese variety "CT" without the spot trait, and the Japanese variety "WJT" with the sex-limited trait, which have dominant naked pupa traits, were newly bred with the purpose of expanding the selection range of combinations between the above six varieties with the sex-limited trait and varieties with the naked pupa trait. This experiment aimed at development of a hybrid combination with the traits of both naked pupa and sex-limited, utilizing the above varieties for recombinant protein production.

Materials and Methods

The silkworm variety "T" for breeding material and its characteristics

The silkworm variety "T" was provided by Sysmex Corporation (https://www.sysmex.co.jp) as the breeding material for the new parent varieties with the naked pupa trait. It is a successor of the variety (Yamazaki *et al.,* 1997) that has been preserved for many years at the Institute of Biological Sciences, Katakura Industries Ltd, and is said to have derived from the variety with the strong naked pupa trait *(Nd)* (Ariga *et al.,* 1951; Nakano, 1951), but its history is somewhat unclear. This variety has larval spots, and there were reports that the percentage of silkworms showing complete naked pupal morphology without cocoon layers in mulberry leaf-fed rearing was around 60 % (the emergence rate of complete naked pupae). Therefore, before using this variety as breeding material, we investigated the cocooning condition of the variety by rearing it for all larval stages on an artificial diet 'Silkmate S for Juvenile silkworms of the original strains' manufactured by Nosan Corporation (https://www.nosan.co.jp). To confirm the mode of inheritance of the naked pupa trait of the variety "T", F_1 and BF_1 crosses were performed between this variety and the Chinese silkworm variety "TC8" that forms normal cocoons with thick cocoon layers owned by our institute, and the situation of the emergence of normal and non-normal cocoons (cocoons with thin-layers or completely naked pupae) was investigated (Table 1).

All larvae (1,342) hatched from the provided layings of the variety "T" were reared, and after excluding larvae with poor feeding on the artificial diet, 650 mature larvae were randomly selected and transferred to cocoon frames.

While investigating the cocooning condition, individuals of the silkworm that formed normal cocoons were not considered, but many incompletely exuviated pupae (Figure 1A) were observed. On the other hand, there was a large difference in the thickness of cocoon layer of individuals that formed thin cocoon layers.

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Figure 1: Morphological abnormalities in the pupae of silkworm varieties with the naked pupa trait

A. Incompletely exuviated pupae.
B. Morphological abnormalities of the abdomen (a) and head (b) sites in the pupae.

The segregation pattern of normal and non-normal cocoons in the F_1 and the BF_1 generations showed that all silkworms in the F_1 generation did not make normal cocoons, and in the BF_1 generation, in which females of the F_1 generation were crossed with males of the silkworm variety "TC8", the separation ratio of normal to non-normal cocoons was almost 1:1 (χ2-test, $p>0.05$).

The variety "T" with the naked pupa trait was crossed with the normal varieties owned by Institute of Sericulture and Silk Science, The Dainippon Silk Foundation, namely the Japanese variety "WJ" with

Table 1: Performance of F_1 and BF_1 hybrids between the silkworm variety "T" with the naked pupa trait and the normal Chinese silkworm variety "TC8" with the thick cocoon layer

Crossing [1] (♀×♂)	Number of larvae in the 4th instar [2]	Number of mounted larvae [3]	Healthy pupae		Non-healthy pupae		Percentage of normal cocoons relative to the number of healthy pupae	χ^2-test in BF_1 genetation (1:1)[5]
			Number of normal cocoons	Total number of both thin cocoons and naked pupae[4]	Number of incompletely exuviated pupae	Total number of both pre-pupal larvae and dead pupae		
T	-	650	0	609[6]	37	4	0	-
TC8	480	459	442	0	3	14	100	-
T×TC8 (F_1)	480	468	0	422	28	18	0	-
TC8×T (F_1)	480	474	0	442	25	7	0	-
(TC8×T)×T (BF_1)	480	452	0	398	41	13	0	-
(TC8×T)×TC8 (BF_1)	480	449	217	193	23	16	52.9	p=0.236>0.05

[1] Each parent silkworm variety and each crossing in all stages were reared on an artificial diet 'Silkmate S for Juvenile silkworms of the original strains' manufactured by Nosan Corporation. The amount of artificial diet fed was 11.0 g/larva for the parent silkworm variety "T", 18.0 g/larva for the large parent silkworm variety "TC8", and 15.0 g/larva for each crossing during the 5th instar stage.

[2] The number of larvae was adjusted on 24 hours after the beginning of rearing in the 4th instar.

[3] Both the larvae of poor growth due to poor feeding on the artificial diet and non-exuviated larvae in the 5th instar stage were excluded, and only well-developed larvae were transferred to the cocooning frames.

[4] Most pupae were healthy, but some pupae with minor morphological abnormalities in areas, such as the thorax and head were included.

[5] The separation ratio of normal cocoons to non-normal cocoons in the BF_1 generation. Observed significance level of χ^2- test, (1:1 ; p= 0.05) .

[6] The number shows the total number of cocoons (494) with thin layers, cocoons (36) with ultra-thin layers, and naked pupae (79).

Visual sorting revealed that the percentage of pupae with the thin cocoon layer to the number of healthy pupae (609 pupae) was 79.2 (494 pupae), the percentage of pupae with ultra-thin cocoon layer was 7.8 (36 pupae), and the percentage of completely naked pupae that did not form any cocoon layer was 13 (79 pupae).

the sex-limited trait, and the Chinese silkworm variety "C" without the spot trait. From these crossed progenies, the Japanese variety "JT" without the spot trait, the Chinese variety "CT" without the spot trait, and the Japanese variety "WJT" with the sex-limiting trait, which have the dominant naked pupa trait, were allowed for breeding (Figures 2 and 3).

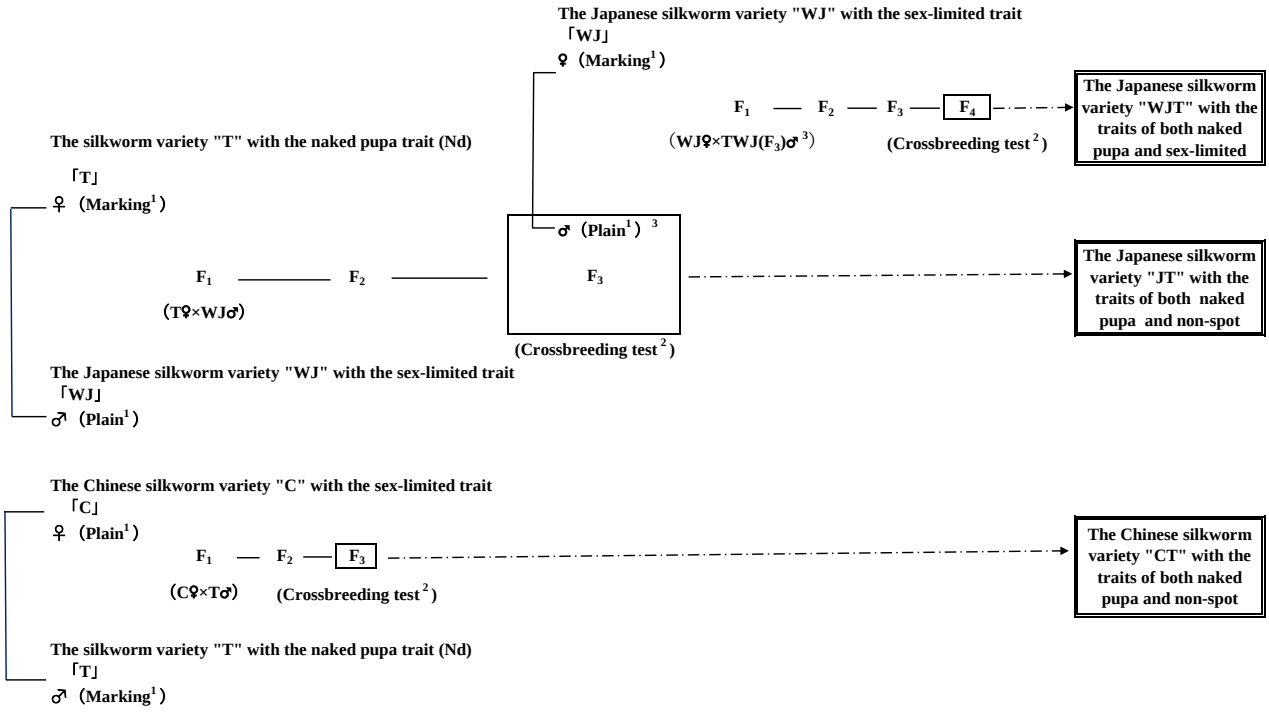

Figure 2: Pedigree of the parent silkworm varieties with the naked pupae trait

[1]"Marking" indicates silkworms with spots, while "Plain" indicates silkworms without spots;

[2]Crossbreeding test to confirm homozygosity of the naked pupal gene;

[3]The males without spots of the F_3 generation of the crosses between the variety "T" and the variety "WJ" were used in the crosses.

A B C

Figure 3: Newly bred silkworm varieties with the naked pupa trait on 5th day in the 5th instar stage

A. The Japanese silkworm variety "JT" without the spot trait (marking);

B. The Chinese silkworm variety "CT" without the spot trait (marking); C The Japanese silkworm variety "WJT" with the sex-limited trait:

a. A female larva with spots; b. A male larva without spots.

During spring to late autumn, the 1st to 3rd instar silkworms of each generation were reared on the artificial diet 'Silkmate S for Juvenile silkworms of the original strains' manufactured by Nosan Corporation, while the 4th to the 5th instar silkworms were reared on mulberry leaves. In the winter and early spring when mulberry leaves were not available, the silkworms were reared wholly on artificial diet throughout the larval stages.

Cross-breeding test for verification

To confirm homozygosity for the naked pupa gene, a cross test (Kuroda, 1995) was performed on the F_3 generation of varieties "CT" and "JT" during the early winter rearing period (December 2014). For the variety "WJT", this was done in the F_4 generation during the early spring rearing period (February 2016). The testing methods followed the previously reported methods used in the breeding of "Ryokken 1 gou" (Iida *et al.*, 2007) and "Ryokken 2 gou" (Iida *et al.*, 2018).

Initially, 40 layings (a group of eggs laid by a single female moth) were selected for the variety "JT", 47 layings for the variety "CT" and 31 layings for the variety "WJT", which were

at the intermediate stage of breeding, based on egg-laying ability. Each laying was divided into two and one of them was kept as a successor of the laying to be selected based on the results of the verification cross tests. Each group of the larvae hatched from each of the other halved layings was reared and checked to see if any individuals made normal cocoons on each group after mounting. Only the groups, in which individuals did not make normal cocoons, were subjected to a verification cross test. The test was conducted on each group of larvae hatched from each of 16 layings of the variety "JT", 20 layings of the variety "CT", and 12 layings of the variety "WJT". In each test group, 12 randomly selected male moths emerging from the group were mated with 12 female moths emerging from the variety that forms normal cocoons, or with the sexes reversed. In the F_1 generation, 1/4 of each laying was cut from 12 layings and these 12 segments were pasted on one sheet. The larvae hatched from the 12 segmented layings on the sheet were reared. The number of larvae was adjusted on 24 hours after the beginning of rearing in the 4th instar stage, to 480 larvae for both the varieties, "JT" and "CT", and 250 larvae of each sex for the variety "WJT" with the sex-limited trait per group. After their mounting, the presence of normal cocoons in each group was examined to determine the homozygosity of the naked pupa gene in the test laying in the F_3 or the F_4 generation of each variety.

New parental silkworm varieties with the naked pupa trait and their characteristics

1) Breeding process and characteristics of the Japanese silkworm variety, "JT" with traits of both naked pupa and non-spot (Figures 2 and 3A).

To breed the Japanese silkworm variety with traits of both naked pupa and non-spots, a female of the silkworm variety "T" with the traits of both naked pupa and spot was crossed with a male without a larval spot, of the Japanese silkworm variety 'WT' with the sex-limited trait during the spring rearing period (May 2014) (Figure 2). Subsequently, in the F_2 generation, only individuals, that did not have a larval spot and did not form normal cocoons, were selected and crossed. In the F_3 generation, 40 layings were selected based on egg-laying ability, and each laying was divided into two, one of which was used for the verification cross test to confirm the homozygosity of the naked pupa gene. Each group of larvae hatched from each of the 40 halved layings was reared and mounted during the winter rearing period (November 2014), and the results confirmed no normal cocoons in 16 groups. And to confirm the homozygosity of the naked pupa gene, moths emerging from each of the 16 groups were crossed with moths emerging from the normal silkworm variety "BNg" with a thick cocoon layer. During the subsequent spring rearing period (May 2015), each group of larvae hatched from each of the 16 test layings made by crossing with the variety "BNg" and each group of larvae hatched from each of the 16 layings in the F_3 generation, that had been divided into two and reserved as successors, were reared simultaneously. After mounting, the absence of normal cocoons in only one group confirmed the homozygosity of the naked pupa gene, so the F_4 generation eggs were produced by sib mating using moths from the group of larvae hatched from the halved laying that had been preserved as a successor of the F_3 generation used in this selected group. This strain was passed down for two successive generations and named "JT" as the prototype of the Japanese silkworm variety with traits of both naked pupa and non-spots. Since the next generation, each group of larvae hatched from each of 2 to 4 layings was reared during the spring, summer, and autumn rearing periods. Then, in each season, either group selection based on pupal size and the high proportion of both complete naked pupae and healthy pupae, or individual selection from each group based on complete naked pupae, pupal health, and pupal size was carried out, and this strain was passed on to the next generation.

The variety "JT" can be reared for all larval stages on the artificial diet 'Silkmate S for Juvenile silkworms of the original strains' manufactured by Nosan Corporation. The color of hibernating eggs is dark gray with greenish tint, and that of newly hatched larvae is dark brown. The 5th instar larvae are pale blue and have a normal body shape without a larval spot (Figure 3A). The pupae are either completely naked or have very thin sericin cocoon layers that can be torn off by hand (Figure 4).

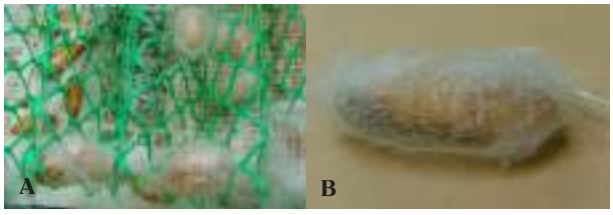

Figure 4: Observation of cocooning on the 7th day after mounting in the silkworm varieties withthe naked pupa trait

A. Naked pupae and pupae with thin cocoon layers on the cocooning frame. B. A pupa with a thin cocoon layer.

2) Breeding process and characteristics of the Chinese silkworm variety"CT"with traits of both naked pupaand non-spots (Figures 2 and 3B).

To breed the Chinese silkworm variety with traits of both naked pupa and non-spots, a female of the Chinese silkworm variety "C" without the spot trait was crossed with a male of the silkworm variety "T" with the traits of both naked pupa and spot, during the spring rearing period (May 2014) (Figure 2). Similar to the breeding method of the variety "JT", in the F_2 generation, only individuals, that did not have a larval spot and did not form normal cocoons, were selected and these were crossed. In the F_3 generation, 47 layings were selected based on egg-laying ability, and each laying was divided into two, one of which was used for the verification of cross tests to confirm the homozygosity of the naked pupa gene. Each group of the larvae hatched from each of the 47 halved layings was reared and mounted during the winter rearing period (November 2014), and the results confirmed no normal cocoons in 20 groups. And to confirm the homozygosity of the naked pupa gene, moths emerged from each of the 20 groups were crossed with moths emerged from the normal silkworm variety "BCg" with a thick cocoon layer, for each group. During the early spring rearing period (February 2015), each group of larvae hatched from each of the 20 test layings made by crossing with the variety "BCg" was reared. After mounting, the homozygosity for the naked pupa gene was confirmed in 2 groups by the absence of normal cocoons in each group. During the spring rearing period (May 2015), each group of larvae hatched from each of 2 halved layings (preserved as successors of the F_3 generation), which were selected based on the results of rearing tests conducted during the previous rearing period to confirm genetic homozygosity, was reared. After mounting, one group was selected based on the high proportion of both completely naked pupae and pupae with ultra-thin cocoon layer within the group, and F_4 generation eggs were produced by sib mating. This strain was passed down for two successive generations and named "CT" as the prototype of the Chinese silkworm variety with traits of both naked pupa and non-spots. Since the next generation, each group of larvae hatched from each of the 2 to 4 layings was reared during the spring, summer, and autumn rearing periods. Then, in each season, either group selection based on large pupae and the high proportion of both complete naked pupae and healthy pupae, or individual selection from each group based on complete naked pupae, pupal health, and pupal size was carried out, and this strain was passed on to the next generation. The variety "CT" can be reared throughout the larval stages on the artificial diet 'Silkmate S for Juvenile silkworms of the original strains' manufactured by Nosan Corporation. The color of hibernating eggs is wisteria gray, and that of newly hatched larvae is blackish brown. The 5th instar larvae are pale blue in color and have a rather thick body without larval spots (Figure 2B). The pupae are either completely naked or have very thin sericin cocoon layers that can be torn off by hand (Figure 4).

3) Breeding process and characteristics of the Japanese silkworm variety "WJT" with the traits of both naked pupa and sex-limited (Figures 2 and 3C).

During the spring rearing period (May 2015), to breed the Japanese variety with the traits of both naked pupa and sex-limited, a female of the Japanese silkworm variety "WJ" with sex-limited trait was crossed with a male from the F_3 generation of the crosses between the variety "T" and the variety "WJ", for which the homozygosity of the naked pupa gene, was confirmed (Figure 2). That is, a male moth from a group of larvae hatched from one halved laying, that was preserved as successors to the F_3 generation of the silkworm variety "JT" and confirmed homozygous for the naked pupa gene, was used for this cross (Figure 2). In the F_2 and the F_3 generations, only individuals that were completely naked pupae or had ultra-thin cocoon layers were selected (both female and male pupae), and crossed. In the F_4 generation, 31 layings were selected based on egg-laying ability, and each laying was divided into two, one of which was used for the verification cross test to confirm the homozygosity of the naked pupa gene.

Each group of the larvae hatched from each of the 31 halved layings was reared and mounted during the early spring rearing period (February 2016), and the results confirmed no normal cocoons in 12 groups. And to confirm the homozygosity of the naked pupa gene, moths emerging from each of the 12 groups were crossed with moths emerging from the normal silkworm variety "BNg" with a thick cocoon layer, for each group. During the spring rearing period (May 2016), each group of larvae hatched from each of the 12 test layings made by crossing with the variety "BNg", was reared. After mounting, the homozygosity for the naked pupa gene was confirmed in 11 groups by the absence of normal cocoons in each group. During the summer rearing period (July 2016), each group of larvae

hatched from each of the 11 halved layings (preserved as successors of the F_4 generation), which were selected based on the results of rearing tests conducted during the previous rearing period to confirm genetic homozygosity, was reared. After mounting, one group was selected based on the high proportion of both completely naked pupae and pupae with ultra-thin cocoon layer within the group, and the F_5 generation of eggs were produced by sib mating. This strain was passed down for three successive generations and named "WJT" as the prototype of the Japanese variety with traits of both naked pupa and sex-limited. Since the next generation, each group of larvae hatched from 4 to 6 layings was reared during the spring, summer, and autumn rearing periods. Then, in each season, either group selection based on large pupae and the high proportion of both complete naked pupae and healthy pupae, or individual selection from each group based on complete naked pupae, pupal health, and pupal size was carried out, and this strain was passed on to the next generation. The variety "WJT" can be reared for all larval stages on the artificial diet 'Silkmate S for Juvenile silkworms of the original strains' manufactured by Nosan Corporation. The color of hibernating eggs is light gray with greenish tint and that of newly hatched larvae is dark brown. In the 4th and the 5th instar larvae, the sexes can be easily distinguished by the presence / absence of spots in female and male larvae, respectively (Figure 3C). The 5th instar larvae are pale blue and have a normal body shape. The pupae are either completely naked or have very thin sericin cocoon layers that can be torn off by hand (Figure 4).

Development of silkworm hybrids and investigation of their characteristics

Eleven hybrids with both the traits of naked pupae and sex-limited were created by crossing the newly developed naked pupal varieties "JT", "CT" and "WJT" with normal varieties without the naked pupa trait,

Table 2: The characteristics of silkworm hybrids with the naked pupa trait

Hybrid[1]		Hatchability % (Mean ± s.d.) (n=10)	Number of larvae at the beginning of rearing in the 1st instar stage	Percentage of grown larvae in the 1st instar stage[2]	Percentage of molted larvae in the 4th instar stage[3]	Survival rate in the 4th instar stage[4] (%)	Number of larvae in the 4th instar stage[5]	Feeding amount of artificial diets in the 5th instar stage(g/larva)	Duration (day . hour) 5th instar stage	Duration (day . hour) total	Number of mounted larvae	Pupation rate[6] (%)	Pupal weight[7] (g/pupa)	Concentration of hTF protein[8] μg/g (male pupae)
Kinshu × Shouwa[9]	♀	98.4±0.7	382	100	-	-	300	13.2	7・04	24・01	298	96.3	2.31	-
	♂												1.69	269
WC3×JT	♀	98.1±0.9	1130	100	95.8	96.7	150	8.0	6・00	23・12	149	100	1.72	-
	♂						250	7.2	6・00	23・07	250	99.2	1.35	329
WR×JT	♀	98.9±0.6	1020	100	95.5	97.4	150	8.0	6・00	22・00	150	100	1.73	-
	♂						250	7.2	6・00	21・12	250	97.2	1.47	333
WJT×WC3	♀	94.7±2.5	1096	99.8	91.9	95.9	150	8.0	6・00	24・00	150	100	1.63	-
	♂						250	7.2	6・00	23・12	250	99.6	1.31	315
WJT×WR	♀	95.4±2.7	1004	100	95.0	97.3	150	8.0	6・00	22・00	150	100	1.77	-
	♂						250	7.2	6・00	21・12	250	97.6	1.48	324
WJ7×CT	♀	96.2±3.3	1111	100	94.0	97.4	150	8.0	6・00	23・22	149	99.3	1.93	-
	♂						250	7.2	5・12	22・22	248	98.0	1.47	278

[1]Each test hybrid in all stages was reared on an artificial diet 'Artificial diet for Juvenile silkworms of the hybrids' manufactured by Sysmex Corporation.

[2]Percentage of grown larvae in 24 hours after the beginning of rearing in the 1st instar.

[3]Percentage of larvae that have completed their 3rd molt and entered the 4th instar.

[4]The total survival rate of the 4th instar larvae and those that did not grow to the 4th instar stage relative to the total number of larvae at the beginning of rearing in the 1st instar was investigated at 24 hours of the 4th instar.

[5]The number of larvae in each hybrid was adjusted on 24 hours after the beginning of rearing in the 4th stage, 150 and 250 larvae of average size were selected for female and male, respectively.

[6]The pupation rate is the ratio of the number of healthy pupae to the number of mounted larvae.

[7]The weight of 100 pupae was examined by sex and the average value of each was calculated.

[8]To evaluate the productivity of the recombinant protein hTF, 100 male pupae were tested for each hybrid.

[9]"Kinshu × Shouwa" was tested as a control with males and females reared together.

owned by our Institute. The production tests on recombinant proteins in each of the hybrids were carried out by Sysmex Corporation, and 5 hybrids (Table 2) were selected based on a quantitative evaluation of recombinant protein human tissue factor (hTF), one of the essential proteins involved in human blood coagulation by Enzyme-Linked Immunosorbent Assay (ELISA). To investigate the characteristics of each of the selected 5 hybrids, they were reared for all larval stages on the artificial diet 'Artificial diet for Juvenile silkworms of the hybrids' manufactured by Sysmex Corporation, in a clean room in the Institute of Sericultural Science and Technology. The number of larvae for each hybrid was adjusted on completion of 24 hours of the 4th instar; 150 and 250 medium-size larvae were selected for female and male, respectively. The surviving larvae were sorted into the 4th instar larvae and those that did not grow to the 4th instar stage, *i.e.,* the 4th instar and the non-4th instar groups. Each group was counted, and the percentage of the 4th instar larvae (including the number of the 4th instar larvae selected for the test) to the number of larvae at the beginning of rearing in the 1st instar stage, and the total survival rate relative to the number of larvae at the beginning of rearing in the 1st stage were examined. After adjustment for rearing numbers in the 4th instar larvae, the larvae were reared separately for males and females, and the feeding amount of each sex was 2.7 g/larva for females and 2.2 g/larva for males. Based on the preliminary tests, the feeding amount of each test hybrid during the 5th instar stage was fixed at 8.0 g/larva for females and 7.2 g/larva for males.

As the purpose of this study was to utilize pupae and since a few larvae died in the larval stage after adjustment for the number of the 4th instar larvae in all test hybrids, the pupation rate in respect of the number of larvae mounted was examined. One hundred pupae of each sex were also weighed. In addition, for each hybrid, 100 male pupae were used to evaluate the productivity of the recombinant protein hTF.

Results

The characteristics / preferences of silkworm hybrids with sex-limited and naked pupa traits, developed with the purpose of producing recombinant proteins are depicted in Table 2 and Figure 5.

A

B

Figure 5: The practical silkworm Hybrid "MR" with the traits of both naked pupae and sex-limited that were developed for the production of recombinant proteins

A. Larvae on the 4th day in the 5th stage: a. A female larva with spots; b. A male larva without spots; B. Male pupae on the 6th day after mounting.

Initially, five silkworm hybrids with traits of both naked pupa and sex-limited were selected from 11 hybrids created, based on the evaluation of the productivity of recombinant protein hTF (human tissue factor) in pupae. That is, ①"WC3 (the female of the Chinese silkworm variety with the sex-limited trait) × JT (the male of the Japanese silkworm variety with the traits of both naked pupa and non-spot)", ②"WR (the female of the Chinese silkworm variety with the sex-limited trait) × JT, ③"WJT (the female of the Japanese silkworm variety with the traits of both naked pupa and sex-limited) × WC3, ④"WJT × WR", and ⑤"WJ7 (the female of the Japanese silkworm variety with the sex-limited trait) × CT (the male of the Chinese variety with the traits of both naked pupa and non-spot)" were selected, and examined for their characteristics (Table 2).

The hatchability of the hybrids of "WC3 × JT" and "WR × JT" cross, both of which use the Chinese varieties as their mother parents, were higher than those hybrids of "WJ7× CT", "WJT × WC3", and "WJT × WR", where all of

them used Japanese varieties as their mother parents. The growth rates examined at 24 hours after the beginning of the feeding in the 1st instar stage with the artificial diet for all hybrids in this study were found to be very high. The number of both the 4th instar larvae and surviving larvae that did not grow to the 4th instar stage were examined and the results showed that both the percentage of the of larvae reaching the 4th instar and the total survival rate in "WJT × WC3" were slightly lower than those of other hybrids, but for the total duration of larval stage, both sexes in the hybrid took maximum number of days among the tested hybrids.

The pupation rate in relation to the number of mounted silkworms in each sex was estimated. The results showed that the pupation rate of females was more than 99 % in all hybrids, which is a good trend. But, in males, though it was lower than that of females in all hybrids, the rates exceeded 97 %, indicating that the pupation rates of these five hybrids were fairly good at practical level. Comparisons among the hybrids showed that the hybrids with the component variety,"WC3" had a slightly higher rate than those with "WR". The average pupal weight per 100 pupae tended to be slightly more for both males and females in the hybrids with the variety "WR" than "WC3". Under the test rearing conditions, the female pupae of the hybrid "WJ7 × CT" was the heaviest among the tested hybrids at 1.93 g, followed by "WJT × WR (1.77 g)", "WR × JT (1.73 g)","WC3 × JT (1.72 g)", and "WJT × WC3 (1.63 g)". On the other hand, the hybrid "WJT ×WR" had the heaviest male pupae at 1.48 g, followed by "WJ7 × CT (1.47 g)","WR × JT (1.47 g)","WC3 × JT (1.35 g)", and "WJT × WC3 (1.31 g)". In addition, the productivity of recombinant protein human tissue factor (hTF) in male pupae was also examined by testing 100 male pupae of each hybrid, and the results showed that the productivity of hTF in each hybrid was different, but all test hybrids were more productive than the control, "Kinshu × Shouwa". The highest hTF production per gram of male pupae was found in "WR × JT" with 333 µg, followed by "WC3 × JT (329 µg)","WJT × WR (324 µg)","WJT × WC3 (315 µg)" and "WJ7 × CT (278 µg)".

Regarding the condition of each hybrid after mounting, differences were observed in the percentage of completely naked pupae and the thickness of the cocoon layer formed by each combination, but none of the hybrids formed normal cocoons, and even when a cocoon layer was present, it was thin and could be easily torn off with a fingertip. Based on the above results, "WJT×WR", was selected as the hybrid for recombinant protein production using only males because the males were heavier and more hTF productive, and furthermore, this mating combination was more efficient in egg production through reversible mating.

The hybrid "WR×WJT" was produced by reversing the crossbreeding form, and its feeding habit on an artificial diet, pupation rate, pupal weight, and other characteristics were investigated, and the productivity of the recombinant protein, hTF in the male pupae was evaluated. The results were comparable to those of the hybrid "WJT × WR" (unpublished), so the hybrid "WJT × WR" with the traits of both naked pupa and sex-limited was named "MR" as the practical silkworm hybrid (Figure5).

The characteristics of the hybrid "MR" are as follows. The hybrid can be reared for all larval stages on artificial diets, which were developed for rearing crossbred silkworms, such as 'Artificial diet for Juvenile silkworms of the hybrids (for the 2nd instar silkworms)' and 'Artificial diet B for Juvenile silkworms of the hybrid' manufactured by Sysmex Corporation as well as the artificial diet 'Silkmate 2S' manufactured by Nosan Industry Co. However, when the 5th instar silkworms of both sexes were reared on the same amount of feed as the normal hybrid "Kinshu × Shouwa", unhealthy pupae such as incompletely exuviated pupae, malformed pupae, and even larval mortality occurred at a significant level (unpublished). As a counter measure, limited feeding is an effective way to obtain a practical level of pupation rate. The color of the hibernating eggs is light gray with greenish tint, although there is some variation in the color of the eggs depending on the maternal variety used. The color of newly hatched larvae is dark brown. In the 4th and 5th instar larvae, the sexes can be easily distinguished by the trait of larval spots. Females of the 5th instar larvae are pale blue, with rather thick body and spots, while males are pale blue, with rather narrow body and no spots (Figure 5A). Some males and females form completely naked pupae, and others form a very thin sericin cocoon layer that can be torn off by hand (Figure 5B). The proportion of individuals with the thin cocoon layer varies depending on the composition of the artificial diet fed to them. The percentage of individuals with this thin cocoon layer was approximately 76 % when reared for all stages on an artificial diet

'Artificial diet for Juvenile silkworms of the hybrids (for the 2nd instar silkworms)' and approximately 88 % when reared on an artificial diet 'Silkmate 2S'.

Discussion

In this study, by using the silkworm variety "T" with dominant naked pupa trait as the breeding material, three new varieties *viz.,* the Japanese varieties, "JT" and "WJT" and the Chinese variety "CT" which have dominant naked pupa traits, were bred, and furthermore, the practical silkworm hybrid "MR" with both the naked pupa trait and the sex-limited trait was developed for the recombinant protein production.

Naked pupae are individuals of the silkworms that pupate without making cocoons, and there are two major reasons for its occurrence: ① degeneration of the silk glands due to mutation and ② acquired abnormalities in the silk glands. A strain with strong naked pupa trait *(Nd)* was isolated from "Ou 16 gou (European No. 16) " and the naked pupa is caused in this case by an abnormality in the development of the silk gland cells, *i.e.,* by an irregular shape of the silk gland cells with the abnormal secretion of silk materials (silk thread component) (Ariga *et al.,* 1951; Nakano, 1951). This strain is characterized by a mixture of individuals in which a few are with their middle and posterior silk glands degenerated, resulting in naked pupae due to the inability to release silk material, and individuals that release a small amount of sericin to form a thin cocoon layer, however, subsequent moth development and egg-laying are normal, and the genetic traits are completely dominant. Furthermore, it has been considered that the reason why this strain pupates normally without excessive nitrogen, even though the amount of nitrogen excreted as cocoon silk is low, is that the amount of uric acid excreted is higher than that of normal silkworms (Kobayashi *et al.,* 1980).

In contrast, another strain with weak naked pupa trait *(Ndb)* of only about 50 % expression rate was isolated from "Nichi-111". This strain is reported to be prone to silk gland abnormalities caused by adverse environmental factors, such as certain strong odors, resulting in naked pupae, which all die before the moths emerge because the silk glands remain without decomposition in the pupae. As mentioned above, the silkworm variety "T", which is believed to be derived from the strain with strong naked pupa *(Nd),* was used as breeding material for the three new varieties with the naked pupa trait, *viz.,* "JT", "CT", and "WJT". In the breeding process of all the varieties, there were some degree of mortality in the juvenile stage, while many incompletely exuviated pupae (Figure 1A) were observed after mounting. In this case, the percentage of incompletely exuviated pupae to the number of mounted larvae was about 7-22 %. In addition, among the pupated population, about 23-40 % of individuals with morphological abnormalities in the abdomen and other parts of the body (Figure 1B) were noticed, although there were various degree of differences. Furthermore, among the moths that emerged, some individuals were observed to have issues related to mating due to deformed wings and distorted bodies. Therefore, during the breeding process of these three varieties, the individual selection was repeated by focusing on the thinness of the cocoon layer and the elimination of malformed pupae. As a result, the selection effect was realised, and the proportion of cocoons with a thick sericin cocoon layer, strongly deformed pupae and incompletely exuviated pupae, which were more in the initial breeding of each variety, got reduced. In addition, the number of individuals that were able to metamorphose into moths, mate, and lay eggs normally, while having the morphology of completely naked pupae or very thin cocoon layers, increased significantly, and it was concluded that three newly bred varieties could be used as the parent varieties that constitute the cross combination for the development of the hybrids with the naked pupa trait. As described in the Results section, there were four other candidates of hybrids for the hybrid "MR (WJT × WR)" selection: "WC3 × JT", "WR × JT", "WJT × WC3", and "WJ7× CT". And initially, using both male pupal weight and hTF productivity as indicators, we intended to narrow down to "WJT × WR" and "WR × JT" as candidates for recombinant protein production.

Usually, when a breeder selects the hybrid to be the target of development, both the efficiency and ease of egg production should be considered along with the results of the characterization of each tested hybrid. Therefore, the hybrids of both "WJT × WR" and "WJT × WC3", which were composed of two-parent varieties with sex-limited traits, were selected as new candidates because it was possible to produce eggs without waste through reversible mating. However, in the silkworm

egg production, in addition to the efficient production of eggs utilizing reversible mating, it was necessary to avoid disease caused by pebrine protozoa in the eggs to be produced, and it was recommended that all the original varieties constituting the hybrids be reared for all stages on an artificial diet. Therefore, it was essential to use an original variety that eats well on an artificial diet for the hybrids, and since the variety "WC3" had a poor feeding performance on an artificial diet (artificial diet 'Silkmate S for Juvenile silkworms of the original strains' manufactured by Nosan Corporation), the hybrid "WJT×WR" was finally selected and named "MR".

In the hybrid "MR", when the total amount of the artificial diet to females and males during the 5th instar was fed at 8.0 g/larva and 7.2 g/larva, respectively, the average weight of female pupa was 1.77 g and that of male pupa was 1.48 g. On the other hand, in the normal hybrid "Kinshu × Shouwa" which was reared with mixed sexes, the pupal weight of females was 2.31 g and that of males was 1.69 g when the total amount of the artificial diet during the 5th instar was fed at 13.2 g/larva (Table 2). Preliminary tests had shown (unpublished) that hybrids with naked pupal traits tended to produce incompletely exuviated and malformed pupae when fed with the same amount of the artificial diet (standard amount) as the hybrid "Kinshu × Shouwa" during the 5th instar. Therefore, to obtain a practical pupation rate, "MR" was reared on 39 % less diet for females and 45 % less diet for males than the hybrid "Kinshu × Shouwa" were reared. As a result, the pupal weight of the female was equivalent to about 73 % of that of the hybrid "Kinshu × Shouwa" and 88 % of that of the male (Table 2). These results indicate that the hybrid "MR" with the naked pupa trait has a higher feed efficiency relative to pupal weight than the normal hybrid "Kinshu × Showa" because little protein is used to make the cocoon layer. Observation of the cocooning condition of "MR" showed that both sexes did not only have completely naked pupae, but also a mixture of individuals that formed a thin cocoon layer. However, these cocoon layers were all of sericin that were thin enough to be easily torn off by hand.

To conclude, the hybrid "MR" with the naked pupa trait developed in this study can be easily distinguished between male and female silkworms by the larval spots and can remarkably save time and labor needed for the process of extracting the pupae from the cocoons. This hybrid is currently used year-round in the bio-reagent development division of Sysmex Corporation, and is reared in a sterile environment at all stages on an artificial diet. It is utilized to produce recombinant proteins as raw materials for clinical and biotech reagents.

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CROSS-TRANSMISSION OF A MICROSPORIDIAN PATHOGEN FROM MUGA SILKWORM, *ANTHERAEA ASSAMENSIS* HELFER TO ERI SILKWORM, *SAMIA RICINI*

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Abstract

Pebrine, a devastating parasitic disease caused by microsporidia (*Nosema* sp.) is one of the impending challenges for the development of sericulture, including the wild silkworm culture in the North-eastern part of India. The Indian golden silkworm, locally called muga silkworm, *Antheraea assamensis* suffers heavily from pebrine disease. However, the effect of this disease is negligible in the case of eri silkworm, *Samia ricini*. In this study, we investigated whether *Nosema* spores isolated from *A. assamensis* could cause pebrine disease in *S. ricini.* Inoculation of microsporidia from *A. assamensis* to *S. ricini,* confirmed the case of cross transmissibility of pebrine disease between the two Saturniid species. The negative impact of *Nosema* infection was observed in all the metamorphic stages of *S. ricini,* such as larvae, pupae and moths. Cross-transmission of *Nosema* sp. was further confirmed by sequencing of an internal transcribed spacer region in which 100 % similarity was found between the pathogens. This finding necessitates the development of new strategies for the management of pebrine disease in the social ecosystem where both muga and eri silkworms are reared by farmers in Northeast India so that the crop loss due to pebrine disease in wild silkworms could be minimized.

Key words: Cross-transmission, ITS sequencing, *Nosema* sp., pebrine, *Samia ricini.*

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Introduction

Eri silk derived from *Samia ricini* and muga silk derived from *Antheraea assamensis* Helfer are two of the most important silks produced in the North-eastern region of India. About 90 % of eri silk in India is produced in the North-East region of India, mostly in the state of Assam (85 %) (Chakrabarty *et al.,* 2012). Its close relative from the Saturniidae family, muga silkworm, *A. assamensis* is unique in its ability to produce the glittering golden coloured 'muga silk' and is available only in the North-Eastern States of India. Eri silkworms are reared indoors similar to mulberry silkworms and muga silkworms are reared outdoors, on trees. The outbreak of various microbial diseases *viz.,* pebrine, flacherie and muscardine are the major constraints encountered in the silk industry (Subrahmanyam *et al.,* 2019; Goswami *et al.,* 2021). It is reported that *S. ricini* is more disease tolerant compared to *A. assamensis* (Chakravorty and Singh, 2005). Eri silkworm is hardy and can withstand extreme climatic conditions (Prasad and Saha, 1992), while the muga silkworm is susceptible to various diseases that cause heavy crop loss (Thangavelu and Sahu, 1983).

Nosema sp. of microsporidians are opportunistic pathogens that infect and cause the catastrophic disease, pebrine (also known as microsporidiosis or pepper disease), in silkworms. This often results in enormous crop losses of about 36 % and has a very visible negative economic impact on sericulture (Nataraju *et al.,* 2005; Subrahmanyam *et al.,* 2019). Based on ten major morphological characteristics of the spores under electron microscopy, three species of *Nosema* that infect wild silkworms and are common in Eastern and North-Eastern India have been named *Nosema ricini* from *S. ricini, N. assamensis* from *A. assamensis,* and *N. mylitta* from *Antheraea mylitta* (Chakrabarti and Manna, 2006).

The periodic occurrence of pebrine disease in the rearing field indicates the possibility of cross infection of pebrine spores from the other alternate hosts. The perpetual incidence of microsporidian infection in silkworm may be due to various sources of secondary contamination or cross infection from the alternate hosts (Ishihara and Iwane, 1991). It has been observed that most of the farmers raised silkworm host plantations, such as som (*Persea bombycina*), soalu (*Litsea monopetala*), castor (*Ricinus communis*) and kesseru (*Heteropanas fragrans*) in the same farm and conducted muga silkworm rearing in the som and soalu plants and at the same time harvest castor and kesseru leaves for eri silkworm rearing which have a possibility of cross transmission of pebrine pathogen from muga to eri silkworm (Das *et al.,* 2014). The existence of more than one species in the same habitat and presence of disease in any one of them may lead to cross transmission.

Nosema sp. are reported to be specific about their host (Sengupta and Griyaghey, 1981). However, besides *Nosema bombycis* Nagaeli, 4-6 other microsporidia belonging to *Nosema, Pleistophora* and *Thelohania* genera were isolated from Mulberry silkworm (Fujiwara, 1980; Sato *et al.,* 1981); *N. bombycis* infecting *Bombyx mori* is also shown to infect 20 different lepidopteran insects (Machay, 1957). The *Nosema* spores of Tasar silkworm, *Antheraea mylitta,* also infect and cause disease in Mulberry silkworm (Patil, 1993) and non-pathogenicity of *Nosema* spores of *A. mylitta* to Mulberry silkworm has also been reported (Sengupta and Griyaghey, 1981). *Nosema* sp. affecting *A. pernyi* does not infect the Mulberry silkworm (Jolly, 1986).

Understanding the possibility of cross-infection of microsporidia causing pebrine in muga to eri is necessary because it is almost a regular practice in Assam to rear muga and eri simultaneously without knowing the adverse effects of cross-transmission. The study of cross-transmission of microsporidian spores has been previously examined from Mulberry, Eri and Tasar to Muga (Chakrabarti and Manna, 2008) and non-Mulberry silkworm to Mulberry silkworm (Chakrabarti and Manna, 2009). Cross-infectivity of pebrine disease from Muga to Eri silkworm was reported (Das *et al.,* 2014), however, systematic documentation parameters, such as symptoms and relationship of spores with larval mortality, cocoon number, *etc.,* were not taken into consideration. The present study on cross-infectivity of pebrine disease from muga to eri silkworm revealed positive transmission with exhibition of classical symptoms of pebrine disease, mortality of larvae, pupae and adult moths. This report deals with the detailed observation of the symptoms exhibited by the cross-infected larvae, impact of *Nosema assamensis* infection on larval weight, length, mortality, cocoon number, shell weight and pupae formation confirming the pathogenicity of the spores.

Materials and Methods

Isolation of *Nosema* spores from muga silkworm

To isolate *Nosema* spores, diseased muga mother moths were collected and homogenized in 0.8 % K_2CO_3 solution. The homogenate was allowed to settle for 3 minutes and filtered through cotton or muslin cloth and the filtrate was collected. Filtrate was checked for pebrine spores under a light microscope. The filtrate was centrifuged at 3000 rpm for 5 minutes and the pellet was suspended in water. The smear was observed under a microscope (600x) for pebrine spores. The shape, size, luster and Brownian movement were used as indices for the identification of pebrine spores.

Purification of *Nosema* spores

The spores were purified using the Percoll Gradient method as discussed earlier (Sato and Watanabe, 1980; Subrahmanyam *et al.,* 2019). The purified spores were suspended in 0.8 % NaCl solution and stored at 4^0 C for further experiment.

Inoculation of microsporidia of *A. assamensis* (muga silkworm) to *S. ricini* (eri silkworm)

Third instar eri silkworm larvae were collected and grouped into three replications for cross-infection treatment (T1, T2 and T3). For each treatment, 50 silkworms were taken (150 larvae in total for three treatments). Similar beds were prepared for uninfected control (C1, C2, C3). A concentration of 1.5 x 10^8 spores ml^{-1} of the purified spore solution was smeared on castor leaves and fed to the treatment lots whereas in the uninfected control, castor leaves washed with distilled water were fed.

Rearing of silkworm

The rearing beds were maintained with regular cleaning to avoid fungal infection and healthy castor leaves were collected each day and fed. Optimum conditions with temperature of 28–30 ^{0}C and relative humidity of 70-80 % were maintained. Mortality due to pebrine in dead larvae was confirmed by the presence of *Nosema* spores through microscopic examination.

Treated larvae were observed for the presence of pebrine symptoms, such as peppered spots, non-uniform growth of larvae, sluggishness, *etc.* They were noted and compared with control. Pre- and post-cocoon parameters, such as weight of twenty larvae, delayed spinning, cocoon number, shell weight, pupae formation, moth emergence, fecundity and silk content (in g), were noted and the data were statistically analyzed in R studio (R CoreTeam, Vienna, Austria. http://www.r-project.org).

Confirmation of transmission through ITS sequencing

The experiment of cross-transmission was confirmed by sequencing of the internal transcribed spacer region (ITS) located in the rRNA gene (LSUrRNA-ITS-SSUrRNA-IGS-5S) (Huang *et al.,* 2004). Microsporidia originally collected from pebrine infected *A. assamensis* (*Nosema* sp. M1 and M2) was compared with microsporidia collected from *S. ricini* during cross-transmission (*Nosema* sp. C1 and C2). PCR reaction was performed with primers in a total reaction volume of 20 µL. Primer pair ILSUF/S33R (Huang *et al.,* 2004) was used to amplify internal transcribed spacer (ITS) in microsporidia infecting *A. assamensis* that yielded an amplicon size of ~500 bp. Cloned microsporidia gene product was purified by QIAquick PCR Purification Kit (Qiagen, USA, www.qiagen.com) and was sequenced. Raw nucleotide sequences were edited using BIOEDIT application (https://bioedit.software.informer.com) and further the sequences were aligned pair-wise using online Clustal W tool in MEGA version 11.0 (Kumar *et al.,* 2018). The sequences were submitted to NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with accession numbers OP628235, OP628236 and OP628237.

Results and Discussion

A set of experiments were conducted to investigate if the *Nosema* pathogen causing pebrine disease

in *A. assamensis* is able to infect *S. ricini.* In these experiments, *Nosema* sp. originally isolated from *A. assamensis* was found to be highly infective and pathogenic to *S. ricini* as the range of larval mortality was significant (P<0.01) in all the cross-infected larvae. Mortality was recorded significantly higher (99 %) in larvae under cross-infection with the inoculum size of 1.5 x 10^8 spore ml^{-1}. Out of 150 total larvae treated with *Nosema* sp., only 1 female emerged as an adult moth.

Rate of infection

The infection rate in *Nosema*-inoculated eri silkworms was very high, and disease symptoms began to appear in 4th instar infected larvae. Mortality of the larvae was first noticed on the 6th day post inoculation in treated larvae (Figure 1), which were tested positive for pebrine spores. The number of dead larvae per day kept increasing whereas in control, some degree of mortality was recorded on the 9th day, which was not progressive (Figure 1). The dead larvae of uninfected control were also tested and found to be pebrine free. The majority of the mortality in the inoculated worms occurred in the fourth and fifth instars, particularly while moulting.

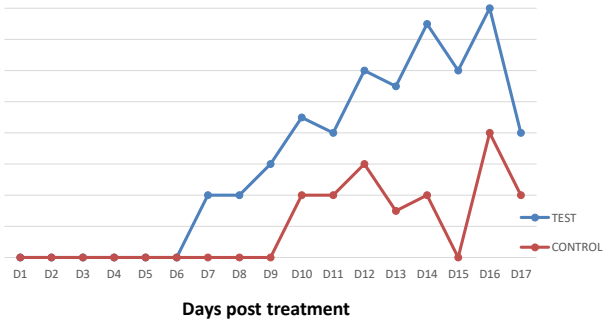

Figure 1: Day-wise mortality due to cross-infection. Test: *Nosema* sp. inoculated eri silkworm larvae (cross-infected) in T1, T2, T3; Control: Healthy uninfected eri silkworm larvae in C1, C2 and C3.

Larval symptoms during *Nosema* infection

The first signs of pebrine disease in *S. ricini* were delayed moulting and larval mortality. Dead worms in the fourth instar had intact moulted skin, as well as vomiting of gut juice and loose excreta. Infected larvae curl up and the body gets stained in its own gut fluid, which was examined under a microscope and spores were detected as undergoing active multiplication in their gut.

One of the most noticeable symptoms among the infected larvae during their delayed moulting was length heterogeneity. In contrast, uniform larval length was observed in the uninfected control. Also, the treated silkworms showed poor appetite and slower motility (sluggishness), which also affected their growth compared to that of control. Other symptoms included the appearance of black spots, first noticed on the integument of mature 5th instar larvae (Figure 2). The black pepper-like spots are considered to be hypodermal cells, which become enlarged and vacuolated upon infection, and blackened due to the formation of melanin pigment (Subrahmanyam *et al.*, 2019).

The present findings are in line with the statements that pebrine infected larvae do not show external symptoms until the disease is far advanced (Jolly, 1986). At the advanced stage, larvae become sluggish and show symptoms, such as poor appetite, retarded growth and development, irregular moulting, paler appearance, shrunken body and flaccid texture.

Figure 2: Formation of black spots on larval integument of infected (*Nosema* sp.) eri silkworm larvae at 5th instar.

Effect of *Nosema* infection on larval weight

A range in measurement of larval weight was observed in the *Nosema* infected 5th instar silkworms (Figure 3).

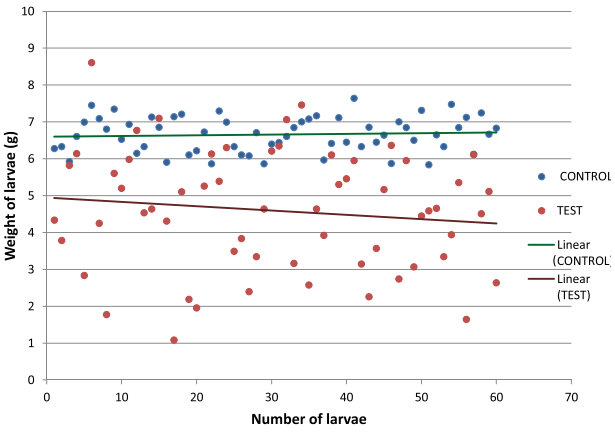

Figure 3: Effect of *N. assamensis* infection on mature eri larvae (p-value = 3.521e-06).

The wider range recorded in larval weight might be due to the existence of different instar larvae in the same rearing bed because of the impaired moulting process. Whereas, in uninfected control, the weight of larvae were in the range of 6-8 g comparatively being stable than those of infected larvae. In contrast, majority of infected worms showed wider range from 2.0 to 6.0 g in mature larval stage indicating the poor appetite and sluggishness as a result of slow growth in the heavily infected larvae whereas, in control the weight ranged between 6 and 8 g showing the uniformity in growth. This indicates a significant difference (P<0.01) among the weight of the larvae in control and infected. The crushed infected dead larvae were examined under a phase contrast microscope, which revealed the presence of dense mature spores, confirming cross-infection (Figure 4).

Cocoon characteristics

Spinning of the cocoon began on the 9th day of the rearing in control silkworm larvae, whereas spinning was delayed by two days in *Nosema* sp. infected eri silkworm larvae. Figure 5 shows the comparison between cocoon number and cocoon shell weight obtained in respect of control and test. In treatment, the shell weight displayed a wider range (0.1-0.5 g) and there was a decrease in the number of cocoons formed (41 cocoons formed out of 150 treated larvae), whereas 117 cocoons were formed in control (out of 150 larvae). The shell weight also showed a significant difference (Figure 5, p-value < 2.2e-16). The cocoons of the control were well formed with high silk content (32.74 g from 117 cocoons), whereas some of the cocoons in test were deformed, one naked pupa was formed in T2 and, incomplete cocoon formation was also observed due to larval mortality while spinning. The silk content in the test was 11.07 g from 41 cocoons.

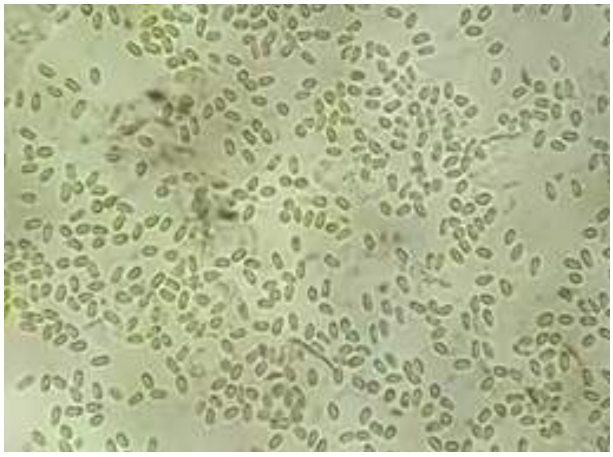

Figure 4: Phase contrast microscopic image (600X) of *Nosema* spores isolated from pebrine infected eri silkworm larvae.

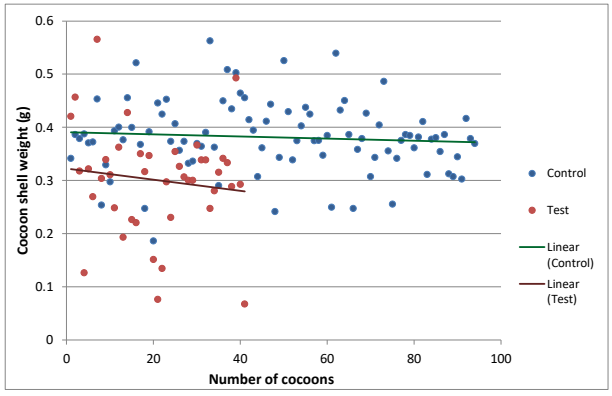

Figure 5: Comparison of shell weight and number of cocoons in control and test. Test: *Nosema* treated eri silkworm larvae (cross-infected) in T1, T2 and T3; Control: Healthy uninfected eri silkworm larvae in C1, C2 and C3; p-value < 2.2e-16.

Pupa formation

Incomplete pupa formation was observed in pebrine infected silkworm larvae (T1, T2 and T3) (Figure 6). This could be due to higher level of multiplication of *Nosema* spores. In control, complete pupal formation was observed. This corroborates the findings that infected pupae are flabby, lusterless with softened abdomen and in cases of severe infection, they even fail to metamorphose into adults (Singh and Saratchandra, 2003).

Figure 6: Comparison of eri pupal development between uninfected control (C) and *Nosema* sp. cross-infected treatment (T).

Emergence

Table 1 shows the comparison between control and test in respect of moth emergence. The moth emergence in control samples started on the 28th day. In total, 81 adult moths emerged out of 117 cocoons and the remaining 36 did not emerge. However, only one female moth out of 150 larvae emerged from 41 cocoons formed on the 31st day post treatment. The emerged moth had clubbed wings and distorted antennae with unfertilized eggs protruding from its gut which is a clear indication of pebrine infection.

Table 1: Comparison between number of moth emergences in uninfected (Control) and infected (Test) eri cocoons

	Control (C1+C2+C3)	Test (T1+T2+T3)
Total number of cocoons	117	41
Total number of moths emerged	81	1
Number of male moths emerged	41	0
Number of female moths emerged	40	1
Total number of non-emerged moths	36	40

Fecundity

After emergence, in the uninfected control group, the male and female moths underwent natural coupling and females started laying eggs from the next day. The larvae hatched on the 7th day after laying. The fecundity in control was found to be higher than the benchmark. Whereas, in the infected test, only one female moth emerged which laid unfertilized eggs.

Confirmation of transmission through DNA sequencing

In order to confirm that the infection caused by pathogen in eri silkworm is indeed due to *Nosema* sp. isolated from muga silkworm, we sequenced

ITS regions of both the pathogens obtained from muga and also from cross-infected eri samples. DNA primer pair ILSUF/S33R yielded an amplicon size of ~500 bp which corroborates with the finding of approximately same amplicon size of 501 bp using the primer pair ILSUF/S33R (Subrahmanyam *et al.,* 2019). Microsporidian pathogen of muga silkworm, *A. assamensis* was identified as *Nosema assamensis* based on ITS sequence analysis. The sequence analysis revealed a 100 % similarity between ITS regions of *Nosema* sp. M1 isolated from *A. assamensis* to that of *Nosema* sp. C1 isolated from *S. ricini* indicating both the pathogens are same (Figure 7). Thus, the cross transmission of *Nosema* spores from *A. assamensis* to *S. ricini* was confirmed.

Observation of all the metamorphic stages from larvae to adult moths in both control and test revealed that the uninfected control group larvae had healthy lifespan completing all the stages without any major crop loss, whereas, the infected larvae failed to survive with the pebrine infection and displayed heavy crop loss without any continuation of generation. Similar to our observations, higher larval and pupal mortality as well as shorter life span of *Ostrinia nubialis* (European corn borer) was noticed upon *N. pyrausta* infection (Kramer, 1959). Infected moths of field collected European corn borer larvae had a shorter life span and the percentage of infected larvae that survived to emerge as adults were lower (Zimmack *et al.,* 1954).

Cross infectivity of *Nosema* was studied previously in different lepidopteran pests like Turnip moth (*Agrotis segetum*) and Army worm (*Spodoptera exigua)* (Kashkarova, 1981). In an earlier cross-infectivity study, microsporidia associated with *Pieris brassicae* showed definite cross infectivity to silkworm, *Bombyx mori* L. wherein silkworm larvae inoculated with the *Nosema* sp. died before reaching the moth stage (Rather *et al.,* 2019). Similar kind of results were found with *Nosema* sp. isolated from *Spodoptera littoralis* (Egyptian cotton leafworm) which was found to be cross-infective to *Adoxophyes orana, Agrotis ipsilon, Chilo suppressalis, Hyphantria cunea* and *Pieris rapae crucivora,* although its

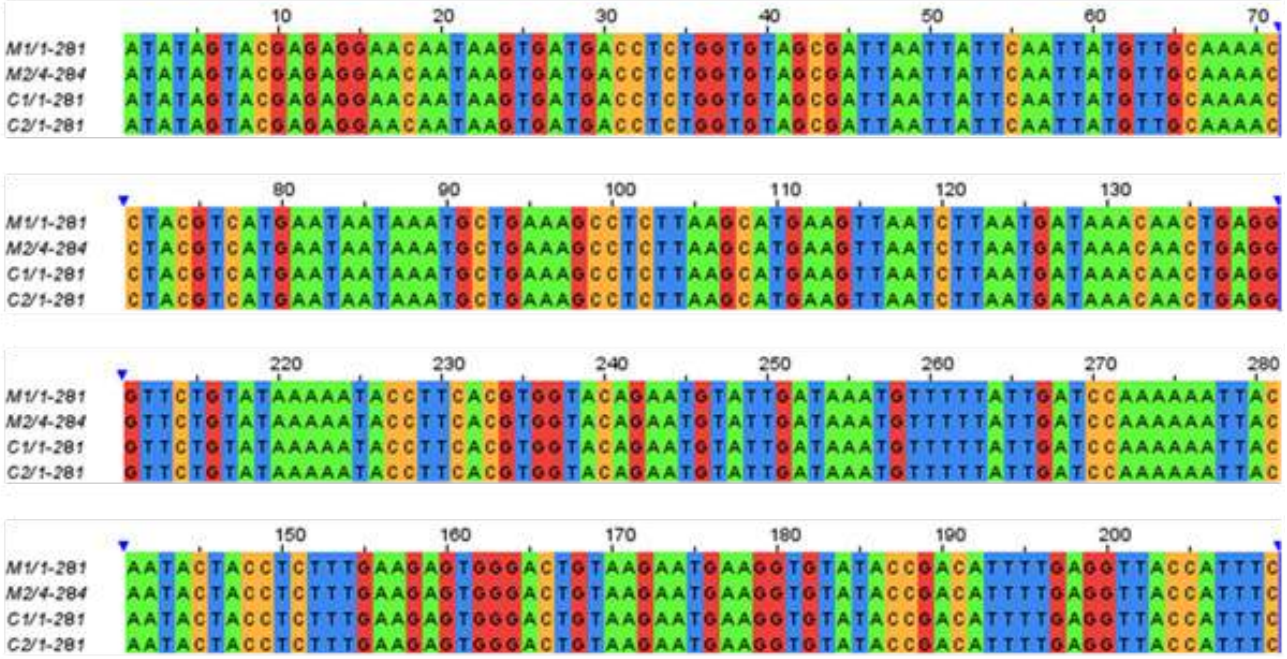

Figure 7: Pair-wise sequence alignment of ITS region of *Nosema* sp. M1 and M2 were isolated from *A. assamensis* and *Nosema* sp. C1 and C2 were isolated from *S. ricini* using MEGA version 11(https://www.megasoftware.net/). A 100 % sequence similarity is found between ITS of the both microsporidian pathogens.

pathogenicity to some of these insects was relatively weak with infection percentage of 11, 22, 58, 30 and 33, respectively. But, *Bombyx mori, Leucania separata, Mamestra brassicae,* and *Plutella maculipennis* did not get infected (Watanabe, 1976). Cross-infectivity of microsporidian pathogens were also observed in apiculture wherein *N. bombi* of bumble bee species can infect honey bee, *A. mellifera* (Fantham and Porter, 1913; Kudo 1924; Showers *et al.,* 1967).

Our previous studies confirmed *Nosema* sp. of *A. assamensis* as "true *Nosema* group" which has shown close phylogenetic relation with other *Nosema* sp. of wild silkworms with significant bootstrap values (>50 %) (Subrahmanyam *et al.,* 2019). Hence, it may be inferred that despite the evolutionary divergence of eri and muga, the positive results of cross-transmission shows that *Nosema* is able to affect closely related organisms proving its simultaneous evolution along with the host organism.

From the current study, it is apparent that, wherever the rearing of eri and muga silkworm are conducted simultaneously, that zone is in risk of cross-transmission. A study of cross-infected pebrine due to spores from eri to muga silkworm in natural condition revealed 22.6 % mortality (Mistry, 1979). We can confirm the vice versa from the present finding with a higher mortality rate. Repeated incidence of microsporidia infection in silkworm may be due to various sources of secondary contamination or cross-infection from other alternate hosts (Ishihara and Iwano, 1991). We anticipate that microsporidian infection from muga rearing fields may possibly spread *Nosema* spores to nearby eri silkworm host plantation, such as castor and kesseru. Therefore, it is recommended to undertake separate zones for muga and eri culture or follow different rearing schedules / seasons. Proper management measures, such as disinfection of rearing trays as well as proper washing of foliage by clean water before feeding it to the eri larvae can be practiced to minimize the risk of cross-transmission of the disease.

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SILK UNION (BACKED) FABRICS – A PROMISING PRODUCT FOR THE SILK APPAREL SECTOR PART II: AIR PERMEABILITY, WATER VAPOUR PERMEABILITY AND WICKING PROPERTIES OF SILK UNION (BACKED) FABRICS

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Abstract

Silk union (backed) fabrics in both Soft silk and Dupion silk varieties with varied fabric construction parameters and different material for back were developed to cater to the production of ladies blouses and garments. These fabrics were evaluated and characterised. The results of characterisation and evaluation of Silk union (backed) fabrics with respect to fabric Hand *i.e.,* Total hand value (THV) and their suitability for ladies wear for different seasons are provided in Part – I of this publication. In continuation of this, the Silk union (backed) fabrics were characterised and evaluated with respect to the transmission properties *viz.,* air permeability, water vapour permeability and wicking. The analysis of the results reveal that Soft silk and Dupion silk (backed) fabrics woven with sateen weave stand out as the best, with respect to water vapour permeability and wicking followed by twill and plain weave fabrics. In the case of air permeability, plain weave fabrics have higher permeability than twill and sateen weave fabrics. Therefore, it is evident that by changing fabric weave while keeping all other structural parameters constant, significantly influences two out of the three transmission properties *viz.,* air permeability and wicking of these fabrics but not water vapour permeability. Whereas, change in the material for backing does not influence any of the three transmission properties. Hence, fabrics developed with silk on the face and either Cotton or Modal for the backing especially with sateen weave can be a promising product for ladies wear and the silk apparel sector as a whole in the near future.

Key words: Air permeability, backed fabrics, Dupion silk, fabric hand, Modal, Soft silk, water vapour permeability and wicking.

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Introduction

The main purpose of clothing is to maintain normal body temperature and to protect the body against varying external conditions. Another important purpose is that of decoration. No matter how efficient a particular garment may be in protecting the body it must at the same time appeal to the aesthetic sense of the wearer. Only under extreme climatic conditions, for example in the polar region, is the appearance of a fabric subjugated to the prime purpose of protection (Morris, 1953). Clothing comfort is dependent upon the low stress mechanical, thermal and moisture transfer properties of the fabrics. There is a general agreement that air and moisture transmission through textiles has a great influence on the thermo-physiological comfort of the human body, which is maintained by perspiring both in vapour and in liquid form (Li, 2001).

Fabric construction parameters, such as fabric sett, cover factor and weave, considerably change the performance of the fabric particularly in respect of low stress mechanical properties (Kawabata and Masako Niwa, 1989). Comfort properties are influenced by changes in the structural parameters, and woven fabrics with high porosity have better permeability and wicking properties (Hakan Ozdemir, 2017).

The review of literature revealed that some research has been carried out with respect to evaluation and characterisation of pure silk fabrics, wool, cotton, linen and silk union fabrics (Ron Postle and Gu Ping, 1994; Sharma *et al.,* 2000; Behra, 2007; Behra and Mishra, 2007) but, there is little information available on the development and characterisation of Silk union (backed) fabrics. With this backdrop, Silk union (backed) fabrics in both Soft silk and Dupion silk varieties with varied fabric construction parameters and different material for back were developed to cater to the production of ladies blouses and garments. These fabrics were evaluated and characterised. The results of characterisation and evaluation of Silk union (backed) fabrics with respect to fabric Hand *i.e.,* Total hand value (THV) and their suitability for ladies wear for different seasons are given in Part – I of this publication (Jameela Khatoon *et al.,* 2020). The present study is aimed at characterisation and evaluation of transmission properties *viz.,* air permeability, water vapour permeability and wicking of the Silk union (backed) fabrics.

Materials and Methods

Varieties of Soft silk and Dupion silk (backed) fabric samples were developed using good quality mulberry raw silk and Dupion silk yarn reeled on Automatic reeling machine (ARM) and good quality Cotton (combed and mercerized) and micro Modal yarn. The yarns were prepared in different plies, twist levels and shades. The details of the structural parameters of Soft silk and Dupion silk (backed) fabrics are given in Table 1.

Table 1: Structural parameters of Soft silk (backed) & Dupion silk (backed) fabrics

Fabric particulars/Weave	Plain	Twill	Sateen
Ends per inch	200	200	200
Picks per inch	80	100	100

Twelve samples of fabric (six single and six in two layers) in both Soft silk and Dupion silk varieties with varied fabric construction parameters and different material for back to cater to the production of ladies blouses and garments were developed. The samples developed in single layer were considered for this study. The codes for Soft silk and Dupion silk fabrics woven with Cotton back and Modal back are provided in Table 2.

Table 2: Fabric codes of Soft silk & Dupion silk fabrics woven with Cotton & Modal backing

Fabric type	Single layer (With Cotton backing)		
	Plain	Twill	Sateen
Soft silk	SS-PB-C	SS-TB-C	SS-SB-C
Dupion silk	DUP-PB-C	DUP-TB-C	DUP-SB-C
	Single layer (With Modal backing)		
Soft silk	SS-PB-M	SS-TB-M	SS-SB-M
Dupion silk	DUP-PB-M	DUP-TB-M	DUP-SB-M

These fabric samples were subjected to testing of structural, functional, aesthetic, low stress mechanical and comfort properties objectively by adopting standard methods for testing under controlled environmental conditions. From the data generated, the Silk union (backed) fabrics were characterised with respect to general and transmission properties *viz.,* air permeability, water vapour permeability and wicking properties.

Results and Discussion

I. Soft silk fabrics

IA. General properties: The data on Fabric weight and thickness of Soft silk fabrics is given in Table 3.

Table 3: Fabric weight & thickness of Soft silk fabrics

Sl. No.	Fabric code	Weight (g)	Thickness (mm)
1	SS-PB-C	123	0.259
2	SS-TB-C	147	0.368
3	SS-SB-C	144	0.380
4	SS-PB-M	121	0.256
5	SS-TB-M	143	0.365
6	SS-SB-M	143	0.377

(i) Fabric weight: It can be observed from Table 3 that the weight of the fabric samples ranged between 121 and 147 grams. Soft silk fabrics woven with plain weave are lighter than the fabrics woven with twill and sateen weave. The differences among weight of fabrics with plain, twill and sateen weave are statistically significant at 95 % level of confidence [One way Anova -F(2,27)=977.906, p=0.000]. Whereas, the difference between the fabrics woven with twill and sateen weave was not significant (p=0.051). There was no significant difference in weight between the Soft silk fabrics of similar weave woven with cotton and modal for the backing [One way Anova- F(1,28)=0.298, p=0.590].

(ii) Fabric thickness : As seen in Table 3, the fabric thickness ranged between 0.256 and 0.380 mm and the difference in thickness is statistically significant at 95 % level of confidence among the fabric samples with different weaves [One way Anova - F(2,57)=3285.069, p=0.000]. Whereas, there is no significant difference in thickness between the Soft silk fabrics of similar weave woven with cotton and modal for the backing [One way Anova - F(1,58)=0.057, p=0.813]. The reason could be the use of similar yarn and fabric structural parameters adopted to facilitate the comparison between different varieties of fabrics.

I B. Transmission properties of Soft silk fabrics

The results of fabric transmission properties *viz.,* air permeability, water vapour permeability and wicking of Soft silk (backed) fabrics are given in Table 4.

Table 4: Transmission properties of Soft silk fabrics

Sample code	Air permeability (CFM)	WVP Index	Wicking height (cm)	
			Warp	Weft
SS-PB-C	15.62	99.66	7.22	9.34
SS-TB-C	9.70	99.03	9.56	12.62
SS-SB-C	11.45	98.34	14.22	14.40
SS-PB-M	14.34	101.03	8.38	9.72
SS-TB-M	10.32	103.61	10.74	12.58
SS-SB-M	11.42	102.60	10.68	13.66

(i) Air permeability

It can be observed from Table 4 that the Soft silk fabrics woven with plain weave have higher Air permeability compared to the fabrics woven with twill and sateen weave and the difference is statistically significant at 95 % level of confidence [Anova-F(2,57)=62.885, p=0.000]. This can be explained by the difference in the weight and the thickness of the fabrics. Also plain weave fabrics were woven with less weft density than the twill and sateen fabrics. Increase in weft density

results in the decrease in porosity. Therefore, plain weave fabrics have more porosity than the twill and sateen weave fabrics. This could perhaps be the reason for plain weave fabrics having higher air permeability due to higher porosity compared to the twill and sateen weave fabrics.

It is also observed that the difference in air permeability between the Soft silk fabrics woven with Cotton and Modal is statistically not significant at 95 % level of confidence [Anova-F(1,58)=0.124, p=0.726]. This may be due to the uniformity in the yarn and fabric structural parameters which were kept constant to facilitate the comparison between different varieties of fabrics. This implies that the change in weave has a significant influence on the air permeability of these fabrics whereas the change in material for the backing does not.

(ii) Water vapour permeability

As seen in Table 4, the water vapor permeability index of Soft silk woven fabrics ranges from 98.34 to 103.61. The difference in the water vapor permeability index of Soft silk woven fabric woven with different weaves is statistically not significant at 95 % level of confidence [Anova-F(2,15)=2.701, p=0.100]. The difference between the fabrics woven with different material for backing is also statistically not significant at 95 % level of confidence [Anova-F(1,16)=0.953, p=0.348]. Therefore, there is no significant difference in water vapor permeability of the Soft silk fabrics woven with different weaves and different materials for backing.

(iii) Wicking

It can be observed from Table 4 that Sateen weave fabrics woven with either Cotton or Modal for the backing have higher wicking height in both warp-way and weft-way compared to plain and twill weave fabrics. This could be attributed to the fact that length of the yarn floats in sateen weave fabrics is higher compared to twill and plain weave because of less yarn interlacement resulting in less yarn crimp which offers less resistance to the movement of the fluid and hence, results in more wicking height compared to the other two fabrics.

Warp-way wicking: There is no statistically significant difference at 95 % level of confidence [Anova-F(2,3)=4.248, p=0.133] in the wicking height (warp-way) among the fabrics woven with different weaves.

Weft-way wicking: There is statistically significant difference at 95 % level of confidence [Anova-F(2,3)=91.464, p=0.002] in the wicking height (weft-way) among fabrics woven with different weaves.

Whereas, the difference between wicking in both warp-way and weft-way of the fabrics woven with different material (Cotton & Modal) for backing is statistically not significant at 95 % level of confidence [Anova-F(1,4)=0.033, p=0.865] and [Anova-F(1,4)=0.005, p=0.947], respectively.

II. Dupion silk fabrics

II A. General properties: The data on Fabric weight and thickness of Dupion silk fabrics is given in Table 5.

Table 5: Fabric weight and thickness of Dupion silk fabrics

Sl. No.	Fabric code	Weight (g)	Thickness (mm)
1	DUP-PB-C	119	0.254
2	DUP-TB-C	139	0.360
3	DUP-SB-C	140	0.396
4	DUP-PB-M	118	0.260
5	DUP-TB-M	138	0.365
6	DUP-SB-M	140	0.385

(i) Fabric weight: It can be observed from Table 5 that the weight of the fabric samples ranged between 118 and 140 grams. Dupion silk fabrics woven with plain weave are lighter than the fabrics woven with twill and sateen weave and the difference is statistically significant at 95 % level of confidence [One way Anova -F(2,27)=7780.500, p=0.000]. There was no significant

difference in weight between the Dupion silk fabrics of similar weave woven with cotton and modal for the backing [One way Anova- F(1,28)=0.032, p=0.859].

(ii) Fabric thickness: As seen in Table 5, fabric thickness ranged between 0.254 and 0.396 mm and the difference in thickness is statistically significant at 95 % level of confidence among all the fabric samples woven with different weaves [One way Anova - F(2,57)=2703.318, p=0.000]. There was no significant difference between the Soft silk fabrics of similar weave woven with cotton and modal for the backing [One way Anova - F(1,58)=0.000, p=1.000]. The reason could be the use of similar yarn and fabric structural parameters adopted to facilitate the comparison between different varieties of fabrics.

II B. Transmission properties of Dupion silk fabrics

The details of the transmission properties *viz.*, air permeability, water vapour permeability and wicking are given in Table 6.

Table 6: Transmission properties of Dupion silk fabrics

Sample code	Air permeability (CFM)	WVP Index	Wicking height (cm)	
			Warp	Weft
DUP-PB-C	16.68	89.43	7.20	8.58
DUP-TB-C	13.30	98.20	9.88	12.90
DUP-SB-C	15.41	94.98	11.26	13.06
DUP-PB-M	14.90	98.43	7.84	8.80
DUP-TB-M	15.27	76.28	9.43	13.04
DUP-SB-M	15.99	100.03	11.36	14.30

(i) Air permeability

It can be observed from Table 6 that the Dupion silk fabrics woven with plain weave have higher Air permeability compared to the fabrics woven with twill and sateen weave and the difference is statistically significant at 95 % level of confidence [Anova-F(2,57)=5.290, p=0.008]. This can be explained by the difference in the weight and the thickness of the fabrics. Also plain weave fabrics were woven with less weft density than the twill and sateen fabrics. Increase in weft density results in the decrease in porosity. Therefore, plain weave fabrics have more porosity than the twill and sateen weave fabrics. This could perhaps be the reason for plain weave fabrics having higher air permeability due to higher porosity compared to the twill and sateen weave fabrics.

It is also observed that there was no significant difference between the Dupion silk fabrics woven with Cotton and Modal at 95 % level of confidence [Anova-F(1,58)=0.454, p=0.726]. This may be due to the uniformity in the yarn and fabric structural parameters which were kept constant to facilitate the comparison between different varieties of fabrics. This implies that the change in weave has a significant influence on the air permeability of these fabrics whereas, the change in material for the backing does not.

(ii) Water vapour permeability

As seen in Table 6, there are no statistically significant differences among water vapour permeability of the Dupion silk fabrics woven with different weaves at 95 % level of confidence [Anova-F(2,15)=0.174, p=0.842] and different material for backing [Anova-F(1,16)=4.937, p=0.041]. Therefore, there is no significant difference in water vapour permeability of the Dupion silk fabrics woven with different weaves and different material for backing.

(iii) Wicking

It can be observed from Table 6 that Sateen weave fabrics woven with either Cotton or Modal for the backing have higher wicking height in both warp-way and weft-way compared to plain and twill weave fabrics. This could be attributed to the fact that length of the yarn floats in sateen weave fabrics is higher compared to twill and plain weave because of less yarn interlacement resulting in less yarn crimp which in turn offers less resistance to the movement of the fluid and hence, results in more wicking height compared to the other two fabrics.

Warp-way wicking: There is statistically significant difference at 95 % level of confidence [Anova-F(2,3)=69.639, p=0.003] in the wicking height (warp-way) among the fabrics woven with different weaves.

Weft-way wicking: There is statistically significant difference at 95 % level of confidence [Anova-F(2,3)=54.463, p=0.004] in the wicking height (weft-way) among fabrics woven with different weaves.

Whereas, the difference between wicking in both warp-way and weft-way of the fabrics woven with different material (Cotton & Modal) for backing is statistically not significant at 95 % level of confidence [Anova-F(1,4)=0.004, p=0.954] and [Anova-F(1,4)=0.058, p=0.822], respectively.

Conclusion

From the analysis of the results, the following inferences can be drawn:

- Change in weave has significant influence on the air permeability whereas, the change in material for the backing does not in the case of both Soft silk and Dupion silk fabrics.
- Change in weave or material for backing does not significantly influence water vapor permeability of both Soft silk and Dupion silk fabrics.
- Change in weave does not significantly influence warp-way wicking height but does significantly influence weft-way wicking height of Soft silk fabrics. Whereas, it does significantly influence both warp-way and weft-way wicking height of Dupion silk fabrics.
- Change in material for backing does not significantly influence both warp-way and weft-way wicking in the case of both Soft silk and Dupion silk fabrics.

Further, amongst the varieties of Soft silk and Dupion silk (Backed) fabrics developed with different weaves, and different material for backing, fabrics with sateen weave stand out as the best with respect to water vapour permeability and wicking followed by twill and plain weave fabrics. In the case of air permeability, plain weave fabrics have higher permeability than twill and sateen weave fabrics. Therefore, it is evident that by changing fabric weave, while keeping all other structural parameters constant, significantly influences two out of the three transmission properties *viz.*, air permeability and wicking of these fabrics but not water vapour permeability. Whereas, change in the material for backing does not influence any of the three transmission properties. Hence, fabrics developed with silk for the face and either Cotton or Modal for the backing especially with sateen weave can be a promising product for ladies wear and the silk apparel sector as a whole in the near future.

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STANDARDIZED EMBRYO ISOLATION METHODOLOGIES: KEY STEPS FOR DEVELOPING EFFICIENT EGG HANDLING TECHNIQUES IN MUGA SILKWORM SEED SECTOR

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Abstract

The potency and vitality of Muga silkworm, *Antheraea assamensis* (Helfer) eggs could efficiently be determined through embryo isolation methodologies, which help to develop egg handling techniques in Muga seed sector. Mulberry silkworm (*Bombyx mori* L.) embryo isolation techniques *viz.,* Hot water and Potassium hydroxide (KOH) methods were adopted in the present embryo isolation study and standardized with slight modifications as suitable for Muga silkworm eggs. The studies revealed that the hot water method is more suitable for early embryonic stages *i.e.,* from 24 h (1st day) to 96 h (4th day), whereas KOH method is the easiest one for isolation of mid *i.e.,* 120 h (5th day) and late *i.e.,* from 144 h (6th day) to 216 h (9th day) embryonic stages or up to hatching stage 240 h (10th day).

Key words: Embryonic stages, hot water method, KOH method, Muga silkworm.

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Introduction

Silkworm egg is the most important attribute of sericulture from the commercial point of view. In the past decade, many technologies were developed for handling Mulberry silkworm (*Bombyx mori* L.) eggs (Nakada, 1932; Takami and Kitazawa, 1960; Yokoyama, 1973; Tazima, 1978; Datta, 1988; Reddy *et al.,* 1997; Rao *et al.,* 1998; Reddy *et al.,* 2003). Egg preservation or handling techniques are incomplete without the study of silkworm embryo. Utilization of embryonic studies for developing silkworm egg handling techniques aimed at preservation, incubation and synchronization of hatching is a known technology in sericulturally advanced countries (Tazima, 1978; Reddy *et al.,* 2003; Sarkar *et al.,* 2012). Thus, the silkworm embryo isolation technology plays an important role in embryonic studies. Earlier reports revealed that the majority (80 %) of the Muga eggs received by the farmers are of poor quality (Borpuzari, 2010) and production of the Muga silk ultimately depends upon

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good quality eggs, because only superior quality eggs can ensure a good harvest and healthy crop (Tikader *et al.,* 2013). Egg handling technologies may help in maintenance and supply of good quality eggs to the farmers but very limited information is available on this aspect of Muga silkworm (*Antheraea assamensis* Helfer) (Goswami *et al.,* 2013). In the present study, an attempt was made to standardize the Muga silkworm embryo isolation methodologies for early, mid and late age eggs, which may help in developing various egg handling techniques in Muga Silkworm.

Materials and Methods

Muga silkworm eggs differ from those of mulberry silkworm, *B. mori* L. by bigger size and outer thick chorion with gummy substance. For isolating different Muga silkworm, *A. assamensis* (Helfer), embryonic stages from eggs, Hot water method standardized by Reddy *et al.* (2003) and Potassium hydroxide method (KOH method) described by Tazima (1978) for *B. mori* L. were adopted and modified as relevant for Muga silkworm eggs.

Hot water method

Muga silkworm eggs were collected from the ovipositional device, Kharika and placed inside the nylon netted bags after surface sterilization. These bags carrying the eggs were dipped in hot water at 65 - 78 °C for 5 – 10 minutes depending on the age of the embryo; dipping in hot water helps to arrest/fix the specific embryonic stage growth at that particular hour/day. The water temperature (65-70 °C) and dipping duration (5-6 minutes) should be lowered for early embryonic stages (Figure 1). For the mid embryonic stages, the water temperature of 70 - 75 °C and dipping duration of 6-8 minutes was monitored. Water temperature of 77-78 °C and dipping duration of 8 -10 minutes was regulated carefully for late embryonic stages. After treatment, the eggs were dried at room temperature and glued on to an egg sheet and dried in shade for 30 minutes after firm fixation of eggs on the egg sheet; eggs were then dissected skillfully under a dissection microscope using a sharp blade. The chorion (outer shell) was removed from the upper surface of the egg and collected the embryo along with the yolk into the petri dish (Figure 1). The yolk material and embryonic layers were cleaned very gently and carefully and isolated embryos were cleaned with distilled water and preserved in 75 % alcohol for further studies. This dissection methodology is very helpful to identify the early embryonic stages *i.e.,* from 20 to 100 h. Fifth day onwards, KOH method can be applied for quick and easy isolation of the Muga embryo.

Potassium hydroxide method (KOH method)

For isolation of early embryonic stages, the KOH method is difficult and not suitable. At constant temperature (70 – 75 °C), mid and late age Muga silkworm eggs were boiled in 2-4 % Potassium hydroxide (KOH) solution for 1 to 6 minutes depending on the age of the embryo. The concentration of KOH solution was inversely proportional to the boiling time for mid and later embryonic stages. KOH solution dissolves the hard chorion (outer shell) of the eggs and releases the egg contents. Released egg contents were carefully transferred to a petri dish and by squirting the hot water (65 to 70 °C) gently over the released contents of the eggs, embryos were isolated. Further, embryos were cleaned under dissection microscope and preserved in 75 % alcohol for further embryological studies (Figure 2). This method is proved the most useful for easy isolation of later embryonic stages (6^{th} to 9^{th} day), but extreme care must be taken for mid embryonic stages (5^{th} day) so that higher concentration of KOH does not dissolve the embryos.

Results and Discussion

Among the two methodologies, KOH Method is difficult to apply for early Muga eggs *i.e.,* day-1 to day-4 because KOH solution disintegrates these stage embryos very fast when compared to mid (day 5 and day 6) and late embryonic stages (day 7- day 10). But, KOH method is the most suitable one to isolate Muga embryos very easily from day 6 to day 10 Muga eggs (Figure 2). Though hot water method is practically more difficult than KOH method, it is the more appropriate one for isolation of early Muga silkworm embryonic stages from day 1 to 4. Hot water method can be applied in all embryonic stages but it is time consuming and slightly more difficult than KOH method. After experimental trials for isolation of Muga embryos, the KOH method is found the best for isolation of mid and late embryonic stages of Muga eggs. In both the methodologies, cleaning of

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yolk material and amniotic layer is crucial and requires perfection to isolate the embryo under dissecting microscope.

Embryological studies paved the way for development of egg handling techniques for domestic silkworm, *B. mori* L. It is clearly evident that egg preservation techniques are incomplete without the embryological studies (Reddy *et al.,* 2003). In seed production, if incubation is the last step, it forms the first step in silkworm rearing. Incubation is an important seed handling activity which should always be followed by black box technique. Circadian rhythmic studies along with chronological developmental studies of embryos helped a lot in development of incubation technique (Tanaka, 1966; Ayuzawa *et al.,* 1972; Joy and Gopinathan, 1995). Pathologists also consider proper preservation and incubation of silkworm eggs as an important prophylactory measure in preventing the occurrence of silkworm diseases (Reddy *et al.,* 1997). Ayuzawa *et al.* (1972) observed that silkworm seed should be preserved under the best ecophysiological conditions so as to exploit its full potential. For development of approximate 30 days preservation schedules for pure multivoltine races of mulberry silkworm (Tayade *et al.,* 1987; Verma and Chauhan, 1996), chronological studies of their embryos, paved the way. Thus, embryo isolation technology proves to be extremely important in developing egg handling techniques in silkworm seed sector. The present study is an initial step towards standardization of embryo isolation methodologies for developing egg handling techniques in Muga seed sector.

Conclusion

In future, Muga embryo isolation methodologies, may give scope to preparation of Muga embryonic chart; from day 1 to day 10. This chart would facilitate identification of sensitive and appropriate embryonic stages that are suitable for developing Muga egg preservation and incubation technologies. These embryological developmental studies in Muga eggs may also give scope for circadian rhythmic studies, which may lead to solve the practical problems involved in grainages by developing synchronized and uniform hatching techniques for Muga eggs.

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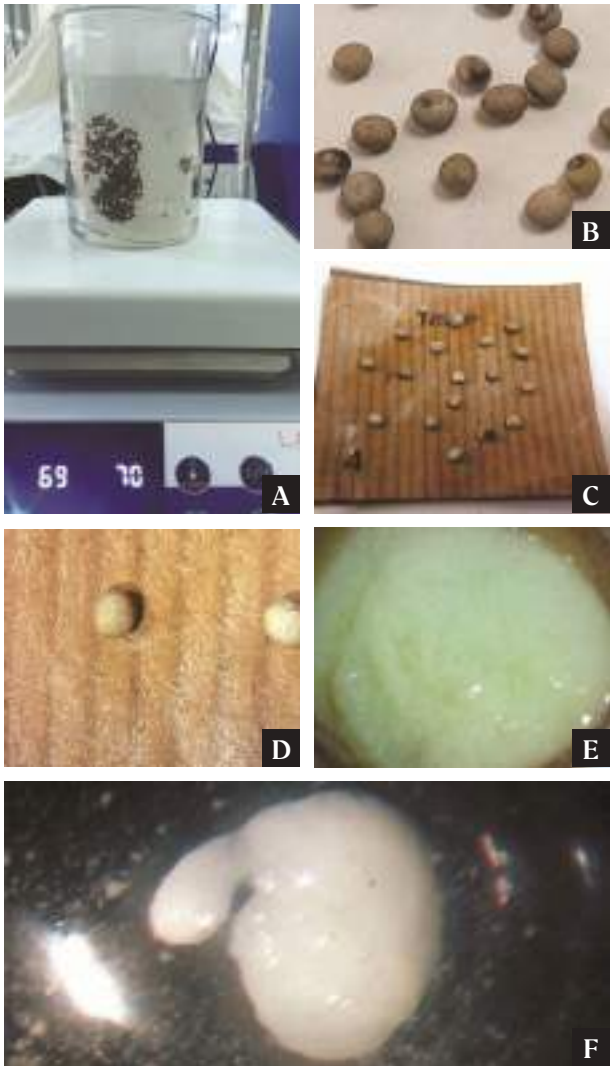

Figure 1 A-F: Hot water method for isolation of early Muga embryonic stages: A. Dipping of nylon netted bags carrying Muga Eggs in hot water at 69-70 °C for 5 min; B. Drying of eggs on clean surface for a few minutes; C. Eggs glued on the egg sheet and dried for 30 min for firm fixation; D. Eggs focused under dissection microscope; E. Egg dissected on the upper surface and embryo with yolk, identified on the ventral side; F. Early embryonic stage with head formation stage.

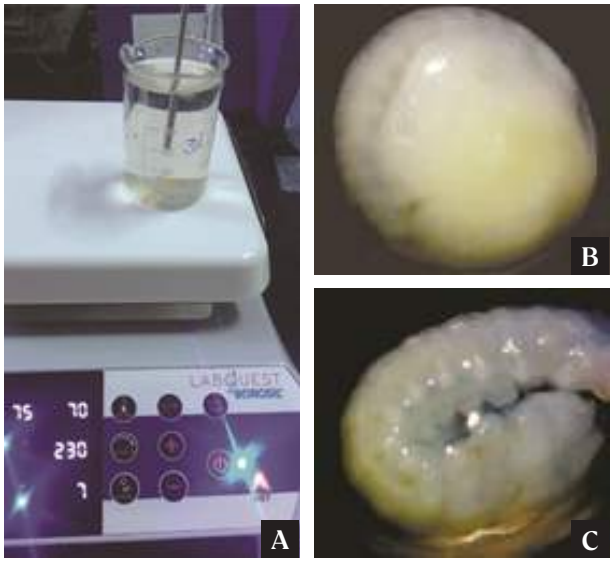

Figure 2 A-C: KOH method for isolation of late Muga embryonic stages: A. Boiling of 6th day Muga eggs in 3 % KOH for 5min; B. Embryo along with the yolk content; C. 6th day embryo isolated from the yolk material under dissection microscope.

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PHYSIO-CHEMICAL PROPERTIES OF SOAP ENRICHED WITH SERICIN

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Abstract

Sericin, the silk protein has specific features, such as gelling and moisture absorption abilities as well as anti-oxidant and anti-bacterial properties. It has got wide applications in industries, such as cosmetics, nutraceuticals, medical, pharmaceuticals, *etc.* The bio-compatibility, bio-degradability and wettability of sericin facilitate development of cosmetic products not only for skin but for nails and hair also. This study was taken up to explore the usage of sericin in cosmetic application where its utilization can help in generating extra revenue for the stakeholders. Hence, we investigated the potential of sericin to act as an active ingredient in bathing soaps. Sericin extracted from cut cocoons of both mulberry and eri varieties using HTHP method were tested. Glycerin was replaced by adding sericin and it was observed that added sericin imparted moisturizing property to the soaps. Sericin as having emulsifying property also, showed enhanced foam height. With its hygroscopic nature and easy water-solubility, sericin will help the skin to absorb the amino acids present in it.

Key words: Amino acid, anti-aging, bio-compatibility, bio-degradability, hygroscopic.

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Introduction

The main constituents of silk *i.e.,* fibroin and sericin account for about 75 % and 25 % of the total silk weight, respectively (Freddi *et al.,* 2003; Teli and Rane, 2011). Efforts have been on for many decades to recover and reuse sericin discarded by silk industry. Though considered as a waste, it can act as a natural bio-polymer in various applications (Gupta *et al.,* 2014). The gelling, moisture absorption, anti-oxidant and anti-bacterial properties being possessed by sericin has made it a commercial resource in various industries in recent years. Sericin has got wide applications in industries, such as cosmetics, medical, pharmaceuticals, *etc.* (Rangi and Jajpura, 2015). In this context, special silkworm races producing more percentage of sericin were reared in Japan for studying the properties of sericin for cosmetic use.

Many researchers have reported the use of sericin in cosmetics industry. It is mentioned that bio-compatibility, bio-degradability and wettability of sericin allow development of cosmetic products containing sericin not only for skin but for nails and hair also (Barajas Gamboa *et al.,* 2016). Gel prepared with 1.5- 2.0 % of sericin gives a silky and smooth feeling

to skin by forming a proactive and moisturizing surface on top most layer of skin. The moisturizing property of silk sericin gels is investigated by different techniques, such as skin impedance measurement, hydroxyproline content, trans-epidermal water loss (TEWL), and scanning electron microscopy (SEM) (Padamwar *et al.,* 2005).

A Lot of research has been carried out to check the suitability of sericin for cosmetic application. Many patents are also available mentioning the use of sericin in preparation of soaps and various skin care formulations. A patent by Unilever mentions the development of personal care composition comprising 0.1 to 5 % sericin having specific structure which delivers benefits, *viz.,* anti-aging, hydration, anti-inflammation, anti-oxidant and anti- pollution (Huang *et al.,* 2018).

A US patent discloses skin cleansing products which includes glycerinated soap and hard soap comprising sericin protein from *Thai silk,* as a main ingredient. The invention claims that products are safe for use and help nourish the skin so as to smoothen and soften it. The prepared soaps were inspected by a skin irritation test and found that it does not cause irritation and therefore can be used for unisex and all ages (Meesilpa, 2004).

Growing scope of sericin has made natural based product manufacturers to start selling sericin suitable for various cosmetic formulations. Thailand based Specialty natural products, France based Sollice Biotech and Switzerland based DSM are examples of such companies. Specialty natural products is a Thai Botanical Herbal extract manufacturer which sells sericin extract liquid from *Bombyx mori,* suitable to be used for shampoo, hair conditioner, skin moisturizer and shower gels (Sericin Extract Liquid - Specialty Natural Products https://www.snpthai.com/en/product/sericin-extract-liquid/). Sollice Biotech is a hi-tech tested cosmetic ingredients supplier which provides a large variety of cosmetic actives with a wide action range. SILK SERICIN® is pure sericin silk proteins supplied by the company which is entirely bio-compatible and bio-degradable providing moisturizing, anti-aging, anti-oxidant and soothing effect to skin. The sericin supplied by this company is a slightly beige coloured powder suitable for facial and body care with a recommended dose of 0.1-2 %.

International market has been flooding with skin care products containing sericin. Indian market is also realizing the scope and gaining grounds in this area. One can buy silk nourishing body wash, silk protein face wash, silk shampoo and silk conditioner from line of cosmetic products of Fab India Pvt Ltd (https://www.fabindia.com/searchresults?&search=silk&category-filter=Beauty). Lux® silk sensation soap bar and Lux® soft touch soap bar are two soaps from Unilever having sericin as one of the ingredients.

This study was taken up to explore the possibilities of value addition to sericin to generate extra revenue for the stakeholders. Apart from mulberry silkworm *Bombyx mori,* eri silkworm, *Philosamia ricini* is yet another completely domesticated species and studies have shown that mulberry silk contains ~30 % sericin while eri silk, ~15 % (Prasong *et al.,* 2009). So, in this study, both mulberry and eri sericin were taken as active ingredients for preparation of the soap.

Materials and Methods

Materials

Sericin enriched soap preparation

Sericin required for the study was extracted from bivoltine mulberry (Gupta *et al.,* 2014) and Eri cut cocoons using HTHP technique (Sreenivasa *et al.,* 2018). The sericin liquor thus obtained was filtered, dried and converted into powder form. To prepare soaps, Karnataka Soaps and Detergents Limited (KSDL), Bengaluru was contacted and their base soap was taken as reference. Glycerin (1 %) is usually added to reference base soap to get moisturizing property and in this study, glycerin was replaced with 1 % mulberry sericin and 1 % eri sericin. Physio-chemical properties were compared among four soaps (Base soap, base soap with 1 % glycerin, base soap with 1 % mulberry sericin and base soap with 1 % eri sericin) to ascertain the effect of sericin. A characteristic floral/musky note of fragrance was also added to the soaps.

Methods

The sampling and testing of the soaps was done at Karnataka Soaps and Detergents limited using IS 286:1978 standard (BIS, 1978). The methods substantially correspond to various ISO standards.

Total fatty matter (TFM)

Total fatty matter was determined by IS method corresponding to ISO 685 Analysis of soap: Determination of total alkali content and total fatty matter content (*ISO 685-2020*).

Free caustic alkali and free fatty acid

The IS method followed for estimation of free caustic alkali corresponds to ISO 685 Analysis of soap: Determination of total alkali content and total fatty matter content (*ISO 685-2020*). To measure the free caustic alkali present in soap, 10 g of the soap sample was dissolved in 100 ml of ethyl alcohol using a reflux condenser. Amount of free caustic alkali was measured using the following equation:

$$\text{Free caustic alkali (as NaOH), per cent by mass} = 100 \text{ X } \frac{4VN}{M}$$

where, V = volume in ml of standard sulphuric acid or hydrochloric acid used, N = normality of standard sulphuric acid, and M = mass in g of the material taken for the test.

To detect the amount of free fatty acid, same procedure was followed except for the addition of barium chloride solution and titration was done against standard sodium hydroxide solution. The amount of free fatty acid was calculated using the following equation:

$$\text{Free fatty acid, per cent by mass} = 100 \text{ X } \frac{28.25V1N1}{M}$$

where, V1 = volume in ml of standard sodium hydroxide solution used, N1 = normality of standard sodium hydroxide solution, and M = mass in g of the material taken for the test.

Alcoholic insoluble

The method followed is based on digesting the material in alcohol and calculating the dry weight of the remains. It is similar to ISO 673 Analysis of soaps: Determination of foreign matter of low solubility in ethanol (*ISO 673:1981*).

Chloride as NaCl

The test method adopted for estimation of chlorides corresponds to ISO 457 Analysis of soap: Determination of chlorides, test method (*ISO 457:1983(en)*). To calculate the presence of chloride in soap, 10 g of the soap was dissolved in 250 ml hot water. The following equation was used to calculate the amount of chloride present in soap in percentage by mass:

$$\text{Chlorides (as NaCl), per cent by mass} = 100 \text{ X } \frac{14.6 \text{ (S-B)/ N}}{M}$$

where, S = volume in ml of standard silver nitrate solution required for the material, B = volume in ml of standard silver nitrate solution required for the blank, N = normality of standard silver nitrate solution, and M = mass in g of the material taken for the test.

Moisture / volatile matter

The method used to calculate the moisture and any other volatile material contained in soap correspond to ISO 672 Analysis of soaps: Determination of moisture and volatile matter (*ISO 672-1968*). A simple oven method was used where; 5 g of the soap was kept in an oven at a temperature of 105 ± 2 °C in a petri dish till constant weight. The following equation was used to calculate the moisture content in the soap:

$$\text{Moisture and volatile matter content, per cent by mass} = 100 \text{ X } \frac{m}{M}$$

where, m = loss in mass in g of the material after drying, and M = mass in g of the material taken for the test.

Glycerin content

Similar to ISO 1066 Analysis of soaps: Determination of glycerol content (*ISO 1066-1975*), glycerin is estimated from acidimetric titration of formic acid produced by the reaction between glycerol and periodic acid (Pohle *et al.,* 1949). The glycerin content was calculated using the below mentioned formula:

$$\text{Glycerol, per cent by mass} = (T - T1) \times \frac{1.1511}{M}$$

where, T = volume in ml of 0.05 N sodium hydroxide solution required for test, T1 = volume in ml of 0.05 N sodium hydroxide solution used for blank, and M = mass in g of the sample taken.

Lauric acid content

Lauric acid content was calculated using liquid gas chromatography (Tatineni Spandana, 2019). Lauric acid was extracted in methanol from 10 g of the soap. The methanol layer was dried to get residues of lauric acid. Lauric acid and glycerol were separated using Sodium hydroxide. Lauric acid crystals were separated, dried and dissolved in methanol. The solution was filtered and was analyzed by gas chromatography.

Titre

It is the highest temperature reached when the mixed fatty and rosin acids obtained from soap are crystallized under the conditions of the test. Titre is generally taken to represent the solidification point of the mixed fatty and rosin acids, although they actually solidify over a range of temperature.

Foam height

The soap samples were used to form lather in water and the time taken for the foam-to collapse was measured using a stop watch (Abubakar Zauro, 2016). Five grams of the grated soap was added to 100 ml of 300 ppm hard water and was blended on low speed for 60 seconds. Then the lather was poured into a graduated cylinder and measured immediately after levelling off the top surface of the foam. In India, soap bars are deemed to conform to the standard requirements of a particular grade greater than or equal to 280 ml for grade 1, 240 ml for grade 2, and 200 ml for grade 3 (Ross and Miles, 1941).

pH of the soap

The pH of all the soap samples was measured using a pH meter at a dilution of 1 % of soap in distilled water (Mendes *et al.,* 2016).

Results and Discussion

The results obtained from the analysis of different physio-chemical parameters of the different soaps are given in Table 1.

TFM value tells us about the harshness of the soap towards skin. Higher TFM confirms that soap is highly hydrating and is less damaging to the skin and do not cause dryness to skin after bathing. Less TFM soap captures all the moisture in the skin making it dry. On the basis of TFM values, bathing soaps are categorized into three grades, Grade 1: soaps with a minimum of 76 % TFM, Grade 2: soaps with a minimum of 70 % TFM and Grade 3: minimum 60 % of TFM (Arasaretnam and Venujah, 2019). Table 1 show that the base used for preparations by KSDL is of grade 1 with a TFM value of 80 %. When 1 % glycerin was added to the base in the reference sample, the TFM value of the soap was found to be 79.31 %. When mulberry sericin was added to the soap base instead of glycerin, the TFM value got slightly reduced to 78.56 % but in both the cases, adhering to grade 1. But addition of eri sericin to the soap base further reduced the TFM to 70.86 % lowering its grade to level 2.

Table 1: Physio-chemical properties of different soaps

Particulars	Without glycerin	With glycerin	With mulberry sericin	With eri sericin
pH of soap	9.94	9.92	9.99	9.98
Colour of the soap	Off white	Off white	Off white	Beige
Odour of the soap	Characteristic floral with musky note	Characteristic floral with musky note	Characteristic floral with musky note	Characteristic floral with musky note
Total fatty matter, % by mass	80.01	79.31	78.56	70.86
Free caustic alkali as NaOH, % by mass	0.039	0.032	0.039	-
Free fatty acid as oleic acid, % by mass	-	-	-	0.57
Alcoholic insoluble, % by mass	1.17	1.24	2.44	2.38
Chlorides as NaCl, % by mass	0.78	0.73	0.62	0.61
Moisture & volatile matter, % by mass	11.05	10.94	11.31	10.88
Glycerin content, % by mass	0.15	0.96	0.21	0.22
Lauric acid content, % by GLC	13.69	14.11	15.65	13.14
Titre (^{0}C)	40.5	40.5	40.5	40.5
Foam height (ml)	450	440	500	500

Total alkalinity is a measurement of all alkaline substances in the soaps *i.e.,* primarily carbonates, bi-carbonates and hydroxides in addition to other substances. Free caustic alkali is one of the parameters that determine the abrasiveness of any given soap on skin. Presence of free caustic alkali in soap mostly results from improper or incomplete saponification (Onyekwere, 1996). In all soaps in the study, the free caustic alkali value was less than 0.05 % indicating complete saponification. Just like absence of free caustic alkali, soaps were free from free fatty acid as oleic acid.

Alcoholic insoluble is a parameter used to determine the purity of soap (Popescu *et al.,* 2011). It is the measure of non-soap ingredients known as builders or fillers, such as Sodium silicate, Sodium phosphate, Sodium carbonate and minor constituents, such as

bleachers, whitening agents and fluorescing agents in the finished product. The soap with high value of alcoholic insoluble suggests that it contains high level of impurities (Ogunsuyi and Akinnawo, 2013). The alcoholic insoluble value of soap base and soap having glycerin is 1.17 % and 1.24 %, respectively. But when 1 % of sericin was added to the soap, it increased to 2.44 % and 2.38 %, respectively for mulberry and eri as sericin is insoluble in alcohols.

The determination of presence of chlorides in soap is important as excess amount of chlorides causes soaps to crack (Taiwo *et al.,* 2008). The amount of chlorides as NaCl present in prepared soaps ranges from 0.78 to 0.61 % only. As only in one soap 1 % glycerin was added, that only had 0.96 % glycerin content. Since all the soaps have common base, they have shown the same titre value of 40.5 ^{0}C.

Moisture content is used in assessing the shelf-life of a product. If the moisture content of the soap is too high, it would lead to reaction of excess water with un-saponified fat to give free fatty acid and glycerol on storage (Adane, 2020). The moisture content of the soaps prepared in the study fall within the limits mentioned in Encyclopedia of Industries Chemical analysis (10 – 15 %).

The natural pH of skin surface is slightly acidic (Braun-Falco and Korting, 1986). The soap, in contact with water undergoes a hydrolysis reaction, releasing the alkali contained in these products and increasing the skin pH to 10-11 (Volochtchuk *et al.,* 2000). pH alterations in skin caused by the use of different types of soaps can damage the skin resulting in skin dryness (Stamatas *et al.,* 2010). Most of the soaps in market have a pH within the range of 9-10. The soap samples prepared in the study have also shown similar pH range. The pH of the soap depends upon the pH of the soap base; here, the pH of the soap base was 9.9 which was unaltered on addition of sericin.

Foam height of soap base was found to be 450 ml but, on addition of sericin, it increased to 500 ml. Sericin has inherent emulsifying properties which could be responsible for the increase in the foaming ability of the soaps.

Conclusion

The sericin is hygroscopic in nature and easily water-soluble making it easy for the skin to absorb the amino acids present in it. Addition of small amounts of sericin in soap can enhance the nutrition and elasticity of the skin. The experimental results show that the addition of sericin has no negative effect on the soap but it enhances the foaming property of it. Thus, silk sericin shows the potential to act as an active ingredient in soaps.

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EFFECT OF PRUNING HEIGHT AND TRAINING METHODS ON CASTOR (NBR1) TO MAINTAIN AS PERENNIAL BUSHY TREE FOR ERI SILKWORM REARING

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Abstract

Eri silkworm, *Samia ricini* is primarily reared on Castor *(Ricinus communis)* and many secondary host plants. The non-blooming red (NBR1) is one of the most suitable castor varieties for eri rearing. For eri silkworm rearing, foliage production is more important than seed production and hence, training of castor plants to small bushy trees of perennial nature with more foliage production would be more advantageous over annual crop. The effect of pruning height and training methods on the quality leaf production of castor (NBR1) was studied at Eri Silkworm Basic Seed Farm, Central Silk Board, Topatoli, Assam to ascertain the adaptability of castor plants as perennial trees. The treatments included four pruning heights (basal cut at 20 cm, 50 cm, 100 cm and 150 cm). Results evidently showed that the highest of 26.82 kg leaf biomass /tree was obtained from pruning height of 150 cm, followed by 100 cm (15.75 kg/tree), the yield from 50 cm and 20 cm pruning heights were on par and significantly higher than that of annual castor plants. Around 4800 kg leaf can be harvested in a year from one acre of annual castor plantation, whereas a well maintained, trained castor perennial garden can yield up to 10000 to 12500 kg. Training methods aimed at maintaining a proportioned small bushy castor tree with robust branches and dense canopy of leaves would serve the need of regular leaf harvest for eri farmers in a long way.

Key words: Eri silkworm, perennial castor, pruning, semi-woody plants, training.

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Introduction

Castor *(Ricinus communis)* is the most prefered host plant of eri silkworm *Samia ricini* (Donovan). Castor plant grows naturally over a wide range of geographical regions and similarly can be cultivated under diverse physical and climatic regimes. It is a warm season crop, cultivated in tropical, subtropical and temperate regions.

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A frost free growing period of 140-190 days is required for castor cultivation. It is grown in poor sandy to rich alluvial soil texture and red sandy loam to heavy black clayey soil under well drained conditions. Castor plant absorbs toxic heavy metals from soil. So, it can be grown for remediation of polluted soils (Kiran and Prasad, 2017). Castor can tolerate a pH of 8.0, even though it prefers slightly acidic conditions (pH of 5.0 to 6.5). In alkaline and saline conditions, the soil structure and soil physical properties will become an important limiting factor for growth.

Castor being a semi-woody, evergreen herbaceous plant, can be trained to a bushy shrub or a small tree. Originally, castor is of perennial nature and possesses indeterminate growth habit (Liv *et al.,* 2012). Castor is generally grown as an annual crop for oil seed production. The goal of tree training in castor is primarily to have a sturdy bush stature with increased longevity and produce more branches with good foliage suitable for eri silkworm rearing, allowing the rearers to harvest the leaves of required maturity for different instars. Lim *et al.,* 2007 suggested that castor leaves in the same plant can have widely variable leaf life span, which can be influenced by many environmental factors. The factors, such as diseases, severe drought stress, high temperature, salt stress, and high plant population influences the quality of leaf production. Hairy caterpillars, semilooper, jassids. whiteflies and thrips are important pests and in late winter, leaf blight and leaf spot diseases affect leaf yield (Sarmah *et al.,* 2011). The wild castor surviving as weed along roadside in the tropical climate can be a valuable resource of germplasm for resistance to pest, diseases and climatic variation (Salihu *et al.,* 2014).

Basically, castor plant produces long straight woody stems of 25 to 30 feet height, generally, without much branches if maintained as perennial. This pattern of foliage growth in long straight stems often poses difficulty in regular leaf harvest for eri silkworm rearing. If the plants are retained for the second season, the quality of the foliage deteriorates and production of small size leaves gets initiated. To overcome this bottleneck, repeated seed sowing year after year is practised by eri rearers to get short statured castor plants with quality leaves. Hence, cost of castor cultivation becomes high and also makes it labour intensive.

Studies on training castor to perennial bush for enhancing leaf production is scarce. Experiments were conducted at Eri Silkworm Basic Seed Farm, Central Silk Board, Topatoli, Assam to study the adaptability of castor plant (NBR1) as perennial tree. The present paper discusses in detail the pruning height and types of pruning as adaptable for perennial castor cultivation meant for eri silkworm rearing. The study also envisages to bring in a new package of practices for cultivating perennial castor focussing on enhanced leaf production suitable for ericulture.

Materials and Methods

The experiment was conducted at Eri Silkworm Basic Seed Farm, Central Silk Board, Topatoli, Assam, during the year 2021-22 to study the response of pruning in NBR1 castor varieties to different pruning heights and training methods suitable to yield good quality leaf biomass for eri silkworm rearing. The castor seeds were sown at recommended spacing of 2 X 2m and the plants were maintained as per recommended package of practices for eri rearing. Before initiating the pruning trials, the castor plant was allowed to grow to a height of 3.5-4 m with a strong healthy woody stem with good girth of 8-12 cm diameter during the first two years. In the third year, pruning experiments were conducted. Castor maintained as annual plants were taken as control. The treatments included four pruning heights (basal cut at 20 cm, 50 cm, 100 cm and 150 cm). The data on the yield of castor leaves obtained were analysed using simple descriptive statistics.

The main objective of training castor plant is to develop a strong framework of scaffold branches and increase the longevity of the tree. All the necessary cultural/ agronomic practices essential for training the castor plants were attempted as per standard techniques (Wade and Westerfield, 2020). The non-blooming red (NBR1) varieties were selected for the study as they are the most preferred one for eri silkworm rearing. The adaptability of castor as perennial tree along with pruning and training methods to maintain a proportioned small bushy tree were assessed. The training methods that were found feasible for optimum leaf production were also standardised and recommended.

Results and Discussion

The results evidently showed significant differences in castor leaf yield among the treatments of pruning cut heights (Table 1). The highest of 26.82 kg biomass/tree was obtained from pruning height of 150 cm, followed by 100 cm (15.75 kg/tree), the yield from 50 cm and 20 cm pruning heights were on par but significantly higher than those of annual castor plants.

Under the natural conditions, the stem of NBR1 castor variety is multi-branched, wherein the primary branches give rise to secondary branches. Woody stems are usually solid to a considerable height (~60 cm) and further develops hollow stems. Woody solid stem portions are generally well formed in soil having maximum organic matter. In the present study, it is observed that the plants in which scaffold branches arise within 0.7-0.9 m height from the ground level can form a small tree stature if pruned and trained properly. Previous studies by Govindan *et al.,* 2003, showed a significant difference in plant height and internode length among different castor genotypes grown for eri silkworm rearing. Plant height is controlled by the inherent genetic constitution of the plant. The intensity of apical dominance is high in most of the castor varieties. In NBR1 castor variety also, high apical dominance suppressed the growth of their lateral buds until the second growing season. Pruning helped to destroy the apical dominance temporarily and promoted the growth of lateral buds.

Proper judicious removal of plant parts, such as shoots, spurs and leaves, nipping away of terminal parts *etc.* helped to maintain tree structure. Pruning and training improved assimilation in main trunk and promoted side branches with healthy leaves. Also, the required short bushy stature was attained. Planned pruning cuts (Wade and Westerfield, 2020), such as heading and thinning were adopted during the second year. Best season for heading in castor was identified as winter coinciding with January and February months in Assam. Summer and rainy seasons should be avoided.

Table 1: Effect of pruning heights on leaf yield of castor plants (NBR1)

Pruning height (cm)	1st Harvest (kg)	2nd Harvest (kg)	3rd Harvest (kg)	4th Harvest (kg)	Total annual yield (kg)
150	7.85 ± 0.41	7.22 ± 0.39	6.39 ± 0.79	5.37 ± 1.03	26.82 ± 2.14
100	4.14 ± 0.78	4.02 ± 0.56	3.84 ± 0.38	3.75 ± 0.69	15.75 ± 1.72
50	1.61 ± 0.35	1.69 ± 0.31	1.56 ± 0.17	1.93 ± 0.23	6.79 ± 0.05
20	2.01 ± 0.17	1.91 ± 0.38	2.14 ± 0.26	2.03 ± 0.29	8.08 ± 0.33
Control	0.51 ± 0.13	0.53 ± 0.07	0.55± 0.09	0.40 ± 0.04	1.99 ± 0.10
CD at 5 %	0.56	0.49	0.54	0.74	1.59

Each value (Mean ± SD) represents pooled data of 3 replications.

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Heading and thinning cuts should go hand in hand in castor plant training. It is observed that the pruning cuts stimulated the shoot growth in different ways. Heading stimulated regrowth near the cut (Figure 1).

Figure 1: Shoot growth near pruning cut

It is the most invigorating type of pruning cut and resulted in thick compact growth. Such pruning is commonly adopted in mulberry crop grown for *Bombyx mori* silkworm rearing (Pawan *et al.*, 2017). Vertical stems exhibited vigorous shoot growth within 4 to 6 inches of the pruning cut. Further shoot growth was farther away from the pruning cut on limbs having a 45° to 60° angle from the vertical limb (Figure 2).

Thinning cuts assisted to keep the plant in the shape of a small tree bush. In this process, the irregular and unproductive branches coming out of the canopy were removed from the point of its origin and no re-growth was allowed to occur from the cut ends. Thinning did not invigorate more shoot formation, but regulated the distribution of available nutrients to the selected healthy shoots for quality leaf production as desired for an eri silkworm host plant. Thinning cuts can be performed in all seasons. This cultural operation permitted more sunlight to the canopy, helped the tree to maintain a balanced vigour and aided to obtain optimum yield of superior quality castor leaves.

Figure 2: New shoot growth initiated farther away from pruning cuts

Figure 3: Castor plants trained to bushy perennial tree

Another important cultural practice that ensured quality castor leaf production is clipping of inflorescence as and when produced. The net assimilation rate of castor plants was 1.1 mg cm^{-2} day^{-1} in the vegetative period compared with 0.6 mg cm^{-2} day^{-1} in the reproductive phase (Aires *et al.,* 2011). Since castor seeds are good source of oil, most of the nutrients from soil and photosynthates are utilised in the seed formation process. Clipping the inflorescence on time ensures quality leaf production by avoiding huge diversion of assimilates for formation of castor beans with rich oil content.

Around 4800 kg of leaf can be harvested in a year from one acre of annual castor plantation of NBR1 variety (Singh, 2015). A well maintained, trained castor perennial garden can yield up to 10000 to 12500 kg leaf (Table 2).

Holistic nutrient management of soil is vital to obtain satisfactory leaf yields. Precise inputs are to be provided based on soil test results. The tap root of the perennial castor can go even up to the depth of 5–6 m in the soil. The lateral roots are spread up to 1 m laterally. Hence, the spacing has to be more than 2 m between the plants and 3 m between the rows considering the canopy size. The long petioles of castor leaves are another important factor that limits the high plant density.

Table 2: Recommendations for perennial castor (NBR1)

Spacing	2.5 m between plants in a row and 3m between rows.
No. of plants	539 trees per acre; 1333 trees per ha.
Land preparation	Well ploughed land with good drainage, formation of ridges and furrows (for low lying areas).
Intercropping	Amenable for intercropping with legumes, and vegetable crops.
FYM	6 kg in three split doses per tree per year. Every castor leaf harvest should be followed by ½ cft FYM application.
NPK fertilizers	90:45:45 kg per ha; N 67 g/ plant, P 33 g/plant, K 33 g/plant. Increased fertilizer application often resulted in taller plants with bigger trunks. Hence, optimization of nutrient supplement for leaf initiation is required based on soil test results.
Irrigation	After every leaf harvest, 2-3 rounds of irrigation is required for new leaf initiation.
Pruning	Heading is done during winter season after one year of stem growth with at least 60 cm height of thick woody solid stem. Thinning can be done all through the year.
Optimum leaf yield	7.5 -8 kg leaves per harvest/single trained tree. An average leaf yield of 22 to 25 kg/tree in four harvests in a year. 10000 to 12500 kg/ acre/ year.
Eri rearing capacity/ year	800-1000 DFLs/ year.

Castor plant response to water stress differs significantly depending on the intensity and duration of stress and stage of development. The moisture requirements of castor at different growth stages are not uniform. The osmotic adjustment in castor and leaf composition varies with moisture stress (Babita *et al.,* 2010). A mild water stress leads to inflorescence/ raceme initiation instead of leaf production. Therefore, every leaf harvest should be followed by irrigation and nutrient supplementation. Application of organic manures, such as vermicompost, FYM and compost increases the leaf productivity. The quality of leaf biomass plays a significant role in the growth of the silkworm and ultimately in the economic traits of cocoons (Jayaramaiah and Sannappa, 2000; Chandrappa *et al.,* 2005). The study also revealed that the leaf quality of perennial bushes was found as good as that of annual plants with respect to eri silkworm rearing (unpublished). The woody stems were found susceptible to borer pests and termite attack. Chemical control is not an option for pest management in silkworm host plantations. Therefore, mechanical removal of infected stumps from the garden is recommended.

Conclusion

This article provides insights on impact of different pruning heights and other techniques useful for training of castor plants to a foliage rich small bushy tree. The results are promising with quantitatively and qualitatively higher leaf yield for eri silkworm rearing. The present study opens up the scope for establishment of castor plantation of perennial tree stature with more leaf production for eri silkworm rearing. A fairly deep, well-drained soil with good organic matter is the prime requirement for maintaining perennial castor tree garden. Nevertheless, these perennial castor trees reduce the cost of cultivation on land preparation, repeated seed sowing, gap filling, weed management *etc.* Further research priority should be placed on improved castor breeds adaptable to pruning to high yielding perennial bushy trees.

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PRACTICES OF TASAR COCOON COOKING ADOPTED FOR WET REELING TO ATTAIN BETTER PRODUCTIVITY AND RECOVERY OF SUPERIOR QUALITY RAW SILK

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Abstract

Tasar green cocoon lots dried using CSTRI conveyor drier and by conventional method of sun drying were considered the material for the present study. The dried cocoons were cooked by adopting new methods of pressurized cooking and vacuum permeation, and conventional method of open pan cooking to prepare them for wet reeling. All the batches of cocoons were reeled on CSTRI wet reeling machine. In addition, the traditional method of cooking was also followed wherein the cocoons were reeled on dry reeling machine. The yarns produced were tested for quality parameters as per international standard testing methods. The data generated on reeling and testing of silk were analyzed statistically. By adopting conveyor hot air drying of tasar cocoons in association with pressurized cooking/vacuum permeation cooking technique, significant improvement in productivity, reelability, silk recovery (*i.e.,* less number of cocoons per kg silk) and quality of silk yarn can be achieved as compared to the practices of conventional method of sun drying and open bath cooking of tasar cocoons. The silk produced can be effectively used as warp for production of fabric.

Key words: Multi-end, pressurised cooking, productivity, raw silk recovery, vacuum permeation, wet reeling.

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Introduction

Cooking is the process which facilitates softening of the cocoons for easy unwinding of filament during reeling, to enable high recovery of quality silk. Tasar cocoon shells are very hard due to its sericin being compounded with calcium oxalate and tannin and hence, is very difficult to get softened. Use of certain chemicals along with pre-treatment of water permeation are required to achieve this. Tasar cocoon cooking demands specialized treatments for the softening procedure as water or chemicals used do not penetrate easily into it. Many studies have been conducted in this direction by using 1. Proteolitic enzymes, 2. Soda, 3. Soap and Soda, 4. Soda and Hydrogen Peroxide, 5. Hydrogen

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Peroxide and Soap, 6. Citric acid, 7. Sodium Perborate, 8. Ethylene di-amine *etc.* (Das *et al.,* 1993; Sonwalkar, 1993; Gulrajani *et al.,* 1997; Gahalot, 2010). Kariyappa and Somashekar (2003) and Kariyappa and Subhas (2021) have reported that the tasar cocoons dried in conveyor drier and cooked thereafter for wet reeling has improved yarn recovery, reelability and quality of silk. Udaya *et al.* (2010, 2018) had reported that plain water cooking followed by vacuum permeation treatment with chemicals has increased cooking efficiency, reeling performance and yarn quality to a certain level. All these methods have yielded certain results comparable to those of conventional cooking method but, there is still a lot of scope for further improving the efficiency to the desired level.

Many studies have addressed the aspects of cooking of mulberry cocoons by use of vacuum permeation chamber and pressurised cooking technology. Hariraj *et al.,* 2005, have reported that vacuum permeation treatment before cooking of long stored bivoltine cocoons is found to soften the sericin by wetting the shell layers uniformly thereby improving the water absorption and reeling characteristics. Subhas and Somashekar (2003 a, b, c,) have reported the effect of retting temperature and permeation treatment on reeling performance of Indian bivoltine hybrid cocoons and it is found that these treatments have significant influence on the cocoon cooking condition, which in turn influences the reeling and yarn quality characteristics.

Presently, the reelers are cooking nearly 150-200 no. of tasar cocoons per day in an open vessel or in pressure cooker using chemicals in a batch-wise process (batch of about 100 cocoons) which is time consuming and results in uneven / under cooking of cocoons. It also results in batch to batch variation, bursting of cocoons and thereby increase in waste percentage *etc.* To overcome these problems, there is a need to develop a proper tasar cocoon cooking technology for handling large quantity. Keeping in view the facts stated above, the present work was proposed to study the influence of vacuum treatment / pressurised cooking technology on effectiveness of cooking of tasar cocoons in achieving better productivity, higher reeling performance and quality of silk.

Materials and Methods

Tasar Daba cocoons (30000 no.) of 'A' grade, purchased from Raw material bank Chaibasa, Jharkhand, were divided into two batches; one batch was dried on CSTRI conveyor drier and the other one was sun dried and were subjected to experimental trial.

Traditional method of cooking

Tasar cocoons were cooked in an earthen pot using sodium bicarbonate (10 gpl). The cocoons were cooked by immersing them in boiling chemical solution in earthen pot for one hour. After boiling, the pot was covered overnight, with straw at the top to retain warmth and moisture. After soaking overnight, cocoons were taken out, spread on ash bed or gunny bag to remove excess water. After this, the cooked cocoons were always kept moist using wet cloth wrapping and then deflossed individually and reeled on dry reeling machine.

Open pan method of cooking

Tasar cocoons were taken in cage, boiled in plain water for 45 minutes in an open pan and after that, the cooking bath temperature was allowed to drop to 70 °C. The cocoons were then taken out and soaked in the following solution at an initial temperature of 70 °C for 1 h.

Sodium silicate (8 gpl) + Sodium carbonate (10 gpl) + Hydrogen peroxide (12 gpl)

The cocoons were then deflossed individually by hand and reeled on wet reeling machine by wet reeling principle.

Pressurised cooking method

Cooking trials were carried out by varying retting, steaming, cooking and adjustment duration using CSTRI Pressurised cooking chamber. Some trials involved the procedure of boiling in EDTA while cooking. After the pressurised cooking, cocoons were soaked in a solution consisting of different

combination of chemicals *viz.,* Sodium carbonate, Sodium bicarbonate, Hydrogen peroxide and Sodium Silicate at different concentrations as given Table 1. The initial temperature of soaking bath was maintained at 70 °C and soaking time varied from 30 minutes to overnight / one day / two days. Subsequently, the cocoons were deflossed individually by hand and reeled on wet reeling machine by wet reeling principle.

Table 1: Conditions for tasar cocoon cooking using CSTRI Pressurized cocoon cooking chamber

Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
Cooking											
Boiling in EDTA 5 gpl (min)									3	3	3
Retting at 80 °C (min)	2	2	2	2	2	2	2	3	3	3	3
Steaming (min)	10	10	10	10	10	10	10	5	5	5	5
Boiling (min)	5	5	5	5	5	2	2	2	2	2.5	2.5
Steaming (min)								5	5	10	10
Boiling (min)								5	5	5	5
Setting (min)							5				
Sprinkle cold water to reduce temperature (96-86 °C)		√	√	√	√	√	√	√	√	√	√
Soaking											
Sodium carbonate (gpl)	6	4	2	8	4	4	4	4	4	4	4
Sodium bicarbonate (gpl)	4				4		4	4		4	
Sodium silicate (gpl)	5	4	2	8	5	12	5	5	4	5	4
H_2O_2 (ccpl)	5	15	12	15	5	12	5	5	12	5	12
Soaking duration (h)	12	2	36	1	12	1	12	12	1	12	12

T1-T11 are cooking treatments by using pressurised cooking chamber by varying treatment durations, chemical concentrations, chemical combinations and soaking durations.

Vacuum permeation cooking method

Cooking trials were also conducted using vacuum permeation chamber at vacuum pressure of 300 mm Hg for 3 cycles, which is followed by cooking of cocoons in boiling water for 10 minutes, setting for 5 minutes and then sprinkling cold water to bring down the temperature to 70 °C for adjustment. Then the cooked cocoons were soaked in a solution consisting of different combinations of chemicals *viz.,* tasar plus, Sodium carbonate, Sodium bicarbonate, Hydrogen peroxide and Sodium silicate at varying concentrations as given in Tables 2 and 3. The initial temperature of soaking bath was maintained at 70 °C and soaking duration varied from 30 minutes to overnight / one day / two days. After completion of soaking, the cocoons were deflossed individually by hand and reeling was carried out on wet reeling machine by wet reeling principle.

Table 2: Conditions for tasar cocoon cooking using vacuum permeation equipment

Treatment	P1	P2	P3	P4
Cooking				
Permeation at 300 mm Hg pressure (cycles)	3	3	3	3
Cooking duration (min)	10	10	10	10
Setting (min)	2	2	2	2
Sprinkle cold water (min)	2	2	2	2
Soaking				
Tasar plus (gpl)	8			
Sodium carbonate (gpl)		4	4	4
Sodium bicarbonate (gpl)			4	4
Sodium silicate (gpl)	5	4	5	5
H_2O_2 (ccpl)	5	5	5	5
Soaking duration (h)	12	12	12	36

Table 3: Cooking conditions with vacuum permeation

Treatment	P5	P6	P7	P8	P9	P10	P11	P12
Cooking								
Permeation at 300 mm Hg pressure (cycles)	3	3	3	3	3	3	3	3
Cooking duration (min)	10	10	10	10	10	10	10	10
Setting /adjustment time (min)	2	2	2	2	2	2	2	2
Sprinkle cold water (min)	2	2	2	2	2	2	2	2
Soaking								
Sodium carbonate (gpl)	4	4	4	3	3	3	3	3
Sodium bicarbonate (gpl)	4	4	4	3	3	3		
Sodium silicate (gpl)	4	4	4	3	3	3	3	3
H_2O_2 (ccpl)	5	5	5	5	5	5	5	5
Soaking duration (h)	12	36	48	12	36	48	12	48

P1-P12: Cooking conditions for tasar cocoons in vacuum permeation equipment, by varying treatment durations, chemical concentrations & combinations and soaking durations.

Under each trial, 100 cocoons were cooked and reeled on wet reeling machine by maintaining 6 cocoons per end. Standard conditions were maintained in all the cases during cooking and reeling. The data were recorded in respect of silk weight and waste weight (defloss, reeling and pelade waste) in each case. Based on the experimental data, different post cocoon parameters were calculated, *i.e.,* cooking efficiency, raw silk recovery percentage, waste percentage and reelability percentage. Yarn produced by adopting the above cooking and reeling methods were subjected to quality testing. The yarns were tested on serigraph for tensile properties (bundle strength), *i.e.,* tenacity and elongation, and for cohesion, on Duplan cohesion tester. The data generated during reeling and testing were analysed statistically using SPSS package.

Cooking efficiency: After cooking, the soft, burst open and hard cocoons were counted in each case and calculated the cooking efficiency as follows (Subhas and Somashekar, 2003 a, b, c).

$$\text{Cooking efficiency (\%)} = \frac{\text{No. of cocoons softened after cooking}}{\text{No. of cocoons taken for reeling}} \times 100$$

Reeling and reelability

Reeling was carried out on wet reeling machine with 4 ends, adopting the following Reeling parameters / condition:
Reeling speed: 100 m/min
Croissure length: 8 cm
Number cocoons/end: 6

Number of ends reeled: For experimental reeling, 2 ends; for mass reeling, 4 ends in wet reeling machine. Further, mass reeling was also conducted on CSTRI multi-end reeling machine, with 5 ends.

Reelability (%)

In order to assess the reeling characteristics, reelability test was carried out during tasar cocoon reeling process. The cocoons used for the study were counted before reeling and the no. of cocoons casted during reeling was noted down with the help of casting counter. Based on the results, reelability of the cocoons was calculated as follows (Subhas and Somashekar, 2003 a, b, c).

$$\text{Reelability (\%)} = \frac{\text{Number of cocoons taken for reeling}}{\text{Number of casting}} \times 100$$

Raw silk Recovery percentage

Raw silk recovery percentage is based on the quantity of tasar silk recovered from the cocoons through the reeling process and was calculated on the basis of shell weight of the cocoons (Subhas and Somashekar, 2003 a, b, c).

$$\text{Raw silk recovery (\%)} = \frac{\text{Reeled tasar silk weight}}{\text{Total shell weight}} \times 100$$

Silk waste percentage

During the reeling of the cocoons, some amount of silk waste is generated consisting of the outer layer mass defloss waste, reeling waste and the last un-reelable pelade waste, expressed as percentage on reeled silk weight (Subhas and Somashekar, 2003 a, b, c).

$$\text{Silk waste (\%)} = \frac{\text{Total silk waste generated}}{\text{Reeled tasar silk weight}} \times 100$$

Tensile properties

Tensile properties of tasar reeled silk *i.e.,* tenacity (gpd) and elongation (%) were evaluated as per the IS 15090 standard (BIS, 2002) on serigraph, for bundle strength and elongation. Tenacity of raw silk is its tensile strength or the amount of tensile stress a silk yarn can withstand before breaking and elongation of raw silk is the amount that it can stretch when pulled to the breaking point. Silk specimens were tested for 25 revolutions each and 15 readings were taken.

Cohesion

The objective of this test is to determine the degree of agglutination of silk filaments forming the thread. The raw silk was subjected to mechanical abrasion

and number of strokes required to split the filaments was observed on Duplan cohesion tester. Cohesion properties of tasar reeled silk were evaluated as per IS 15090 standard. Ten readings were taken for each sample and the average number of strokes was expressed as cohesion of raw silk.

Table 4: Reeling performance of cocoons cooked with pressurised technique

Parameter	Treatment													
	C T1	C T2	C T3	C T4	C T5*	C T6	C T7	C T8	S T8	C T9	C T10	S T10	C T11	S T11
R.S.R. (%)	72.8	73	67	64.7	75.4	74.7	72.5	74.9	63.9	64.3	75.4	68.5	65.7	62.9
W. (%)	27.2	27	33	35.3	24.6	25.4	27.5	25.2	36.1	35.8	24.6	31.5	34.3	37.1
S.W. (g)	123	128	105	86.1	139	139	132	139	126	134	141	126	127	111
W.W. (g)	39.0	41.7	60.4	47	52.8	45.2	44.7	46.6	71.5	73.8	52	57.7	66.1	65.4
No. of cocoons required to produce 1 kg silk	815	784	956	1161	718	721	758	721	791	754	711	797	790	904
Tenacity (gpd)	2.6	2.7	2.5	2.6	2.7	2.5	2.6	2.7	2.4	2.6	2.7	2.3	2.7	2.4
Elongation (%)	28.7	25.4	27	28.7	27.3	25.3	23.6	24	20.9	24.8	34.6	31	31	25.7
Cohesion (strokes)	20.2	24.4	11	19.8	32.8	14.6	25.6	19.6	13.4	23.4	47	19.6	28.9	18.2
Reelability (%)	52.3	52.6	41.1	38.4	65.1	55.3	51.6	54.1	38.8	60.2	6.15	42.4	42.6	41.7

*All parameters are significant ($p<0.05$); R.S.R. = Raw silk recovery (%), W. = Waste (%), S.W. = Silk weight, W.W. = Waste weight, C = Conveyor dried, S = Sun dried.

Table 5: Reeling characteristics of cocoons cooked under vacuum permeation

Parameter	Drying / Cooking treatment												
	C P1	C P2	S P2	C P3	C P4	C P5	C P6	C P7	C P8	C P9	C P10	C P11*	C P12
R.S.R. (%)	76.8	77.2	64.9	75	78.3	73.9	74.6	75.9	77.3	81.3	78.2	78.9	77.2
W. (%)	23.2	22.8	35.1	25	21.7	26.1	25.4	24.1	22.7	18.7	21.8	21.1	22.8
S.W. (g)	128	153.8	119.5	146.2	151.7	148	148	158	163	159	153	169	161
W.W. (g)	38.6	45.5	64.6	41.9	36.3	52.4	50.3	50.7	49.6	36.6	42.7	43.7	47.6
No. of cocoons required to produce 1kg silk	782	650	839	684	659	675	678	633	613	629	654	591	622
Tenacity (gpd)	2.5	2.8	2.5	2.6	2.7	2.63	2.75	2.52	2.65	2.64	2.57	2.75	2.64
Elongation (%)	26.8	24.4	24.5	30.2	25.3	31.9	26.6	26	24.7	27.9	26.4	24.1	27.9
Cohesion (strokes)	26.4	28.6	17.2	28.4	29	23.8	24.8	25.2	27.5	26.6	25.2	28.8	25.5
Reelability (%)	61.2	67.6	55.2	64.4	62.1	54.8	41.7	59.7	64.6	63.5	59.7	65.6	64.5

* All parameters are significant ($p<0.05$); C = Conveyor dried, S = Sun dried.

Table 6: Comparison of reeling performance of cocoons subjected to new and traditional methods of cooking

Parameter	Cooking method			
	Traditional	Open pan	Pressurised cooking	Permeation cooking
Cooking efficiency (%)	72	86	94	96
R.S.R. (%)	42.3	52.0	75.4	78.9
W. (%)	57.7	48.0	24.6	21.9
Reelability (%)	23.3	45.5	64.6	65.6
No. of cocoons required to produce one kg silk	1288	1138	718	613
Tenacity (gpd)	2.1	2.6	3.1	2.9
Elongation (%)	20	23	25	24
Cohesion (strokes)	0	21	33	29

Table 7: Comparison of reeling performance of new and traditional methods of cooking (ANOVA)

		Sum of Squares	df	Mean Square	F	Sig.
Cooking efficiency (%)	Between Groups	1070.917	3	356.972	71.394	0.000
	Within Groups	40.000	8	5.000		
	Total	1110.917	11			
R.S.R. (%)	Between Groups	2905.560	3	968.520	1827.396	0.000
	Within Groups	4.240	8	.530		
	Total	2909.800	11			
W. (%)	Between Groups	2790.149	3	930.050	506.150	0.000
	Within Groups	14.700	8	1.838		
	Total	2804.849	11			
Reelability (%)	Between Groups	3676.667	3	1225.556	735.333	0.000
	Within Groups	13.333	8	1.667		
	Total	3690.000	11			
No. of cocoons required to produce one kg silk	Between Groups	949204.667	3	316401.556	219.914	0.000
	Within Groups	11510.000	8	1438.750		
	Total	960714.667	11			
Tenacity (gpd)	Between Groups	2.000	3	.667	8.000	0.009
	Within Groups	.667	8	.083		
	Total	2.667	11			
Elongation (%)	Between Groups	48.917	3	16.306	4.659	0.036
	Within Groups	28.000	8	3.500		
	Total	76.917	11			
Cohesion (strokes)	Between Groups	1653.667	3	551.222	200.444	0.000
	Within Groups	22.000	8	2.750		
	Total	1675.667	11			

Results and Discussion

Pressurised cooking

Totally, 14 cooking treatments were undertaken by varying retting, steaming, cooking durations and chemical concentration. It is observed that cooking treatment T5 (Table 4) associated with conveyor dried cocoons has shown the highest raw silk recovery percentage, less waste percentage and less number of cocoons required to produce one kg silk compared to sun dried cocoons and other trials. Treatment T5 has resulted in reelability of 65.1 %, raw silk recovery of 75.45 %, less number of cocoons (718) required to produce one kg silk, tenacity of 2.7 gpd, elongation percentage of 27.3 and cohesion of 33 strokes (Table 4). The statistical analyses indicate that cooking treatment T5 has given significantly better results than others and it is significant at 5 % level. Based on the above trials, protocol for cooking of tasar cocoons for wet reeling using pressurised cooking technique was standardized.

Vacuum permeation cooking

In total, 13 cooking treatments were conducted and the results show that cooking treatment of P11 (Table 5) using conveyor dried cocoons has offered the highest raw silk recovery percentage, less waste percentage and less number of cocoons required to produce one kg silk compared to sun dried cocoons and other experimental trials. P11 has recorded 78.9 % raw silk recovery, 65.6 % reelability percentage, less no. of cocoons (591) required to produce one kg silk, tenacity of 2.75 gpd, elongation percentage of 24.1 and cohesion of 29 strokes as shown in Table 5. From the statistical analysis, it is revealed that cooking treatment P11 has significantly higher values than other trials at 5 % level. Based on the above trials, protocol for cooking of tasar cocoons for wet reeling using Vacuum permeation cooking was standardized.

Comparison of reeling performance of cocoons subjected to new and traditional methods of cooking

Conveyor dried cocoons were cooked by four different methods *i.e.,* traditional, open pan, vacuum permeation technique and pressurised cooking technology. The results of post cocoon parameters for different cooking methods adopted were recorded and are presented in Table 6. It is observed that cooking method of pre-treatment of vacuum permeation shows the highest cooking efficiency, raw silk recovery percentage, reelability percentage, less waste percentage and less number of cocoons required to produce one kg silk compared to pressurised cooking, open bath and traditional methods of cooking. The tasar yarn produced by adopting the pre-treatment of pressurised cooking technology has resulted in higher tenacity, elongation and cohesion strokes. From the statistical analysis (Table 7), it is observed that there is no significant difference between pre-treatment of vacuum permeation cooking method and pressurised cooking, in parameters, such as cooking efficiency, reelability, raw silk recovery, number cocoons required to produce one kg silk, tenacity, elongation and cohesion strokes.

These post cocoon parameters of cocoons subjected to pre-treatment of vacuum permeation and pressurised cooking technologies are significantly (at 5 %) better than those of traditional and open pan methods. This is due to the fact that the pressurized cooking and vacuum permeation treatment help the water forcibly reach every layer of the cocoon shell and cross over points of filaments.

After cooking, cocoons were soaked in chemical solution which easily and uniformly flows into all the layers of filament inside the shell due to pre-treatment of pressurized cooking and vacuum permeation. This facilitates uniform softening of the cocoons and

hence, results in better cooking efficiency, reelability and raw silk recovery percentage. Due to uniform cooking of tasar cocoon shells under these cooking conditions, the silk filaments were reeled with less breakage during reeling with the least number of castings compared to traditional and open pan methods, leading to significant increase in reelability. Significant increase in reelability in the case of pre-treatment of pressurized cooking and vacuum permeation has contributed in achieving high raw silk recovery, less waste % and reduced number of cocoons per kg silk production as compared to those of traditional methods.

Traditional cooking process, due to its prolonged and improper procedure generates more number of burst cocoons compared to pressurized and vacuum permeation cooking whereas, hard uncooked cocoons are more in open pan method wherein the cooking efficiency is reduced significantly. However, the cooking performance is comparatively better in open pan as compared to traditional method. Less number of cocoons required to produce one kg silk in the case of pressurised cooking and vacuum permeation cooking methods than other methods is due to higher raw silk recovery and less waste generation.

Tenacity and elongation of silk yarn reeled from cocoons subjected to pressurized and vacuum permeation cooking methods are significantly higher than those of traditional and open pan cooking methods and this may be attributed to the damage caused to the silk fiber due to prolonged alkali treatment during the cooking in the case of traditional and open pan methods. Further, in open pan/traditional method of cooking, cocoons are not softened as much as of pressurized cooking and vacuum permeation cooking which increases filament unwinding tension and total reeling tension during reeling which affects and reduces the elongation of silk yarn.

Cohesion of tasar reeled yarn obtained is significantly better for pressurized and vacuum permeation cooking methods as compared to those of the traditional and open pan methods. This could be attributed to the better and uniform swelling and softening of sericin of cocoon filaments in all the layers of cocoon shell under the former conditions. Very poor cohesion in open pan is due to under and non-uniform cooking of cocoons and very poor/no cohesion in the yarn produced from traditional method of cooking is due to prolonged and improper cooking of cocoons in alkali solutions leading to complete removal of sericin in the filament.

It is to be noted that when cocoons are cooked properly, the sericin of the filament will be swollen and softened to the required level. When these filaments from the cocoons pass through the croissure of good length during reeling, the required agglutination of the filaments in the raw silk thread will take place and results in good cohesion of raw silk. Whereas, when the cocoons are under cooked, sericin of the cocoon filaments will not be swollen and softened to the required level, leading to poor agglutination of the filaments in the raw silk thread. This results in poor cohesion of raw silk, even though sufficient binding pressure is exerted on the thread by putting a good croissure length.

Mass trial

Based on the results of the above mentioned experiments, the best cooking conditions were identified for cooking the conveyor-dried cocoons by adopting vacuum permeation technique and pressurised cooking technology and mass trials were conducted for four hours. The cocoons cooked under optimized conditions, were deflossed and reeled on CSTRI wet reeling machine and multi-end reeling machine maintaining five cocoons per end and the results were compared with those of traditional method of open pan cooking. The detailed results are presented in Table 8.

Table 8: Mass cooking and reeling trials using Wet and Multi-end reeling machines

		Reeling machine		
		Wet reeling		Multi-end
Trial	Open pan cooking	Vacuum permeation cooking (P11)	Pressurised cooking (T5)	Vacuum permeation cooking
Production per 4 h (g)	273	510	525	720
No. of cocoons	300	550	550	550
S.W. (g)	326	695	644	720
W.W. (g)	214	295	346	270
RSR (%)	60.44	70.41	65.05	72.27
W. (%)	39.56	29.59	34.95	27.73
No. of cocoons required to produce 1 kg silk	920	791	854	763
Tenacity (gpd)	2.60	2.78	2.72	3.08
Elongation (%)	25.4	28.8	29.23	27.80

Table 9: Economics of wet reeling to produce one kg tasar silk in Multi-end reeling machine

#	Particulars	Rate ( )	Quantity	Amount ( )
1	Cost of cocoons	3.2 per cocoon	750 no.	2400
2	Water		31 l	
3	Soda 4 gpl	55 per kg	124 g	8
4	Sodium silicate	100 per kg	124 g	13
5	Hydrogen peroxide	100 per liter	155 cc	16
6	Electricity			
a.	Multi-end reeling machine 1 HP (for 6 h)	9 unit	1*0.745*6*9	40
b.	Re-Reeling 1hp (1 h)	9 unit	1*0.745*1*9	7
c.	Electric boiler 9 KW (4 h)	9 unit	9*4*9	324
7	Labour	300 per day	2 no.	600
A	Total cost of production			3408
B	Cost of waste	500 per kg	269 g	135
C	Cost of production (A-B)			3273
D	Selling price of raw silk			5000
	Profit (D-C)			1727

1HP=0.745 kilowatt (kw), unit price: 9 per kwh

Table 10: Economics of wet reeling to produce one kg tasar silk in wet reeling machine

#	Particulars	Rate ( )	Quantity	Amount ( )
1	Cost of cocoons	3.2 per cocoon	750 no.	2400
2	Water		31 l	
3	Soda 4 gpl	55 per kg	124 g	8
4	Sodium silicate	100 per kg	124 g	13
5	Hydrogen peroxide	100 per liter	155 cc	16
6	Electricity			
a.	Wet reeling machine 1/2 HP (for 8 h)	9 unit	1/2*0.745*8*9	27
b.	Re-Reeling 1hp (for 1 h) 9 per unit (KW)	9 unit	1*0.745*1*9	7
c.	Electric boiler 9 KW (for 4 h)	9 unit	9*4*9	324
7	Labour	300 per day	2 no.	600
A	Total cost of production			3395
B	Cost of waste	500 per kg	269 g	135
C	Cost of production (A-B)			3260
D	Selling price of raw silk			4500
	Profit (D-C)			1240

From the mass trial results, it is observed that by adopting conveyor drying of cocoons and cooking with pressurized or vacuum permeation technique, remarkably high raw silk recovery to the extent of 70-72 % and significantly better productivity *i.e.,* 500-525 grams per person per 4 h in wet reeling machine and 650-720 grams per person in multi-end reeling machine can be achieved which are significantly better than those of open pan method. Further, the number of cocoons required to produce one kg silk can be reduced significantly.

At present, with the best existing method in the wet reeling, productivity being achieved is 220-273 grams per person per 4 h and recovery is to the tune of 55-60 %, number of cocoons required per kg silk is 920-1000. But, with the adoption of conveyor drying and new technology of cocoon cooking for wet reeling, it is possible to achieve higher level of production *i.e.,* 500-525 grams per person per 4 h, raw silk recovery of 70-72 % and also to further reduce the requirement of number of tasar cocoons to produce one kg silk, to about 750-850 from the existing rate of 1000-1200 cocoons.

Techno-economics of wet reeling

The techno-economic assessment of the new technology to produce one kg tasar silk by wet reeling is shown in Tables 9 and 10 for multi-end reeling and wet reeling machines, respectively. In the wet reeling of tasar cocoons, the cost of chemicals required to produce one kg silk is 37 for both the machines and the labour cost (2 labourers) works around 600 per kg silk. Electrical energy cost in the case of multi-end reeling is 40 per kg while in the case of wet reeling machine, it is 27 per kg. Total cost of production including cocoon cost, per kg Tasar silk comes to 3,408 in the case of multi-end and 3,395 in the case of wet reeling machine. During wet reeling of tasar cocoons, 269 grams of waste was generated per kg silk,

worth 500 per kg. The selling price of multi-end and wet reeled yarn is 5000 and 4500 per kg, respectively. The respective profit per kg silk yarn is 1,727 and 1,240.

Conclusion

By adopting conveyor hot air drying technology in association with new techniques of cocoon cooking *viz.,* pressurized cooking with CSTRI pressurised cooking machine or CSTRI vacuum permeation, it is possible to achieve better reeling performance in multi-end or wet reeling machine and produce superior quality tasar silk which can be used for warp. Interestingly the tasar cocoons processed by utilizing the new techniques could be reeled on multi-end reeling machine just like mulberry with good productivity and recovery of quality silk yarn (50 denier). The yarn didn't present slubs, neps knots and hence, can be directly used as warp in power loom and handloom to be woven into fabric of assured quality.

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INCIDENCE, NATURE OF DAMAGE AND CULTURAL MANAGEMENT OF *ARIOPHANTA LAEVIPES* MULLER, A MOLLUSC PEST OF MULBERRY NURSERY

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Abstract

Mulberry leaf is the sole food of silkworm, *Bombyx mori* L. which produces the finest natural silk in the world. It has been observed that 60 % of the cost of production of silk cocoons is accounted for mulberry cultivation alone. Hence, the quality of mulberry leaf and the climatic conditions together hold a highly decisive role in the success of silk crop. The present paper reports the occurrence of *Ariophanta laevipes* Muller, a mollusc pest causing damage to mulberry nursery at P2, Basic Seed Farm (BSF), Sheeshambara, Dehradun, Uttarakhand, India. *A. laevipes* is being reported as a pest on mulberry saplings for the first time from Uttarakhand. Several ways and means are resorted to, for the management of mollusc pests, the prominent one being by using pesticide. But with the increasing threat of environmental pollution and human health hazard attached to the use of pesticides, the practical as well as eco-friendly approach to check mollusc population is by cultural means. The description of the mollusc species, nature of damage and cultural management practices are discussed in the present paper.

Key words: *Ariophanta laevipes,* cultural management, incidence, mollusc, mulberry.

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Introduction

It is inevitable in sericulture that mulberry leaf fed to silkworms is ensured pest free to have higher productivity of silk. Mulberry is grown under a wide range of climatic conditions and is often affected by a large number of pests. The present paper reports the occurrence of *Ariophanta laevipes* Muller causing damage to mulberry nursery at P2, Basic Seed Farm (BSF), Sheeshambara, Dehradun. *A. laevipes* Muller is an air-breathing land snail species, a terrestrial pulmonate gastropod mollusc which comes under the family, Ariophantidae. Generally, the mulberry leaves

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infested by various species of snails are not suitable for silkworm rearing as they produce a sticky mucus layer both in wet and dry conditions. Silkworm show an aversion to feed these leaves, resulting in wastage of large quantity of leaves without getting converting into silk cocoons (Avhad *et al.,* 2013; Nath and Misra, 1980).

The pest causes ponderable damage to plant and lowers the crop yield. The experimental farm comes under humid and subtropical zone and is situated in the geographical position of north latitude 30°20'12'' and east longitude 77°53'31'' with an average annual rainfall of 1485 mm.

Due to its perennial nature and luxuriant foliage throughout the year, mulberry plant provides food and shelter for a variety of insect and non-insect pests. So far, over 300 insects and non-insect pests have been reported on one or the other part of the mulberry plant (Rangaswami *et al.,* 1978; Kotikal, 1982; Reddy and Kotikal, 1988; Sharma and Sharma, 1989; Sengupta *et al.,* 1991; Subba Rao and Mitra, 1995; Ramakant *et al.,* 2006; Raheem *et al.,* 2014).

The snail is a soft bodied animal which belongs to the class Gastropoda of the phylum Mollusca. The body of the snail is asymmetrical, spirally coiled and enclosed in protective calcareous left-handed shell. Mostly, the land snails are nocturnal but following a rain, they may come out of their damp, dark hiding place during the day. The snails are predominantly phytophagous. Mead (1961) has mentioned that some of the land snails are destined to be serious pests in the future. Many number of land snails have been reported as pests in India (Raut and Ghose, 1984; Ramakrishna *et al.,* 2010; Sreenivasa *et al.,* 2019).

The present paper provides a description of mollusc species, *A. laevipes* Muller, nature of damage on mulberry and its cultural management practices.

Materials and Methods

Regular observations were made on mulberry nursery at P2, BSF, Sheeshambara, Dehradun and the nature of damage caused to saplings and percentage infestation were recorded. The percentage incidence was calculated on the basis of number of plants damaged out of total number of plants observed in a particular nursery bed.

Results and Discussion

Habit and habitat

Molluscs are restricted to moist habitats. They hide during the day in holes, grass dumps *etc.* and become active at night. The snails aestivate or hibernate during adverse conditions. The aestivating / hibernating snail population is of great significance as this population becomes responsible for restocking an infested area. With the onset of monsoon, the snails which have survived the stress and strain of adverse climatic conditions during aestivation / hibernation become active again and resume their biological activity. The fallen leaves, debris, animal dung and dead snails serve as food for molluscs.

The eggs are laid in moist places. The cluster contains 50-100 eggs depending on the age of snail. Snail rehabilitates itself by rebuilding its population spread all over again and becomes a menace.

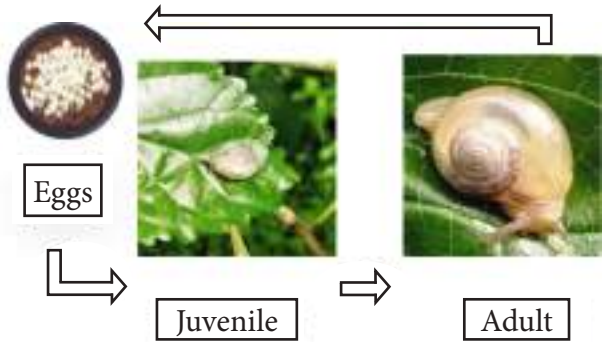

Figure 1: Life cycle of *A. laevipes* Muller

The incidence of *A. laevipes* Muller was observed from July to September, 2021 at P2, BSF, Sheeshambara, Dehradun and was also observed to decrease with sunshine period particularly from the month of September. The incidence was also observed higher under flood irrigation than drip irrigation as reported by Narendra Kumar *et al.* (2011). Snails start eating the leaf from one side and make holes on the leaf surface. The young snails prefer tender leaves and stem and

avoid hard portions, while the older ones attack young as well as grown up saplings thereby inflicting damage to vasculature, weaken stems and expose shoots to spreading over to newer areas should be checked. A large number of chemicals, such as arsenics, copper compounds, common salt, chlorinated hydrocarbons,

Table 1: Incidence of *A. laevipes* Muller on mulberry

Nursery bed	Total no. of plants per bed	No. of plants		% incidence
		Infested	Not infested	
1.	200	5	195	2.5
2.	200	0	200	0
3.	200	6	194	3
4.	200	4	196	2
5.	200	4	196	2
6.	200	7	193	3.5
7.	200	3	197	1.5
8.	200	5	195	2.5
9.	200	3	197	1.5
10.	200	7	193	3.5
11.	200	4	196	2
12.	200	3	197	1.5
13.	200	5	195	2.5
14.	200	5	195	2.5
15.	200	0	200	0
16.	200	2	198	1
Total	**3200**	**63**	**3137**	**1.97**

other infections. The nature of damage caused by *A. laevipes* Muller was observed diverse. The percentage damage by mollusc species to mulberry saplings is shown in Table 1.

Management

There are several ways and means for the management of mollusc pests but the aim is to reduce their population below the economic injury level, to avoid upset of natural balance. In this regard, its organophosphates, carbamates and metaldehyde are successfully used to control mollusc infestation. Metaldehyde (2.5 %) has been recommended as the most suitable treatment for control and management of snails (Srivastava, 1992; Narendra Kumar *et al.,* 2011) and the recommended concentration is safe to silkworm as well as mulberry plants (Sreenivasa *et al.,* 2016). Besides, there are diverse management options including, physical (collection and destroying by immersing in 25 % salt solution), cultural (field sanitation) and biological (release of natural enemies) measures. Jayashankar *et al.* (2013) reported that

application of Bordeaux mixture may reduce the population. On the contrary, the use of chemical pesticides poses a threat of environmental pollution as well as potential human health hazard. Effective cultural means have been evolved to check mollusc population to reach the status of pest which includes; collection and destruction of mollusc pests and their egg masses, location and destruction of day-hide outs of the snail not only during the season of activity but also during the season of inactivity, location and destruction of the hibernating / aestivating pockets, along with the mollusc hiding therein. This is very important, because during hibernation / aestivation, large number of molluscs remain accommodated in smaller areas, and if these be destroyed, then very little number will be left behind to become active at the onset of monsoon and naturally the damage will be less.

Surveillance on the molluscs management should be a regular and continuous process. As mentioned already, it should not be limited to a few months during the period of maximum activity but also to be continued during the season of inactivity by locating and destroying the hibernating pockets as it is this population which builds up on the onset of monsoon.

The molluscs can be collected and destroyed at any time between dusk and dawn. Clear weeding, and removing of the refuse is also recommended to check the snail population from crossing the threshold level. The mollusc pests are not harmful to warm blooded animals and other soil fauna. They are also not injurious to crops and above all, they cause the least pollution in the environment. However, since the management practices followed involve several man-hours or man-days, extensive research has to be carried out to explore the inter-relationship between the snail and its natural enemies and the population dynamics which would serve to formulate new and impactful strategies for management of the snail menace.

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TROPICAL TASAR SILKWORM (*ANTHERAEA MYLITTA* DRURY) IN SOUTH-EASTERN KARNATAKA: FIRST REPORT

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Abstract

An abundant natural population of tropical Tasar silkworm (*Antheraea mylitta* Drury) was identified recently in Channapatna area of Karnataka. The host plant was predominantly Almond trees. There was striking variability in qualitative and quantitative traits within the population. The cocoon color was of two types, *i.e.,* whitish grey and yellowish grey, while four types of wing color polymorphism were observed in both male and female moths. Wide range of variation was recorded in larval weight (36.05 – 44.26 g), pupal weight (5.0 - 13.5 g), peduncle length (2.0 - 6.5 cm), cocoon weight (6.5 - 15.5 g), cocoon length (35.9 - 47.8 mm) and breadth (20.5 - 29.4 mm), shell weight (1.0 - 2.6 g) and shell percentage (15.38 - 16.66). This seems to be the first time *A. mylitta* population is conspicuously seen in Karnataka with such striking intra-population variability, suggesting the need for detailed bioecological studies followed by devising strategies for *in situ* conservation of the population.

Key words: *Antheraea mylitta* Drury, Almond trees, Channapatna, ecorace, Tasar silkworms.

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The tropical Tasar silkworm, *Antheraea mylitta* Drury, has been freely interbreeding in nature for centuries, and the fauna is highly heterogeneous. As the insect has established itself in various forms of ecological populations (ecoraces) in different geographical niches of the country, depending on food plants and micro-environmental conditions available to them, the species exists in the form of nearly 44 ecoraces and is distributed over different states of India (Suryanarayana and Srivastava, 2005). Tasar sericulture is practiced in six major Indian states, *viz.,* Bihar, Orissa, Chhattisgarh, Telangana, West Bengal, and Uttar Pradesh, and is widely distributed in moist deciduous, semi-evergreen, dry deciduous, and tropical

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dry deciduous forests in India (Reddy *et al.,* 2015). Tasar culture is still quite alien to the state of Karnataka. However, recently (date: 27-02-2023), a small cluster of Tasar silkworm population was observed on Almond trees (*Terminalia catappa*) near the Channapatna town area (Channapatna – Halaguru main road) (Figure 1).

Figure 1: A view of the reporting area along with its google co-ordinates

Channapatna is a city and taluk headquarters in Ramanagara district, South-eastern Karnataka, India. It is located on the Bangalore - Mysore highway. It is about 55 km from Bangalore and 80 km from Mysore. Channapatna has an average elevation of 739 metres, temperature of 25.7 °C, relative humidity of 69 %, and annual rainfall of 931.58 mm. The wet season is muggy and overcast; the dry season is partly cloudy, and it is hot year round.

Figure 2: Tasar cocoons spotted on the Almond tree

Eggs **Larva** **Pupa**

Figure 3: Different life stages of the Tasar silkworm

Table 1: The variability in quantitative traits of the Tasar silkworm population

Parameter	Range
Larval weight (g)	36.05 – 44.26
Peduncle length (cm)	2.0 – 6.5
Pupal weight (g)	5.0 – 13.5
Cocoon weight (g)	6.5 – 15.5
Cocoon breadth (mm)	20.5 – 29.4
Cocoon length (mm)	35.9 – 47.8
Shell weight (g)	1.0 – 2.6
Shell percentage	15.38 – 16.66

Figure 4: Morphological view of Tasar cocoons

The dry deciduous (thorny scrub) forest patch is quite close by to the Channapatna town. Interestingly, the Tasar silkworm population was observed the highest (200 to 800 cocoons per plant) on the Almond trees (Figure 2) and miniscule (2 to 5 no.) on the other host plants (Arjun, Asan, Jamun, and Ber) present near

the Almond trees. The eggs, larvae, and pupae (Figure 3) were also observed on the same trees. A wide range of variability was recorded in the population with respect to larval weight, pupal weight, peduncle length, cocoon size, shell weight and shell percentage (Table 1). Cocoon color polymorphism was observed in the population as whitish grey and yellowish grey (Figure 4) while four types of wing color polymorphism were noticed in both male (Figure 5) and female (Figure 6) moths.

Figure 5: Polymorphism of wing color in male moths

Figure 6: Polymorphism of wing color in female moths

Almond is one of the tertiary host plants of the tropical Tasar silkworm (Jolly *et al.,* 1974). The presence of the highest population on Almond plants suggests that they are the most preferred host plants for Tasar moths to lay eggs. It is also interesting that the population is present only on the roadside plants and not on interior layout trees, garden trees, or farm land trees. This indicates that the Tasar silkworms are eaten away by birds in the undisturbed areas. On the contrary, majority of the worms remain untouched as birds are less frequent on the roadside Almond plants probably due to vehicular disturbance. These were planted as avenue trees by the forest department along both the sides of the road for about 6 km distance. Since this gives a clear indication of the presence of Tasar silkworm on this stretch, further study on this is essential. Conservation of Tasar silkworm population in this area may lead to establishment of Tasar silkworm genetic resources in the new area and commercial exploitation can be thought of, in the future. An explorative survey in the Channapatna area for Tasar silkworm populations would delineate the extent of the population availability in the region. It would be appropriate to have collaborative efforts among Central Silk Board, the State Sericulture Department, and the Forest Department for raising awareness about Tasar silk production, its documentation and conservation of the area.

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NEWS CORNER

International Training in Silk Industry

A) Training on Sericulture and Silk Industry

The training on **Sericulture and Silk Industry** was conducted at Central Sericultural Research and Training Institute, Central Silk Board, Mysore for a period of four weeks from 6th November to 3rd December 2022. The training was fully sponsored under the Indian Technical Economic Cooperation (ITEC) Programme of Ministry of External Affairs, Govt. of India. Twenty six trainees from 10 countries participated on different aspects of sericulture and silk industry, which include; mulberry cultivation, silkworm rearing, silkworm egg production, Chawki rearing, post cocoon technology, *etc.* Besides the practical oriented training activities, the trainees were exposed to various field developmental activities of silk industry at farmers' level. Visits to historical and other important places was also part of the training programme.

B) Post - Cocoon Technology

The training on "Post - Cocoon Technology" was conducted at Central Silk Technological Research Institute for a period of four weeks from 8th January to 4th February 2023. Twenty participants from 17 countries participated in the training successfully. The training was sponsored under the Indian Technical Economic Cooperation (ITEC) Programme of Ministry of External Affairs, Govt. of India. The training covered the various aspects of post - cocoon technology, such as silk reeling, wet processing, dyeing and printing, weaving, *etc.* Extensive field visits to the sericulture areas and facilities in and around Bangalore and Mysore were undertaken with a view to demonstrate the successful model of commercial sericulture practice developed in India.

'The Chronicles of Silk'

'The Chronicles of Silk' developed by ISC is ready for subscription by interested persons. The book was released during the 26th ISC congress held at Romania during 7-11 September 2022. The book reveal the hitherto unknown story about the origin of Wild Silk in South Asia by 2400 BCE which happens to be contemporary with the mulberry silk originated in China. While the mulberry silk spread to other areas by 2nd century BCE, the wild silks were extensively used in the Indo European cultural settlements as early by 2400 BCE. The story of wild silk industry is deeply intermingled with the development of Indian population consequent upon the migration of population from steppe regions to South Asia. The wild silk materials were later transacted to the ancient civilizations of Mesopotamia, Persia, Pacific, Egypt *etc.* which conclusively prove the narrations of silk mentioned in the ancient Sanskrit scriptures.

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INTERNATIONAL SERICULTURAL COMMISSION
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7th APSERI Congress

The organizers of the 7th Congress of Asia-Pacific Congress of Sericulture and Insect Biotechnology (APSERI) notified the event to take place from August 18 to 22, 2023 in Zhenjiang, Jiangsu province, China. The Congress is designed to initiate a scientific research network in Asia and Pacific Rim for international academic and industrial institutions and professionals in silkworm and insect biotechnology.

For more details, please check the first announcement of the Congress at:
https://swjs.just.edu.cn/2023/0302/c4707a319606/page.htm

INTERNATIONAL SERICULTURAL COMMISSION
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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.

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