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Research Paper

IN VITRO EVALUATION OF NOVEL FUNGICIDE MOLECULES AGAINST *CEROTELIUM FICI* CAST. (ARTH.) CAUSING BLACK LEAF RUST OF MULBERRY

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ABSTRACT

Commercial sericulture largely depends on mulberry silk production by the silkworm, *Bombyx mori* L. Superior quality mulberry leaves as feed is inevitable to produce silk of high grade and yield. Consumption of infected or diseased leaves by silkworm leads to poor larval growth making them prone to different diseases. The major foliar diseases are leaf rust, leaf spot, powdery mildew, leaf blight and bacterial blight. In view of the destructive nature of leaf rust incidence in mulberry and the consequent adverse effects on silk cocoon crop, the present investigation was carried out to conduct an *in vitro* evaluation of novel fungicide molecules against *Cerotelium fici,* the causative agent. Out of fourteen fungicides evaluated, Ametoctradin 27 % + Dimethomorph 20.27 % SC, a combi-product was found highly effective at all the concentrations tested and showed the least spore germination (0.62 %) at 0.1 %. Similarly, Chlorothalonil, a non-systemic fungicide which is currently recommended for management of leaf rust of mulberry showed 2.06 % spore germination at 0.3 %, which was on par with other effective fungicides. The effective novel fungicide molecule could be used for the management of leaf rust after evaluation in field condition and bio-assay with silkworm. Since it possesses a combination of both systemic and contact mode of actions, could be recommended as an alternative to the existing fungicide in the leaf rust disease management.

Key words: *Cerotelium fici,* fungicides, leaf rust, mulberry.

INTRODUCTION

Mulberry (*Morus* spp.) has prime importance in the sericulture industry as it is the sole feed of silkworm (*Bombyx mori* L.). It has been cultivated as a long-term plantation for decades and pruned several times in a year for its foliage. Globally, India is the major consumer of silk fabrics and second largest raw silk producer after China. The industry provides regular dependable income on a consistent basis for a wide range of age groups and social strata (Okhandiar and Kumaresan, 2019; Arunakumar *et al*., 2021).

The quality and yield of mulberry leaf decides the quantum of silkworm rearing, its healthy and robust growth which reflects in qualitative and quantitative cocoon yield and ultimately the raw silk production. Although the leaf quality is a specific character of mulberry variety, it is adversely influenced by the improper soil and climatic conditions, improper agronomic inputs and the outbreak

of diseases and pests. The various varieties of mulberry under cultivation in India differ greatly in their adaptability to different soil types and climatic conditions, leaf quality and leaf yield as well as resistance to diseases. Under optimum agronomical inputs, the expected average leaf yield and quality of popular mulberry varieties at farmers' field could not be achieved. The reasons for this yield gap are several, which include biotic and abiotic factors, sociological and economical factors. Among all, the biotic factors are of major significance in determining the quality and leaf yield.

The major foliar diseases of mulberry are leaf rust [*Cerotelium fici* (Cast) Arth.], leaf spot [*Cercospora moricola* (Cooke) Sacc.], [*Setosphaeria rostrata* (Wakker) Boedijin] (syn. *Exserohilum rostratum*), shot hole leaf spot [*Nigrospora sphaerica* (Sacc.) Mason], powdery mildew [*Phyllactinia corylea* (Pers) Karst], leaf blight [*Alternaria alternata* (Fr. Keisaster) or *Fusarium pallidoroseum*], and bacterial blight [*Pseudomonas syringage* p.v. *mori* (Boyer and Lambert) Stevens, *Xanthomonas campestris* pv. *mori* (Pammel) Dowson] (Sengupta *et al.,* 1990; Philip *et al.,* 1994; Gunashekhar *et al.,* 1995; Arunakumar *et al.,* 2019 a; Arunakumar *et al.,* 2019 b). Of these, leaf rust is the most alarming foliar disease during winter season, mature leaves are more prone to the attack and cause 10-15 % leaf yield loss besides deteriorating the leaf quality (Sengupta *et al.,* 1990; Philip *et al.,* 1994; Raju and Chowdary, 2013). It reduces the leaf yield (20-25 %) as well as the contents of moisture, protein, reducing sugars and total sugars in leaves (Prasad *et al.,* 1993). There are two major types of rust disease *viz.,* black rust and red rust which are common in mulberry. Black rust is caused by *Cerotelium fici* (Cast.) Arthur and also known as *Peridiospora mori* Barclay (Prasad *et al.,* 1993) and red rust is caused by *Aecidium mori* Barclay. This disease causes up to 15 % leaf loss (Teotia and Sen, 1994; Pratheeshkumar *et al.,* 2000).

Economy of sericulture is severely affected by the foliar diseases (Mir *et al.,* 2013). Further, feeding the silkworms with diseased leaves affect the commercial characters of the cocoons (Sullia and Padma, 1987). Therefore, a keen vigilance along with appropriate and timely control measures need to be implemented to avoid quantitative and qualitative leaf yield loss in mulberry. Use of fungicides is an alternate method of managing plant diseases in the absence of resistant cultivar. Fungicides *viz.,* Bavistin (Carbendazim 50 % WP) and Kavach (Chlorothalonil 75 % WP) have been recommended for the control of the leaf rust disease which could contain the disease by 70 % and 50 %, respectively (Philip *et al.,* 1994). Further, it has also been reported that continuous use of recommended fungicide may lead to development of resistance against it by pathogens (Gangawane, 1997).

Evaluation of new fungicides for efficacy against the disease, evaluation of proven fungicides in different localities and development of spray schedules against susceptible cultivars is a continued process. In the absence of resistant cultivars, use of fungicides is an absolutely essential tool particularly when there is a sudden outbreak of the disease (Padule *et al.,* 1988). Various effective new fungicides have been introduced in the market for the control of foliar diseases in agricultural crops. But, these fungicides have not yet been tested against the mulberry leaf rust disease. Keeping all these points in view, an *in vitro* evaluation of novel fungicide molecules was carried out by collection of uredospores of *C. fici* from the infected leaves of highly susceptible mulberry plants.

MATERIALS AND METHODS

Collection and separation of uredospores from highly susceptible mulberry plants

The leaves of mulberry showing typical symptoms of the leaf rust were collected from mulberry gardens of Molecular Biology Lab-I, Central Sericultural Research and Training Institute (CSRTI), Mysuru during the year 2016-17. The fungal pathogen (*C. fici*) being a biotroph, cannot be cultured in artificial media and hence, the spores were collected from the naturally infected leaves of mulberry.

The following protocol was followed to separate the uredospores from the diseased leaves.

1. Identification of highly susceptible mulberry plants in mulberry garden.

2. Collection of mulberry leaves severely infected by *C. fici* (leaf rust).

3. Collection of fungal spores from single pustule by using paint brush into a fold of aluminium foil.

4. Incubation of fungal spores at 15-20 °C for further study.

in vitro evaluation of novel fungicide molecules against *C. fici*

The efficacy of fungicides against *C. fici* was assessed *in vitro* by using spore germination technique at different concentrations (Chaudhary and Chaudhari, 2013).

The urediniospores suspension was prepared separately in sterile water to obtain 4×10^8 urediniospores per ml. Then a drop of spore suspension was mixed with one drop of fungicidal solution in a cavity slide to achieve the required concentration. In each treatment, three replications were maintained. Slides were incubated at 22-24 °C for 24 h. The observation on spore germination was recorded 24 h after incubation under microscope at 40 X magnification. An untreated control was maintained with sterile water. Per cent urediniospores germination was calculated by using the formula described by Vincent (1947) and the data obtained were analyzed statistically.

Chemical preparation

Stock solution of the fungicides (100 ml) were prepared and from this, dilutions of 0.05, 0.1 and 0.2 % were prepared. For the Chlorothalonil fungicide, 0.1, 0.2 and 0.3 % concentrations were prepared and distilled water was used as untreated control (Table 1).

Table 1: Details of fungicides tested *in vitro*

Sl. No.	Common name	Trade name
1	Propiconazole 25 % EC	Tilt 25 % EC
2	Hexaconazole 5 % EC	Contaf 5 % EC
3	Tebuconazole 250 % EC	Folicure 250 % EC
4	Captan 70 % + Hexaconazole 5 % WP	Taquat 75 % WP
5	Tebuconazole 50 % + Trifloxystrobin 25 % WG	Nativo 75 % WG
6	Pyroclostrobin 23.6 % EC	Headline 23.6 % EC
7	Azoxystrobin 250 % SC	Amistar 250 % SC
8	Thiophanate methyl 70 % WP	Roko 70 % WP
9	Ametoctradin 27 % + Dimethomorph 20.27 % SC	Zampro 47.27 % SC
10	Cymoxanil 8 % + Mancozeb 64 % WP	Curzate 72 % WP
11	Zineb 68 % + Hexaconazole 4 % WP	Avatar 72 % WP
12	Fenamidone 10 % + Mancozeb 50 % WG	Sectin 60 % WG
13	Tricyclazole 75 % WP	Baan 75 % WP
14	Chlorothalonil 75 % WP	Kavach 75 % WP

RESULTS AND DISCUSSION

The use of fungicides has become an inevitable measure in the management of plant diseases particularly in mulberry in the absence of resistant cultivars to *C. fici*. Foliar sprays give maximum protection against foliar diseases. Use of chemicals is the most common and effective method especially to control the mulberry foliar diseases. The chemicals either inhibit the germination, growth and multiplication of the pathogens or kill them. These chemicals are generally used for direct protection of leaves from the infection or eradication of the pathogens that had already infected the plant.

in vitro evaluation of novel fungicide molecules against *C. fici*

The tested fungicides showed significant differences in their efficacy to impact uredospore germination. Results on effect of different fungicides on the uredospore germination are presented in Table 2.

Table 2: *in vitro* evaluation of novel fungicides against *C. fici*

Sl. No.	Treatment	Concentration (%)	Uredospores germination (%)				
			0.05	0.10	0.20	0.30	Mean
1	Propiconazole 25 % EC		22.2	12.28	9.02		14.5
2	Hexaconazole 5 % EC		39.22	26.37	26.57		30.72
3	Tebuconazole 250 % EC		17.03	5.23	1.53		7.93
4	Captan 70 % + Hexaconazole 5 % WP		16.49	9.0	6.15		10.54
5	Tebuconazole 50 % + Trifloxystrobin 25 % WG		15.4	10.83	9.89		12.04
6	Pyroclostrobin 23.6 % EC		25.51	19.84	19.88		21.75
7	Azoxystrobin 250 % SC		22.37	12.62	9.63		14.87
8	Thiophanate methyl 70 % WP		11.74	10.03	8.92		10.23
9	Ametoctradin 27 % + Dimethomorph 20.27 % SC		2.01	0.62	1.27		1.3
10	Cymoxanil 8 % + Mancozeb 64 % WP		2.55	4.38	1.04		2.66
11	Zineb 68 % + Hexaconazole 4 % WP		8.23	5.02	4.3		5.85
12	Fenamidone 10 % + Mancozeb 50 % WG		9.63	5.88	1.06		5.52
13	Tricyclazole 75 % WP		8.81	2.91	0		3.91
14	Chlorothalonil 75 % WP		-	19.41	12.32	2.06	11.26
15	Control		95.71	96.15	95.64		95.83
	Comparing the means of			SEm ±	CD at 5 %		
	Treatment (A)			1.21	3.39		
	Concentration (B)			0.54	1.52		
	A X B			2.09	5.88		

In general, it is revealed that all the tested fungicides resulted in significantly less per cent spore germination than that of control. Among all the treatments, Ametoctradin 27 % + Dimethomorph 20.27 % SC was found highly effective at all the concentrations tested. On the other hand, Tricyclazole 75 % WP showed 0.0 % urediniospores germination at 0.2 % concentration, which was the least value recorded among the fungicides tested under three different concentrations. At 0.1 % concentration, significant and the least urediniospores germination (0.62 %) was recorded by Ametoctradin 27 % + Dimethomorph 20.27 % SC, which is followed by Tricyclazole 75 % WP (2.91 %) and Cymoxanil 8 % + Mancozeb 64 % WP (4.38 %). At 0.05 % concentration also, Ametoctradin 27 % + Dimethomorph 20.27 % SC showed the least per cent spore germination (2.01 %), which is statistically on par with the remaining concentrations (0.1 & 0.2 %). The lowest mean spore germination was recorded with the treatment of Ametoctradin 27 % + Dimethomorph 20.27 % SC (1.3 %) followed by Cymoxanil 8 % + Mancozeb 64 % WP (2.66 %) and Tricyclazole 75 % WP (3.91 %) among the tested fungicides and as against the mean of control (95.83 %) (Table 2).

Similarly, Tofoli *et al.* (2016 a) reported that Ametoctradin 30 % + Dimethomorph 22.5 % (1.25 L .ha^{-1}) provided significant control of late blight and stated that it is a new alternative for the management of potato late blight. The fungicide, Ametoctradin is also a new substitute for the control of oomycetes. Belonging to a new class of pyrimidylamines (FRAC 45), it is characterized as being a powerful respiratory inhibitor of complex III, cytochrome bc1 at QO site, stigmatellin binding subsite (Gold *et al.,* 2009; Merk *et al.,* 2011; FRAC, 2016).

The promising results of the present study in respect of Ametoctradin in combination with Dimethomorph were also highlighted by Reimann *et al.* (2010), Tegge *et al.* (2012) and Tofoli *et al.* (2016 a). Likewise, Tofoli *et al.* (2016 b) also emphasized the high potential of these mixtures to promote yield in potato fields affected by late blight.

Apart from Ametoctradin having a distinct mechanism of action and a compatible toxicological profile, it is important to highlight that this fungicide has a high degree of stability and affinity with waxy layers of the leaf surface, which allows high levels of protective, curative, and anti-sporulant action; longer-lasting protection; and rain resistance (Gold *et al.,* 2009; Merk *et al.,* 2011; Tofoli *et al.,* 2012; Tofoli *et al.,* 2014). Given the technological innovation presented by the new chemical and the results found in this study, it is concluded that a combi-product Ametoctradin 27 % + Dimethomorph 20.27 % SC is also found effective in inhibition of uredospores germination for the first time.

Significant and maximum spore germination was observed for treatments, Hexaconazole at 0.05 % (39.22 %) and Pyraclostrobin at 0.05 % (25.51 %) indicating their ineffectiveness on a comparative scale. But results contradictory to this were reported earlier by Sreekantappa and Naik (2004) who noted that non-systemic mancozeb (0.15 %) and systemic fungicide hexaconazole (0.025 %) inhibited spore germination of *C. fici* to the maximum extend *i.e.,* up to 77.4 and 91.3 per cent, respectively under laboratory condition. Hence, we have every reason to believe that the leaf rust causing fungus, *C. fici* might have developed resistance against the hexaconazole fungicide due to its continuous usage. It has been reported that continuous use of recommended fungicide could lead to development of resistance against it by the pathogen, a situation which threaten the continued use of effective chemical control against the disease (Gangawane, 1997).

Of the non-systemic fungicides, Chlorothalonil at 0.3 % showed the least per cent spore germination (2.06 %). Similar results were obtained by Padule and Kaulgud

(1994) wherein the fig rust could be effectively controlled by Chlorothalonil at 0.2 % to the extent of 85 % followed by Copper oxychloride (0.4 %). Similarly, two sprays of Captafol and Chlorothalonil at 0.2 % each reduced the leaf rust (*C. fici*) severity up to 50 % and increased leaf yield of mulberry by 28 % (Gunasekhar *et al.,* 1995). Benagi (1991) evaluated eight fungicides *in vitro* on uredospore germination of *P. arachidis* and reported that Propiconazole, Tebuconazole, Chlorothalonil, Oxadaxil and Diclobutrazole were effective.

Conclusion

Out of fourteen fungicides evaluated, a combi-product of novel fungicide molecules, Ametoctradin 27 % + Dimethomorph 20.27 % SC at 0.1 % was found highly effective and showed the least spore germination (0.62 %). This could be a promising alternative to the presently recommended fungicide and be introduced in the disease management system after evaluation in field condition and bio-assay with silkworm. Similarly, a non-systemic fungicide, Chlorothalonil at 0.2 % which has already been recommended for management of leaf rust is found still effective in inhibition of spore germination and hence, can be continued for use in the chemical management of leaf rust disease in mulberry.

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Research Paper

CHARACTERIZATION OF SERICIN EXTRACTED FROM ERI SILKWORM, *PHILOSAMIA RICINI* L.

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ABSTRACT

Sericin is an abundant, underutilised waste / by-product of silk industry. It is a protein extracted from the silk filament. There have been many efforts to explore the utilization of silk sericin for developing value-added products in the bio-medical, pharmaceutical, cosmetic, and food industries. While enough work has been done on the characterization of mulberry silk sericin, eri silk sericin still remains an unexplored area. Suitable technical knowhow is also the need of the hour to support utilization of eri sericin especially in bio-technology related industries. The present study was carried out to characterize eri sericin and compare it with that of mulberry. Results reveal that the method of extraction plays a vital role on the degumming efficiency. Eri sericin is rich in calcium and magnesium, making it difficult to process. Protein content of eri sericin is less than that of mulberry sericin and UV spectral analysis has shown that quality of protein in mulberry sericin is superior to that of eri. SDS-PAGE disclosed that sericin extracted using HTHP technique has a molecular weight range from 17 to 245 kDa. Seventeen amino acids including three essential ones constitute the protein structure of eri sericin.

Key words: Amino acid, eri sericin, heavy metal, mulberry sericin, protein content.

INTRODUCTION

Vanya (wild) or non-mulberry silks represent the finest aspects of India's rich textile culture. Apart from mulberry silkworm *Bombyx mori,* eri silkworm, *Philosamia ricini* is yet another domesticated species among the wild silkworms. Eri silk is commercially produced mainly by two species, namely *Samia cynthia ricini* and *Philosamia ricini* which are reared on castor *(Ricinus communis)* and kesseru *(Heteropanax fragrans)* and produce white or brick-red cocoons (Devaiah *et al.,* 1985; Reddy *et al.,* 1989). Abundant availability of castor foliage in the rural areas of north-eastern states *viz.,* Assam, Meghalaya, Nagaland *etc.,* makes ericulture a subsidiary occupation for rural and tribal people of the hilly region (Saratchandra, 2003). In India, the major quantum of eri silk is produced in north-eastern states but other states *viz.,* West Bengal, Bihar, Odisha and Madhya Pradesh also offer their share of eri silk cocoons (Suryanarayana, 2005). Because of its domestication and multi-voltine nature, eri silk is becoming more popular and in the last two decades, the production of eri silk has increased 2.5 fold (Central Silk Board, 2020).

Eri cocoons are neither stifled nor the pupae killed in hot water like mulberry silkworms. Hence, eri silk is named *Ahimsa silk*! (Kariyappa *et al.,* 2007). The unique characteristics of eri silk has made it demanding

and during the last decade, its production has increased from 2460 to 6910 metric tons (CSB, 2020) and is expected to rise further. Consequently, there would be an increase in the availability of sericin-containing waste water from eri sericulture in the coming days.

Eri silk is different from mulberry silk in terms of its physical properties and composition. Studies have shown that eri silk contains only half the amount of sericin of mulberry silk. Mulberry contains ~30 % of sericin while eri silk possesses only ~15 % (Prasong *et al.,* 2009). Sericin is a water soluble glycoprotein layer that covers the fibroin present in the cocoon. Sericin layer protects the pupae from the different environmental conditions, natural calamities, and predators especially for non-mulberry silkworms. The strong adhesive nature of this protein is attributed to the hydrogen bonding capability of the enormous number of hydroxyl amino acids present in it. Mulberry sericin has lot of scope in non- textile applications. While sufficient amount of studies have been taken up on characterization of mulberry sericin, eri sericin still needs to be explored. Hence, the present study is an attempt to characterize eri sericin and compare it with that of mulberry.

MATERIALS AND METHODS

Materials

White and red eri cocoons as shown in Figure 1 were procured from the local cocoon market of *Bodoland,* Assam. Firstly, the cocoons were cleaned to remove debris if any and were then cut open to remove the pupae. Cut cocoon shells were used for the extraction of sericin. As Eri is a wild silk, where the quality of cocoons varies with the external conditions where they were raised, the results obtained are likely to vary slightly from lot to lot. All the chemicals used in the study were of analytical grade and the experiments were carried out with deionised water.

Methods

Extraction of sericin

Degumming of cocoons was carried out under high temperature (at 130 ^{0}C for 30 min) and high pressure using CSTRI Eri Eco degumming machine developed by CSTRI (Sreenivasa *et al.,* 2018). After this, cocoons were squeezed to collect the sericin liquor. Figure 1 represents the flow diagram of the extraction process. Unlike the traditional method of degumming, no chemicals were used in this process. Subsequently, sericin was converted into powder form by tray drying technique.

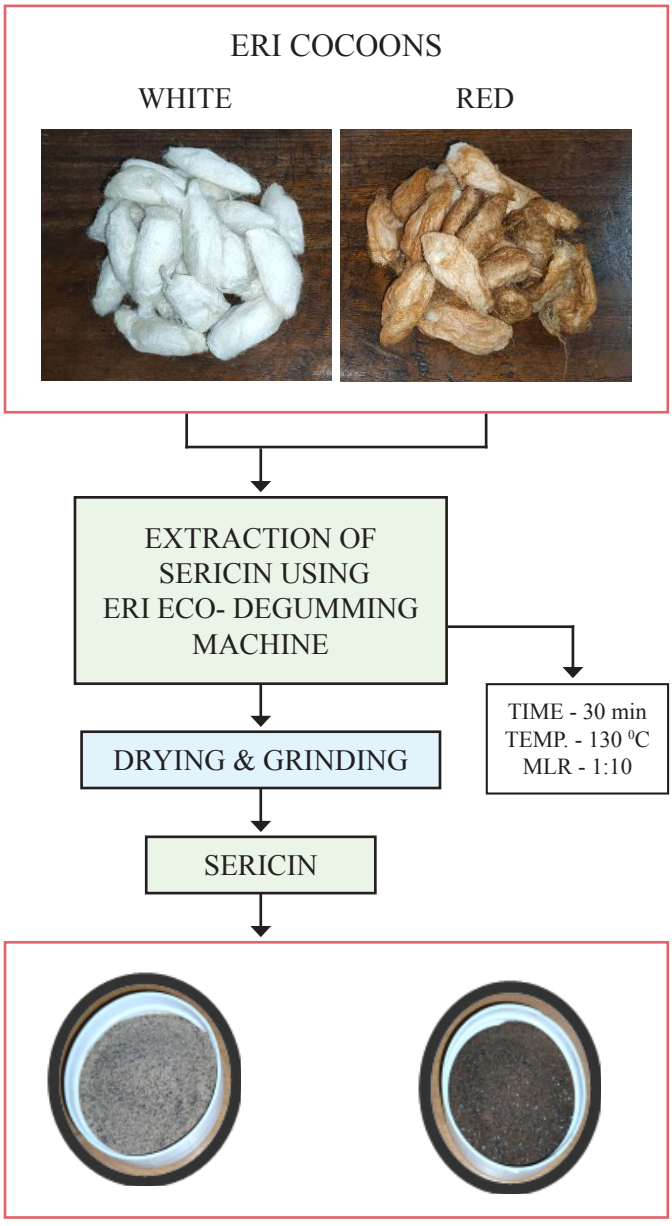

Figure 1: Flow diagram representing sericin extraction process

Degumming loss per cent

Degumming loss was calculated by measuring the weight loss % of cocoons after degumming using the following formula:

$$\text{Degumming loss \%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Where, initial weight is the weight of un-degummed cocoons and final weight is the weight of cocoons after degumming.

Heavy metal content (In-house test method ORG/ MOA/17)

A known weight of sericin powder was taken in a pre-weighed silica crucible and placed in muffle furnace at 600 ^{0}C for two hours. The residue obtained was digested in 10 N Nitric acid and made up to 25 ml in a calibrated standard flask. The solution was analysed on ICP-MS for the identification and quantification of heavy metals.

Ash content (FSSAI Manual 2016, In-house test method)

A known weight of sericin powder was taken in a pre-weighed silica crucible and placed in muffle furnace at 600 ^{0}C for two hours. The percentage of ash content was calculated from the residue weight.

Nitrogen and protein content

IS 7219:2005 standard prescribes Kjeldahl method for the determination of total nitrogen content which was then converted into protein content of sericin samples as per the standard.

UV Spectral analysis

UV spectra from 220 to 320 nm were recorded for both white and red eri sericin samples and compared with the spectrum of mulberry sericin. A-ratio was also calculated from the spectra. A-ratio signifies the purity of proteins and is calculated by measuring the ratio of absorbance of the sample at 280 nm and 260 nm. Since nucleic acids absorb UV radiation at 260 nm and proteins, at 280 nm, A-ratio above 1.8 typically corresponds to a sample that is free of contamination. Abnormal A- ratios usually indicate that a sample is contaminated by residual phenol, guanidine, or other compounds.

SDS PAGE analysis

SDS-PAGE analysis was performed to check the molecular weight of sericin according to Laemmli (Gamo *et al.,* 1977) using gradient resolving gel. The electrophoresis was carried out for five hours at 90V after which it was stained with Coomassie brilliant blue. After destaining, molecular weight was estimated by comparing with the molecular markers.

Amino acid analysis

Amino acid analysis was performed according to the method of Bidlingmeyer *et al.,* 1984 *i.e.,* Pico-Tag amino acid analysis system. Soluble aliquots containing 20 mg of protein was hydrolysed for 24 hours under vacuum at 110 ^{0}C using 6 M boiling HCl. The amino acid analysis was carried out using a three-step procedure. In the first step, protein samples were acid hydrolyzed to free amino acids. Amino acids were modified by PITC in the second step and the last step included the separation of the modified amino acids by RP-HPLC.

RESULTS AND DISCUSSION

Degumming loss per cent

Degumming loss of 14.60 and 13.60 % was obtained for HTHP technique and conventional method, respectively. HTHP technique has the advantage of reduced duration of degumming time *i.e.,* 30 min whereas the conventional method takes an hour. Also, as no extra chemicals are used in HTHP technique, recovery of sericin becomes easy. Hence, the results show that degumming can be done efficiently using HTHP technique.

Heavy metal content

The results of heavy metal content of mulberry and eri sericin are shown in Table 1. It is revealed that presence of cadmium and arsenic is below detection limit in mulberry and less than 1 mg/kg in eri sericin samples. Presence of copper and zinc is more than 10 mg/kg and presence of lead, chromium and nickel is less than 3 mg/kg in eri sericin. It is observed that eri sericin possesses more amount of heavy metals, compared to that of mulberry. High amount of calcium (2 %) and magnesium (0.2-0.5 %) present in eri silk makes it difficult for processing and gives a stiff feel to the silk. This is the reason eri sericin requires higher temperature for degumming when compared with mulberry silk.

Table 1: Estimation of heavy metals in mulberry and eri sericin

Heavy metal (mg/kg)	Mulberry sericin	Eri sericin	
		White	Red
Copper	1.4	16.3	15.8
Cadmium	BDL of 0.1	BDL of 0.1	0.1
Lead	BDL of 0.1	1	1.1
Chromium	0.8	2	2.7
Arsenic	BDL of 0.1	0.31	0.7
Zinc	19.2	21	33.5
Calcium	6407	22372	23693
Magnesium	821	2756.5	5797
Nickel	1	1.6	1.4

Ash content

Our results show that ash content of mulberry sericin is 4.2 % (Table 2) which is in accordance with the earlier results in this line (Wu *et al.,* 2007). The ash content directly depends upon the presence of foreign and mineral matter present in the substance. As eri sericin is rich in calcium and magnesium, it shows high % of ash content. In comparison to white eri sericin, the red eri sericin has more amount of ash as the latter contains higher amount of calcium and magnesium than white eri sericin.

Nitrogen and protein content

The protein content in any matter is directly proportional to the amount of nitrogen in it. The quantity of protein in a particular matter can be determined by multiplying the % of nitrogen present in it by 6.25. Hence, more the amount of nitrogen in the matter, more is the protein content. The results of nitrogen and protein content in the samples under study are shown in Table 2. These results are in agreement to those reported by Gupta *et al.,* 2014. The nitrogen and protein content was less in white eri sericin which was further lower in red eri sericin.

Table 2: Comparison of ash and protein content of mulberry and eri sericin

Parameter	Mulberry sericin	Eri sericin	
		White	Red
Total Ash (%)	4.20	12.89	20.94
Nitrogen (%)	14.13	13.03	10.61
Protein (%)	88.31	81.44	66.31

UV Spectral analysis

UV spectra of mulberry and eri samples are depicted in Figure 2. Amino acids give characteristic peak at 275 nm which is present in all the three spectra but the shape of the curve is different for different samples. A-ratio which is the ratio of absorbance value at 280 nm and 260 nm wavelength, indicating the degree of contamination in the samples was also calculated from the spectra.

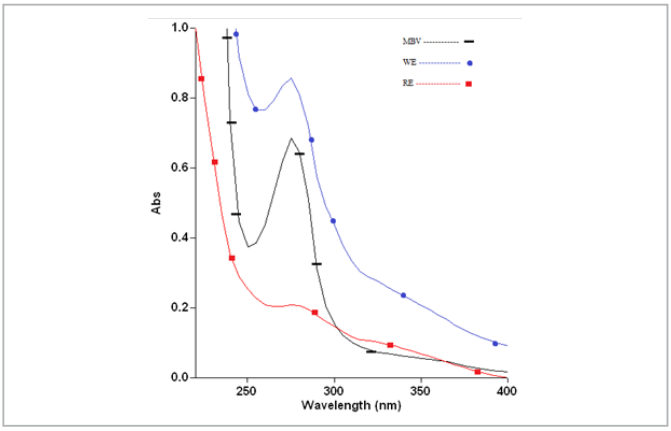

Figure 2: UV Spectra of mulberry (MBV), white eri (WE) and red eri sericin (RE) samples

Our results recorded A-ratio of 1.49, 1.09 and 0.98 for mulberry, white and red Eri sericin samples, respectively. A-ratio of the protein is a direct indicator of its quality. The ratio of eri sericin is observed much lower in comparison to mulberry sericin indicating the superior quality of sericin extracted from mulberry. The presence of flavonoid and phenolic compounds in red eri sericin is behind the least value of A-Ratio, as lower A-ratios usually indicate that a sample is contaminated by residual phenol, guanidine, or other compounds.

SDS PAGE analysis

The data on molecular weight distribution of the sericin samples evaluated using SDS PAGE analysis are shown in Figure 3. Sericin extracted using HTHP technique was compared with native sericin. Molecular weight of sericin varies in the range of 20-400 kDa. Native eri sericin shows bands at 250, 130, 35 and 17 kDa. But no bands were observed for sericin extracted using HTHP technique. It is observed that when HTHP or autoclave methods were used for extraction of sericin, the samples give a smear in SDS-PAGE.

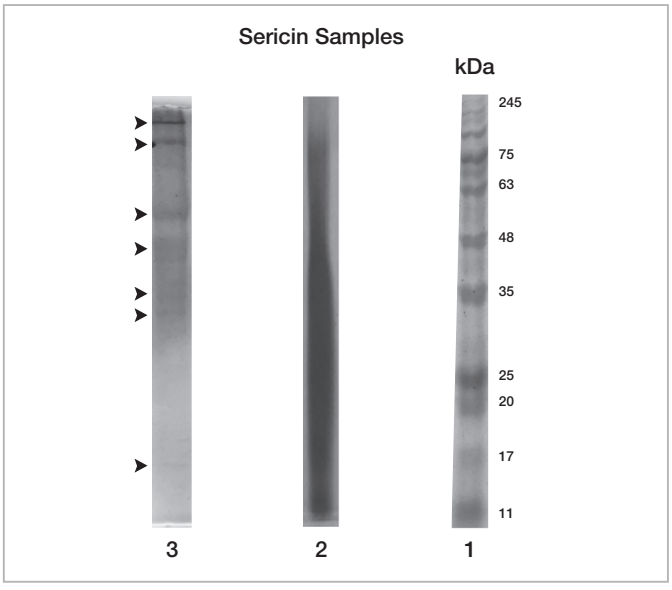

Figure 3: SDS PAGE analysis of sericin samples (1 - Marker, 2 – HTHP extracted eri sericin, 3 - Native eri sericin)

Table 3: Amino acid composition of eri sericin in comparison to mulberry sericin

Amino acid	Eri sericin (%)	Mulberry sericin* (%)
Serine	31.64	21.56-37.3
Asparagine + Aspartic acid	14.60	14-20.82
Glycine	13.82	8.23-23.2
Glutamine + Glutamic acid	8.06	3.3-7.77
Threonine	7.26	3.8-8.57
Alanine	4.86	1-6.7
Tyrosine	4.34	3.41 -4.78
Lysine	4.30	1-10.2
Histidine	4.05	1.27-1.87
Arginine	2.02	2.8-11.95
Valine	1.86	1-6.31
Leucine	1.08	0.6-4.67
Isoleucine	0.99	0.94 -1.16
Phenylalanine	0.87	0.84-1.17
Cysteine	0.25	0.24-0.39

* Miguel and Álvarez-López, 2020; Wu *et al.,* 2007

It is evident from the data shown in Table 3 that the sericin powder is rich in serine (nearly 30 %), asparagine + aspartic acid and glycine. Fair amount of essential amino acids *viz.,* histidine, lysine and threonine are also present in sericin. Sericin is low in sulphur containing amino acids, such as cysteine. The amino acid profile obtained for eri sericin is in similar line to that of mulberry sericin.

Conclusion

Our results have characterised a few physicochemical properties of eri sericin with slight variations from that of mulberry. Mostly discarded earlier as waste product during silk processing, eri sericin in the recent years is turning into a lucrative business component, thanks to its application in cosmetic and bio-medical fields. Eri sericin recently finds widespread applications in cosmetic industry, as anti-oxidant and anti-apoptotic compound, as a support for enzyme immobilization, as dietary supplement, and also as bio-material for cell culture, drug, and gene delivery. Present results qualify the compound a promising candidate to be used in various applications.

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Research Paper

INFLUENCE OF METHOD OF TASAR COCOON DRYING ON REELING PERFORMANCE AND QUALITY OF TASAR SILK

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ABSTRACT

In an attempt to detect a highly efficient protocol for drying Tasar green cocoons, an experiment was set up with three cocoon drying schemes *viz.,* CSTRI conveyor hot air dryer, batch type hot air dryer and sun drying. The dried cocoons of all these sorts were then cooked and reeled on wet reeling principle by maintaining same cooking conditions. The yarns produced were tested for quality parameters through international standard testing methods. The results drew a conclusion that by adopting conveyor hot air drying of Tasar cocoons in association with optimum cooking, significant improvement in reelability and silk recovery *(i.e.,* less number of cocoons per kg of silk) can be achieved for delivering superior quality of Tasar silk to be used for warp/weft.

Key words: Conveyor, moisture loss, reelability, Tasar, yield.

INTRODUCTION

In India, Tasar silk is mainly produced in the states of Jharkhand, Chhattisgarh and Odisha, besides small quantities from the states of Maharashtra, West Bengal, Bihar and Andhra Pradesh. Tasar culture is the main stay for many a tribal community in India. Presently, 2.01 lakh ha of Tasar plantation are available for rearing and about 137000 families are engaged with the industry. Annual production of Tasar cocoons is around 24181 Lakh numbers out of which 3136 MT (8.75 % of total silk production) of Tasar silk is being produced (CSB, 2019-20). Tasar is copperish colored and coarse silk, the reeled Tasar silk is used to produce high value products *viz.,* sarees, shirting, dress materials, over coat, suitings, furnishings, interiors *etc.* It is less lustrous than mulberry silk, but has its own feel and appeal.

Tasar cocoon production is seasonal and commercial (reeling) crops are raised during November –December. The reelers purchase the cocoons during the season and have to store it for the entire year for reeling purpose. For storing Tasar cocoons for longer duration, cocoons have to be dried to the required level to remove the moisture and avoid moth emergence as well as fungus attack. The cocoon drying will also bring in uniformity in the sericin quality and hence, facilitate uniform cooking, reeling and production of high quality silk.

The Tasar cocoons being thick shelled, hard and compact with bigger pupae inside make the moisture removal process difficult. It's highly difficult to dry up large quantities of Tasar cocoons available during a particular season through sun dying, steam stifling and batch type hot air dryer drying as all these methods have their own

limitations. In the sun drying method, cocoons would be exposed daily to scorching sun light /UV rays. Sun drying requires longer duration of more than months to dry up the cocoons and is also labor oriented. Still, this is practiced by the reelers due to lack of proper drying technology and infrastructure. During the longer period of drying process, considerable loss of cocoons may occur due to various causes, such as moth emergence, attack of rodents, insects and micro organisms and damages due to rain.

Steam stifling kills the pupae but cannot remove the moisture completely and hence, storage of steam stifled cocoons is not possible as they are prone to fungus attack. Hence, hot air drying is the most suitable option for drying the Tasar cocoons. Two existing technologies using hot air as a source of drying are in practice *i.e.,* batch type and conveyor type dryers. Batch type of dryer is suitable only to dry small quantum of Tasar cocoons and towards this, 50 kg/100 kg capacity ovens are available. Therefore, the mass scale drying technology *i.e.,* conveyor drying developed by CSTRI would be suitable for drying the Tasar cocoons.

CSTRI has developed 4 band conveyor hot air dryer (2 ton capacity/per day) for mulberry cocoons (Subhas *et al.,* 2020) and the same shall be utilized to dry large quantity of Tasar cocoons *i.e.,* 1,60,000 to 1,80,000 numbers per day, which help the reeler to keep their dried Tasar cocoons for longer duration for their conversion. In the conveyor dryer, all the parameters of cocoon drying *viz.,* temperature, duration of drying and exhaust control can be controlled effectively due to mechanization.

There are quite a number of studies on the influence of drying temperature profile on degree of drying and its influence on reeling and quality performance. Hariraj and Somashekar (2001 a, b) have reported that both temperature profile and duration of drying have significant effect on the degree of drying of hot air dried cocoons. Subhas and Somashekar (2008) observed that degree of drying and cocoon cooking conditions have significant influence on reelability, raw silk recovery and quality of raw silk. It is reported that hot air stifling of cocoons could offer higher stifling efficiency, raw silk recovery and quality of silk than sun drying (Hegazy and ABD-Elrahman, 2006). Singh (2011) could detect that quality of the cocoons dried in the solar dryer was at par with those dried in the conventional electrical oven in terms of the silk yield and strength of the yarn and consumption of electricity. Wei *et al.* (2018) have recommended that temperature range of 120-125 °C is optimum for drying of cocoons. Recent works show that raw silk recovery from electrically hot air stifled muga cocoons is significantly higher than those of flame smoke stifling (Mishra *et al.,* 2020). Sreenivasa *et al.* (2001) have reported the superiority of non-conventional energy for stifling of Tasar cocoons using black cloth over that of direct sun light drying, in terms of raw silk recovery, reelability and waste percentage.

From the literature, it could be found that most of the works were pertaining to mulberry cocoons only. Information including published literature available on drying of Tasar cocoons using conveyor drying is scanty. Hence, the present study was taken up to optimize temperature profile for drying of Tasar cocoons on conveyor hot air dryer and to ascertain the effect of drying on the cooking and reeling efficiency and quality of raw silk as compared to the case of batch dryer and sun dried cocoons.

MATERIALS AND METHODS

Raw material

a. Tasar green cocoons of commercial crop purchased from Raw material Bank (RMB), Chiabasa were used for the study. Out of this, 20000 cocoons were used for conveyor drying trials (2 Ton capacity) and the rest of 10000 cocoons were utilized for taking up trials on batch type dryer (50 kg capacity). In total, three trials each were conducted on conveyor and batch type hot air dryers.

b. In addition, 5000 numbers of sun dried cocoons from the same lot were also purchased from Raw material Bank (RMB), Chiabasa.

Drying conditions

1. The Tasar cocoons were dried in batch type hot air dryer (50 kg capacity) by adopting the following temperature and duration profile.

Treatment	Temperature profile	Duration (h)
T1	115 °C-2 h, 105 °C-2 h, 90 °C-1 h, 75 °C-1 h, 60°C-1 h	7
T2	115 °C-1 ½ h, 105 °C-1 ½ h, 90 °C- 1 h, 75 °C-1 h, 60 °C-1 h	6
T3	115 °C-1 h, 105 °C-1 h, 90 °C-1 h, 75 °C-1 h, 60 °C-1 h	5

2. The Tasar cocoons were dried in CSTRI conveyor hot air dryer (4 band) by adopting the following temperature profile with three different durations *viz.,* 6 h (T4), 6 h 30 minutes (T5), and 5 h 40 minutes (T6).

Temperature profile of conveyor dryer	
Band number	Temperature (°C)
1st	115
2nd	100
3rd	85
4th	70

3. The Tasar cocoons meant for the third trial were divided into six lots and dried in batch type dryer. For both the dryers, the cocoons were weighed initially before drying and after drying for every two hours up to 8 h and after that, every one hour drying trial was also taken up for a duration of 2 h to know the kinetics of drying (Table1).

The moisture loss due to drying was calculated as percentage of the original green weight.

$$\text{Moisture loss (\%)} = \frac{\text{(Initial weight - final weight)}}{\text{Initial weight}} \times 100$$

Cooking conditions

The hot air dried cocoons were subsequently cooked according to the following recipe which has been standardized after taking up several trials.

Dried Tasar cocoons (100 no.) were boiled in the solution of EDTA (5 gpl) for 10 minutes and then allowed to cool down for 30 minutes. They were then transferred to solution/recipe containing the following chemicals.

Sodium carbonate 2 gpl
Sodium silicate 2 gpl
Hydrogen peroxide (30 %) 12 cc per liter

The initial temperature of bath was maintained at 70 °C and cocoons were soaked in the recipe for 30-40 minutes. The cooked cocoons were individually de-flossed by hand and then taken for reeling.

Reeling conditions

One hundred cocoons per lot were taken for conducting the reeling performance and for all trials, same cooking and reeling conditions were maintained. Three replications were taken for each lot. The cooked cocoons were deflossed individually by hand. The deflossed cocoons were reeled on Tasar CSTRI wet reeling machine by maintaining 6 cocoons per end. Temperature and reeling speed of the reeling bath was maintained at 45 °C and 60 rpm, respectively.

Data: During the reeling process, number of cocoon castings were recorded along with weight of raw silk produced, weight of defloss waste, basin waste and pelade waste and the following parameters were calculated.

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

$$\text{Raw silk recovery (\%)} = \frac{\text{Weight of raw silk}}{\text{(Weight of silk + Weight of total waste)}} \times 100$$

Weight of total waste = Weight of (defloss waste + reeling waste + pelade waste)

$$\text{Waste (\%)} = \frac{\text{Weight of total waste}}{\text{(Weight of silk + Weight of total waste)}} \times 100$$

$$\text{Number of cocoons required to produce one kg of silk} = \frac{\text{Number of cocoons taken for reeling (100)}}{\text{Weight of silk (g) from 100 cocoons}} \times 1000$$

Yarn testing

Tensile and elongation properties of reeled Tasar silk yarns were measured by using Instron tensile tester model no. 5000R using IS 1670-1991 testing method.

Statistical analysis

The data obtained from the experiments were analyzed through one way analysis of variance (ANOVA) *i.e.,* general linear model, multivariate post hoc, the least significant difference, descriptive statistics and regression analysis using SPSS 11.5.

RESULTS AND DISCUSSION

1. Effect of drying duration on moisture loss

The results of the moisture loss of Tasar cocoons dried under different durations of time are provided in Table 1 and Figure 1. The moisture loss is observed to vary significantly as the duration of drying increased. The moisture loss percentage during initial four hours of drying were 24.71, 41.34, 48.35 and 53.42 % whereas, variations in moisture loss percentage between 8th, 9th and 10th h of drying were not significant. Results indicate that the maximum moisture from pupae and cocoon shell is removed during the first 2 to 6 h of drying. The curve plot of moisture loss percentage verses drying duration indicate that the curve is best fit to polynomial function. From the curve, it is observed that maximum drying takes place during the initial 4 hours followed by a marginal drying that leads to a constant rate of drying. This may be because the pupa, which contains about 79 % moisture, loses the maximum amount of moisture during the initial hours of drying, as the initial temperature is higher. It is evident that the drying duration has significantly influenced the moisture loss percentage (Table 2).

Table 1: Degree of drying of Tasar green cocoons under different temperature profiles and durations of drying

Duration (h)	Temperature profile	Moisture loss (%)
2	115 °C	24.71
4	115 °C-100 °C	41.34
6	115 °C-100 °C-85 °C	48.33
8	115 °C-100 °C-85 °C-70 °C	53.42
9	115 °C-100 °C-85 °C-70 °C-70 °C	54.39
10	115 °C-100 °C-85 °C-70 °C-70 °C-70 °C	54.26

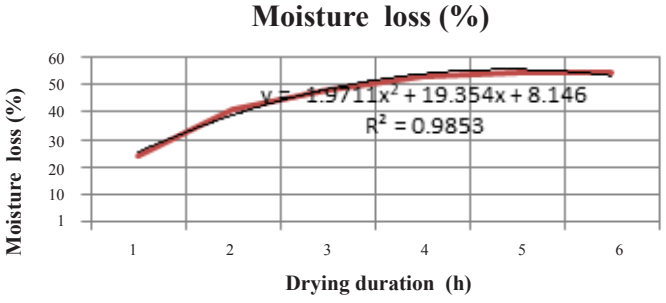

Figure 1: Influence of drying temperature profile on moisture loss percentage

Table 2: Effect drying duration on moisture loss percentage

ANOVA					
	df	SS			
Regression	1	589.28	MS	F	Significance F
Residual	4	84.77	589.28	27.81	0.006
Total	5	674.05	21.19		

2. Effect of drying temperature profiles on moisture loss (%)

Table 3 depicts data on the impact of different treatments *viz.,* use of batch type and conveyor hot air dryers with different drying temperature profile on the moisture loss (weight loss) of the Indian Tasar cocoons. The treatment, T1 in batch type and T5 in conveyor dryers with resultant moisture loss percentage of 56.30 % and 51.25 %, respectively have brought about maximum removal of moisture which indicates that major amount of moisture is removed from the pupae and cocoon shell under the corresponding cocoon drying temperature profiles.

Table 3: Impact of drying Tasar cocoons under different temperature profile

Treatment	Hot air dryer	Temperature profile	Total duration (h. min)	Moisture loss (%)
T1	Batch type	115 °C-2 h,105 °C-2 h, 90 °C-1 h, 75 °C-1 h, 60 °C-1 h	7.00	56.30
T2	Batch type	115 °C-1 ½ h, 105 °C -1 ½ h, 90 °C-1 h, 75 °C-1 h, 60 °C-1 h	6.00	44.64
T3	Batch type	115 °C-1 h, 105 °C-1 h, 90 °C-1 h, 75 °C-1 h, 60 °C-1 h	5.00	46.12
T4	Conveyor	115 °C-100 °C-85 °C -70 °C	6.00	43.93
T5	Conveyor	115 °C-100 °C-85 °C -70 °C	6.30	51.25
T6	Conveyor	115 °C-100 °C-85 °C -70 °C	5.40	45.39

Thus, it is evident that when a higher initial temperature is used for drying, it removes the moisture effectively from the cocoon shell and pupa apart from the killing of pupa and uniform hardening of the sericin. Under the temperature profile, T2 and T4, the cocoons are dried to the extent of 44.64 and 43.93 %, respectively in 6 h of drying, indicating that moisture is not removed to the required level, which may be due to the fact that moisture coming out of pupa accumulates in the inner most part of the cocoon shell and slowly diffuses through the shell depending up on the temperature corresponding to heating in the dryer. In the case of temperature profile of T2, T3, T4 and T6, the moisture loss is on lower side due to lower duration of drying. This is because the higher thickness of Tasar cocoon shell demands more time and higher temperature to allow the water vapor to evaporate outside easily. The study indicates that initial temperature of drying shall be in the range of 115 °C and duration, from 6 h 30 min to 7 h to achieve required moisture loss in the case of Tasar cocoons. Further, from the results, it is observed that, there is no much difference between T1 and T5 in terms of moisture loss.

3. Effect of drying temperature profiles on reeling characteristics

a. Reelability percentage

The reeling performance of Tasar cocoons dried under different treatments *viz.,* sun drying, batch type and conveyor hot air dryers by subjecting to different temperature profiles is presented in Table 4. T5 (conveyor dried) lot of cocoons presented significantly higher reelability than batch type dryer treated and sun dried cocoons. Among the rest, reelability of T1 (batch type) was found significantly higher than that of T2 & T3 (batch type) and sundried cocoons. The reelability of sun dried cocoons was in general, significantly lower than that of other treatments. But, no significant differences could be recorded between T2 & T3 and T4 & T6. Comparatively better reelability of the conveyor dried cocoons is attributed to uniform drying of the cocoons and hence, hardening of sericin to the required level. Low reelability of sun dried cocoons is due to more hardening due to degradation of sericin of cocoons due to exposure to Ultraviolet rays of sun light for a longer period. Sun dried cocoons have higher degradation of heavy chains and greater prevalence of light chains and degradation of peptide bonds in the composition due to ultraviolet rays of sun light. From the Analysis of Variance (Table 5), it is observed that differences in the reelability of the cocoons *i.e.,* between sundried and batch dryer dried, sun dried and conveyor dryer dried and batch dryer dried and conveyor dryer dried are statistically significant at 1 % level. This is because all surfaces of the cocoons are subjected to hot air and hence, effective uniform drying of the cocoons is achieved in conveyor hot air dryer.

Table 4: Influence of drying temperature profile on Tasar cocoon reeling performance

Method of drying		No. of cocoons	Reelability (%)	Silk weight (g)	Waste (g)	Raw silk recovery (%)	Waste (%)	Cocoons required to produce 1 kg silk (No.)
Sun dried	S1	100	42	104.45	74.78	58.28	41.72	957
Batch type hot air dryer	T1	100	57	119.27	61.02	67.86	32.14	843
	T2	100	53	114.34	54.27	66.59	33.41	880
	T3	100	51	114.99	67.79	63.01	36.99	873
Conveyor hot air dryer	T4	100	55	120.85	59.01	67.19	32.81	828
	T5	100	62	127.32	48.86	72.06	27.94	787
	T6	100	58	126.03	68.87	65.04	34.96	794

b. Raw silk recovery and waste percentage

The present results show that raw silk recovery percentage of cocoons dried in conveyor dryer (T5) and batch type dryer (T1) are significantly higher than that of sun dried cocoons (Table 4). These treatments have displayed correspondingly higher reelabilty percentage too. Sun dried cocoons exhibited the lowest reelability which in turn has reduced raw silk recovery. The Analysis of Variance (Table 5) reveals that the differences in the raw silk recovery of the cocoons between treatments *viz.,* sun dried and batch dryer dried, sun dried and conveyor dryer dried and batch dryer dried and conveyor dryer dried are statistically significant at 1 % level. The cocoons dried under conveyor dryer facilitated uniform cocoon cooking leading to enhanced reeling efficiency, silk recovery and quality of silk.

In this context, it is to be noted that waste percentage of sun dried cocoon (S1) is significantly higher compared to other treatments. The waste is significantly the lowest in the case of conveyor dryer dried cocoons (T5) and batch type dryer (T1). Low reelability increases the dropping of cocoons during reeling catering to repeated end findings from dropped cocoons, thus increasing the waste percentage of the cocoons. In the case of waste percentage also, the differences between that of sun dried and batch dryer dried, sun dried and conveyor dryer dried and batch dryer dried and conveyor dryer dried are statistically significant at 1 % level (Table 5). This is because the ultra violet rays of sun light inflict certain degree of damage *i.e.,* reduce the strength of silk, harden the sericin and even change the color of the cocoons to yellow, eventually causing a reduction in the reelability of the cocoons and more waste generation.

Table 5: Analysis of variance results of reeling performance of differently dried cocoons

Variable	Comparison	Sum of Squares	df	Mean Square	F	Sig.
Reelability (%)	Between groups	706.476	6	117.746	85.264	0.000**
	Within groups	19.333	14	1.381		
	Total	725.810	20			
Silk weight (g)	Between groups	1439.818	6	239.970	4.819	0.007**
	Within groups	697.210	14	49.801		
	Total	2137.028	20			
Waste (g)	Between groups	1879.557	6	313.259	16.416	0.000**
	Within groups	267.152	14	19.082		
	Total	2146.709	20			
Raw silk recovery (%)	Between groups	448.947	6	74.825	14.335	0.000**
	Within groups	73.074	14	5.220		
	Total	522.022	20			
Waste (%)	Between groups	448.947	6	74.825	14.335	0.000**
	Within groups	73.074	14	5.220		
	Total	522.022	20			
Cocoons required to produce 1 kg silk (No.)	Between groups	79379.327	6	13229.888	5.433	0.004**
	Within groups	34088.914	14	2434.922		
	Total	113468.241	20			

** - Significant at 1 % level

c. Number of cocoons required to produce one kg silk

Renditta *i.e.,* the amount of cocoons (kg) required to produce one kg of silk is also an important parameter denoting the quality of cocoons. The number of cocoons required to produce one kg of Tasar silk varied when different methods of drying were adopted. They were 787, 843 and 957, respectively for conveyor dryer, batch type dryer and sun drying (Table 4). It is noteworthy that by adopting conveyor drying, 18 % of cocoons can be saved to produce one kg of silk as compared to sun drying. This could be attributed to the uniform drying of the cocoons to the required level and the consequent better reelability accomplished in conveyor dryer. From the Analysis of variance (Table 5), it is observed that differences in the number of cocoons required to produce one kg of silk between those of sun dried and batch dryer dried, sun dried and conveyor dryer dried and batch dryer dried and conveyor dryer dried are statistically significant at 1 % level.

d. Tenacity

Our results indicate that tenacity of Tasar silk produced from conveyor dryer treated cocoons was significantly higher than that of batch dryer and sun dried cocoons and it is attributed to uniform drying with required temperature and duration profile (Table 6). Due to uniform drying, some of the random coil in amorphous region might have been converted into beta structure and hence, the higher tenacity. Further, from the results, it is observed that sun rays have severely affected the tenacity of the yarn and hence, it is the lowest for sun dried lot compared to other drying methods. It is worth mentioning here that the silk being very sensitive to UV rays of the sun, gets degraded of its micro-structure significantly resulting in reduction in tenacity.

e. Elongation percentage

Cocoons subjected to conveyor dryer treatment presented more of elongation percentage as compared to that of batch type dryer and sundried cocoons (Table 6), which is statistically significant at 1 %.

g. Young's modulus

Young's modulus/Initial modulus is the initial part of a stress/strain curve and describes the ability of a yarn to resist elastic deformation under load. It describes a material's propensity to retain its shape, even when it is being stretched, pulled, twisted, or compressed. Young's modulus of Tasar silk yarn out of conveyor dried cocoons was significantly better than that of yarn produced from batch type dryer treated and sun dried cocoons.

Table 6: Influence of drying temperature profile on reeled Tasar silk yarn quality parameters

Type of drying		Maximum load (gf)	Tenacity (gf/d)	Elongation (%)	Young's modulus (gf/den)	Energy max-load (kgf mm)
Sun dried	S1	139.0	2.1	19.7	47.80	3.73
Batch type hot air dryer	T1	238.3	3.5	22.6	114.39	7.96
	T2	172.4	2.7	21.7	80.30	4.36
	T3	162.2	2.3	24.4	53.60	4.21
Conveyor hot air dryer	T4	209.7	3.0	30.8	64.80	6.71
	T5	264.4	3.5	26.3	114.01	6.80
	T6	221.0	3.2	27.9	133.97	5.53

Conclusion

The present investigation has established that maximum drying of Tasar cocoons takes place during the initial 4 hours followed by marginal drying and subsequently attaining a constant rate of drying. Our results also point out that about 6 h 30 min to 7 hours of drying duration under temperature profile of 115 to 70 °C is required for drying the Tasar cocoons to the optimum level. Sun drying of Tasar cocoons of course has certain disadvantages that bring about an adverse impact on the reeling performance *viz.,* reelability, raw silk yield and quality characteristics of Tasar silk. This experiment has come out with the major finding that conveyor hot air dried cocoons give significantly better raw silk yield and less waste percentage as compared to the treatments of sun drying and batch type dryer. Obviously, the cocoons dried in batch type dryer offered more favorable results than sun dried cocoons. Since the Tasar cocoon production is seasonal, conveyor hot air dryer will be a quite useful and essential option for mass scale drying of cocoons and saving of labor cost to a significant extent is an added advantage.

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Research Paper

DETERMINATION OF PUPAL SEXUAL SIZE DIMORPHISM IN TASAR SILKWORM, *ANTHERAEA MYLITTA* DRURY (LEPIDOPTERA: SATURNIIDAE) FROM INDIA USING DISCRIMINANT FUNCTION ANALYSIS

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ABSTRACT

Tasar silkworm, *Antheraea mylitta* Drury pupa has no significant morphological dimorphic features except genital openings. Female pupa possesses a genital slit longer than that of male. However, the adults exhibit dimorphic features in antennae, wing colouration, size of the abdomen *etc.* We evaluated sexual size dimorphism (SSD) in pupa of *A. mylitta* based on the morphometrics of a set of 22 measurements. The z test revealed that certain selected puparial characters were highly significant ($P \leq 0.05$). Discriminant Analysis & Principal Component Analysis (PCA) (multivariate analysis) categorized the significant characters. A few characters were found significant in univariate and multivariate analyses which are of great utility in differentiating the sexes of Daba ecorace, such as pupal weight, cocoon weight, shell weight, length of pupa and length of abdomen. These morphometrics will provide enough and accurate ways of diagnosing the sexes in the pupa of the tasar silkworm which is a highly essential procedure followed during the process of seed production and bioassay.

Key words: Discriminant analysis, Multivariate analysis, Principal component analysis, pupal morphometrics, sexual size dimorphism.

INTRODUCTION

Tropical tasar silk is produced by the larvae of *Antheraea mylitta Drury* (Saturniidae: Lepidoptera). Being wild in nature, they are commercially reared outdoor, on its primary food plants *viz., Terminalia tomentosa, T. arjuna* and *Shorea robusta.* India is the largest producer of tropical tasar silk in the world with annual raw silk production of 2977 MT. Tasar culture has been a livelihood option for 1.25 lakhs of tribal families residing by the edge of forest in Northern and North-East India (Reddy *et al.,* 2015). Creation of rural employment, alleviation of poverty and elevation of socio-economic status of tribals are unique features of Indian tropical tasar industry (Suryanarayana *et al.,* 2005). There are 44 ecoraces reported of which ecorace, Daba is commercially exploited in major tasar growing states, such as Jharkhand, Chattisgarh, Odisha and Madhya Pradesh.

Sexual dimorphism, the differences in appearance between males and females of the same species, such as in colour, shape, size, and structure, are caused by the inheritance of one or the other sexual pattern in the genetic material. Sexual dimorphism is of interest in entomological studies, since in many cases, either the differences between sexes are not obvious or the individuals are very small; thus, identification of discriminating characters allows easy determination of sexes. In Tasar silkworm, sexual size dimorphism (SSD) followed by their separation at pupal stage is important particularly in the fields of seed production, bioassays, sex ratio estimation *etc.* SSD at adult stage is quite easy and can be done based on a few characters, such as antennae shape, size of the abdomen, wing colouration, wing length and characteristics of genitalia. Female adults are usually larger and heavier than males. Our study was aimed to answer some of the fundamental questions about sexual dimorphism in the tasar silkworm, *A. mylitta,* such as 1. Does sexual dimorphism exist in Tasar silkworm? If it does, can this be quantified? 2. What characters contribute the most towards dimorphism at pupal stage? 3. Up to what degree of accuracy, the tasar pupae can be classified as a male or a female?

MATERIALS AND METHODS

The diapausing cocoons of tasar silkworm ecorace, Daba were collected from asan trees of different locations via Ranchi and Kharsawan (Jharkhand) and Balaghat (Madhya Pradesh) during February 2021. Thirty two such samples were selected from each study area for each sex, and measurements of selected characters were made manually. Sexual size dimorphism (SSD) is poorly defined in tasar silkworm. So, 16 characters & 6-character ratios were used to define the sex using morphometrics in a comprehensive manner (Table 1, Figure 1).

Tasar pupal measurements were carried out with a sliding caliper wherever possible and sterozoom microscope (Olympus make 2010). Descriptive statistics with means and standard error was computed for each measurement in both the sexes. For evaluation of significance of mean, univariate statistics (z test) was used; two sample means of the characters used were subjected to z test in MS Excel-2010 at 5 % and 1 % probability levels. Per cent difference of the measurement was calculated using the expression: [(mean measurement value of female – mean measurement value of male) ÷ mean measurement value of male] x 100. The sexual dimorphism in size was assessed by sexual dimorphism index (SDI) or Storer's index, in which a larger value indicates greater sexual dimorphism (Storer, 1966). SDI was calculated as the mean value of female divided by the mean value of male for each measurement. Ranks are allotted based on SDI. To explore the variations and refine their significance towards sexual dimorphism, multivariate analyses, such as Discriminant Analysis & Principal Component Analysis (PCA) were carried out using R Studio Software version 3.9.1 (2019), SAS (2021) & PAST software 4.0 (Hammer *et al.,* 2001).

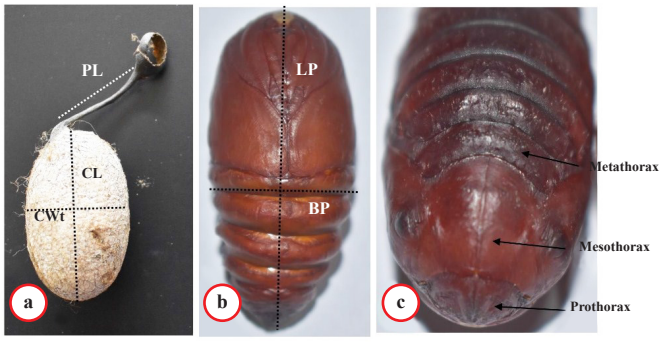

Figure 1: Morphometric measurements considered for detecting sexual dimorphism in *Antheraea mylitta* pupa. a. *Antheraea mylitta* cocoon (100x), b & c *Antheraea mylitta pupa* (150x).

RESULTS

Morphology of tasar cocoons: The dusty white and poultry egg-shaped cocoons are constructed on the host plant with a ring and peduncle & pupae of both the sexes are brown. The observations on the studied Daba ecorace revealed that male pupae are smaller than the female ones (Figure 2). There was only one apparent morphological difference present in pupal stage. Male pupae have short abdomen and the male genital pore is present on the 9th sternum. Female pupae are larger with a larger abdomen and possess genital slit (Figure 2 f). However, morphometric variations were significant as exhibited by the mean values and the z test of significance. The z test (two sample mean) showed that most of the characters selected were significant. Peduncle length (PL), length of prothorax (LPt) & character ratios & shell percentage were found to be non-significant in discriminating Daba BV cocoons collected from Ranchi, Jharkhand. PL, breadth of prothorax (BPt), character ratios & shell percentage were found to be non-significant in discriminating Daba BV cocoons collected from Kharsawan, Jharkhand. PL, LPt, BPt, length of mesothorax (LMt), length of metathorax (LMet), character ratios & shell percentage were found to be non-significant in discriminating Daba BV cocoons collected from Balaghat, Madhya Pradesh. PL, LPt, BPt, character ratios & shell percentage were found to be non-significant in discriminating the cocoons of Daba TV collected from Balaghat, Madhya Pradesh (Univariate z test). Thus, PL & the character ratios, such as length of pupa versus breadth of pupa (LP:BP), length of prothorax, mesothorax and metathorax versus breadth of prothorax, mesothorax and metathorax and shell percentage were found non-significant in all studied locations of Daba ecorace (Table 1 i & ii).

Sexual Dimorphism Index (SDI) for each measurement was calculated. The median of SDI for 22 measurements was 1.06 (Daba, Ranchi), 1.13 (Daba, Kharsawan), 1.09 (Daba, Balaghat) and 1.12 (Daba, TV). If the value is less, it indicates a relatively low level of sexual difference; greater than the median, it indicates a high level of sexual difference for the particular location, as the morphometrics are influenced by the local environment. Table 1 displays a few measurements which are relatively with high level of sexual difference for Daba, Ranchi, Kharsawan, Balaghat and Daba, TV. These measurements were pupal weight, cocoon weight, length of abdomen, shell weight, length of pupa *etc.*

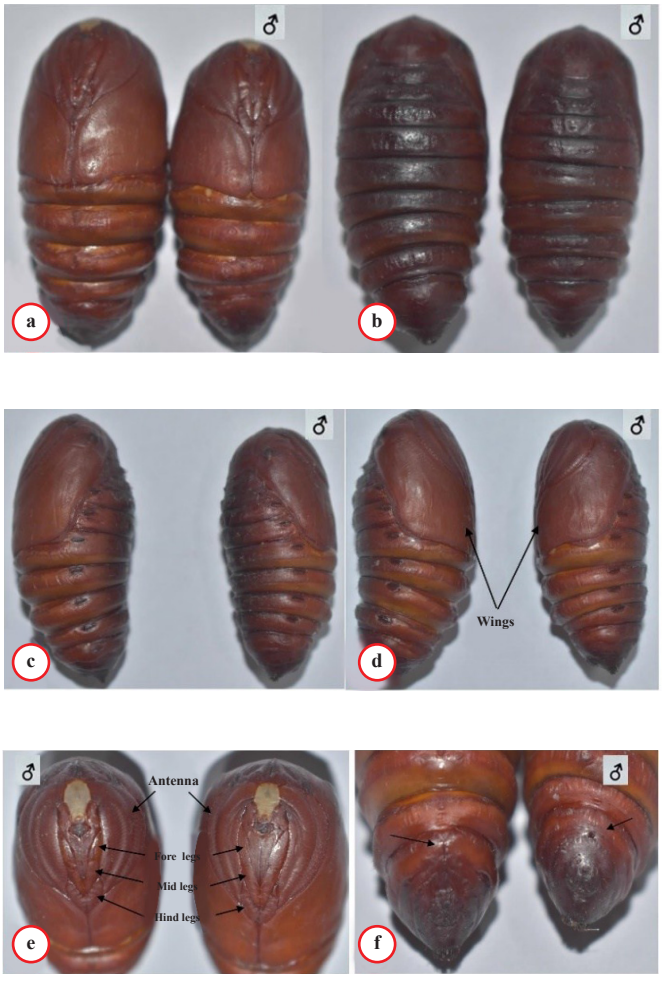

Figure 2: Sexual dimorphism in *Antheraea mylitta* pupa a. Dorsal view, b. Ventral View, c & d. Lateral view, e. Antenna and legs in pupa, f. Genital slit in female and genital pore in male.(150x).

Table 1 (i): Descriptive statistics of the Tasar silkworm pupa: z statistics, dimorphic ranks among both the sexes & Sexual Dimorphism Index

Location	Daba BV, CTRTI, Ranchi, Jharkhand										Daba BV, CTRTI, Ranchi, Jharkhand									
	Female (μ)			Male (μ)			Z	% Difference	SDI	Rank	Female (μ)			Male (μ)			Z	% Difference	SDI	Rank
Character	μ ± SE	Range	Shapiro Wilk	μ ± SE	Range	Shapiro Wilk					μ ± SE	Range	Shapiro Wilk	μ ± SE	Range	Shapiro Wilk				
CWt	15.4 ±0.37	10.23 -20.37	0.98	11.61 ±0.12	10.36-13.32	0.98	9.82*	32.9	1.33	2	15.2±0.3	12-20	0.96	10.4 ±0.14	9.0-12.0	0.88	14.41*	45.5	1.46	2
SWt	2.56±0.08	1.5-3.77	0.98	2.27±0.04	1.71-2.72	0.97	3.05*	12.5	1.12	4	2.1±0.04	1.6-2.5	0.91	1.8±0.04	1.5-2.2	0.83	4.61*	15.4	1.15	8
PL	5.53±0.2	3.3-7.9	0.96	5.38±0.28	3.1-11.2	0.80	0.43NS	2.8	1.03	16	5.3±0.36	3.1-11.2	0.85	4.6±0.23	2.6-7.4	0.95	1.52NS	14.1	1.14	10
PWt	12.7±0.3	8.73-16.4	0.99	9.18±0.1	8.22-10.93	0.97	11.04*	38.3	1.38	1	13.1 ±0.28	10.1-17.5	0.97	8.4±0.15	7.0-10.0	0.96	14.84*	55.1	1.55	1
CL	5.82±0.05	5.0-6.3	0.95	5.47±0.04	4.9-5.9	0.92	5.58*	6.4	1.06	9	5.6±0.06	4.7-6.4	0.96	5±0.06	4.0-5.3	0.84	7.61*	13.2	1.13	11
CW	3.61±0.03	3.1-4	0.96	3.38±0.02	3.1-3.7	0.93	5.58*	6.6	1.07	8	3.3±0.04	2.8-3.6	0.92	3±0.06	1.9-3.4	0.72	4.18*	9.3	1.09	15
LP	4.84±0.04	4.2-5.3	0.95	4.33±0.03	3.9-4.6	0.95	10.25*	11.8	1.12	5	5.3±0.08	4.5-6.5	0.97	4.6±0.04	4.2-5.1	0.95	7.75*	14.6	1.15	9
BP	2.36±0.03	2.2-2.8	0.85	2.11±0.02	2-2.3	0.86	8.06*	11.7	1.12	6	2.3±0.03	1.9-2.7	0.94	2±0.04	0.9-2.3	0.72	5.65*	15.4	1.15	7
LPt	0.63±0.01	0.6-0.7	0.60	0.62±0.01	0.6-0.7	0.44	1.94NS	3.0	1.03	15	0.6±0.02	0.4-0.7	0.83	0.5±0.01	0.4-0.7	0.84	2.79*	10.6	1.11	14
BPt	1.15±0.01	1.1-1.3	0.71	1.1±0.01	1-1.2	0.65	4.5*	5.1	1.05	14	1.1±0.02	0.9-1.3	0.90	1.0±0.04	0.7-1.9	0.71	1.84NS	8.6	1.09	16
LMt	1.06±0.01	1-1.2	0.76	1±0.0001	1-1		5.58*	6.3	1.06	10	1.1±0.02	0.9-1.3	0.90	0.9±0.02	0.7-1.1	0.91	4.36*	13.0	1.13	12
BMt	1.05±0.01	1-1.2	0.73	0.99 ±0.0001	0.9-1.0	0.26	4.34*	6.0	1.06	12	1.2±0.02	0.8-1.4	0.91	1.0±0.02	0.7-1.2	0.92	6.43*	20.8	1.21	5
LMet	0.38±0.01	0.3-0.4	0.44	0.36±0.01	0.3-0.4	0.61	2.26	6.0	1.06	11	0.4±0.01	0.2-0.6	0.83	0.3±0.01	0.2-0.3	0.66	5.39*	29.0	1.29	3
BMet	1.46±0.02	1.4-1.7	0.73	1.32±0.01	1.2-1.5	0.80	7.29*	11.2	1.11	7	1.4±0.02	1.1-1.6	0.93	1.3±0.02	1.1-1.4	0.88	3.01*	6.9	1.07	17
LT	2.08±0.02	1.9-2.3	0.91	1.98±0.01	1.9-2.1	0.79	4.61*	5.2	1.05	13	1.7±0.03	1.4-2.0	0.92	1.5±0.02	1.3-1.8	0.93	4.19*	10.7	1.11	13
LA	3.55±0.04	3.1-4	0.93	3.15±0.02	2.9-3.5	0.94	8.49*	12.7	1.13	3	3.6±0.07	2.8-4.5	0.97	3.1±0.03	2.8-3.4	0.94	7.23*	16.5	1.17	6
LP : BP	2.06±0.02	1.79-2.3	0.97	2.06±0.02	1.77-2.25	0.95	0.04NS	0.1	1.00	18	2.3±0.05	1.9-3.3	0.90	2.4±0.10	1.8-5.2	0.45	-0.58NS	-2.6	0.97	19
LPt : BPt	0.55±0.01	0.5-0.64	0.87	0.56±0.01	0.5-0.64	0.71	-1.14NS	-2.0	0.98	19	0.5±0.02	0.4-0.8	0.93	0.5±0.02	0.3-0.7	0.92	-0.23NS	-1.1	0.99	18
LMt : BMt	1.01±0.01	1-1.1	0.33	1.01 ±0.0001	1-1.11	0.26	0.31NS	0.2	1.00	17	0.9±0.02	0.6-1.4	0.92	1.0±0.03	0.7-1.4	0.88	-1.76NS	-7.0	0.93	21
LMet : BMet	0.26 ±0.001	0.2-0.29	0.79	0.28±0.01	0.21-0.33	0.85	-1.33NS	-4.7	0.95	20	0.3±0.01	0.2-0.5	0.92	0.2±0.01	0.1-0.3	0.95	3.64*	21.0	1.21	4
LT: LA	0.59±0.01	0.54-0.71	0.90	0.63±0.01	0.54-0.7	0.98	-5.25NS	-6.6	0.93	21	0.5±0.01	0.4-0.7	0.91	0.5±0.01	0.4-0.6	0.95	-1.42NS	-4.2	0.96	20
Shell percentage	16.51 =±0.29	11.3-19.3	0.92	16.52 ±0.29	11.3-19.3	0.97	-7.06NS	-15.7	0.84	22	13.6 ±0.31	10-19.2	0.95	17.1 ±0.42	13.0-22.2	0.96	-6.66NS	-20.4	0.80	22

Table 1 (ii): Descriptive statistics of the Tasar silkworm pupa: z statistics, dimorphic ranks among both the 'Sexual Dimorphism Index'

Location	Daba BV, Balaghat, Madhya Pradesh										Daba TV, Balaghat, Madhya Pradesh									
	Female (μ)			Male (μ)			Z	% Difference	SDI	Rank	Female (μ)			Male (μ)			Z	% Difference	SDI	Rank
Character	μ ± SE	Range	Shapiro Wilk	μ ± SE	Range	Shapiro Wilk					μ ± SE	Range	Shapiro Wilk	μ ± SE	Range	Shapiro Wilk				
CWt	13.3 ±0.22	11.1-15.8	0.97	8.53±0.19	6.10-10.30	0.94	16.54*	55.8	1.56	3	12.10 ±0.27	8.1 -15.3	0.97	7.65±0.21	5.2 -10.1	0.95	12.94*	58.3	1.58	2
SWt	2.1±0.06	1.4-2.8	0.95	1.35±0.05	0.80-2.00	0.95	9.36*	56.0	1.56	2	1.58 ±0.06	1.0-2.2	0.95	1.06±0.04	0.6-1.6	0.95	7.22*	48.8	1.49	3
PL	4.2±0.36	1.0-9.5	0.87	3.67±0.29	1.60-7.50	0.88	1.07NS	13.4	1.13	7	5.87 ±0.40	2.9-12.5	0.91	4.99±0.29	3.0-9.2	0.93	1.78NS	17.5	1.18	7
PWt	11.1 ±0.22	8.3-13.5	0.98	7.13±0.18	4.80-8.30	0.91	14.06*	55.8	1.56	4	10.39 ±0.25	7.5-13.3	0.98	6.43±0.27	0.8-9.5	0.86	10.88*	61.8	1.62	1
CL	5.3±0.05	4.8-6.1	0.94	4.75±0.04	4.30-5.20	0.96	8.27*	11.8	1.12	9	5.17 ±0.06	4.5-6.0	0.96	4.28±0.05	3.8-5.0	0.90	11.25*	20.7	1.21	6
CW	3.5±0.03	3.1-3.9	0.94	3.08±0.04	2.20-3.50	0.77	8.38*	13.1	1.13	8	3.46 ±0.03	3.1-4.0	0.94	3.05±0.04	2.8-3.9	0.62	7.30*	13.3	1.13	10
LP	4.6±0.08	4.1-6.2	0.86	3.99±0.04	3.50-4.40	0.95	6.93*	15.4	1.15	5	4.52 ±0.05	4.0-5.1	0.93	3.88±0.05	3.0-4.4	0.87	9.34*	16.4	1.16	8
BP	2.2±0.02	2.0-2.5	0.93	2.02±0.04	1.70-3.10	0.66	4.61*	10.8	1.11	10	2.11±0.08	1.8-4.4	0.34	1.91±0.03	1.6-2.2	0.95	2.47**	10.3	1.10	13
LPt	0.6±0.01	0.4-0.7	0.82	0.57±0.01	0.40-0.60	0.61	-0.40NS	-1.1	0.99	18	0.59±0.01	0.5-0.7	0.75	0.73±0.17	0.5-6.0	0.20	-0.84 NS	-19.6	0.80	22
BPt	1.0±0.02	0.7-1.2	0.82	1.01±0.01	0.90-1.10	0.77	0.92NS	2.2	1.02	16	1.07±0.01	0.9-1.2	0.75	1.07±0.01	1.0-1.1	0.58	-0.23 NS	-0.3	1.00	18
LMt	0.9±0.02	0.6-1.1	0.87	0.90±0.01	0.80-1.00	0.80	1.67NS	4.9	1.05	13	1 ±0.01	0.9-1.2	0.83	0.90±0.03	0.4-1.1	0.69	3.49*	11.5	1.11	12
BMt	1.3±0.02	1.0-1.6	0.95	1.24±0.02	1.10-1.40	0.83	3.27*	7.8	1.08	12	1.43±0.02	1.2-1.6	0.91	1.32±0.02	1.2-1.8	0.75	3.59*	8.0	1.08	14
LMet	0.4±0.01	0.3-0.4	0.60	0.35±0.01	0.30-0.40	0.64	1.25 NS	4.5	1.04	14	0.40±0.01	0.3-0.6	0.69	0.32±0.01	0.3-0.4	0.44	7.00*	27.7	1.28	4
BMet	1.3±0.03	1.1-2.1	0.73	1.15±0.02	0.90-1.60	0.86	3.88*	13.9	1.14	6	1.36±0.03	1.2-1.8	0.83	1.21±0.01	1.0-1.6	0.89	4.30*	12.4	1.12	11
LT	1.6±0.01	1.4-1.7	0.80	1.48±0.02	1.20-1.90	0.86	5.75*	9.7	1.10	11	1.59±0.03	1.2-1.8	0.93	1.50±0.02	1.1-1.7	0.84	2.70*	6.0	1.06	16
LA	2.3±0.07	1.7-2.9	0.93	1.36±0.06	0.80-2.10	0.94	10.05*	67.3	1.67	1	1.43±0.03	1.1-1.8	0.94	1.14±0.04	0.7-1.7	0.96	5.98*	25.2	1.25	5
LP: BP	2.1±0.03	1.7-2.7	0.88	1.99±0.03	1.35-2.41	0.94	1.46 NS	3.3	1.03	15	2.18±0.04	1.2-2.5	0.72	2.04±0.03	1.6-2.4	0.94	2.71*	7.0	1.07	15
LPt : BPt	0.6±0.02	0.4-0.9	0.90	0.57±0.01	0.40-0.67	0.87	-0.69 NS	-2.5	0.98	19	0.55±0.01	0.5-0.7	0.91	0.68±0.15	0.5-5.5	0.21	-0.83 NS	-18.8	0.81	21
LMt : BMt	0.7±0.02	0.5-0.8	0.96	0.73±0.01	0.62-0.91	0.94	-0.95 NS	-2.6	0.97	20	0.71±0.01	0.6-0.9	0.90	0.68±0.02	0.3-0.8	0.80	0.83 NS	3.0	1.03	17
LMet : BMet	0.3±0.01	0.2-0.4	0.96	0.31±0.01	0.23-0.44	0.92	-1.98 NS	-8.0	0.92	21	0.30±0.01	0.2-0.5	0.87	0.26±0.01	0.2-0.4	0.86	3.31*	14.1	1.14	9
LT: LA	0.7±0.03	0.6-1.0	0.89	1.15±0.05	0.67-1.88	0.86	-7.09 NS	-35.8	0.64	22	1.13±0.03	0.9-1.6	0.92	1.37±0.05	0.9-2.3	0.90	-3.88 NS	-17.6	0.82	20
Shell percentage	15.8 ±0.41	12.3-21.7	0.93	15.90 ±0.60	10.64 -28.99	0.86	-0.07 NS	-0.3	1.00	17	13.03 ±0.35	8.8-17.1	0.98	14.07 ±0.56	7.6 -23.1	0.97	-1.58 NS	-7.4	0.93	19

* Significant at 5 % α, ** highly significant at 1 % α and NS non-significant.

CWt- Cocoon weight, SWt- Shell weight, PL- Peduncle length, PWt- Pupal weight, CL-Cocoon length, CW- Cocoon width, LP- Length of pupa, BP- Breadth of pupa, LPt- Length of prothorax, BPt- Breadth of prothorax, LMt-Length of mesothorax, BMt-Breadth of mesothorax, LMet-Length of metathorax, BMet-Breadth of metathorax, LT-Length of thorax, LA-Length of abdomen.

Principal Component Analysis (PCA) and Discriminant Analysis were conducted separately for measurements. PCA revealed that the first three Principal Components (PC1–3) accounted for most of the total variation (Table 2), while the other components were less significant in explaining variations. Eigen vector loadings of Principal Component(s) were compared with characters and their ratios. Those characters and ratios with maximum loading (positive and negative) are considered to be the major sources of variation. Thus, PCA showed that cocoon weight, pupal weight and shell percentage to be significant in all the studied locations. The scatter plots generated through PCA depicts the sexual dimorphism in the tasar silkworm with overlaps (Figure 3). The results of cross-validation by Linear Discriminant Analysis (LDA) correctly identified up to 94.92 % of specimens to males and females (Table 3). Wilk's lambda, Pillai's trace, Hotelling Lawley & Roy's root are found to be significant in discriminating the sexes (Table 4).

Table 2: Morphometrics of tasar silkworm *(Antheraea mylitta)* pupa of Daba ecorace: Proportion of variation and variable coefficients of the first three eigen vectors for PCA

Location of the ecorace	Daba BV, CTRTI, Ranchi, Jharkhand			Daba BV, Kharsawan, Jharkhand			Daba BV, Balaghat, Madhya Pradesh			Daba TV, Balaghat, Madhya Pradesh		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Proportion of total variation (%)	70.62	18.54	10.01	74.1	12.3	10.3	52.1	32.8	13	54.22	27.82	11.56
Character												
CWt	0.650	0.387	-0.032	0.601	0.191	0.292	0.718	0.145	-0.100	0.675	0.186	-0.161
SWt	0.056	0.181	-0.015	0.024	0.094	0.107	0.100	0.115	-0.010	0.066	0.099	-0.050
PL	0.003	0.077	0.997	0.065	0.723	-0.685	0.115	0.044	0.984	0.093	0.461	0.879
PWt	0.590	0.216	-0.017	0.580	0.254	0.278	0.626	0.056	-0.096	0.635	0.132	-0.155
CL	0.062	0.049	-0.014	0.076	0.028	0.077	0.075	0.036	0.017	0.112	0.016	-0.014
CW	0.035	0.033	-0.008	0.035	0.004	0.012	0.056	0.016	-0.009	0.057	-0.012	0.022
LP	0.081	0.020	0.004	0.087	0.021	0.050	0.092	-0.001	0.084	0.096	0.034	0.018
BP	0.039	0.008	0.007	0.037	0.004	-0.017	0.034	0.006	0.011	0.035	0.003	-0.028
LPt	0.004	0.006	-0.001	0.009	0.007	0.004	-0.001	0.007	0.004	-0.005	-0.021	0.035
BPt	0.009	0.003	-0.006	0.009	0.022	0.010	0.004	-0.001	0.015	0.000	0.001	-0.004
LMt	0.011	0.005	-0.006	0.018	-0.006	0.008	0.012	0.003	0.012	0.017	0.006	-0.007
BMt	0.011	0.003	-0.010	0.024	0.001	0.006	0.017	0.004	-0.012	0.019	0.004	0.005
LMet	0.003	0.002	0.006	0.008	0.001	-0.003	0.002	0.000	-0.001	0.010	0.004	-0.005
BMet	0.022	0.007	-0.013	0.009	-0.001	0.003	0.020	0.018	0.020	0.019	-0.004	0.000
LT	0.019	0.013	-0.001	0.025	0.003	0.011	0.021	0.009	0.002	0.010	0.012	0.000
LA	0.065	0.018	0.008	0.062	0.018	0.039	0.135	0.015	0.034	0.045	0.006	0.011
LP:BP	0.001	0.003	-0.004	-0.005	0.002	0.054	0.010	-0.006	0.028	0.017	0.012	0.030
LPt:BPt	-0.001	0.003	0.002	0.002	-0.002	-0.003	-0.003	0.008	-0.006	-0.004	-0.019	0.034
LMt:BMt	0.001	0.003	0.005	-0.006	-0.008	0.002	0.000	0.000	0.015	0.003	0.003	-0.008
LMet:BMet	-0.002	0.001	0.007	0.004	0.001	-0.002	-0.003	-0.004	-0.004	0.004	0.004	-0.004
LT:LA	-0.006	0.001	-0.002	-0.001	-0.002	-0.003	-0.064	0.012	-0.023	-0.043	0.012	-0.018
Shell percentage	-0.456	0.872	-0.065	-0.524	0.604	0.587	-0.165	0.979	-0.023	-0.310	0.850	-0.410

Table 3: Confusion Matrix: Predicted group classification accuracy

Predicted Group	Daba female Ranchi	Daba male Ranchi	Daba female Kharsawan	Daba male Kharsawan	Daba female Balaghat	Daba male Balaghat	Daba Female TV	Daba male TV	Total
Daba female Ranchi	29	3	0	0	0	0	0	0	32
Daba male Ranchi	0	32	0	0	0	0	0	0	32
Daba female Kharsawan	0	0	30	2	0	0	0	0	32
Daba male Kharsawan	0	0	0	32	0	0	0	0	32
Daba female Balaghat	0	0	1	0	30	0	1	0	32
Daba male Balaghat	0	0	0	0	0	32	0	0	32
Daba Female TV	0	0	0	0	0	0	31	1	32
Daba male TV	0	0	0	0	0	4	1	27	32
Total	29	35	31	34	30	36	33	28	256
94.92 % correctly classified									

Table 4: Multivariate statistics and F approximations for testing the significance of canonical correlations between variables

Test	Value	F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.0004781	21.1898	154	1532.3	<.0001*
Pillai's Trace	3.6503637	11.5418	154	1631	<.0001*
Hotelling-Lawley	33.723363	49.3576	154	1142.4	<.0001*
Roy's Max Root	25.254884	267.4722	22	233	<.0001*

DISCUSSION

Our study supports the view that the sexual dimorphism in tasar silkworm could be gauged through variations in pupal size (Baig *et al.,* 2015). The morphometrics and their ratios varied significantly between sexes. These were validated through z- test and multivariate analyses to overcome anomalies that might arise from host and environment. The analysis involving univariate statistic (z test) showed that morphometrics of certain pupal characters were significant in assessing the sexes in the species studied. The multivariate analyses (PCA, LDA) summarize these significant characters and exemplify the values for determining the dimorphism in the puparia. We are in thus support of the opinion that sexual dimorphism in tasar silkworm at pupal stage is mainly size and weight correlated (Baig *et al.,* 2015). In gypsy moth *Lymantria dispar,* female pupae weigh more than males (Babaei *et al.,* 2009). Two pairs of tubercles present on the sterna of segments IX and X of the female pupae are useful in differentiating female from male pupae of *Conopomorpha cramerella* (Lepidoptera: Gracillaridae) (Posada *et al.,* 2011). Such visible morphological variations are lacking in tasar silkworm except the genital openings. *Hyphantria cunea* (Lepidoptera: Erebidae) has its genital slit longer in females (Tuncer

and Aker, 2017) as also observed in tasar silkworm. Discriminant analysis is the most commonly used statistical method for sex discrimination. It is used to analyse a set of axes that offer the maximum possibility of discrimination between two or more groups and is, generally, easy to use. PCA was developed by Pearson as a geometric interpretation in statistics. Results show that PCA can be used to differentiate between the sexes, as a tool employed for sex determination. PCA results are critically dependent on the data processing and selection of variables. The first principal component, usually, accounts for as much of the variability in the data as possible, and each subsequent component accounts for as much of the remaining variability as possible. It is essential to systematically attempt different combinations of components when visualizing a data set. Two or three principal components are usually considered sufficient for plotting purposes. In the PC1 and PC2 plot, the groups appeared to overlap, and there was no clear distinction between them (Figure 3).

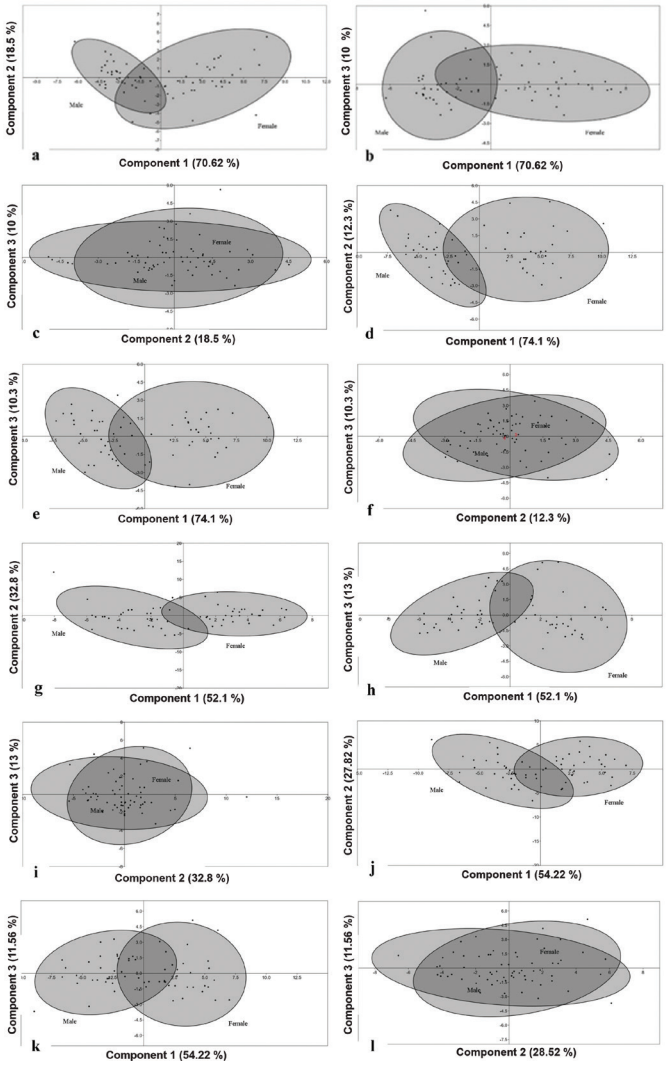

Figure 3: Scatter plots generated through PCA using pupal morphometric characters for differentiating sexes of *Antheraea mylitta* Daba ecorace. a-c – Daba BV, Ranchi ; d-f –Daba BV, Kharsawan; g-i –Daba BV, Balaghat; j-l – Daba TV, Balaghat.

When other components were plotted against each other, they produced even more mixed and complicated results. For instance, when PC2 and PC3 were plotted, with several vectors of negative value. The discriminant analysis from the cross-validation results revealed that about 94 % of the sexes can be accurately identified which shows an acceptable level of sex identification. LDA differentiated the sexes with acceptable overlaps (Figure 4).

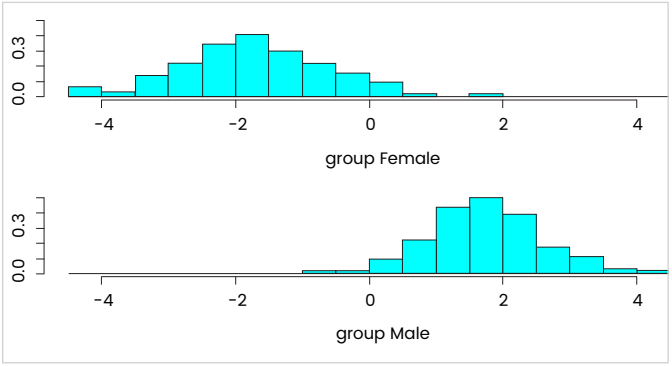

Figure 4: Grouping of sexes of *Antheraea mylitta* Daba ecorace at pupal stage through discriminant analysis.

The canonical analysis showed a clear differentiation of sexes in 4 populations with overlaps (Figure 5). A few characters are found significant in univariate and multivariate analyses which are of great utility in differentiating the sexes of Daba ecorace, such as pupal weight, cocoon weight, shell weight, length of pupa and length of abdomen. In conclusion, this technique is simple, quick, cost effective and accurate for sex determination which is always population specific.

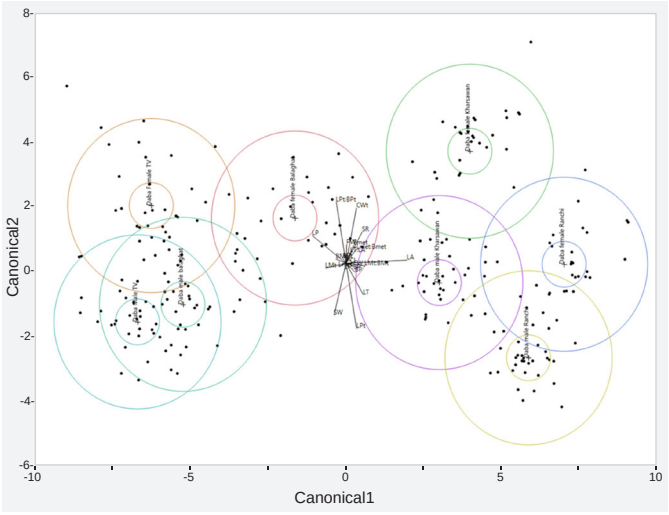

Figure 5: Grouping of sexes of *Antheraea mylitta* Daba ecorace through Canonical discriminant analysis.

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Research Paper

ASSESSMENT OF STATISTICAL SOFTWARE TO ANALYZE GENETIC DIVERSITY IN MULBERRY GERMPLASM

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ABSTRACT

Analysis of genetic diversity in the pool of mulberry germplasm using information on molecular markers is vital for understanding evolutionary processes at the population and genomic level. Various software programs are available for assessing genetic diversity. Most of them are freely accessible online (*via* the internet) and others are used offline. Many perform similar tasks, with the main differences being in the user interface, platform, type of data input and output. But, choosing the right one depends heavily on individual preferences. Genetic diversity analysis is a step-wise process through which existing variations like individuals or groups are identified using a specific statistical method or combination of methods. It is expected that the identical variations would form a pattern of genetic relationship useable in grouping the germplasm accessions. Software designed for SSR data analyses were chosen in this study for estimating diversity at the genetic level. Three statistical software programs, NTSySpc, DARwin and an online tool DendroUPGMA were used and compared for analyzing the same dataset. Different software computational methodologies, input formats and the results obtained from the SSR markers are discussed in this study.

Key words: Genetic diversity, mulberry, SSR, statistical software.

INTRODUCTION

The importance of plant genetic diversity (PGD) is now being recognized as a specific area that provides an opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, including farmer-preferred traits (yield potential, large seed, *etc.*) and breeders preferred traits (pest and disease resistance, photosensitivity, *etc*). Plant breeders have introduced desirable genes and eliminated the undesirable ones, altering the process of underlying heredity principle for several decades (Narain, 2000). Molecular markers play a vital role in identifying diverse germplasm and hence, it is essential to know the best and easy to use software for analyzing the genetic diversity.

Gathering the key information for accessing the genetic diversity of germplasm especially: (i) allele number per locus; (ii) genotype number per locus; (iii) gene diversity; (iv) partition of the diversity into its components within and between populations; and (v) the genetic distance among the analyzed populations is very important (Pagnotta, 2018). This study focuses on data generated from diverse germplasm of mulberry crop that is

extremely heterogeneous and outbreeding type. Mulberry is an economically important plant used for sericulture; it is the lone source for nourishing domesticated silkworms and a model system to study plant-herbivore interaction, plant-microbe interaction and bio-energy resources. This is because trees can maintain high levels of diversity and accumulate new mutations slowly, but can also display rapid local adaptation (Petit and Hampe, 2006).

Molecular markers allow us to determine the genotypes of individuals and are powerful means of investigating and quantifying genetic diversity, spatial genetic structure, tree mating systems, gene flow and breeding patterns of tree species. Currently, the most frequently used approaches to analyse the genetic variation of populations and individuals are microsatellites or Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphisms (SNPs) and Restriction Site Associated DNA Sequencing (RADseq). Similar to minisatellites, SSRs are sequences of a repeated motif, ranging from 1 to 6 bp and varying in length, between individuals (Tautz *et al.,* 1984). In addition to the capability of PCR based method, several characteristics of SSRs, such as high mutation rates and high levels of polymorphism, co-dominance and random distribution across the genome (Zane *et al.,* 2002), make them a more sensitive tool for revealing population genetic structure and gene flow than the other markers. SSR analysis requires the development of a specific primer pair flanking the individual SSR site. The primers are developed through several methods (Zane *et al.,* 2002; Kalia *et al.,* 2011) and may be unique to the species or transferable between closely related species (Barbara *et al.,* 2007). Till today, a few attempts have been made to characterize the genetic diversity in mulberry by using different molecular markers. These include AFLP based marker analysis (Sharma *et al.,* 2000), RAPD and DAMD profiles (Bhattacharya and Ranade, 2001), ISSR based analysis (Vijayan and Chatterjee, 2003), and genetic polymorphism estimation in mulberry hybrids using RAPD (Lou, 1998) and SSR profiling (Thumilan *et al.,* 2016; Pinto *et al.,* 2018; Arunakumar, 2021). This paper explains how to use different software for the analysis of molecular data considering three different software. DendroUPGMA (Sokal and Michener, 1958) is a straightforward approach to construct a phylogenetic tree from a distance matrix. NTSySpc - Numerical Taxonomy and Multivariate Analysis System (Version 2.0) is a system of programs developed by James Rohlf (1998) that are used to find and display structure in multivariate data. Cluster analysis is the most common use of NTSySpc for performing various types of agglomerative cluster analysis (similarity or dissimilarity matrix). DARwin, Dissimilarity Analysis and Representation for Windows is a software package developed for diversity and phylogenetic analysis based on evolutionary dissimilarities (Perrier and Jacquemoud-Collet, 2006). Standard methods for tree and factorial representation are proposed, and they are enhanced with original and specific approaches, addressing particularly the question of sensitivity to data accuracy. This study aims to describe the most informative and widely used SSR marker genotypic data analysis software and illustrate the methods.

MATERIALS AND METHODS

Input data: The scoring input data (Table 1) were generated from the thirty germplasm accessions of mulberry using 12 SSR markers generating 24 alleles. Input data file format for these three software are different.

DendroUPGMA: A dendrogram construction utility (http://genomes.urv.cat/UPGMA/)

DendroUPGMA is a web server that allows free construction of dendrogram, using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) or WPGMA (Weighted Pair Group Method with Arithmetic Mean) algorithm. A dendrogram is a diagram frequently used to illustrate the arrangement of different clusters produced by hierarchical clustering between similar sets of data. They are frequently used in biology to show clustering between genes or samples. It consists of U-shaped lines that connect data points in a hierarchical tree. The length of lines represents the distance between two data points that are connected (Wilkinson *et al.,* 2009).

Table 1: Molecular data of thirty germplasm generated from 12 different SSR markers

	G_01	G_02	G_03	G_04	G_05	G_06	G_07	G_08	G_09	G_10	G_11	G_12	G_13	G_14	G_15	G_16	G_17	G_18	G_19	G_20	G_21	G_22	G_23	G_24	G_25	G_26	G_27	G_28	G_29	G_30
Marker_01	0	0	0	1	0	0	0	0	1	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0
	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	1
Marker_02	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
	0	1	1	0	1	1	0	0	0	0	0	0	1	0	1	0	0	1	1	1	1	1	1	0	0	0	1	0	1	1
Marker_03	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
	0	1	1	0	1	1	0	0	0	0	0	0	1	0	1	0	0	1	1	1	1	1	1	0	0	0	1	0	1	1
Marker_04	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	0	1	0	0	1	0	1	1
	1	0	1	0	0	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1	0	1	1	0
Marker_05	0	0	1	0	1	0	0	1	1	0	1	0	1	0	1	0	0	1	0	1	1	0	0	1	0	0	0	0	0	1
	1	0	0	0	1	1	0	1	0	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	1	1	1	1	0
Marker_06	1	1	1	1	0	0	1	1	1	0	1	1	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
	1	0	1	1	0	1	1	1	1	1	1	0	1	0	0	1	1	1	0	0	1	1	1	1	1	0	0	1	1	0
Marker_07	0	1	0	1	1	1	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1
	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	0	0	1	0	1
Marker_08	0	1	0	0	0	1	0	0	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	1	1	0	0	1	0
Marker_09	0	1	0	1	0	1	1	0	1	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0
	0	1	0	1	0	1	1	0	1	1	1	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0
Marker_10	1	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0	1
	0	1	0	1	0	1	1	0	1	0	1	1	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0
Marker_11	0	0	0	0	0	1	1	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Marker_12	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	0	1	0	1
	1	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	1	0	0	0	1	0	1

Dendro servers may be sets of variables in a variety of formats. The Dendro UPGMA server calls the number of sets of variables as rows. The number of variables is the number of columns. The data could be in fasta-like format, *i.e.,* a first line that begins with the ">" character and contains the identifier field, and a second line that contains a set of variables separated by tabs or spaces (but not a combination of both). In addition, only one line for each set of variables (separated by spaces) can be used. In this case, the first variable is assumed to be the identifier field.

NTSySpc (Version 2.0): Numerical Taxonomy and Multivariate Analysis System

NTS files are ordinary ASCII files (txt files, not binary files). A file for a data matrix may be prepared with an editor or any word processor that has a txt (non-document)

mode. The NTedit program included with NTSySpc can be used to prepare data files and ensure that they are in the proper format. In addition, NTSySpc can also read data matrices from Excel spreadsheets (XLS files) and trees from Nexus format files.

DARwin: Dissimilarity Analysis and Representation for Windows

DARwin uses its formats for data files but import/export from/to standard formats in the phylogeny are proposed. All files are text ASCII files with Tab as separators; they can be edited with any text editor. They can also be imported or exported directly from spreadsheets like Excel in saving files in text format with Tab as separators. A similarity or a distance matrix is generated in CVX or tab-delimited format.

Mode of operation of statistical software

DendroUPGMA

> Search DendroUPGMA on the Google site (http://genomes.urv.cat/UPGMA/) > Select Dendrogram construction using the DendroUPGMA>Click on DendroUPGMA web >Enter input data into the given box >Select similarity index >Choose the clustering method UPGMA or WPGMA >Then click on submit button (Figure 1).

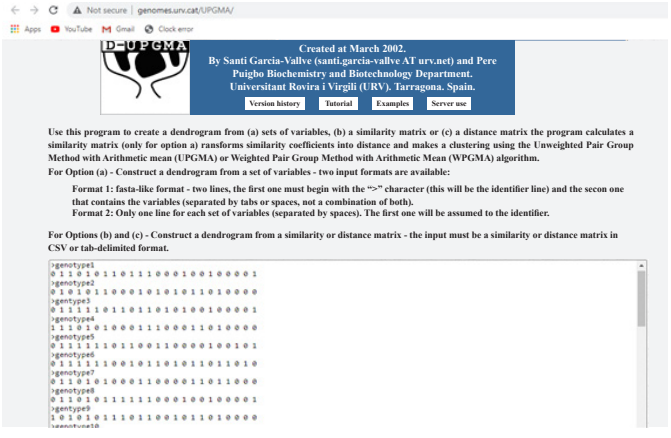

Figure 1: Input data for DendroUPGMA software

NTSySpc (Version 2.0) has two modules:
a) NTedit b) NTSySpc

i) Transfer saved data to NTedit

> Open NTedit program > Click "File" menu then "Import Excel" sub-menu > Use "using DDE" or "using OLE" for opening .xls files in higher and lower versions of Microsoft Excel 2000, respectively > Recall the saved .xls file > Click "File" menu and select "Save file" sub-menu to save the file with arbitrary name with.NTS filename extension >Close NTedit programme (Figure 2).

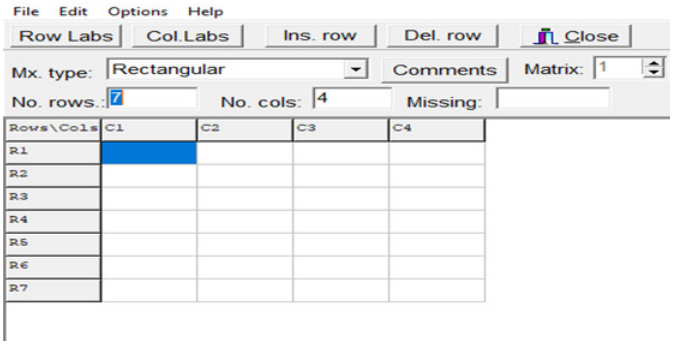

Figure 2: Importing data into NTedit program

ii) Establishing similarity matrix

> Go to "Similarity" tab > Click on "SimQual" toolbar which is specific for making qualitative data like molecular > Recall the previously saved file with. NTS extension in "Input file" field > Tick the box in front of "by rows" part > Choose your desired method for making similarity matrix from the "Coefficient" part > Specify the name and path of your output file in the field "Output file" > Click on "Compute" toolbar

iii) Dendrogram drawing

>Go to "Clustering" tab > Click on "SAHN" toolbar > Recall the previously saved file (containing similarity matrix) in "Input file" field > Specify the name and path of your output file in the field "Output file" > Choose desired method for clustering from the "Clustering method" part (such as DendroUPGMA) > Choose "FIND" option in the field of "In case of ties" part > If you have near 100 individuals, enter

"100" in "Maximum no. ties tress" part > Do not change "Tie tolerance" and "Beta" fields > Click on "Compute" toolbar > Close the "Report listing" windows if the procedure was successful > Click on "plot tree" toolbar > A dendrogram will be drawn.

DARwin software

i) Import molecular data

> Open the DARwin programme >Click on file menu >Then click on the Import data matrix >Select the input data file >Select data type > Click on save data as and type the name of the file (Figure 3).

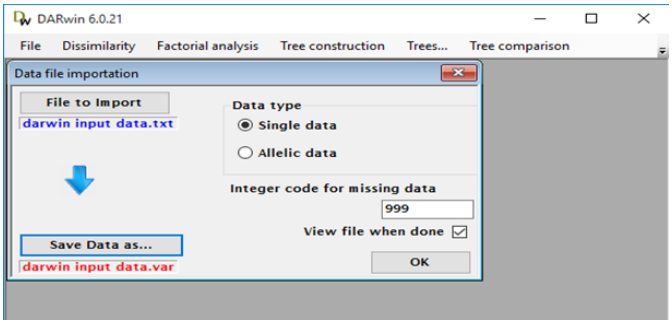

Figure 3: Importing data into DARwin program

ii) Calculate dissimilarity

> Click on dissimilarity men >Select the data type >Select the input file for dissimilarity calculation >Select dissimilarity index select presence/absence >Then click on save dissimilarity as..... >Dissimilarity file is saved as dissimilarity var. File

iii) Fractional analysis on dissimilarity

> Click on fractional analysis menu >Enter no. of axes to edit > Click on the identifier and then select file > Click on save fractional coordinates as..... > Then save the file as AFT file type > Click on ok > Fractional analysis will be drawn.

iv) Tree construction

> Click on tree construction menu >Select any one method >Then select the method option >Select the file for identifier >Then click on save tree as..... >Then click on ok >Tree will be drawn.

RESULTS AND DISCUSSION

The input binary data using different data-file formats of 30 mulberry germplasm were imported into various software packages *viz.,* DendroUPGMA, NTSySpc and DARwin as described in the methods. The software generated the output data into similarity and dissimilarity matrices between the germplasm. In DendroUPGMA, threshold equality is zero per cent and dissimilarity minimum and the maximum value is from 0.15 to 1. In NTSySpc software, the average coefficient of variation ranged from 0.1 to 1.0, which depends on the variation among the variables. Whereas, in DARwin software, tree construction is based on hierarchical clustering and the coefficient of variation values ranged from 0 to 0.1. The NTSySpc and DARwin software allow importing of data from excel files, which makes data management very easy.

The SSR data information from the 30 mulberry germplasm was used to construct the dendrogram using the software DendroUPGMA. It formed two distinct lineages based on degree coefficient ranging from 0.3 to 1.0, and all the mulberry germplasm showed significant variation. SSR analysis grouped the accessions independently into two major clusters, cluster I and Cluster II with 13 and 17 germplasm, respectively (Figure 4). The RAPD and ISSR data were used for the analysis of genetic diversity and relationships in mulberry using the DendroUPGMA software and individual branch was recorded as per the 100 bootstrap replications (Awasthi *et al.,* 2004). Their result revealed that three wild species, namely *M. laevigata, M. serrata* and *M. tiliaefolia,* were genetically distant from the domesticated species. Similarly, Sharma *et al.* (2000) using AFLP markers for diversity study, revealed that there is a strong contrast to the outbreeding and high heterozygosity found in domesticated mulberry species.

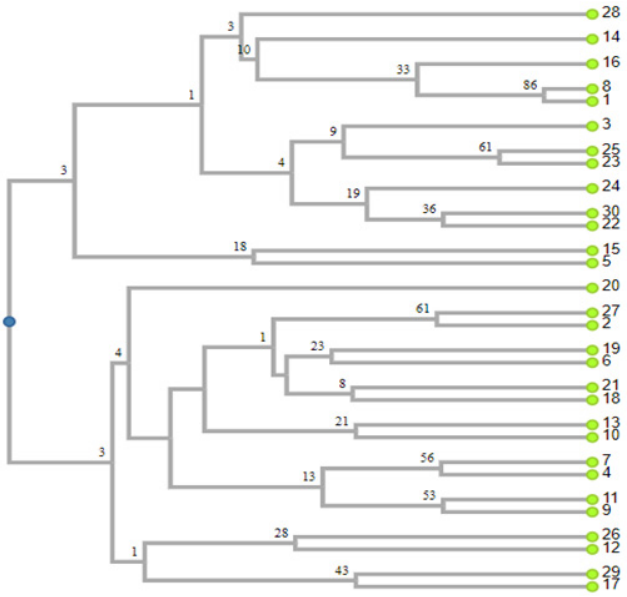

Figure 4: The DendroUPGMA clustering generated based on the simple matching similarity matrix obtained by using 12 SSR markers, illustrating the relative similarity among the 30 accessions of mulberry

DendroUPGMA facilitates the different correlation coefficients, such as Pearson correlation coefficient (r), Jaccard index and Dice coefficient so that we can choose any correlation approach for analysis depending on the data set. However, the molecular binary data set could only analyse through Jaccard or Dice coefficient, both are very similar (particularly dendrogram topology will not differ) and the only difference is in the branch lengths. It computes genetic distance for all pairwise combinations with a fast and simple process although not commonly used more by researchers in publication due to impossibility in alteration of the clustered column which is most important for distinguishing selected variables. Clustering analysis of 26 *Anthurium* cultivars (Srisamoot and Padsri, 2018) was done by using DendroUPGMA online tool, clustering analysis implied that the 26 *Anthurium* cultivars were divided into two major groups. The first group consisted of only foliage *Anthurium* which is a Thai native plant and had a 0.48 genetic similarity index. The second group was divided into three subgroups, Group IIA included five cultivars with 0.58 genetic similarity index, Group IIB included five cultivars, with 0.56 genetic similarity index and Group IIC comprised of thirteen cultivars with 0.66 genetic similarity index. Similarly, the phylogenetic tree created (Yadav *et al.,* 2019) using the DendroUPGMA method through online available software revealed significant differences among the five chickpea cultivars selected for the study for all the parameters. Not only genetically, but physiologically also, the varieties have shown dissimilarity *i.e.,* in flowering habits, biomass and number of branches.

NTSySpc analysis grouped the 30 germplasm into two distinct clusters at the average coefficient of 0.79. The distance coefficient ranged from 0.51 to 0.95 as shown in Figure 5.

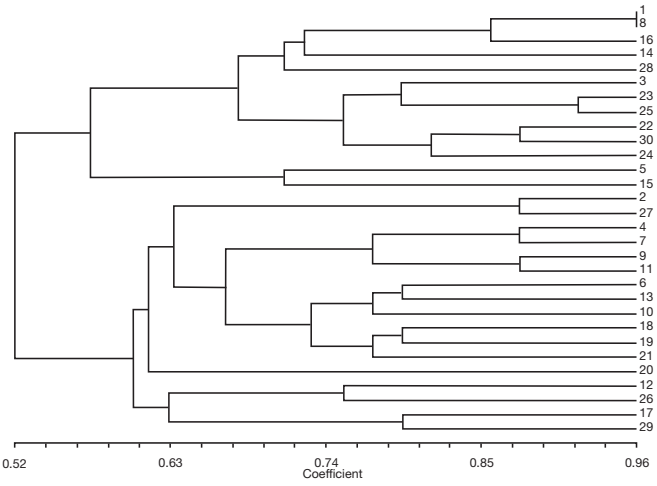

Figure 5: Cluster analysis of 30 germplasm of mulberry based on SSR markers polymorphism using NTSySpc

Cluster I included two sub clusters, namely IA and IB, similarly, cluster II was also divided into two sub clusters, IIA and IIB. Cluster IA consisted of 11 germplasm, whereas, cluster IB had 2 germplasm. The highest number of germplasm *i.e.,* 13 were clustered at cluster IIA, whilst cluster IIB comprised of 4 germplasm. The clustering patterns in the existing analysis gave clear graphical representation of the diverse germplasm. This helps plant breeding researchers to understand and select parents for breeding programs, grouped in different clusters. The advantages of using NTSySpc is that, it has

the ability to load much larger files and it does not append .txt to the ends of file names while saving. Basak *et al.* (2014) separated *Hedychium* flowering plants into 2 main clusters based on different altitudes and intensities of flower aroma using NTSySpc. In the ISSR dendrogram, species of the moist lowlands with strong fragrant flower *(H. coronarium, H. dekianum, H. flavum, H. chrysoleucum, H. stenopetalum, H. spicatum, H. marginatum, H. coccineum, H. aurantiacum)* were combined in cluster I. The strong aroma of the flowers may be an evolutionarily derived trait among the species. A small cluster (cluster II) consisted of species of *H. flavescens* and *H. gardnerianum* that possessed geographic similarity of high altitude and has yellow and mildly fragrant flowers. AFLP marker analysis too closely follows the altitude based on clustering of ISSR marker.

Thirty accessions of mulberry were separated into 16 interactions in the dendrogram generated by DARwin software (Figure 6). Fifteen germplasm were equally distributed between the two groups. More diverse

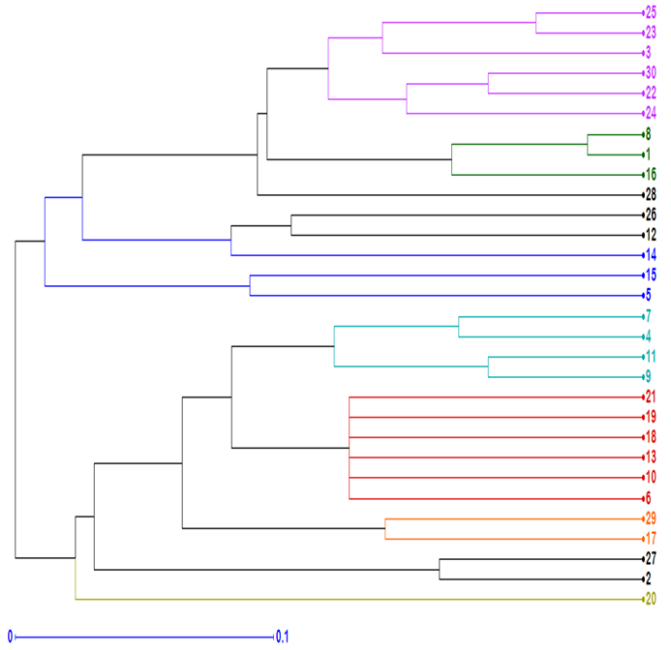

Figure 6: Dendrogram obtained from SSR data of 30 germplasm using DARwin software (Hierarchical clustering)

germplasm when individually grouped within cluster 1 and cluster II, revealed six identical genotypes (6, 10, 13, 18, 19 and 21). Pinto *et al.,* 2018 performed cluster analysis using the data generated by 15 SSR primers against 216 germplasm with Rp >2 by Neighbour Joining method in DARwin software. The germplasm was distinguished into two main clusters and many sub clusters, and these primers resolved 38 popular mulberry cultivars that have a narrow genetic base (Krishnan *et al.,* 2014).

Conclusion

Various online and offline software are available for genetic diversity analysis of different data types acquired from molecular markers (both dominant and co-dominant markers). The decision of usage of statistical software solely depends on character-specific and selected models. Most data analyses will require the use of more than one statistical software due to the distinction of output generated from different software. DendroUPGMA may produce only clustered dendrogram, whereas NTSySpc is enhanced in many ways by taking advantage of the many options available depending upon the type of analysis. Typical applications available in NTSySpc are cluster analysis, ordination analyses and biplots, Principal components analysis, Principal coordinates analysis, Non-metric multidimensional scaling, 2D or 3D plots and comparison of dis/similarity matrices which are only present and more advantageous than the other two software. NTSySpc (V. 2.0) software is relatively easy to use, but it is paid software and bootstrapping is limited. Similarly, DARwin software generates the dendrogram clustered into different ways, such as radial, circled or linear. In addition, DARwin can do bootstrapping analysis and tree construction. It offers several tree types with the different colouring of individual clusters for distinguishing relationships among reference cultivars or samples and this makes it relatively easy to explain the cluster analysis. Hence, DARwin is considered the easiest way to explain the cluster analysis segment.

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Research Paper

EVALUATION OF LEAF NUTRITIONAL QUALITY OF TASAR SILKWORM FOOD PLANT HYBRIDS OF *TERMINALIA ARJUNA* AND *T. TOMENTOSA*

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ABSTRACT

Tasar silk quantity and quality are largely determined by the leaf nutrient quality of food plants. Hence, the present study was envisaged to identify hybrid plants with superior biochemical content from 29 improved *Terminalia arjuna* and *T. tomentosa* plant hybrids, that include both intra and inter-specific crosses. Hybrids were developed in two batches; first batch of 14 hybrids of 4.5 year old, maintained under field condition and second batch of 15 hybrids of 2.5 year old, maintained under pot condition. Single factor analysis of variance revealed significant variation among the test hybrids for all the five biochemical parameters evaluated. Multiple mean comparison through Tukey's test revealed that, among the first batch hybrids, some have shown significantly high values for leaf moisture (70-74.5 %), Chl a (1.68-1.71 mg/g), Chl b (1.37 mg/g), total chlorophyll content (2.66 mg/g), protein content on dry matter basis (12.3 %), carbohydrate on dry matter basis (32.3-34.5 %) and the lowest amount of phenol (1.59 %). Similarly, among the second batch hybrids, some have shown significantly high values for leaf moisture (75.1 %), Chl a (1.52 mg/g), Chl b (0.41-0.42 mg/g), total chlorophyll content (1.94 mg/g), protein content on dry matter basis (20.75 %), carbohydrate on dry matter basis (33.4-34 %) and the lowest phenol content (0.9 %). Overall, in the first batch, 501 x 533 (P4) hybrid performed better in respect of several traits *viz.,* leaf moisture (73.4 %), protein (10.34 %) and carbohydrate content (34.45 %). Similarly, in the second batch, 701 x 614 (P3) hybrid delivered better in terms of leaf moisture (75.1 %), chlorophyll a (1.5 mg/g), chlorophyll b (0.42 mg/g) and total chlorophyll content (1.94 mg/g).

Key words: Nutritional quality, tasar silkworm food plants, *Terminalia arjuna, T. tomentosa.*

INTRODUCTION

In the world sericulture scenario, India is unique for having four types of silk *viz.,* Mulberry, Tasar, Eri and Muga, that includes both wild and domesticated silk. The tasar silk filament being porous in nature, has greater values than other silk, especially mulberry in many aspects, such as natural sheen, luster and feel (Gangwar and Gupta, 2010). Tasar culture is an ecofriendly, forest based industry supporting livelihood of 1.25 lakhs of tribal families residing in forest associated rural areas of Jharkhand, Bihar, Chhattisgarh, Odisha, Andhra Pradesh, Maharashtra, West Bengal and Uttar Pradesh (Reddy *et al.,* 2015). Tasar culture is being practiced in two ways, forest based cocoon collection (subsistence culture) since centuries and intensive silkworm rearing under

systematic/natural plantation (commercial culture) since decades. To facilitate tribal tasar farmers, plantations of *T. arjuna* (Arjun) and *T. tomentosa* (Asan) have been raised in forest land, government waste land and marginal non–agriculture land through several government projects (Gargi *et al.,* 2015).

The quality and quantity of cocoons depend mainly on the nutritional status of food plants upon which they feed (Sahay and Kapila, 1993; Sinha *et al.,* 2002). Leaf quality is one of the major factors that influence the success of tasar crop (Sahay *et al.,* 2001). Agronomic management inputs, such as water, micro and macro nutrients would improve the productivity and leaf quality of food plants (Sahay and Kapila, 1993). However, most of the tasar host plantations are in forest areas, which rarely receive fertilizers and manures due to poor economic status of farmers (Singhvi *et al.,* 2012). Development of superior host plant hybrids with high leaf yield and quality would be an ecofriendly and sustainable approach to obtain better cocoon yield and quality under low input condition.

To facilitate tasar host plant breeding, Central Tasar Research and Training Institute, Ranchi has collected and characterized about 241 host plant germplasm under *ex situ* field gene bank. The genetic variability in *T. arjuna* and *T. tomentosa* have extensively been studied (Kumar *et al.,* 2009, 2010, 2013; Gargi *et al.,* 2010, 2015; Sharma *et al.,* 2011) which revealed considerable variation for leaf yield and biochemical characters. Arjun accessions showing fast growth, short internodal distance, high leaf moisture and protein contents and better rooting ability (more than 80 %) and Asan accessions showing better rearing performance were selected as parents to develop the hybrids (Gargi *et al.,* 2015). In the present study, these hybrids have been evaluated under pot condition for biochemical characters.

MATERIALS AND METHODS

Present study was carried out at Central Tasar Research and Training Institute, Ranchi, Jharkhand. Hybridization was attempted during the year 2012 in 16 cross combinations and during 2014, in 10 cross combinations, and developed 33 hybrids in the first batch and 29 hybrids in the second set. Plants derived from seeds of the same cross combination are genotypically different as their parents are naturally heterozygous and hence, they were treated as separate hybrids. Single plant was evaluated for each hybrid under pot condition. Based on hybrid performance for leaf yield parameters, 14 hybrids were selected from the first batch (5 cross combinations) and 15 hybrids from the second batch (8 cross combinations). These hybrids were assessed for leaf moisture, protein, carbohydrate, phenol and chlorophyll contents. All estimates are means from triplicates. Four and a half year old plants of first batch hybrids were maintained under field condition, while two and a half year old of second batch hybrids were maintained under pot condition, wherein pots were filled with 25 kg of soil mixture (soil:sand:farm yard manure in the proportion of 3:1:1).

For the estimation of protein, carbohydrate and phenol content in hybrid plants, leaf samples were collected from middle portion of the branches and dried in a hot air oven at 60 °C for two days, powdered and used for the analysis. Leaf moisture content was estimated and expressed in per cent.

Estimation of biochemical constituents

Protein content was quantitatively measured by Lowry's method (Lowry *et al.,* 1951). Total leaf carbohydrate content was estimated as described by Hedge and Hofreiter (1962) and phenols content as described by Thimmaiah (1999). Leaf chlorophyll content was estimated in fresh leaf samples collected from fourth node from tip of branch using DMSO (Dimethyl Sulfoxide) method (Hiscox and Israelstam, 1979).

Statistical analysis

Single factor analysis of variance was performed using SPSS 16.0 package. Mean values of all tested biochemical parameters were calculated from triplicates. Mean values of hybrids were compared by employing Tukey's grouping using 'R software'.

RESULTS AND DISCUSSION

Nutritional status of the host plant is decisive in determining the quality and quantity of Tasar silk (Sahay and Kapila, 1993; Sinha *et al.,* 2002) and is one of the major factors that influences success of tasar crop (Sahay *et al.,* 2001). Considering the above fact, parents were selected for the development of hybrids. In the present study, selected hybrids of the first batch (4.5 year old) in field condition and of second batch (2.5 year) in pot condition were evaluated for their biochemical contents. These results were compared with those of their respective parents, which were evaluated at the age of 5 years in a previous study (Suryanarayana *et al.,* 2005). Owing to the wide difference in age between second batch hybrids (2.5 year) and parents (5 year), comparison of biochemical contents is not realistic, however, parental values are indicated wherever required. Moreover, biochemical contents in parents were assessed on fresh leaf basis and hence, their scores stand much lower than that of hybrid values estimated on dry leaf basis. However, relative comparison was made between hybrids and parents.

Single factor analysis of variance in both the first (Table 1) and second batch hybrids (Table 2) revealed significant variation among the test hybrids for all the five biochemical parameters.

Table 1: Leaf biochemical parameters of *Terminalia* spp. hybrids (first batch)

#	Hybrid combination	Leaf moisture (%)	Chlorophyll (mg/g) on fresh weight basis			Protein on dry weight basis (%)	Total carbohydrate on dry weight basis (%)	Total phenol* on dry weight basis (%)
			Chl. a	Chl. b	Total Chl.			
1	501^{y} x 533^{x} (P2)	73.0^{ab}	1.29^{c}	0.37^{ef}	1.65^{e}	7.84^{def}	32.55abc	1.59^{g}
2	615^{x} x 531^{y}(P6)	64.9^{g}	1.22^{d}	0.36^{gh}	1.58^{ef}	8.01^{de}	23.45^{e}	2.52^{de}
3	615^{x} x 531^{y} (P7)	70.3^{def}	1.06^{g}	0.32^{hi}	1.38^{g}	10.76^{b}	32.30^{abcd}	2.27^{e}
4	501^{y} x 533^{x} (P6)	70.5^{cde}	1.13^{f}	0.32^{ghi}	1.46^{g}	8.19^{d}	29.55^{d}	2.36^{de}
5	701^{x} x 531^{y} (P1)	69.6^{def}	1.29^{c}	1.37^{a}	2.66^{a}	7.70^{defg}	30.95^{bcd}	2.97^{b}
6	701^{x} x 531^{y} (P2)	64.5^{g}	0.92^{i}	0.31^{ij}	1.23^{h}	8.43^{d}	29.90^{cd}	2.64^{d}
7	501^{y} x 533^{x} (P4)	73.4^{ab}	1.40^{b}	0.58^{c}	1.97^{c}	10.34^{bc}	34.45^{a}	2.93^{bc}
8	615^{x} x 531^{y} (P1)	72.7^{abc}	1.41^{b}	0.40^{de}	1.82^{d}	6.97^{fg}	7.35^{f}	2.97^{b}
9	501^{y} x 533^{x} (P3)	71.5^{bcd}	1.68^{a}	0.71^{b}	2.39^{b}	7.18^{efg}	24.05^{e}	3.29^{a}
10	501^{y} x 615^{x} (P1)	68.2^{f}	1.21d^{e}	0.35^{gh}	1.56^{f}	12.30^{a}	30.00^{cd}	3.07^{ab}
11	501^{y} x 533^{x} (P5)	69.2^{ef}	1.71^{a}	0.59^{c}	2.31^{b}	8.40^{d}	22.00^{e}	1.97^{f}
12	501^{y} x 533^{x} (P1)	74.5^{a}	1.25^{cd}	0.36^{ef}	1.61^{ef}	5.57^{h}	29.55^{d}	2.41^{de}
13	501^{y} x 701^{x}(P1)	65.4^{g}	1.16^{ef}	0.45^{d}	1.60^{ef}	9.63^{c}	33.50^{ab}	2.65^{cd}
14	615^{x} x 531^{y} (P5)	69.2^{ef}	1.00^{h}	0.27^{j}	1.27^{h}	6.89^{g}	32.50^{abcd}	2.32^{e}
p-value		0.000	0.000	0.000	0.000	0.000	0.000	0.000

^{x}*T. arjuna,* ^{y}*T. tomentosa,* *% Catechol equivalents, data are presented as mean of triplicates; mean values superscripted with the same alphabet(s) are not significantly different.

For leaf moisture content, wide variation was observed among all the experimental hybrids. Multiple mean comparison among hybrids through Tukey's test revealed higher leaf moisture content in four hybrids *viz.,* 501 x 533 P1 (74.5 %), 501 x 533 P4 (73.4 %), 501 x 533 P2 (73 %) and 615 x 531 P1 (72.7 %) in the first batch. Whereas, in the second batch, the highest leaf moisture content was observed in the hybrid, 701 x 614 P3 (75.1 %). It was reported in mulberry (Koul *et al.,* 1996) that, leaf moisture plays a vital role in enhancing leaf nutrition and improves leaf palatability and digestibility in silkworm, which in turn leads to enhanced larval growth and cocoon quality. Increased leaf moisture content enhances feeding efficiency and in turn, growth rate of larva (Sastry *et al.,* 1988). In mulberry, the assimilation and conversion efficiency in silkworm decreases with decreasing dietary moisture (Narayanprakash *et al.,* 1985).

Chlorophyll content revealed considerable variation among hybrid plants. In the first batch hybrids, the range for total chlorophyll, chlorophyll a, and chlorophyll b was 1.26 - 2.66 mg/g, 0.92-1.71 mg/g and 0.27–1.37 mg/g with an average of 1.65 mg/g, 1.25 mg/g and 0.41 mg/g, respectively (Table 1);

Table 2: Leaf biochemical parameters of *Terminalia* spp. hybrids (second batch)

#	Hybrid combination	Leaf moisture (%)	Chlorophyll (mg/g) on fresh weight basis			Protein on dry weight basis (%)	Total carbohydrate on dry weight basis (%)	Total phenol* on dry weight basis (%)
			Chl. a	Chl. b	Total Chl.			
1	533^{x} x 702^{x} (P1)	70.5^{de}	1.04^{f}	0.33^{e}	1.37^{g}	12.06^{f}	34.00^{a}	1.58^{de}
2	819^{x} x 716^{x} (P1)	71.2^{cd}	0.83^{h}	0.24^{g}	1.07^{i}	9.42^{g}	23.65^{ef}	1.24^{f}
3	614^{x} x 701^{x} (P4)	66.9^{i}	0.94^{g}	0.29^{f}	1.23^{h}	13.3^{1e}	33.40^{a}	3.19^{a}
4	533^{x} x 342^{y} (P5)	72.2^{bc}	1.12d^{e}	0.33^{e}	1.45^{f}	15.41^{d}	21.95^{g}	1.63^{cde}
5	701^{x} x 614^{x} (P1)	69.9^{def}	1.26^{c}	0.35^{cd}	1.62^{e}	5.08^{h}	29.00^{bc}	1.66^{bcd}
6	533^{x} x 342^{y} (P9)	68.6^{gh}	1.14^{d}	0.36^{cd}	1.50^{e}	11.77^{f}	29.25^{bc}	1.82^{bcd}
7	819^{x} x 132^{x} (P1)	68.5^{gh}	1.15^{d}	0.33^{e}	1.48^{ef}	11.25^{f}	27.15^{cd}	1.50^{e}
8	533^{x} x 342^{y} (P7)	69.4^{efg}	1.26^{c}	0.37^{bc}	1.63^{e}	9.67^{g}	29.25^{bc}	3.19^{a}
9	533^{x} x 342^{y} (P2)	73.1^{b}	1.10^{e}	0.34^{de}	1.44^{f}	20.75^{a}	25.10^{de}	1.79^{bcd}
10	533^{x} x 702^{x} (P2)	72.6^{bc}	1.16^{d}	0.36b^{cd}	1.52^{de}	16.56^{c}	30.11^{b}	3.08^{a}
11	701^{x} x 614^{x} (P2)	69.6^{ef}	1.15^{d}	0.41^{a}	1.56^{d}	17.95^{b}	21.35^{g}	3.03^{a}
12	504^{x} x 707^{x} (P2)	68.8^{gh}	1.41^{b}	0.37^{bc}	1.78^{b}	13.94^{e}	30.10^{b}	0.90^{g}
13	701^{x} x 614^{x} (P3)	75.1^{a}	1.52^{a}	0.42^{a}	1.94^{a}	12.29^{f}	25.30^{de}	1.71^{bcd}
14	533^{x} x 342^{y} (P4)	68.4^{gh}	1.40^{b}	0.37^{bc}	1.77^{b}	13.51^{e}	21.95^{g}	1.85^{bc}
15	533^{x} x 342^{y} (P3)	67.9^{hi}	1.42^{b}	0.38^{b}	1.80^{b}	16.86^{c}	30.35^{b}	1.89^{b}
p-value		0.000	0.000	0.000	0.000	0.000	0.000	0.000

^{x}*T. arjuna,* ^{y}*T. tomentosa,* *% Catechol equivalents, data are presented as mean of triplicates; mean values superscripted with the same alphabet(s) are not significantly different.

whereas, in the second batch hybrids, it was 1.07-1.94 mg/g, 0.83-1.52 mg/g and 0.24-0.42 mg/g with an average of 1.54 mg/g; 1.20 mg/g and 0.35 mg/g, respectively (Table 2). Higher chlorophyll content was observed in the first batch hybrids in comparison to second batch, which could be attributed to their age *i.e.,* two year older than the latter. Among the hybrids of the first batch, 701 x 531 P1 (2.66 mg/g) has shown the highest total chlorophyll content, which is comparatively superior over both of its parents *i.e.,* acc.701 (2.59 mg/g) and acc. 531 (1.90 mg/g) as reported by Suryanarayana *et al.* (2005); this is followed by two hybrids 501 x 533 P3 (2.39 mg/g) and 501 x 533 P5 (2.31 mg/g), which have shown on par expression with one of their parents, acc. 533 (2.33 mg/g). Similarly, among the second batch hybrids, 701 x 614 P3 (1.94 mg/g) has shown the highest total chlorophyll content, followed by 533 x 342 P3 (1.8 mg/g), 533 x 342 P4 (1.77 mg/g), 533 x 342 P7 (1.63 mg/g), 701 x 614 P1 (1.62 mg/g) and 701 x 614 P2 (1.56 mg/g). In both the batches, majority of hybrids with high chlorophyll content were derived from the crosses involving acc. 533 and acc. 701, which produces high level of chlorophyll *i.e.,* 2.33 mg/g and 2.59 mg/g, respectively (Suryanarayana *et al.,* 2005). The Chlorophyll content forms an important parameter in quantifying the photosynthetic efficiency of plant and hence, it is an essential component while determining the quality of foliage. With respect to chlorophyll a, higher values were reported in 501 x 533 P5 (1.71 mg/g) and 501 x 533 P3 (1.68 mg/g) in the first batch hybrids and 701 x 614 P3 (1.52 mg/g) in the second batch hybrids. These hybrids have shown higher chlorophyll a production than their parents *viz.,* acc. 533 (1.43 mg/g) and acc. 701 (1.51 mg/g) (Suryanarayana *et al.,* 2005). Similarly, higher value of chlorophyll b was observed in 701 x 531 P1 (1.37 mg/g) of first batch hybrids, which is superior to both of its parents, acc.701 (1.03 mg/g) and acc. 531 (0.74 mg/g). Whereas, in the second batch hybrids, 701 x 614 P3 (0.42 mg/g) and 701 x 614 P2 (0.41 mg/g) have shown higher levels of chlorophyll b, but, lower than their parental values as reported in an earlier study (Suryanarayana *et al.,* 2005). Comparatively similar level of chlorophyll content was observed in *T. arjuna* by various authors *viz.,* Lakshmi *et al.* (2013), Deka *et al.* (2015) and Lakshmi and Benarjee (2015).

Leaf protein content on dry matter basis in *Terminalia sp.* hybrids ranged from 5.57 to 12.30 % with an average of 8.44 % in the first batch (Table 1), while it ranged from 5.08 to 20.75 % with an average of 13.32 % in the second batch (Table 2). In mulberry silkworm, it was observed that nearly 70 % of protein content of raw silk, namely fibroin and sericin are biosynthesised directly from mulberry leaf protein and the remaining 30 % protein are sourced from the silkworm body tissues and haemolymph. This indicates the significance of food plant leaf protein level in silkworm nutrition (Ito, 1972; Bose *et al.,* 1991; Sarkar and Fujita, 1994). Among *Terminalia* sp. hybrids, the highest leaf protein was observed in the hybrid, 501 x 615 P1 (12.3 %) in the first batch. Whereas, among the second batch hybrids, 533 x 342 P2 (20.75 %) displayed the highest protein content followed by 701 x 614 P2 (17.75%) hybrid. These hybrids have their parents possessing quite good protein level *viz.,* acc. 701 (21.03 mg/g), acc. 533 (17.17 mg/g) and acc. 615 (14.32 mg/g).

Leaf carbohydrate content estimated on dry matter basis ranged from 7.35 to 34.45 % with an average of 28.01 % in the first batch hybrids (Table 1) and in the second batch hybrids, from 21.35 to 34.0 % with an average of 27.46 % (Table 2). Leaf carbohydrate forms a major source of energy to silkworm besides inducing silkworm to bite leaves (biting factor). Higher levels of carbohydrate content were observed in five hybrids *viz.,* 501 x 533 P4 (34.45 %), 501 x 701 P1 (33.51 %), 501 x 533 P2 (32.55 %), 615 x 531 P5 (32.5 %) and 615 x 531 P7 (32.3%) in the first batch and two hybrids, 533 x 702 P1 (34.0 %) and 614 x 701 P4 (33.4 %) in the second batch. These crosses are involving parents which show relatively high level of carbohydrate than other germplasm accessions *viz.,* acc. 702 (19.54 mg/g), acc. 615 (18.43mg/g), acc.533(15.03mg/g) and acc.701(13.5mg/g).

Among hybrids, the leaf phenol content ranged from 1.59 to 3.29 % in the first batch and from 0.90 to 3.19 % in the second batch. Plants synthesise phenol compounds to resist diseases and insect pests (Goyal *et al.,* 2012; War *et al.,* 2012). On the contrary, insects can detoxify phytophenols substantially by synthesising prophenoloxidases (ProPOs) in foregut (Wu *et al.,* 2015). Generally, plant species with moderate level of phenolics are metabolized through foregut ProPOs, so that they are not absorbed and cause cytotoxic effect over insect. However, higher level of leaf phenolic compounds could cause mortality in tasar silkworm as reported in *Shorea robusta* leaf fed larva (Manjappa *et al.,* 2020). Therefore, balanced level of phenolic compounds is preferred for tasar silkworm rearing. Among the hybrids, lower level of leaf phenol content was observed in 501 x 533 P2 hybrid (1.59 %) in the first batch and 504 x 707 P2 hybrid (0.90 %) in the second batch.

Overall, in the first batch, 501 x 533 P4 hybrid has shown better performance for several traits *viz.,* leaf moisture (73.4 %), protein (10.34 %) and carbohydrate content (34.45 %). Similarly, in the second batch, 701 x 614 P3 hybrid fared better in respect of leaf moisture content (75.1 %), chlorophyll a (1.5 mg/g), chlorophyll b (0.42 mg/g) and total chlorophyll content (1.94 mg/g).

In conclusion, from tasar silkworm nutritional point of view, *Terminalia* sp. hybrids having high leaf yield and high level of leaf moisture, leaf chlorophyll, protein and carbohydrates, and optimum level of phenol is preferred. Hence, outstanding hybrids with respect to nutritional content could be further explored in developing high yielding hybrids with nutritional quality.

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Report

IMPACT OF DIGITALISATION ON FASHION SEGMENT AND SUSTAINABILITY OF INDIAN SILK INDUSTRY

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ABSTRACT

Silk has been the perfect material for fashion designers for centuries. It is an established fact that the fashion industry including silk has been facing various challenges that affect sustainability. These challenges range from high taxation, availability of raw material, constrained market, pollution problems and lack of go green concepts to name a few. Now, the COVID 19 has posed yet another challenge! Central Silk Board has contributed towards *go green* concept since 2000 with a few studies, initiatives and products. In a nut shell, textile industry as a whole is also responsible for global pollution to certain extent. According to a report of 2018 released by the United Nations Economic Commission for Europe, the industry generates 20 per cent of global water waste and sends 21 billion tons of textiles to landfills annually and Silk industry is not an exception. One of the rational ways to reduce this pollution to some extent is Digitalisation and use of Artificial Intelligence. Digitalisation can open up new vistas for business; bring in significant reduction in labour costs, an increase in profit margins, besides localization of products and sustainability of processes. Digitalisation includes the visual representation of clothing built using computer technologies and 3D software that is virtual, true-to life garment visualisation with cutting-edge simulation technologies. Such technology most certainly would help in silk apparel production processes and fashion merchandising. Not only this, digitalising of silk production activities - silk apparel production activities, mass media campaigns on eco-friendly silk products/silk by-products encompassing nutraceutical, bio-medical, cosmetic, pharmaceutical products also add to the global go green concept and thus address sustainability. In fact, with the concerted efforts, the digitized silk fashion world would be adopting a, timeless, effortless, season less doctrine, in order to stimulate growth and sustainability. Digitalisation has never held such prevalence so far, for both the brand and consumer thanks to the pandemic. Enforced by Covid-19, business world today has successfully exploited the digital experiments, graduating from the virtual playground to the consumer interaction. Although in infancy, digitized luxury goods like silk are predicted to become a new and exciting component in the years to come.

Key words: Artificial intelligence, digitalisation, fabric waste, fashion merchandising, global pollution, sustainability, virtual show rooms.

Sustainable fashion is gradually becoming a movement and process of bringing change to fashion products and the system towards ecological integrity. It addresses the entire system of fashion dealing with inter-dependent socio-economical, cultural, ecological, and financial systems. Encompassing the stakeholders - consumers, producers, contemporary and future dwellers, sustainable fashion thus becomes the responsibility of the public and the private sectors. The goal of sustainable fashion must include: creation of flourishing eco-systems and communities through its activity; increase the value of local production/products and prolonging the life span of the materials. Wet processing of textile products causes high level of environmental influence as a result of intensive energy consumption and chemical material-loaded wastewater discharge (Palamutcu, 2017). The fast fashion, amplifies the problems, as it is causing high water consumption and discharge of hazardous chemicals, increase in waste, besides increased violations of human rights, together with significant greenhouse gas emissions.

Environmental concerns and fashion

The textiles and fashion are the industries that negatively affect the environment. As globalization has facilitated cascading lower prices, and collections shifting so fast! Naturally, consumers feel that the fashion is disposable! And for certain, fashion increases the pollution and causes environmental hazards in all the three stages: production, use and disposal.

Mother Nature has the highest impact. Excessive water usage, pollution from chemicals used in dyeing, preparation and the disposal of enormous amounts of unsold/used textiles through incineration or landfill deposits pollute the environment. Adding to it, growing water scarcity is a concern, the enormous usage level of fashion materials is also quite alarming as production of textile materials mostly takes place in the areas where fresh water is already scarce. Only around 20 % of clothing is recycled or reused and huge amounts of fashion product end up as waste in landfills or get incinerated. It has been estimated that in the UK alone, around 350,000 tons of clothing ends up as landfill every year!

Fast fashion– a real culprit!

The temporal aspects of fashion are the most important reasons for the current unsustainable condition of the fashion system; and the main culprit is *fast fashion*-continuous gushing in of new materials into the market! Changing fashions very frequently has brought new consumer behaviour among *see now—buy now!* Now, everyone wants to dress in latest trends. It is a behaviour that made everyone to dress following the latest trends. Fast fashion has increased the material throughput in the system. Fashion brands are now producing almost twice the amount of clothing today compared with before the year 2000. Current fashion-consumption practices result in large amounts of textile waste, most of which is incinerated, landfilled or exported to developing countries.

Transparency and the Supply chain

Transparency in the supply chain has been a controversy for the fashion industry, the current focus on *reduce, reuse, recycle,* is primarily promoted through brand initiatives, and fails to address the global impact of the fashion system.

Sustainable consumption practices

There are negative social and environmental impacts at every stage of the fashion production: It encompasses production, processing, marketing, use, maintenance and even discarding. Enhancing the lifespan of products can be yet another approach to sustainability.

Impact of Textile recycling on fashion sustainability

Digital platforms and digital merchandising are gaining momentum in the fashion market and many brands have emerged with e-commerce that made consumers to experience virtual reality. According to Mc Kinsey

(2019), a huge increase in online sales is expected in the near future with respect to total sales; This trend particularly affects the fashion luxury segment like silk which is expected to represent about 13 % of the total fashion market in the years to come. In recent years, the fashion industry has also been experiencing better marketing thanks to digital promotion. Most fashion executives see investments in ICT and the digitalization of the value chain as big opportunities.

Digitalisation and Artificial Intelligence

Online shopping

In the last few years, online platforms have significantly blown up. Many of them like *Zalando, Amazon,* and *Myntra,* have already been operating in the fashion industry successfully. However, most of the traditional fashion companies are still sceptical about making collaborations with online giant e-commerce players! it might be because they are scared to lose control over their own brand. Large and diverse data bases are turned into rich information thanks to artificial intelligence that could be utilised by the companies to improve the entire supply chain - from design to manufacturing, sales, and advertising.

Importance of social media

Social media is very important in developing e-commerce during this period of economic crisis, the channel of fashion and traditional apparel is undergoing consistent decline, the fashion and online apparel industry is the one that marks the biggest growth with regard to e-commerce sales. In this social media era, the fashion industry is experiencing the growing role of influencers as consumers perceive their lifestyle as more authentic and attractive in terms of wide choice and lesser price comparatively than traditional advertisements.

Smartphone have made many fashion companies to create apps, where customers can visit the website in their mobile devices and buy online directly from the Smartphone. Payments are also done through mobiles only.

As sustainability has long been at the centre of both scientific and institutional debate, the agenda should transform our world and focus on three pillars of sustainable development: economic growth, social inclusion, and environmental protection. As silk is a low volume but high value item, it demands more attention.

Here are a few technologies transforming production and recyclability to make fashion more sustainable, especially in silk sector.

- Virtual sampling reduces waste in both design and product development. It also reduces cost of the material and time related to creating physical samples. This is most important if the material is silk. Silk being a low volume but high value item, digitalisation is a quite option for silk industry.

- Materials commonly used in clothing take considerable resources and often degrade slowly. Some eco-friendly textile alternatives include recycled fibres and those extracted from agricultural waste products, such as leaves and rinds. Natural fibres like hemp, bamboo, banana, pineapple, and ramie are used as alternatives which produce less waste during production, are long-wearing, and bio-degradable.

- On-demand design and manufacturing will play a significant role in the future of fashion. Instead of producing clothes and then selling them, the items are ordered, then produced. The rise of e-commerce and social commerce over the last decade has created an entirely new business model.

- Mobile Body Scanning offers information regarding body size and type. With such accurate information, manufacturers can create garments to fit different body types rather than depending on a proportional scale.

- Mobile 3D body scanning and a three-dimensional image help consumers to immediately determine how clothes fit them. It is *virtual dressing!*

- Incorporating renewable materials and recycling old clothes into new ones can make fashion rotate.

Re-commerce

An estimated USD 500 billion value is lost every year due to clothing that's hardly worn and rarely recycled. Two techniques that combat this waste include up cycling/re cycling and second-hand sales.

Efforts of Central Silk Board

Central Silk Board has been exploring all the possibilities to digitalise its activities/labs/procedures *etc.* It is already identifying the areas/labs/activities where Artificial Intelligence can be used. The possible areas may be as follows:

- Marketing of cocoons, raw silk and silk material.
- All Silk exchanges can adopt E-auction like Ramanagaram cocoon market in Karnataka. Being transparent, accurate, faster and user friendly, AI and digitalisation certainly help traders as well as customers.
- Forecasting of cocoon and raw silk prices can be done by AI.
- Sorting and grading of cocoons including evaluation can be done for Mulberry, Tasar, Eri and Muga as well.
- Grading of silk yarn.
- Fabric quality inspection system.
- Identification of purity of silk sarees and other silk items.
- Assessment of fabric comfort properties.
- Assessment of *zari.*
- Colour formulation for dyeing.
- Colour recipe for printing different silk material.
- Colour grading and fastness testing.
- Evaluation of evenness, cleanliness and neatness of raw silk.

Digitalisation of R&D literature, including research project reports, journals *etc.,* are done and in addition to that, it is planning to have an AI check in the new projects proposed. All the four major components of proposed *Silk Samagra* are interlinked with each other and aimed at a common goal. One of the main objectives of the scheme is Technology translation through *Sericulture Information Linkages and Knowledge System* (SILKS) Portal and, Mobile applications (Press Information Bureau, 2019).

Digital silk fashion could also be the interplay between digital technology and *couture.* Information (ICTs) should be deeply integrated both into the silk fashion industry as well as within the experiences of clients. It should be employed to design silk fashion products, while the industry leverages onto digital technologies impacting silk production, processing, weaving, designing, fashion designing, marketing, and distribution, sales and aftermath. Digital technologies have made it easier for small brands to build awareness and sell to customers, helping them capture a disproportionate share of growth (Johanna Andersson *et al.,* 2019).

Digitalisation could also be extensively used in communication activities with all relevant stakeholders, and contribute towards co-creating the amazing fashion world of silk. Among many applications available to fashion designers especially of silk, to model the fusion of creativity with digital technologies, digital textile printing is worth mentioning here. Here, prints are directly applied to fabrics with printer, reducing 95 % the use of water, 75 % the use of energy, and minimizing textile waste. 3D printing would be an effective technique of sustainable manufacturing as it eliminates the fabric waste generated from cutting and the chemical dangers of silk dyeing. Silk clients can skip fitting rooms and cosmetics counters, and instead, virtually see how they look in specific outfits and makeup *using* kiosks/ mobile phones/ tablets. The customer can scan a QR using a smart phone and open up the story of where, how and by whom a specific piece of silk clothing was made, designed, supply chain and care tips that optimize

longevity and unlock the travels of a garment from silk farms to weaving and tailoring; whether it is eco-friendly or not! We can't have a sustainable fashion industry unless we trace and track the supply chain. Now, many companies are gradually shifting to eco-friendly business plans that use innovative technologies to obtain transparency and product traceability. The other side of this pandemic crisis is about sustainability. The push for the elimination of textile waste has encouraged a few luxury clothing brands to create digital clothing over physical ones. Silk is one of the most opted luxury items. Adding to it, with digital innovation, many are expecting a reduction in seasonal runways and continental travel besides an increase in thoughtful consumption which ultimately minimizes carbon footprint and material waste. Now, the business acumen revolves around conceptual narration, virtual fashion shows, virtual showrooms *etc.* Thanks to social media, now even common people can contribute to co-create the fashion world for silk - shaping tastes, customs, and fashion-related values and more importantly sustainability. Designed for the screen, digital silk has the potential for mass market appeal as it promotes Instagram culture and advocates sustainable production. A platform is aimed at connecting investors who would like to invest in SDG-related themes and projects, and investees who are seeking funding support for their SDG-aligned projects (NITI Aayog).

Mass-market apparel brands and retailers cannot win in the next decade without transforming to a demand-focused model. Apparel companies are optimising and digitising their processes and rethinking inbound logistics. One new strategy is to optimise the apparel production model, including elements, new delivery models around customisation that incline towards sustainability. As consumers become more and more environment conscious, major players in the industry not only keep the wasteful practices *at bay* but are also embracing eco-friendly technologies. Adoption of new digital technologies would certainly benefit everyone in the supply chain, ultimately passing the benefits to consumers.

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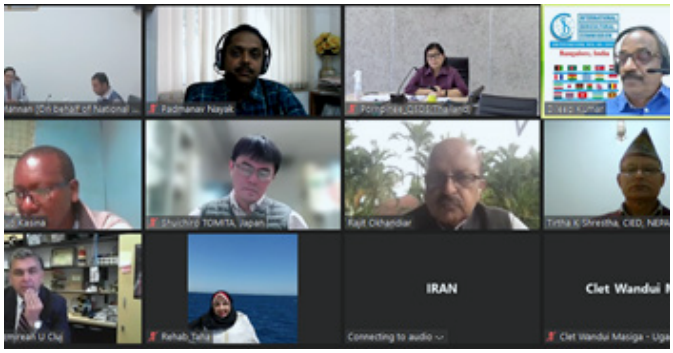
26th ISC Conference

The Conference is the chief governing council of the ISC held once in three years wherein important and critical issues concerning the organization and the silk industry are reviewed and appropriate actions are initiated. Accordingly, the 26th Conference of ISC was held on 14th December 2021 on a virtual mode. The Conference witnessed the participation of delegates from ten member countries.

The Conference unanimously elected Mr. Rajit Ranjan Okhandiar, Member Secretary, Central Silk Board, Bangalore as the Secretary General of ISC for the tenure of three years from 1st January 2022 to 31st December 2024. This is the second time that Mr. Okhandiar has been selected as the Secretary General of ISC, the previous being 1st January 2019 to 31st December 2021.



As part of expanding the reach of ISC to more organizations and individuals associated with the silk industry, the Conference has taken an important decision of reducing the Associate Membership fee from 1st January 2022 as detailed below:

Membership	Existing Fee (US$)	Revised Fee (US$)
Collective Associate Membership	470	150
Individual Associate Membership	175	50

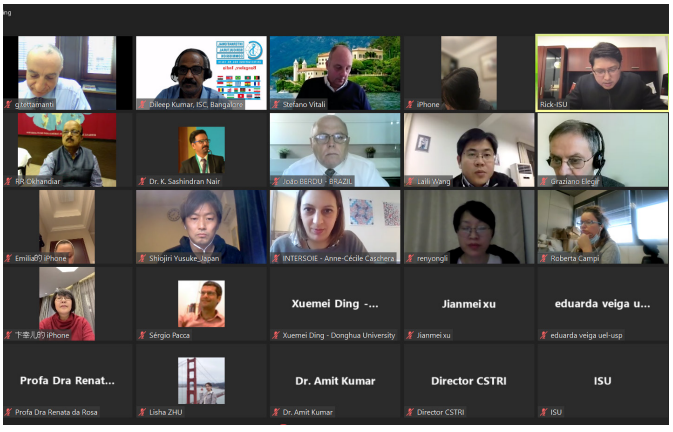
Natural Fibre Coalition - Campaign on Make the Label Count

ISC is a founder member of the global initiative, namely "Natural Fibre Coalition", initiated by International Wool Textile Organization (IWTO) and partnered by organizations of natural fibre industry. The main purpose of the coalition is to campaign with the European Union for including special qualities of natural fibres in the Product Environment Footprint (PEF) methodologies of EU. As part of this, a campaign "Make The Label Count (MTLC)" was launched on 13th October 2021. The alliance has taken steps to protest against the existing PEF methodology of EU wherein the concerns of the natural fibre industry are not addressed.

As a follow up of the founding objectives of the global initiative, a web meeting among its members and experts was held on 10th November 2021. The meeting witnessed the participation of 39 delegates from 22 countries. The lead campaigners have made presentations on the progress of the activities wherein they have lobbied with a few Members of European Parliament and senior officials of EU for including the genuine concerns of the natural fibre industry while finalizing the PEF methodology. The collation has made their visibility in many of the international electronic and print media forums and social media spaces for eliciting opinion in favour of the natural fibre industry. It is reported that the coalition witnessed the enrolment of new members which strengthened the arguments of natural fibre industry with EU.

Working Group Meeting on Life Cycle Assessment (LCA) study on Silk

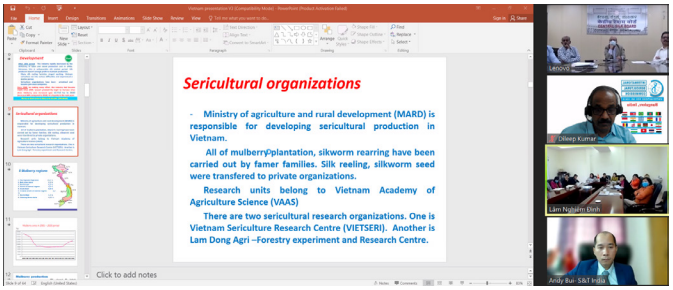
ISC and International Silk Union, China have constituted a Working Group for developing global sustainability standards for silk. As part of this, the LCA study on silk would be undertaken in the important silk producing and consuming countries which would be later aggregated appropriately to derive the global silk standard. As part of the stated objectives, the first web meeting of the LCA Working Group was held on 18th January 2022. The Working Group witnessed the participation of 75 delegates from 18 countries constituting the officials of ISU, ISC, researchers and experts from China, India, Brazil, Italy, France and other countries. The experts have made presentations of the plans afoot for undertaking the LCA studies in their respective countries.

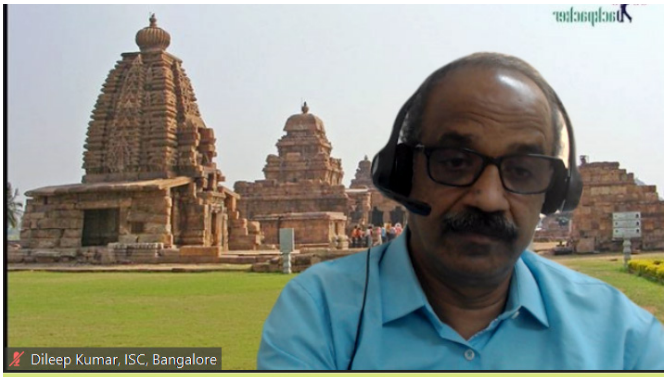


The consensus arrived during the meeting was that the PEF methodology of EU would be used for undertaking the LCA study and the entire study shall be undertaken under the supervision of the Working Group to ensure adoption of common methodologies and guidelines. The Workshop resolved to develop a common frame work and enlist the study under the guidance of the Technical Committee for Apparel and Footwear constituted by the European Commission.

Meeting between Vietnam Sericulture Research Centre (VIETSERI) and Central Silk Board

ISC facilitated an online meeting between Vietnam Sericulture Research Centre (VIETSERI) and Central Silk Board on 29th December 2021 to discuss about the prospects of collaboration for the development of sericulture and silk industry. As part of the meeting, both sides have presented the status of sericulture and silk industry in their respective countries. Further to the discussions held, both sides have agreed to enter into a memorandum of understanding for collaborating in the area of developing improved silkworm breeds and mulberry hybrids. Apart from this, the Indian side has agreed to supply commercial silkworm seed to Vietnam on a consistent basis. Further, the Vietnamese side informed that they would consider enrolling as a Member Country of ISC.





Global Silk Production (in Metric Tonnes)

#	Country	2015	2016	2017	2018	2019	2020
1	Bangladesh	44.00	44.00	41.00	41.00	41.00	41.00
2	Brazil	600.00	650.00	600.00	650.00	469.00	377.00
3	Bulgaria	8.00	9.00	10.00	10.00	10.00	10.00
4	China	1,70,000.00	1,58,400.00	1,42,000.00	1,20,000.0	68,600.00	53,359,00
5	Colombia	0.50	-	-	-	0.50	0.50
6	Egypt	0.83	1.20	1.10	1.25	1.50	1.50
7	India	28,523.00	30,348.00	31.906.00	35,261.00	35,820.00	33,770.00
8	Indonesia	8.00	4.00	2.50	2.50	2.50	2.50
9	Iran	120.00	125.00	120.00	110.00	227.00	270.00
10	Japan	30.00	32.00	20.00	20.00	16.00	16.00
11	Madagascar	5.00	6.00	7.00	7.00	7.50	7.50
12	North Korea	350.00	365.00	365.00	350.00	370.00	370.00
13	Romania	-	-	-	-	0.50	0.50
14	Philippines	1.20	1.82	1.50	2.00	2.00	2.00
15	South Korea	1.00	1.00	1.00	1.00	1.00	1.00
16	Syria	0.30	0.25	0.25	0.25	0.50	0.50
17	Thailand	698.00	712.00	680.00	680.00	700.00	520.00
18	Tunisia	3.00	2.00	2.00	2.00	2.00	2.00
19	Turkey	30.00	32.00	30.00	30.00	5.00	5.00
20	Uganda	-	-	-	-	3.10	3.00
21	Uzbekistan	1,200.00	1,256.00	1,200.00	1,800.00	2,037.00	2,037.00
22	Vietnam	450.00	523.00	520.00	680.00	795.00	969.00
	Total	**2,02,073**	**1,92,512**	**1,77,507**	**1,59,648**	**1,09,111**	**91,765**

Analysis on operation of Chinese cocoon silk industry in 2020 and prospect in 2021

China Silk Association

Affected by the COVID-19 epidemic, the economic operation of China's cocoon and silk industry witnessed dramatic decline in 2020. Benefiting from the effective domestic epidemic prevention and control and improvement of the epidemic situation in foreign countries, the industry revenue and profit indicators began to rebound at the end of 2020. Nevertheless, the deterioration of international trade environment in recent years has brought adverse impacts on China's cocoon and silk industry. China Silk Association has published a series of reports entitled "Analysis of Economic Operation of Cocoon and Silk Industry and Prospect" every year since 2005. It is judged from the industry analysis and experience that in 2021, China's cocoon and silk industry needs actively responding to domestic and international market risks and challenges in the post-epidemic era, remaining committed to the general principle of pursuing progress while ensuring stability, working to the supply-side structural reform of the industry, positively adapting to the new development pattern which gives priority to domestic circulation and promotes positive interplay between domestic circulation and international circulation, and accelerating the adjustment of enterprise product mix and innovation of sales model.

1 Industry performance in 2020

1.1 Production status

1.1.1 Roughly stable cocoon production

According to statistics, the area of mulberry orchard' in China reached 80.78 hm^2 in 2020, representing a year-on-year decrease of 0.04% and an increase of 1.48 hm^2 compared with 2016. The seeding quantity totaled 16,719,100, posting a year-on-year decline of 4.51% and rise of 8% compared with 2016. The yield of silkworm cocoon amounted to 687,200 tons, falling by 4.44% year on year and rising by 10.76% against 2016. The average purchase price of silk worm cocoon was 35.28 yuan/kg, dropping by 22.09% year on year and by 8.02% against 2016. The silkworm cocoon production of China in 2020 is as shown in Tab.1.

Tab.1 Silkworm cocoon production of China in 2020

Serial No.	Regions	Seeding quantity/ 10,000 pieces	Year-on-year/%	Output/t	Year-on-year/%
1	Guangxi	820.00	-0.38	376,500	-0.58
2	Sichuan	215.01	-6.67	83,000	-5.68
3	Yunnan	161.29	-12.50	56,000	-12.50
4	Jiangsu	95.60	-14.89	38,300	-11.14
5	Guangdong	69.75	-14.29	25,000	-16.67
6	Anhui	41.36	10.50	18,200	13.75
7	Zhejiang	45.32	-12.39	17,700	-11.94
8	Shandong	32.59	-11.11	14,900	-23.20
9	Chongqing	38.92	-10.22	12,500	-8.09
10	Shaanxi	30.00	-8.69	11,700	-6.40
11	Jiangxi	24.18	3.17	8,900	2.30
12	Hubei	19.60	-7.41	8,100	2.12
13	Henan	20.30	-0.76	5,700	-3.39
14	Guizhou	14.13	4.70	5,316	5.08
15	Hunan	8.32	-3.27	2,930	-0.68
16	Hainan	2.62	-7.77	1,300	-14.47
17	Shanxi	2.09	-28.17	913	-34.36
18	Gansu	1.33	0.00	219	0.00
Total		1,671.91	-4.51	687,178	-4.44

Note: The above-mentioned data is sourced from the Ministry of Agriculture and Rural Affairs of the People's Republic of China.

Judging from the silkworm cocoon production of China from 2010 to 2020 (Fig.1), although the output in 2020 was lower than that of 2019, the output in the past five years basically remained at around 660,000 tons, the national cocoon output did not show great fluctuation and the overall scale was relatively stable. As inflected by the national average price of silkworm cocoon purchase from 2010 to 2020 (Fig.2), the highest purchase price of silkworm cocoon in China reached 2,369 yuan/50 kg in 2017, yet it saw a decline for three consecutive years in 2018, 2019 and 2020.

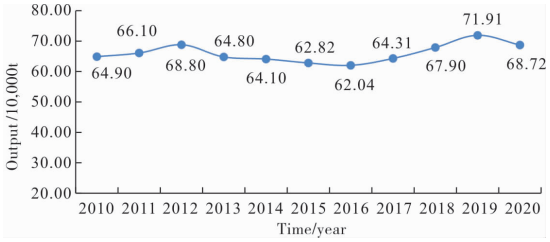


Fig. 1 National output of silkworm cocoons from 2010 to 2020

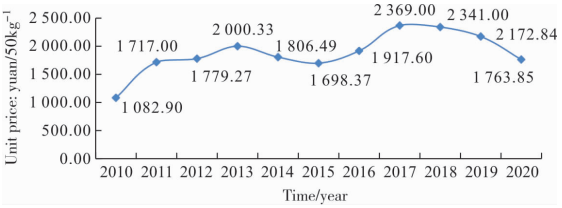


Fig. 2 National average purchase price of silkworm cocoons from 2010 to 2020

Affected by such factors as accelerated process of industrialization in China, the rising cost in breeding and rearing, and labor shortage, the output presented a significant downward trend in the eastern coastal areas of Jiangsu, Zhejiang and Guangdong. Instead, the production of silkworm cocoons in Anhui, Jiangxi, Hubei, Guizhou and other central and western regions rose steadily, effectively offsetting the impact of the reduction in production in the eastern coastal areas. On the whole, the production scale of silkworm cocoons in China remained stable.

1.1.2 Decline in output of major products

As indicated by the statistics of the National Bureau of Statistics of China, the output of major products from enterprises above designated sizedeclined to varying degrees in 2020, as shown in Tab. 2. Among them, silk output totaled 53,400 tons, down 16.21% year on year and 66.29% compared with 2016; the output of silks and satin amounted to 383.5 million m, declining by 18.29% year on year and 42.55% against 2016. The output of silk quilts was 9.24 million pieces, decreasing by 20.89% year on year and 55.45% from 2016. As indicated by the data released by the National Bureau of Statistics of China, the output of raw silk, satin and silk quilts in 2020 dropped by 66.29%, 42.55% and 55.45% respectively against 2016. But according to the China National Silk Association, the artificially high output of major silk products in the past and the widened decline of silk production in 2020 was partly due to the limitation of the statistic scope. In fact, the production data of major products in the past two years were close to the actual output of the industry.

Tab. 2 The output of national silk enterprises above the designated size in 2020

Major products	Unit	2020	Year-on-year/%	% against 2016
Silk	t	53,358	-16.21	-66.29
Spun silk	t	3,132	-32.63	-65.15
Satin	10,000 m	38,350	-18.29	-42.55
Silk quilt	10,000 pieces	924	-20.89	-55.45

Note: The above-mentioned data is sourced from the National Bureau of Statistics of China.

The output of major products in various provinces and cities in 2020 (Tab. 3) indicates that the low overall operating rate of enterprises and the slow recovery of consumer market led to a significant decline in the output of major products in the sector under the impact of COVID-19 at home and abroad. Though the production of raw silk in Guangxi and Sichuan decreased slightly by 14.39% and 0.23% respectively, the two provinces still held a leading position in cocoon silk production. The yield of raw silk in Anhui, Chongqing and Shanxi decreased by less than 10%, and even registered a positive increase in Yunnan, Guizhou and Hubei. Chongqing's satin output increased by 16.31% year on year and the production of silk quilt in Guangdong, Anhui, Guangxi, Xinjiang, Yunnan, Jiangxi and Shanghai even reported a positive increase, with the increase in Guangdong and Guangxi being 105.45% and 82.27% respectively mainly as a result of the increase of domestic home consumption during the outbreaks of the epidemic.

Tab. 3 The output of main silk products in various provinces and cities in 2020

Raw silk (including spun silk)				Silk quilt				Pure silk satin			
Serial No.	Areas	Output/t	Year-on-year/%	Serial No.	Areas	Output/10,000 pieces	Year-on-year/%	Serial No.	Areas	Output/10,000 m	Year-on-year/%
1	Guangxi	19,150	-14.39	1	Jiangsu	261	-8.46	1	Sichuan	17,906	-10.03
2	Sichuan	9,422	-0.23	2	Zhejiang	215	-8.59	2	Zhejiang	10,747	-23.32
3	Jiangsu	5,601	-18.93	3	Shaanxi	99	-13.40	3	Chongqing	2,295	16.31
4	Zhejiang	4,073	-27.83	4	Hunan	73	-6.64	4	Anhui	2,037	-47.91
5	Yunnan	3,127	2.11	5	Shandong	56	-55.01	5	Jiangsu	1,885	-33.26
6	Anhui	3,046	-7.84	6	Henan	56	-10.57	6	Guangxi	1,797	-13.16
7	Jiangxi	2,131	-48.33	7	Guangdong	38	105.45	7	Shandong	1,084	-22.41
8	Chongqing	1,711	-4.14	8	Anhui	32	29.82	8	Yunnan	335	-3.71
9	Henan	1,187	-14.89	9	Hubei	30	-79.17	9	Shaanxi	190	-21.60

Continued Tab. 3

Raw silk (including spun silk)				Silk quilt				Pure silk satin			
Serial No.	Areas	Output/t	Year-on-year/%	Serial No.	Areas	Output/10,000 pieces	Year-on-year/%	Serial No.	Areas	Output/10,000 m	Year-on-year/%
10	Shaanxi	1,041	-49.80	10	Guangxi	19	82.27	10	Guangdong	23	-32.26
11	Guizhou	1,022	15.74	11	Sichuan	19	-56.79	11	Liaoning	21	-57.40
12	Liaoning	565	-50.96	12	Xinjiang	5	11.11	12	Qinghai	20	-75.59
13	Shandong	527	-10.30	13	Yunnan	5	9.93				
14	Guangdong	396	-32.87	14	Jiangxi	4	29.66				
15	Hubei	275	1.67	15	Liaoning	4	-5.04				
16	Shanxi	85	-0.34	16	Shanghai	4	46.69				

Note: The above-mentioned data is sourced from the National Bureau of Statistics of China.

According to the data analysis of the National Bureau of Statistics of China, the output changes of main products in major producing areas from 2016 to 2020 show that with the decrease of silk output in Jiangsu, Zhejiang and other eastern coastal areas year by year, the scale advantage of raw silk output in Guangxi and Sichuan became increasingly prominent. In 2020, Jiangsu's silk and satin output totaled 18.85 million m, down 82.01% against 2016, while Chongqing's silk and satin output amounted to 22.95 million m, up 9.31% compared with 2016. In 2020, the output of silk quilt in Shandong totaled 560,000 pieces, decreasing by 81.24% from 2016, while that in Shaanxi and Hunan respectively increased by 116.87% and 19.85%. With the cancellation of the qualification certification system for fresh cocoon purchase since the issuing of the 13th Five-Year Plan, all links of cocoon silk industry chain were completely marketized, regional cooperation and specialized division of labor were formed among major producing provinces (regions and cities) in the eastern, central and western regions, accelerating the continuous concentration of cocoon silk production to dominant regions. The East-to-West layout was basically formed in domestic cocoon and silk industry, enhancing the industry's agglomeration level and cluster effect.

The latest data sourced from the National Bureau of Statistics of China indicates that from January to March, 2021, the silk output of enterprises above designated size in the entire industry totaled 10,555 t, representing a year-on-year decrease of 1.45%. The output of silk and satin was 87.55 million m, registering a year-on-year decrease of 3.41%. The output of silk quilts amounted to 5.56 million pieces, up 29.78% year on year. As the situation of epidemic prevention and control at home and abroad continued to improve, market demand was released, plenty of spring cocoons were available on the market, silk reeling and weaving enterprises resumed normal operation. Thus, it is expected that the production of major products will witness a steady increase in the first half of 2021, and will basically return to the normal level before the COVID-19 struck in the second half of 2021.

1.2 Industrial economic situation

According to the National Bureau of Statistics of China in 2020, the national silk enterprises above designated size (excluding silk garments and products) achieved operating income of 60.79 billion yuan, decreasing by 12.53% year on year and by 52.28% from 2016. The total profit amounted to 1.646 billion yuan, down 37.65% year on year and 76.31% from 2016. As shown in Tab. 4 and Tab. 5, these enterprises reported a profit of 339 million yuan, 1.136 billion yuan and 170 million yuan in 2020 respectively with regard to silk reeling, silk weaving and silk printing and dyeing, posting a respective year-on-year decline of 62.24%, 24.95% and 24.91%.

Tab. 4 Operating income of enterprises above the designated size in 2016-2020

Main business	Operating income/100 million yuan				
	2020	2019	2018	2017	2016
Silk and finishing	607.90	694.99	805.92	1,203.57	1,273.91
Silk reeling	237.83	291.33	419.66	692.53	774.94
Silk weaving	312.58	335.60	329.11	428.01	419.79
Silk printing and dyeing	57.49	68.06	57.15	83.02	79.18

Note: The above-mentioned data is sourced from the National Bureau of Statistics of China.

Tab. 5 Profits of enterprises above the designated size in 2016-2020

Main business	Profits/100 million yuan				
	2020	2019	2018	2017	2016
Silk and finishing	16.46	26.39	35.44	64.83	69.47
Silk reeling	3.39	8.99	16.59	35.24	41.41
Silk weaving	11.36	15.14	17.19	23.13	23.44
Silk printing and dyeing	1.70	2.27	1.66	6.46	4.62

Note: The above-mentioned data is sourced from the National Bureau of Statistics of China.

Seen from the overall operating conditions of the industry, most cocoon and silk enterprises were faced with difficulties such as poor domestic sales, blocked exports, insufficient production orders, shortage of liquidity and so on, and the downward pressure on the industry economy was increasing under the impact of the epidemic situation at home and abroad since 2020. The comparison of major economic indicators of the industry in 2020 is as shown in Tab. 6.

Tab. 6 Changes in the main economic indicators of the industry in 2020

Main economic indicators	2020 year-on-year increase/%	2019 year-on-year increase/%	Growth rate/%
Total profit	-37.65	-12.18	-25.47
Operating income	-12.53	-4.49	-8.04
Operating cost	-12.23	-4.52	-7.71
Sales expenses, management expenses and financial expenses	-5.7	-10.69	4.99
Deficits	36.66	20.3	16.36
Total liabilities	-0.19	3.94	-4.13
Total loss of loss-making enterprises	97.36	44.72	52.64

Note: The above-mentioned data is sourced from the National Bureau of Statistics of China.

1.3 Export

1.3.1 Sharp decline in export of silk goods

According to the statistics of the General Administration of Customs, export of China's silk goods to taled US $1.071 billion in 2020, posting a year-on-year decrease of 50.62%. Among them, the export of silk goods amounted to US $244 million, 36.23% lower than the previous year; the export of silk and satin totaled US $271 million, dropping by 54.66% year on year; the export of silk garments and products totaled US $557 million, decreasing by 52.46% year on year. Seen from the month-on-month exports, the export of real silk goods declined rapidly in the first quarter, reaching the lowest level in February, being US $38.15 million. After a slight rebound in March, it continued to fall slightly, and gradually picked up from June. In December, the export value amounted to US $103.15 million and began to rebound steadily.

1.3.2 Overall decline in exports to major markets

According to the data analysis of the General Administration of Customs, China's export of real silk goods to major export markets in 2020 all witnessed negative growth year on year (Tab. 7). In terms of export ranking, the EU and the USA remained the main export markets of China's silk goods, with exports of US $307,886,900 and US $207, 337, 400, decreasing by 32.38% and 34.88% respectively. The sum of exports of the EU and the USA accounted for 48.1% of the total exports. Hong Kong of China, Japan and India respectively ranked the third, fourth and fifth, with export value of US $89, 451, 100, US $77,752,000 and US $69,212,300 respectively, all posting a year-on-year decrease of over 30%.

Tab. 7 Export to the main market in 2020

Ranking	Countries and regions	Export/ US $10,000	Year-on-year increase/%	Proportion/%
1	27 EU countries	30,788.69	-32.38	28.74
2	The USA	20,733.74	-34.88	19.36
3	Hong Kong of China	8,945.11	-36.18	8.35
4	Japan	7,775.20	-37.81	7.26
5	India	6,921.23	-57.18	6.46
6	The UK	3,922.26	-34.47	3.66
7	Pakistan	3,670.47	-57.76	3.43
8	South Korea	3,597.84	-40.07	3.36
9	Australia	2,569.45	-19.62	2.40
10	Vietnam	2,129.87	-57.22	1.99

Note: The above-mentioned data is sourced from the General Administration of Customs.

In terms of products for export, the EU, India, Japan, Vietnam and Madagascar ranked the top five in terms of silk products and the export of the top four countries and regions decreased by 21.44%, 56.11%, 47.5% and 61.77%, respectively, while that of Madagascar rose by 310.14% instead. As for satin products, the top five export markets were the EU, Hong Kong of China, Pakistan, India and South Korea, with an respective year-on-year decrease of 47.31% and 38.48% for the EU and Hong Kong of China and over 50% for the other three countries. In terms of clothing and related products, the export to the top five markets including USA, the EU, Hong Kong of China, Japan and the UK declined by 33.48%, 28.94%, 35.06%, 28.14% and 30.68%, respectively.

As indicated by the export to major markets from 2016 to 2020 (Tab. 8), with the rapid rise of the domestic market, the proportion of domestic demand for silk goods witnessed a year-on-

year increase, while that of export decreased year by year since 2016. In 2020, China's top five exports markets (the USA, Italy, Hong Kong of China, Japan and India) concerning silk goods dropped by 51.05%, 45.22%, 54.97%, 51.25% and 76.5%, respectively against 2016.

Tab.8 Export value to the main markets in 2016 – 2020

Countries and regions	Export/US $10,000				
	2020	2019	2018	2017	2016
The USA	20,733.74	47,279.93	52,561.20	39,665.82	42,357.16
Italy	11,634.63	52,974.35	25,613.40	21,460.20	21,240.56
Hong Kong of China	8,945.11	48,243.01	14,630.70	17,184.77	19,863.12
Japan	7,775.20	49,507.46	14,043.10	16,857.85	15,948.07
India	6,921.23	71,902.59	18,513.90	31,042.19	29,448.20

Note: The above-mentioned data is sourced from the General Administration of Customs.

As indicated by the proportion of export to major markets from 2016 to 2020 (Tab.9), the export to the USA and Italy claimed a respective proportion of 19.36% and 10.86% in total exports of silk goods in 2020, rising by 4.93 percentage points and 3.63 percentage points respectively against 2016. The proportion of export to Hong Kong of China and Japan remained basically unchanged, while that to India decreased by 3.57 percentage points.

Tab.9 The proportion of the main markets in 2020 and 2016

Countries and regions	2020		2016	
	Amount/ US $10,000	Proportion/%	Amount/ US $10,000	Proportion/%
The USA	20,733.74	19.36	42,357.16	14.42
Italy	11,634.63	10.86	21,240.56	7.23
Hong Kong of China	8,945.11	8.35	19,863.12	6.76
Japan	7,775.20	7.26	15,948.07	5.43
India	6,921.23	6.46	29,448.20	10.03

Note: The above-mentioned data is sourced from the General Administration of Customs.

Due to the deterioration of international trade environment and the rising of "anti-globalization" in recent years, countries were constantly being stimulated to implement trade protection measures, which led to export enterprises to strengthen their export channels to emerging markets in Southeast Asia, countries along the "the Belt and Road Initiative" and Africa. For instance, in 2016, the export to Nigeria increased by 310.97% year on year. In 2017, the export to Ethiopia, Saudi Arabia and Nigeria grew by 256.5%, 198.4% and 135.64% respectively against the previous year. In 2018, the export to Nigeria read US $284,513,000, which claimed a proportion of 9.62% of total exports, second only to 17.77% of the USA. While export to the major markets such as the USA, Italy and India fell across the board in 2019, the export to Egypt and Ethiopia posted an respective increase of 654.81% and 125.22% year on year.

1.4 Cocoon silk market situation

In the first quarter of 2020, the market panic was exacerbated under the impact of COVID-19, which led to the irrational and rapid decline of the prices related to dried cocoons and raw silk, with the largest drop exceeding 30% compared with the beginning of the year and hitting a new low in the past five years. With the continuous issuance of multiple rescue policies around the country since April 2020, enterprises resumed orderly work and production, and the operating rate of reeling and weaving enterprises grew in Guangxi, Zhejiang, Jiangsu, Sichuan, etc. What's more important, as the domestic cocoon silk circulation and consumer market recovered gradually, the cocoon silk price bottomed out and rose at the end of the second quarter. By the end of 2020, the domestic prices of dried cocoons and 3A grade raw silk were 108,400 yuan/t and 328,500 yuan/t, respectively, which decreased by 6.71% and 11.76% year on year, as shown in Fig.3 and Fig.4.

In 2021, the domestic cocoon silk market continued to rebound and the prices appeared to climb steadily. As of March 31, 2021, the domestic price of dried cocoon and 3A raw silk reached 128,700 yuan/t and 399,900 yuan/t respectively, increasing by 52.85% and 49.44% compared with the lowest price in 2020. Besides, the price of raw silk basically recovered to the highest level since 2019.

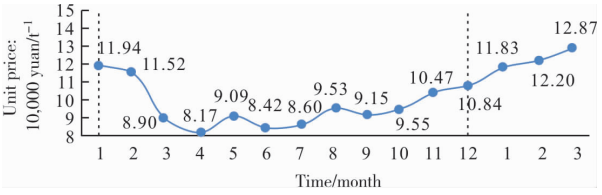


Fig.3 The price trend of dry cocoons from January to December 2020

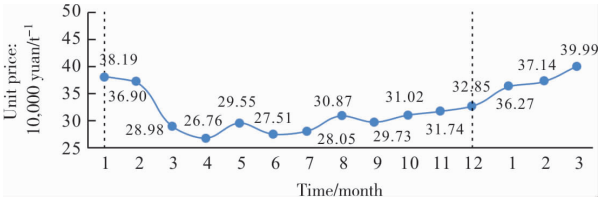


Fig.4 The price trend of grade 3A raw silk from January to December 2020

2 Global silk trade in 2020

China, India, Turkey, Brazil, the USA, Japan, Malaysia, Australia, Thailand and the European Union (27 EU countries) as the most important players in the world silk trade accounted for about 85% of global trade. According to the GTA data, the global

trade volume of silk goods in 2020 registered US $22.782 billion, dropping by 21.17% year on year. Among them, the export totaled US $11.051 billion, down 21.18% year on year while imports amounted to US $11.731 billion, down 20.59% year on year.

2.1 Export

As reflected by the export value of the major export markets in 2020 (Tab. 10), China suffered the biggest decline of 50.26%. The EU, India and Turkey saw similar declines of 16.63%, 16.44% and 13.45%, respectively. It can be seen that silk trade in all countries were affected to varying degrees by the global COVID-19 pandemic.

Tab. 10 The export value of major export markets in 2020

Countries/regions	Export/US $10,000	Year-on-year/%	Proportion/%
The EU	441,575.54	-16.63	39.96
India	278,135.39	-16.44	25.17
Turkey	165,669.00	-13.45	14.99
China	107,118.71	-50.62	9.69
The USA	45,232.70	-22.60	4.09
Thailand	32,746.07	-24.77	2.96
Japan	17,135.62	-14.00	1.55
Malaysia	9,611.77	-4.46	0.87
Brazil	4,602.32	-26.79	0.42
Australia	3,231.08	-8.56	0.29
Total	1,105,058.21	-21.18	100.00

Note: The above-mentioned data is sourced from the China Chamber of Commerce for Import and Export of Textiles.

Judging from the proportions of export value from major export markets in 2020, the export of the EU, India and Turkey increased by 2.47, 1.61 and 1.44 percentage points respectively against 2019. Only China's proportion continued to decline, by 5.67 percentage points year on year. On the whole, the sum of export of the EU, India, Turkey and China reached US $9.925 billion, accounting for 89.81% of the total export, which was roughly the same as last year.

2.2 Import

According to the import of major import markets in 2020 (Tab. 11), the most important consumption markets of silk commodities remained the EU, the USA and Japan, whose import summed to US $9.922 billion, accounting for 84.57% of the total, roughly the same as in previous years. However, the three major markets, namely, the EU, the USA and Japan all saw their imports drop by more than 15%, being 17.6%, 25.45% and 15.8% respectively. Among the emerging markets, only Australia witnessed a decline of less than 10%, at 6.26% in terms of import, while all the rest markets saw declines of more than 18%. Moreover, only China posted a growth as big as 9.24% in regard to silk imports.

Tab. 11 Import value of major import markets in 2020

Countries/regions	Imports/US $10,000	Year-on-year/%	Proportion/%
The EU	481,572.67	-17.60	41.05
The USA	349,848.69	-25.45	29.82
Japan	160,768.29	-15.80	13.70
Australia	51,086.34	-6.26	4.35
India	27,072.48	-42.92	2.31
Thailand	27,122.22	-18.59	2.31
China	26,138.53	9.24	2.23
Malaysia	18,022.08	-36.05	1.54
Turkey	20,257.60	-26.60	1.73
Brazil	11,256.05	-36.12	0.96
Total	1,173,144.96	-20.59	100.00

Note: The above-mentioned data is sourced from the China Chamber of Commerce for Import and Export of Textiles.

3 Prospect of industry development situation in 2021

The political and economic environment at home and abroad has undergone profound and complex changes since the beginning of 2021. The prospect of COVID-19 epidemic is uncertain, and the global economic and trade environment is unstable and uncertain. The risks and challenges facing the industry cannot be ignored.

The current international situation is highly uncertain. The COVID-19 epidemic has yet to be fully brought under control, geopolitical risks, unilateralism and protectionism are on the rise, which has led to the downgraded world economic growth forecasts. According to relevant reports of United Nations Conference on Trade and Development (UNCTAD) and World Trade Organization (WTO), global trade in goods decreased by 5.6% year on year in 2020, marking the biggest year-on-year decline in trade in goods since the international financial crisis in 2008. What's worse, the COVID-19 coupled with the protectionism has seriously disrupted the normal operation of the global economy. Although overseas orders returned to China since the second half of 2020 due to severe outbreaks of COVID-19 in some countries, the tendency to spread international orders outside China would become more apparent as the global industrial chain restructures. In 2021, since the epidemic abroad has yet to be effectively brought under control, the international political landscape is undergoing profound adjustments and economic globalization is facing countercurrents, sluggish consumer demand may become the norm, competition in the international market will inevitably intensify, and risks to economic security and trade environment will persist.

(Please refer to P1-8, Issue 7 of *Journal of Silk* in 2021 for details.)

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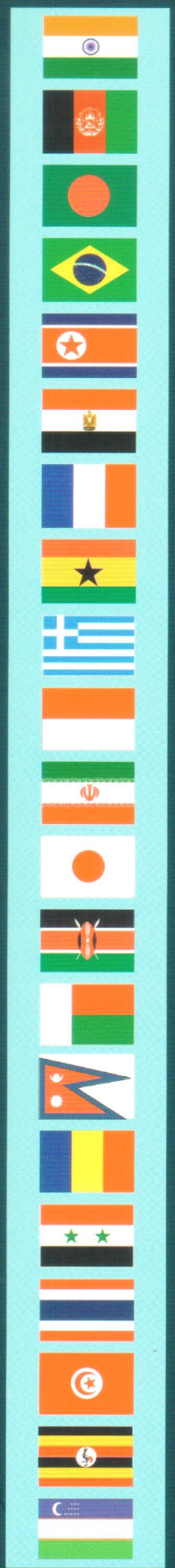

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