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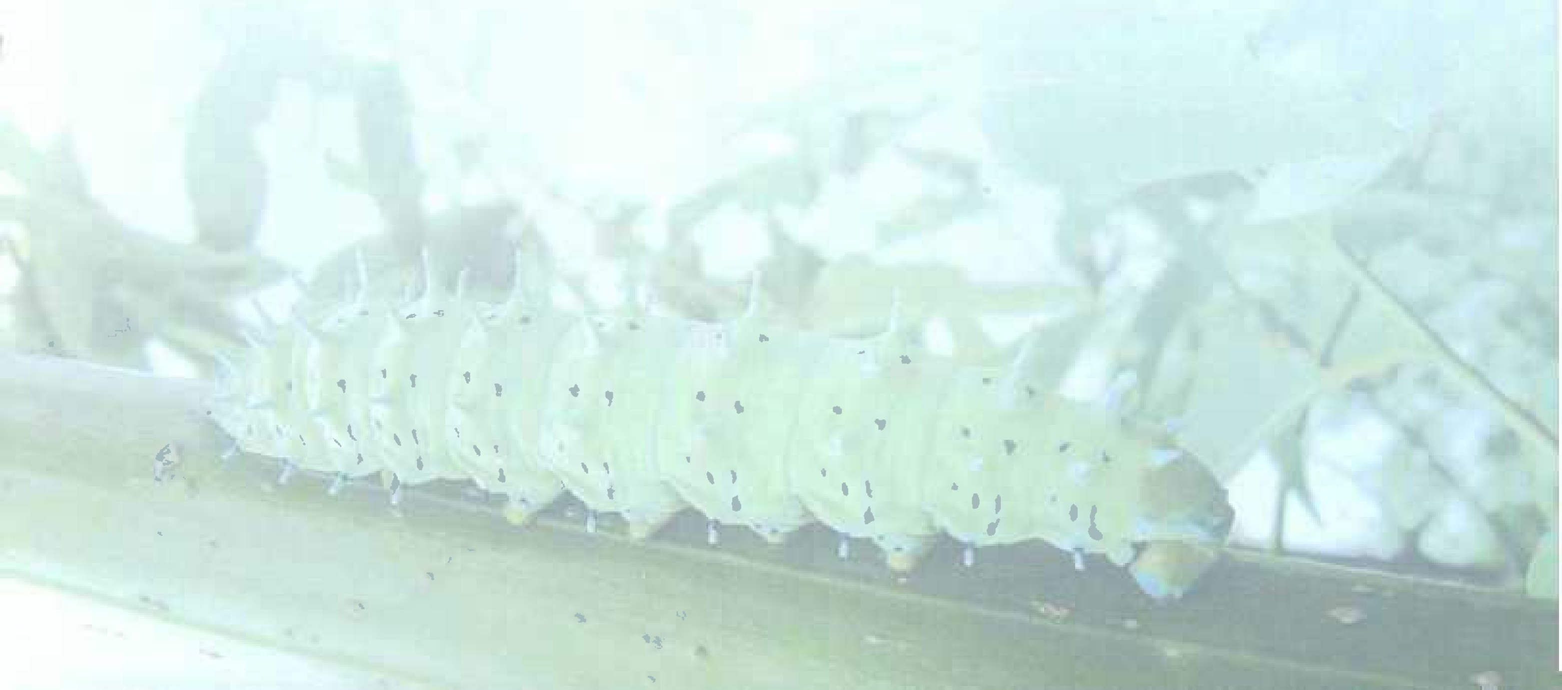

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Research Paper

GENETIC VARIATION IN PHOTOSYNTHETIC PARAMETERS AND SPAD CHLOROPHYLL CONTENT AMONG A DIVERSE SET OF MULBERRY (*MORUS* SPP.) GERMPLASM

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ABSTRACT

Mulberry being one of the fastest growing plants under cultivation, the natural genetic variation existing in the photosynthesis traits can find potential application in designing varieties to meet the challenges of climate change and sustainable farming. In this study, we report the genetic variation in six photosynthetic parameters and SPAD chlorophyll content among 204 diverse set of mulberry germplasm, recorded in two seasons. The coefficient of variation (%) was at par in two recording periods (August, 2015 and November – December, 2105) for net photosynthetic rate – P_N (10.6 and 14.5), stomatal conductance – g_S (25.78 and 28.24), transpiration rate – E (19.43 and 20.19), intercellular CO_2 concentration – C_i (7.05 and 7.10), intrinsic water use efficiency – IWUE (19.33 and 24.13), vapor pressure deficit – VPD (8.26 and 9.24), respectively under moisture saturated regime. The variability in SPAD chlorophyll content ranged from 30.73 to 47.37 in August against 24.60 to 45.47 in November-December with higher mean recorded in the latter season. Positive correlations were observed among P_N, g_S, E and C_i ($p < 0.001$) in both the seasons except in the case of C_i in the second season. P_N, g_S, E and C_i were negatively correlated with IWUE and VPD in both the seasons. The decreased E without much compromise on the P_N under higher VPD and reduced g_S will be the basis for development of moisture stress tolerant varieties. The germplasm with higher IWUE and P_N may confer genetic difference in heat avoidance and may have implication in higher productivity under changing climatic condition. The rate of photosynthesis will contribute significantly in biomass accumulation in mulberry provided other factors *viz*., leaf area, light interception *etc*. remain constant.

Key words: Carbon sequestration, genetic variability, growth, mulberry, photosynthetic traits

INTRODUCTION

Sericulture is a unique agro-based avocation and involves cultivation of mulberry (*Morus* spp.) for rearing of silkworms (*Bombyx mori* L.) for production of the most sought after natural fibre – silk. India stands second in global silk production and is also a major importer of raw silk from China. Mulberry is mainly cultivated for its leaves as a source of nutrition for the silkworm larvae. The plants are grown in the form of bush or small trees by pruning for fresh growth which facilitates the production of quality leaves during silkworm rearing. Of late, there is a shift from the practice of leaf to shoot harvest which has qualitatively improved the silk production and reduced the drudgery besides cost of cultivation. Mulberry is one of the fastest growing plants under cultivation and is rejuvenated by repeated pruning and harvest of the crop. The mulberry varieties respond differently for pruning injuries and display differential ability for photosynthetic fixation of CO_2 and biomass accumulation. Photosynthesis is an important process in re-growth and production of nutritious foliage of mulberry (Satoh, 1982) and influences the plants' ability in CO_2 fixation, biomass allocation to leaves and its overall growth (Dijkstra and Lambers, 1989).

Growth is the key outcome of photosynthesis and the natural variability in the photosynthetic process of a crop germplasm is of critical importance for its genetic improvement. The relationship between photosynthesis and growth is complex, as it is not only linked to rate of photosynthesis but also to the architecture of the plant canopy, leaf area, environment *etc*. Pettigrew and Turley (1998) have reported that narrow genetic variation in cotton photosynthetic components was the main constraint in breeding for increased photosynthesis. Genetic variation in photosynthetic components of cacao contributes to improve productivity and can be used to optimize the performance under specific growing condition (Daymond *et al.*, 2011). Photosynthetic difference between the *Populus* clones germplasm showed correlation between photosynthetic rate and growth when accounted for seasonal variability and tree size (Isebrands *et al.*, 1988). Aspinwall *et al.* (2011) have reported that the genetic difference at leaf level physiological parameters of pine tree germplasm was most consistently related to the productivity.

Photosynthesis is a sensitive light driven CO_2 utilization process affected by various environmental conditions such as temperature (Camejo *et al.*, 2005), stomatal closure due to varying VPD (Pettigrew *et al.*, 1990), nutrient manipulation (Tyree *et al.*, 2009) *etc.* Many environment related factors interactively work together at different levels to influence the photosynthesis (Yang *et al.*, 2002). Pettigrew and Turley (1998) have hypothesized that germplasm which are able to maintain constant photosynthetic rate during different environmental conditions should have more photosynthate for growth and yield than other germplasm.

Mulberry is a heterozygous and perennial C3 plant with enormous diversity in growth related traits. The crop accumulates rapid biomass and therefore, is potentially efficient in carbon sequestration. An assessment of genetic variation in photosynthetic process in germplasm reserves can provide an insight into the potential for utilization of these plant species in mitigation of global warming due to CO_2 accumulation and its consequential effect of climate change (Cherubini *et al.*, 2011). The present study aims to investigate the photosynthetic efficiency and allied component traits in a set of diverse mulberry germplasm to explore the possibilities in designing crop varieties to meet the future challenges in sericultural productivity as well as climate change.

MATERIALS AND METHODS

Plant materials

A diverse set of germplasm (204 entries) sampled from a larger collection (~1000 acc.) with maximum phenotypic diversity and allelic variation was established and maintained in an Augmented Random Block Design (ARBD) with a spacing of 5' × 5' between the entries at Central Sericultural Research and Training Institute (CSRTI), Mysuru, India. Each entry was represented by 4 clonal ramets. The crown heights of the plants were maintained at 3' by pruning. Among the diverse set, 57 entries were exotics and the rest were of indigenous origin. V-1 (indigenous) and Kousen (exotic) were grown as check varieties. The plantation was maintained under standard package of practice including periodic irrigation, application of manure and fertilizers and weed and disease/pest control measures as recommended by the Institute.

Experimental period and soil condition

The data were recorded during two different seasons *i.e.*, August, 2015 and November – December, 2015. The mean temperature, precipitation and humidity were recorded in respective periods (at Meteorological Centre, CSRTI, Mysuru). The soil was red sandy loam with average pH of 7.72.

Light response curve

Photosynthetic response to the different levels of irradiance *i.e.,* 2000, 1500, 1200, 1000, 800, 500, 250 and 100 µmol $m^{-2} s^{-1}$ was measured among 4 genotypes *viz.,* G2, Mysore Local, Kanva-2 and S-36 on fully expanded leaves using Portable Photosynthesis System LI-6400XT (LiCor Inc., USA) to determine the light saturation of photosynthetic rate under soil moisture saturated condition and leaf cuvette temperature maintained at 28°C.

Gas exchange and chlorophyll measurement

About 45-50 days after pruning, net photosynthetic rate [P_N, µmol (CO_2) $m^{-2} s^{-1}$], stomatal conductance [g_S, mol $m^{-2} s^{-1}$], intercellular CO_2 concentration [C_i, µmol (CO_2) mol^{-1}], transpiration rate [*E*, mmol (H_2O) $m^{-2} s^{-1}$] and vapor pressure deficit [VPD, KPa] based on leaf temperature were recorded for all the mulberry entries in three replications, on fully expanded leaves. Soil was moisture saturated during photosynthetic measurements. The PAR (photosynthetic active radiation) (1000 µmol $m^{-2} s^{-1}$) and leaf cuvette temperature were maintained constant as described earlier in all the observations. To reduce the fluctuation in ambient CO_2, air was moved through a protective enclosure. The intrinsic water use efficiency [IWUE, µmol (CO_2) $mmol^{-1}$ (H_2O)] was estimated as per Cregg *et al.* (2000). The relative leaf chlorophyll content was recorded near the middle of the lamina avoiding the midrib, using SPAD-502 Chlorophyll Meter (Minolta, Japan).

Statistical analysis

All the statistical analyses were carried out using mean of 3 independent measurements of gas exchange parameters and chlorophyll content on different ramets. The data were statistically analyzed using analysis of variance (ANOVA). The statistical differences among different parameters were tested with Fisher's least significant difference test ($p < 0.05$). Relationships among the parameters were determined using Pearson's simple correlation test with the predicted coefficient of determination (r^2) value.

RESULTS

Overall, the P_N reached maximum at PAR 1000 µmol $m^2 s^{-1}$ in all the 4 genotypes studied. The P_N has linearly increased with increasing light intensity and reached a saturation point at 1000 µmol $m^{-2} s^{-1}$after which there was no significant increase of P_N with increased PAR (Figure 1). Therefore, PAR 1000 µmol $m^{-2} s^{-1}$ was kept constant for measuring the gas exchange parameters among all the mulberry germplasm. Genetic variation in the photosynthetic parameters was significant across all the mulberry germplasm in August, 2015 as well as in November – December, 2015 (Table 1). The P_N ranged between 9.98 and 27.46 µmol (CO_2) $m^{-2} s^{-1}$ in August, 2015 and from 7.32 to 24.02 µmol (CO_2) $m^{-2} s^{-1}$ in November – December, 2015. Overall, P_N was comparatively higher in August 2015 than that of November – December, 2015 (Figure 2). Similarly, *E* was also higher [1.83 to 8.60 mmol (H_2O) $m^2 s^{-1}$] in August, 2015 than in November – December, 2015 [1.68 to 7.69 mmol (H_2O) $m^2 s^{-1}$].

The variation in g_S was slightly higher in the first data recording period (August, 2015) compared to the second. There was an increase in P_N and also *E* with increasing g_S in most of the germplasm during August, 2015 (92 %) and

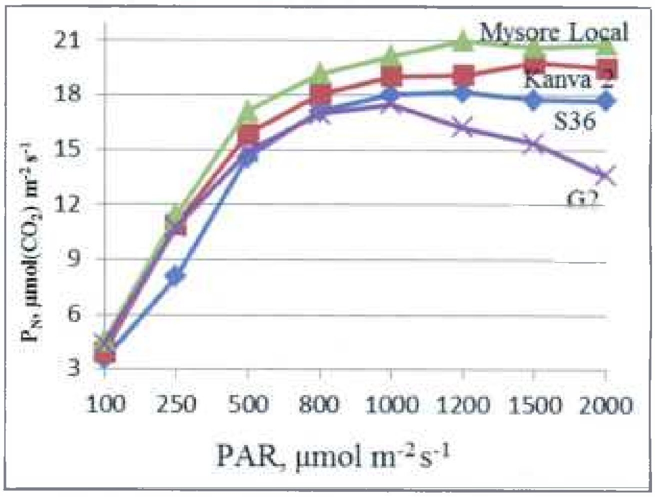

Figure 1: Light response curve - P_N at different photosynthetic active radiation (PAR)

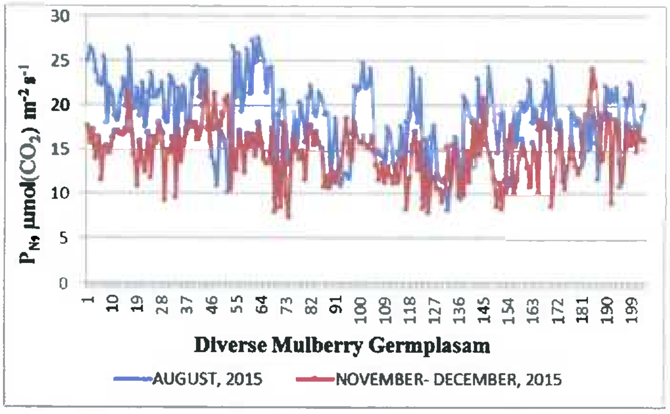

Figure 2: Genetic variation in net photosynthetic rate (P_N) of mulberry germplasm: CV values were 0.60 and 14.50 %, respectively for August 2015 and November – December 2015. The values plotted are means of three replicates in both the seasons. Probabilities according to ANOVA <0.0001 and LSD test at (0.05) are 3.16 and 3.43 in the respective seasons.

November – December, 2015 (82 %). A strong positive correlation was observed between the P_N and g_S (Table 2, Figure 3A and 3B) and very strong positive correlation was observed between *E* and g_S (Table 2, Figure 3C and 3D). In both the seasons, a strong negative correlation was observed between g_S and VPD (Table 2, Figure 3E and 3F).

The variation in the P_N among germplasm to changing C_i level was investigated during both the periods. The average C_i ranged from 206.87 μmol (CO$_2$) mol^{-1} to 310.14 μmol (CO$_2$) mol^{-1} in August, 2015 and from 170.65 μmol (CO$_2$) mol^{-1} to 329.66 μmol (CO$_2$) mol^{-1} in November – December 2015. About 23 % of the germplasm showed a constant P_N for varying g_S and C_i whereas, 45 % recorded higher P_N with increasing g_S and C_i and about 31% germplasm displayed maximum P_N at lower C_i and g_S level during both the periods. There was a weak correlation between P_N and C_i in August, 2015 and no correlation in November – December, 2015. As observed in other crops, a strong positive correlation was observed between C_i and g_S in both the data recordings (Table 2, Figure 4A and 4B).

Across the germplasm, the mean IWUE ranged from 36.75 to 102.53 μmol (CO$_2$) mmol^{-1} (H$_2$O) in August, 2015 and from 28.15 to 132.79 μmol (CO$_2$) mmol^{-1} (H$_2$O) in November – December 2015. A few accessions recorded

Table 1: ANOVA *P*-value, coefficient of variation and least significant difference of photosynthetic parameters in mulberry germplasm

	P_N	g_s	C_i	*E*	IWUE	VPD
	[μmol (CO$_2$) m^{-2} s^{-1}]	[mol m^{-2} s^{-1}]	[μmol (CO$_2$) mol^{-1}]	[mmol (H$_2$O) m^{-2} s^{-1}]	[μmol (CO$_2$) mmol^{-1} (H$_2$O)]	[KPa]
			August, 2015			
Minimum	9.98	0.09	206.87	1.83	36.75	0.22
Maximum	27.46	0.87	310.14	8.6	102.53	2.27
Mean	18.48	0.32	267.34	4.82	63.93	1.49
SD	4.6	0.12	25.27	1.39	16.01	0.33
Pr > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CV %	10.6	25.78	7.05	19.43	19.33	8.26
LSD (0.05)	3.16	0.13	30.44	1.5	19.6	0.2
			November - December, 2015			
Minimum	7.32	0.04	170.65	1.68	28.15	0.83
Maximum	24.02	0.91	329.66	7.69	132.79	2.54
Mean	14.7	0.28	284.59	4.46	57.02	1.53
SD	3.62	0.11	29.2	1.41	19.56	0.28
Pr > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CV %	14.5	28.24	7.1	20.19	24.13	9.24
LSD (0.05)	3.43	0.13	32.43	1.44	22.17	0.22

Table 2: Correlation between photosynthetic parameters among mulberry germplasm ($n = 612$) in two seasons

	P_N	g_S	C_i	E	IWUE	VPD
August, 2015						
P_N						
g_s	0.730**					
C_i	0.201**	0.736**				
E	0.428**	0.820**	0.769**			
IWUE	-0.228**	-0.760**	-0.983**	-0.797**		
VPD	-0.792**	-0.669**	-0.288**	-0.202**	0.290**	
November December, 2015						
P_N						
g_s	0.633**					
C_i	0.059^{NS}	0.683**				
E	0.511**	0.867**	0.644**			
IWUE	-0.095**	-0.699**	-0.939**	-0.7**		
VPD	-0.535**	-0.559**	-0.338**	-0.159**	0.249**	

** Significant at $p < 0.01$; ^{NS}-Not Significant at $p < 0.05$ and $p < 0.01$.

higher IWUE and P_N at reduced C_i and g_S. However, weak negative correlation was observed between P_N and IWUE in the germplasm collection during both the seasons (Table 2). IWUE varied greatly between the germplasm due to the apparent variation of g_S. A strong negative correlation was observed between IWUE and g_S in both the data sets (Table 2, Figure 4C and 4D). There was a strong negative correlation with increasing C_i concentration on IWUE (Table 2) and linear relationship between IWUE and C_i (Figure 4E and 4F).

Leaf SPAD chlorophyll content varied significantly among germplasm ($p < 0.01$), ranging from 30.73 to 47.37 in August, 2015 [LSD at (0.05) was 1.9] and from 24.60 to 45.47 in November – December, 2015 [LSD at (0.05) was 3.31]. The average chlorophyll content was higher in August, 2015 than that recorded in November – December, 2015. Moderate positive correlation was observed between the SPAD chlorophyll content and P_N during August, 2015 ($r = 0.540$; $p < 0.01$) and November – December, 2015 ($r = 0.557$; $p < 0.01$). A linear relationship was observed between SPAD chlorophyll content and P_N (Figure 5A and 5B).

DISCUSSION

The genotypic differences in photosynthetic parameters, such as P_N, E, g_S, C_i and IWUE were studied in a number of crops *viz*., wheat (Morgan and LeCain, 1991), cotton (Pettigrew and Turley, 1998), common bean (Santos *et al*., 2009), reed canarygrass (Brodersen *et al*., 2008), cacao (Daymond *et al*., 2011) *etc*. Most of the studies indicated a narrow genetic variability among the cultivated varieties with higher productivity traits. Though there were many instances of correlation of photosynthetic efficiency with that of yield associated traits, the latter is controlled by many more factors of the plant genotype and environment. In this context, the present study is an effort to assess the genetic variation in attributes related to photosynthesis in mulberry germplasm resources. A significant difference in P_N (with CV, 10.6 % and 14.5 %, respectively in two separate seasons) was observed among the diverse mulberry germplasm, indicating the influence of genetic and abiotic factors on the photosynthetic performance (Reich *et al*., 1999). The higher mean P_N in the first season was due to favorable climatic condition for plant growth (Figure 2). But, in the winter season (November – December), the mulberry plants were affected by abiotic factors influencing growth and productivity (data not shown) and likely to be the reason for decreased mean P_N. However, a few of the germplasm that have maintained consistent P_N in both the seasons, may be due to the genetic potential of these resources which is the least influenced by the environment. However, in majority of the exotic germplasm (mostly of temperate origin), a lower P_N was observed than that of the indigenous ones (tropical) in both the seasons. This can be explained in terms of adaptation of the resources to specific eco-climatic conditions and their photosynthetic response to longer photo period. The adaptive capabilities of tropical and temperate genotypes at warmer temperature differ and consequently, show differences in the rate of photosynthesis. In temperate *Eucalyptus globulus* trees, the study has shown to have limited adjustment under elevated CO_2 and warmer climate (Crous *et al*., 2013).

Transpiration rate varies greatly in plants, both among

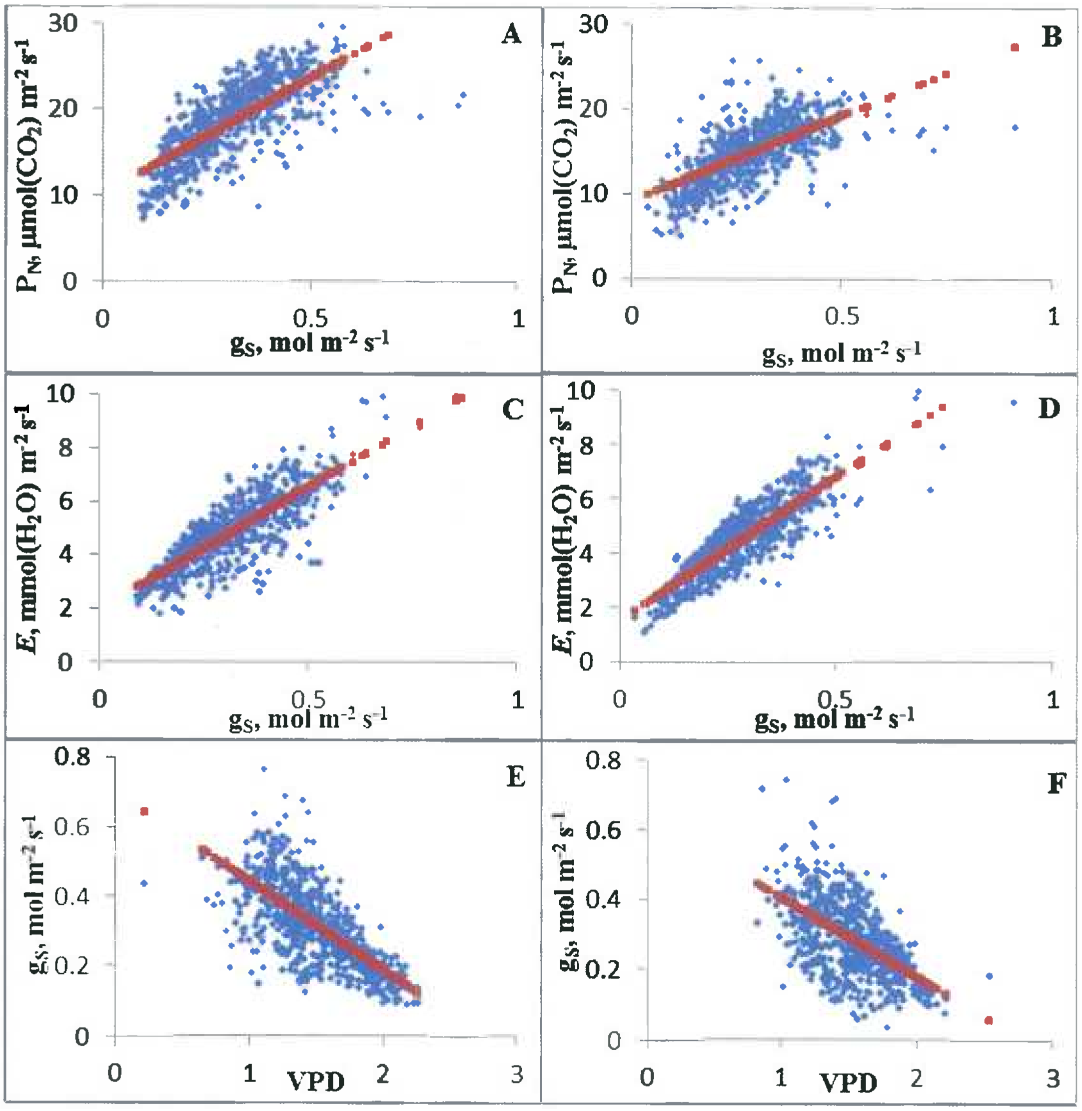

Figure 3: Factors underlying genotypic difference in photosynthetic parameters. A, B - Relationship between P_N and g_s in August, 2015 and November – December, 2015, respectively (the fitted function is $P_N = ag_s + b$, where a = 27.1 and 20.1; b = 9.94 and 8.90; $r^2 = 0.53$ and 0.40, respectivley at $p < 0.01$, <0.05), C, D – Relationship between *E* and g_s in the two respective seasons (the fitted function is $E = ag_s + b$, where a = 9.2 and 10; b = 1.8 and 1.4; $r^2 = 0.44$ and 0.31., respectively at $p < 0.01$, <0.05), E, F – Relationship between g_s and VPD in the two respective seasons (the fitted function is $g_s = aVPD + b$, where a = -0.25 and -0.2; b = 0.69 and 0.6; $r^2 = 0.44$ and 0.3, respectively at $p<0.01$, <0.05). The plotted values are for $n = 612$.

and within species in response to a wide variety of environmental variables and across different spatio-temporal scales (Gilbert *et al.*, 2011). In mulberry, significant difference in *E* (with CV 19.43 % and 20.19 %, respectively in both the seasons) was due to the large difference in g_S and its sensitivity to VPD. This was clearly indicated with the linear relationship between the *E* and g_S in both the seasons (Figure 3C and 3D). Transpiration is linked to the combined influences of climate-driven variables controlling the stomatal function. The decreased transpiration without much compromise on the P_N under higher VPD and reduced g_S will be the basis for development of moisture tolerant varieties. Therefore, a better understanding of the mechanism of transpiration regulation is needed, which could assist physiologists, plant breeders and water resources managers to use the

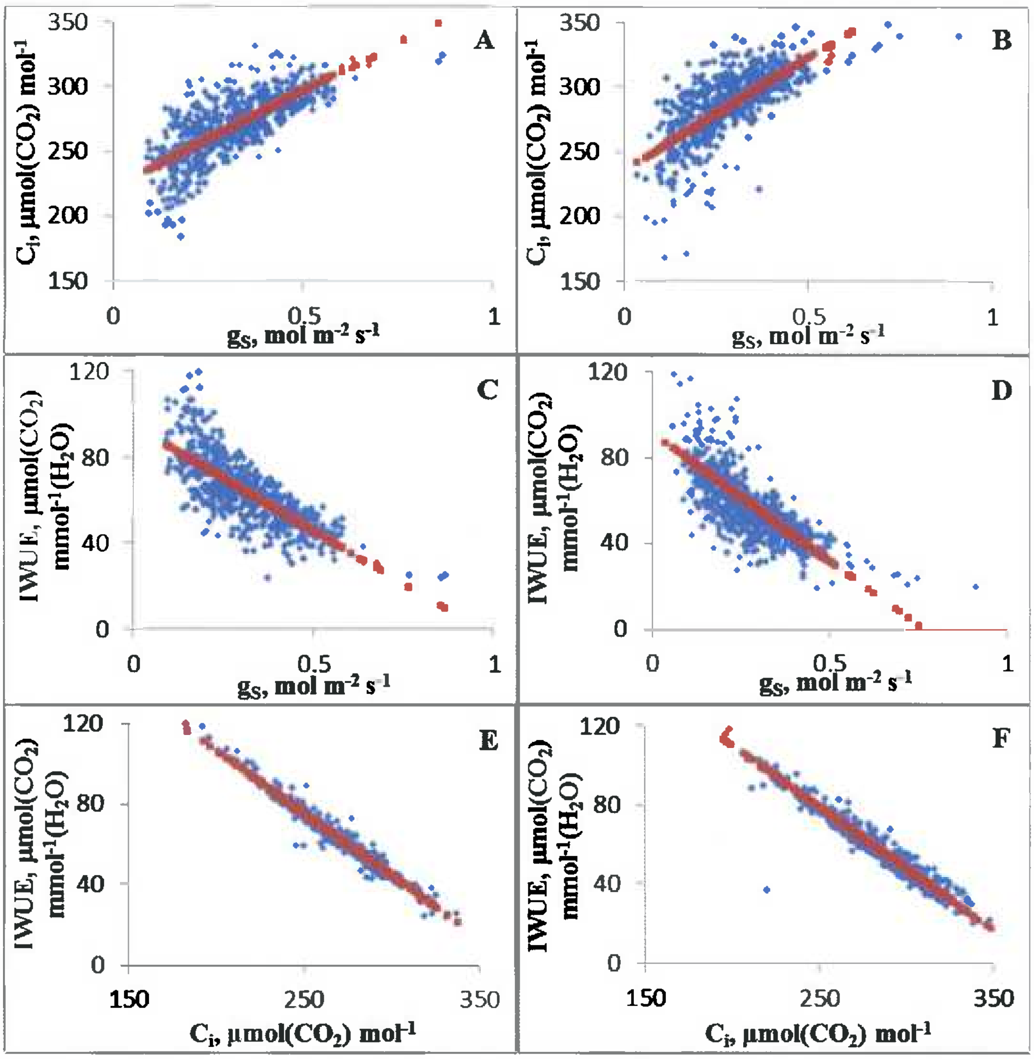

Figure 4: Importance of g_s with other photosynthetic parameters. A, B - Relationship between C_i and g_s in in August, 2015 and November – December, 2015, respectively (the fitted function is $C_i = ag_s + b$, where a = 150 and 175 and b = 221 and 234; $r^2 = 0.54$ and 0.47, respectively at $p < 0.01$, <0.05), C, D - The relationship between IWUE and g_s in the two respective seasons (the fitted function is IWUE = $ag_s + b$, where a = -98.2 and -120 and b = 94.4 and 91.3; $r^2 = 0.58$ and 0.49, respectively at $p < 0.01$, <0.05), E, F - The relationship between IWUE and C_i in the two respective seasons (the fitted function is IWUE = a C_i + b, where a = -0.6 and -0.6 and b = 230 and 236; $r^2 = 0.96$ and 0.88, respectively at $p < 0.01$, <0.05). The plotted values are for *n* = 612.

advantages of transpiration restriction trait.

P_N is regulated by stomatal opening which in turn is influenced by environmental factors. The variation in g_S was nominal across two seasons with significant genotypic variability. The CV (25.78 % and 28.24 %) of g_S in mulberry was the highest among all the parameters in both the seasons and the observed variability in g_S in two seasons was nearly same. Both P_N and *E* were positively correlated with g_S with linear relationship in both the seasons (Figure 3A, 3B, 3C and 3D) indicating the importance of stomatal function in carbon fixation and biomass accumulation under moisture saturated condition (Fischer *et al.*, 1998). Further, Fischer *et al.* (1998) and Richards (2000) studied the importance of g_S at higher temperature under well irrigated condition for controlling the canopy temperature that benefits in maintaining higher P_N. Richards (2000) hypothesized that decreased g_S due to

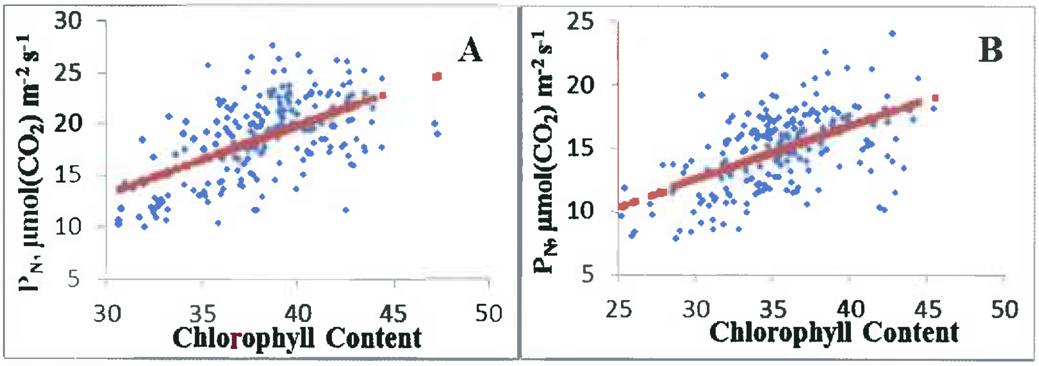

Figure 5: Relationship between the average P_N and SPAD chlorophyll content: (A) August 2015; (B) November – December 2015. The fitted function is P_N = a (Chlorophyll Content) + b, where a = 0.67 and 0.42; b = -7.06 and -0.32; r^2 = 0.31 and 0.30; at $p < 0.05$ in respective seasons. The values plotted are means of three replicates.

VPD or water stressed condition and extra cooling capacity particularly at warmer climate under irrigated condition contributes to the relationship between g_S and plant growth. Therefore, further study on stomatal behavior for the selection of higher and sustained g_S during the growth period under well irrigated condition may be used as a selectable trait for increasing crop productivity. The strong negative correlation between g_S and VPD can be inferred as a result of the constant difference in water vapor concentration between leaf and air (Figure 3E and 3F). Morison and Gifford (1983) observed that VPD > 2 KPa caused decline in transient water potential which in turn drastically reduced P_N and conductance. A similar inference can be drawn in mulberry in both the seasons.

The conductance to CO_2 diffusing into the leaf is variable and regulated by stomata. Therefore, regression coefficient for C_i and g_S was significant in both the seasons (Figure 4A and 4B) predicting a linear relationship between the two photosynthetic parameters. A fraction of 23 % of the germplasm showed consistent P_N under changing g_S and C_i suggesting that these germplasm collections have the potential to function under wide range of CO_2 concentrations. Similar observation has been made in reed canary grass (Brodersen *et al.*, 2008), therefore, it is necessary to understand the trade off between the enhanced P_N and plasticity in stomatal conductance during the fluctuation of CO_2 in the atmosphere. About 45 % of the germplasm displayed higher P_N with increasing g_S and C_i but, at lower C_i, there was a reduction in the P_N probably as a result of RuBP regeneration or electron transport system becoming the limiting factors in the process (Pettigrew and Turley, 1998). About 31 % of the germplasm maintained higher P_N at lower C_i and g_S level in both the seasons, which is indicative of higher photosynthetic activity in the mesophyll tissue (Morgan and LeCain, 1991).

A higher IWUE is indicative of the apparent variation in P_N and g_S or both. The increase in IWUE is the first effect of reduction in g_S indicating a linear relationship between IWUE and g_S (Figure 4C and 4D). Our study has revealed that higher IWUE is achieved by an average P_N with the combination of the lowest mean g_S; on the other hand, lower IWUE is due to higher mean g_S. The results indicated that the effect on IWUE may be due to the genetic variation in g_S rather than the variation in P_N. This was supported by the fact that IWUE and g_S are more negatively correlated than P_N. Therefore, for selection of higher IWUE through reduction of g_S would appear to be a direct proposition. Further, some of the germplasm with higher IWUE and P_N may enhance the capacity of the plant in heat avoidance and may have implication for more productivity under changing climatic condition (Radin *et al.*, 1994) These traits may be important for improving the efficiency of carbon assimilation without sacrificing the productivity (Aspinwall *et al.*, 2011).

Leaf chlorophyll content is a very robust trait and allows a reliable classification of genotypes in different environments. In our study, we inferred a positive relationship between mean total chlorophyll content and mean P_N in both the seasons (Figure 5A and 5B). However, this conclusion cannot be generalized because there were many instances of genotypes having less chlorophyll content recording higher P_N and *vice versa*. These observations suggest that variation in the P_N in response to chlorophyll content is often associated with altered Rubisco level under saturated CO_2 (Stitt *et al.*, 1991) and changes in the light harvesting complex. The decrease in the average P_N and chlorophyll content in the second season may be due to the seasonal stresses. Similar observations have been reported by McGrath and Pennaypacker (1990) while studying the winter wheat, where they reported the effect of leaf rust caused by *Puccinia recondita* on P_N, E, g_S and chlorophyll content. Further, an important question to be investigated is whether the leaf chlorophyll content is associated with stability of mulberry leaf yield. In crops like wheat, the genetic variation of chlorophyll content is well studied and its association with yield has been established (Hamblin *et al.*, 2014).

The role of photosynthesis in contributing towards the increase in yield and biomass production is complex and still not completely resolved. As in any other biological process, photosynthesis is also genetically controlled and the genetic blue print governing the process varies within and between the plant species. The potential of the plant species with variation in photosynthetic traits render them adaptive to the changing environment and to meet the challenges of growing population and demand for increased food, fiber and fodder production. Towards this objective, more investigative efforts are needed to utilize the photosynthetic trait in mulberry crop improvement. Photosynthetic rate is one of the important components and requires a rigorous and extensive measurement regime for high-throughput phenotyping to predict its role in mulberry growth and crop production. Further, this will enable us to understand the regulation of photosynthesis and provide insights into the factors which selectively act on this trait and are required for utilization in molecular crop breeding. The rate of photosynthesis will contribute significantly in biomass accumulation in any plant species provided other factors *viz.*, leaf area, light interception *etc.* remain constant. To break the current yield barriers in any crop, the photosynthetic trait should not be left unexplored.

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REFERENCES

Aspinwall M. J., King J. S., McKeand S. E. and Domec J. C. (2011) Leaf-level gas exchange uniformity and photosynthetic capacity among loblolly pine (*Pinus taeda* L.) genotypes of contrasting inherent genetic variation. *Tree Physiol.*, **31:** 78-91.

Brodersen C., Lavergne S. and Molofsky J. (2008) Genetic variation in photosynthetic characteristics among invasive and native populations of reed canarygrass (*Phalaris arundinacea*). *Biol. Invasions,* **10:** 1317-1325.

Camejo D., Rodríguez P., Morales M. A., Dell'Amico J. M., Torrecillas A. and Alarcón J. J. (2005) High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.*, **162:** 281-289.

Cherubini F., Peters G. P., Berntsen T., Stromman A. H. and Hertwich E. (2011) CO_2 emissions from biomass combustion for bioenergy: atmospheric decay and contribution to global warming. *GCB. Bioenergy*, **3(5):** 413-426.

Cregg B. M., Olivas-Garcia J. M. and Hennessey T. C. (2000) Provenance variation in carbon isotope discrimination of mature ponderosa pine trees at two locations in the Great Plains. *Can. J. Forest Res.*, **30:** 428-439.

Crous K. Y., Quentin A. G., Lin Y. S., Medlyn B. E., Williams D. G., Barton C. V. and Ellsworth D. S. (2013) Photosynthesis of temperate *Eucalyptus globulus* trees outside their native range has limited adjustment to elevated CO_2 and climate warming. *Glob. Change Biol.*, **19:** 3790-3807.

Daymond A. J., Tricker P. J. and Hadley P. (2011) Genotypic variation in photosynthesis in cacao is correlated with stomatal conductance and leaf nitrogen. *Biol. Plantarum*, **55:** 99-104.

Dijkstra P. and Lambers H. (1989) Analysis of specific leaf area and photosynthesis of two inbred lines of *Plantago* major differing in relative growth rate. *New Phytol.*, **113:** 283-290.

Fischer R. A., Rees D., Sayre K. D., Lu Z. M., Condon A. G. and Saavedra A. L. (1998) Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Sci.*, **38:** 1467-1475.

Gilbert M. E., Holbrook N. M., Zwieniecki M. A., Sadok W. and Sinclair T. R. (2011) Field confirmation of genetic variation in soybean transpiration response to vapor pressure deficit and photosynthetic compensation *Field Crop. Res.*, **124:** 85-92.

Hamblin J., Stefanova K. and Angessa T. T. (2014) Variation in chlorophyll content per unit leaf area in spring wheat and implications for selection in segregating material. *PloS One*, **9(3):** e92529.

Isebrands J. G., Ceulemans R. and Wiard B. (1988) Genetic variation in photosynthetic traits among Populus clones in relation to yield. *Plant Physiol. Biochem.*, **26:** 427-437.

McGrath M. T. and Pennypacker S. P. (1990) Alteration of physiological processes in wheat flag leaves caused by stem rust and leaf rust. *Phytopathol.*, **80:** 677-686.

Morgan J. A. and LeCain D. R. (1991) Leaf gas exchange and related leaf traits among 15 winter wheat genotypes. *Crop Sci.*, **31:** 443-448.

Morison J. I. and Gifford R. M. (1983) Stomatal sensitivity to carbon dioxide and humidity a comparison of two C3 and two C4 grass species. *Plant Physiol.*, **71:** 789-796.

Pettigrew W. T., Hesketh J. D., Peters D. B. and Woolley J. T. (1990) A vapor pressure deficit effect on crop canopy photosynthesis. *Photosynth. Res.*, **24:** 27-34.

Pettigrew W. T. and Turley R. B. (1998) Variation in photosynthetic components among photosynthetically diverse cotton genotypes. *Photosynth. Res.*, **56:** 15-25.

Radin J. W., Lu Z., Percy R. G. and Zeiger E. (1994) Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. *Proc. Natl. Acad. Sci. USA*, **91:** 7217-7221.

Reich P. B., Ellsworth D. S., Walters M. B., Vose J. M., Gresham C., Volın J. C. and Bowman W. D. (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology*, **80:** 1955-1969.

Richards R. A. (2000) Selectable traits to increase crop photosynthesis and yield of grain crops. *J. Exp. Bot.*, **51:** 447-458.

Santos M. G., Ribeiro R. V., Machado E. C. and Pimentel C. (2009) Photosynthetic parameters and leaf water potential of five common bean genotypes under mild water deficit. *Biol. Plantarum*, **53:** 229-236.

Satoh M. (1982) Effect of leaves retained at the time of harvest on regrowth, and changes in their physiological activity in mulberry tree. JARQ, **15:** 266.

Stitt M., Quick W. P., Schurr U., Schulze E. D., Rodermel S. R. and Bogorad L. (1991) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' rbcS. *Planta*, **183:** 555-566.

Tyree M. C., Seiler J. R. and Maier C. A. (2009) Short-term impacts of nutrient manipulations on leaf gas exchange and biomass partitioning in contrasting 2-year-old *Pinus taeda* clones during seedling establishment. *Forest Ecol. Manag.*, **257:** 1847-1858.

Yang W. Q., Murthy R., King P. and Topa M. A. (2002) Diurnal changes in gas exchange and carbon partitioning in needles of fast-and slow-growing families of loblolly pine (*Pinus taeda*). *Tree Physiol.*, **22:** 489-498.

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Research Paper

PROCESS OPTIMIZATION OF POLYPHENOL EXTRACTION FROM MULBERRY FRUIT BY ULTRA-SONIC ASSISTED RESPONSE SURFACE METHODOLOGY

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ABSTRACT

A Box–Behnken design of Response Surface Methodology (RSM) with ultra-sonic assisted extraction (UAE) was conducted to analyze the effect of mass concentration range (1-3 g/l), ultrasonic power (60-120W), ultrasonic time (30-50 min), pH range (5-9) and extraction temperature (40-60 °C) on the extraction of polyphenols from mulberry fruit. Plackett-Burman experimental design and steepest ascent experiment were adopted to optimize process conditions. Results indicated that among the selected factors, mass concentration of mulberry fruit, temperature and pH were the most influencing parameters for the extraction of polyphenols. UAE contributed for the high yield of phenol with the mulberry fruit concentration of 2.05 g/l, temperature of 56.8 °C and pH, 8.0. Under the optimal conditions, the total phenol content (TPC) extracted from mulberry fruit can reach up to 7.32 mg GAE/g. The experimental validity illustrated good fit of the optimized parameters for the extraction of polyphenols from mulberry fruit.

Key words: Mulberry fruit, polyphenol extraction, process optimization, RSM, ultra-sonic assisted extraction.

INTRODUCTION

Mulberry fruit is a sorosis; that is composed of a collection of individual fruits (Archens) with light yellow or brown colored seeds. The ripened fruit is fat, succulent, and full of juice, varies greatly from white to black with different color shades upon ripening (Vijayan *et al.*, 2011). In China, mulberries are prolific and are grown widely in cultivation area of up to 18 million m^3 for fulfilling their multipurpose necessities (Jiang *et al.*, 2013). At present, many mulberry-growing areas in China have been focusing on enhancing fruit production and in this direction, new *Morus* species were bred to provide a rich source of phenolic compounds and naturally occurring α-glucosidase inhibitors, which may be protective against human diseases such as sore throat, fever, cancer, chronic disorder, diabetes and cardiovascular diseases (Chen *et al.*, 2011; Yu *et al.*, 2014; Park *et al.*, 2015).

In the previous attempt of our group, amino acids, proteins, total flavonoids, anthocyanins, 1-deoxynojirimycin (DNJ), and polysaccharides were identified in nine mulberry fruit varieties (Sivakumar *et al.*, 2015). Based on our results, Morus J33 variety could serve as a potential source of dietary supplements and therefore, we chose the same variety for our further experiments. In novel extraction principles, optimization of extraction conditions such as temperature, use of supercritical fluids and solvent-to-solid ratio have shown promise for optimizing the release of phenols (Pinelo *et al.*, 2006). The statistical analysis revealed that the optimised conditions such as temperature, sonication power and 4:1 (v/v) ethanol:water ratio yields high total phenolic content (Khan *et al.*, 2010). Other than extraction system involved, temperature and time are important parameters in efficient extraction of phenol content (Thoo *et al.*, 2010). To test the efficiency of solvents used to extract phenols, methanol, ethanol and acetone at three different concentrations *i.e.,* 50, 70 and 90 % were absorbed in tropical fruits (Alothman *et al.*, 2009) For recovery of phenolic compounds, considering the polarity of the extracting solvent and solubility of the compound, we chose ethanol as a solvent since it is efficient for polyphenol extraction (Lapornik *et al.*, 2005; Abozed *et al.*, 2014; Do *et al.*, 2014).

Moreover, as compared to fresh fruits, polyphenol content of dried fruits are expected to be higher due to their low moisture content. Freeze drying and water content of ethanol did not alter the phenol composition (Spigno *et al.*, 2007). The stability of dried fruits has made it possible to prolong their shelf life by preventing the development of some microorganisms responsible for deterioration of fresh fruits (Kamiloglu *et al.*, 2014). UAE offered a higher yield with a shorter time, lower temperature and lower solvent consumption; has a lower energy consumption and higher extraction yield compared with hot water extraction (Ma *et al.*, 2016).

A good extraction method aims not only in achieving a high extraction yield but also contributes to the high biological activity of the product There are four major classes of experimental design: factorial design, combined design, mixture experiments and RSM (Sohrabi and Akhlaghian, 2016) RSM is an effective technique based on sound statistical and mathematical principles and is often used for development, improvement, and optimizing of complex extraction processes and has been successfully used to determine the effects of multiple parameters on response variables and to predict their behavior under given sets of conditions (Kim *et al.*, 2005; Liu *et al.*, 2009; Sumic *et al.*, 2016). RSM is widely used in optimizing extraction process variables, such as phenolics and anthocyanins from different materials (Li *et al.*, 2012).

To determine the suitable extraction method and the best extraction conditions, we have isolated the mulberry fruit extract and optimized the extraction condition in terms of single-factor experiments, Plackett-Burman design (PBD), steepest ascent method, Box-Behnken design (BBD), and response surface methodology (RSM) analysis, in this study. The results are expected to provide a scientific basis and technical support for further study of mulberry phenol extract (MPE). The aim of this study is to provide information on the most recent developments in the field of chemical investigation of polyphenols extracted from mulberry fruit through UAE by using ethanol as suitable extraction solvent to reach good yields with natural antioxidants and to double the extraction efficiency of anthocyanin and polyphenols.

MATERIALS AND METHODS

Materials and chemicals

Ripe mulberry fruits of J33 variety were harvested from the plantation of National Mulberry Orchard (Zhenjiang, PR China). Mulberry fruit was freeze-dried (EYELA FDU-2100, Japan) and ground to powder. Ethanol with analytical grade was obtained from Sinopharm chemical reagent co. Ltd., Shanghai, China.

Preparation of standard and determination of TPC

Ten milligrams of gallic acid was dissolved in 10 ml of ethanol taken in a volumetric flask (1mg/ml). Standard solutions of different concentrations, such as 0.05, 0.1, 0.15, 0.20 and 0.25 mg/ml of gallic acid were prepared in methanol. By using Folin-ciocalteau micro method, the total phenolic content of mulberry extract was determined (Waterhouse, 1999).

Subsequently, 20 µl of extract was mixed with 1.16 ml of distilled water and incubated for 60 seconds. The mixture was blended with 100 µl of 20 % sodium carbonate solution. The mixture was incubated in a shaking incubator for 30 minutes at 40℃ and absorbance was measured at 765 nm (Mohamad *et al.*, 2013).

Plackett-Burman design

The following statistical designs were employed in the extraction of polyphenols from mulberry fruits: Plackett-

Burman design (PBD), the path of the steepest ascent experiment and Box-Behnken design (BBD).

The key feature of PBD is to search for influencing factors in a multivariable system (Feng *et al*., 2010). In this study, PBD was applied to screen the most important factors influencing the extraction of polyphenols. The experimental design (PBD) with symbol code, and level of the variables is shown in Table 1. In PBD, twelve experiments were generated with five significant variables and two blank variables at two levels, -1 for low level and +1 for high level. Each independent variable is represented in two levels, high and low, which are denoted by (+) and (-), respectively. The coded variables under investigation as well as five significant factors such as mass concentration (*A*), ultrasonic power (*B*), extraction time (*C*), pH (*D*) and extraction temperature (*E*), and two blank variables S_1 and S_2, were used in the experimental design. Polyphenol extraction was carried out in duplicate to calculate the average value as the response (in terms of activity of polyphenols). Table 2 represents the design matrix of PB experiments. In addition, 2 center points were added to the variables that could be assigned numerical values.

Table 1: Levels and codes of variables for Plackett-Burman design

Factor and dummy variable	Symbol	Coded variable level	
		-1	1
Mass concentration (g/l)	*A*	1	3
Ultrasonic power (W)	*B*	60	120
Ultrasonic time (min)	*C*	30	50
Dummy variable 1	S_1	-	-
pH value	*D*	5	7
Extraction temperature (°C)	*E*	40	60
Dummy variable 2	S_2	-	-

The path of the steepest ascent experiment

The steepest ascent experiment is known to find out the vicinity of the optimum condition quickly by increasing or decreasing the variables derived from the results of Plackett-Burman design (Gheshlaghi *et al*., 2005). The path of the steepest ascent was parallel to the normal contour line of the model response curve and passed through the center point of the PBD experiment (Thasma Raman Sivakumar *et al*., 2015). The experimental design of the path of steepest ascent is shown in Table 4.

Response surface analysis

In order to optimize main effects, interaction effects and quadratic effects of the influencing parameters to improve the extraction of polyphenol, the response surface methodology was performed with Box-Behnken design (BBD). Based on the results of the PBD and the steepest ascent experiment, three independent variables were chosen including mass concentration (0.5-2.5 g/l), temperature (40-60 ℃) and pH value (5-9), for optimization by Box-Behnken design (BBD), as shown in Table 5. The complete design consisted of 15 combinations including two replicates of the center point (Table 6). Regression analysis was performed on the data obtained from the experimental design. The response function (*Y*) was partitioned into linear, quadratic and interactive components.

The regression coefficients of individual linear, quadratic, and interaction terms were determined according to variable analysis. ($P<0.05$) is considered statistically significant by analysis of variance (ANOVA). The fitted models were subjected to the lack of fit test, which was used to determine whether the models were adequate to describe the observed data. The regression coefficients were used for statistical calculation to generate 3-D surface plots and 2-D contour plots from the fitted polynomial equation (Uluko *et al*., 2013).

Statistical analysis

All the data are presented as mean ± SD. Design-expert software 8.06 (Statease Inc., Minneapolis, USA) and MINITAB® software 16.2.0 (Inc., USA) were used for the experimental design and subsequent regression analysis of the experimental data.

RESULTS

Table 2: Results of Plackett-Burman experiment of the extraction conditions

Run	Mass concentration (g/l) (*A*)	Ultrasonic power (W) (*B*)	Ultrasonic time (min) (*C*)	Dummy variable (*S1*)	pH value (*D*)	Extraction temperature (°C) (*E*)	Dummy variable (*S2*)	Total phenol (mg/g)
1	-1	1	-1	-1	-1	1	1	5.37
2	-1	-1	-1	1	1	1	-1	3.56
3	1	1	1	-1	1	1	-1	4.73
4	1	1	-1	1	-1	-1	-1	3.63
5	1	-1	1	-1	-1	-1	1	2.78
6	1	-1	-1	-1	1	1	1	4.08
7	-1	1	1	-1	1	-1	-1	5.32
8	1	1	-1	1	1	-1	1	3.86
9	-1	1	1	1	-1	1	1	4.48
10	1	-1	1	1	-1	1	-1	3.59
11	-1	-1	-1	-1	-1	-1	-1	5.79
12	-1	-1	1	1	1	-1	1	6.03

Standard gallic acid curve

Gallic acid was used as a standard compound and the total phenols were expressed as gallic acid equivalents using the following linear equation based on the calibration curve:

$$Y=0.0648X+0.0216, R^2=0.9974;$$

where Y is the absorbance and X is the concentration as gallic acid equivalents (mg/ml).

Optimization by Plackett–Burman design

Plackett-Burman design has been widely used by many researchers as a screening method to determine the significant factors in experiment design (Xiao *et al.*, 2010). Twelve runs were carried out to analyze the effects of these seven variables (including a dummy variable) on extraction of phenol. Table 2 represents the extraction efficiency of polyphenols with the yield ranging from 2.78 to 6.03 mg/g (maximum extraction). Three variables (mass concentration, temperature and pH) were chosen for further investigation based on the high yield of polyphenols. Regression analysis determined that the variables, mass concentration (*A*) ($P=0.106$), temperature (*B*) ($P=0.636$), and pH (*C*) ($P=0.695$) having the lowest values of the tested variables could be selected for response surface optimization (Table 3). The ultrasonic power and extraction time played main roles in the extraction of polyphenol (in terms of its activity).

Table 3: ANOVA for the evaluation of the regression model in Plackett Burman design

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	7	6.649	6.649	0.94986	0.79	0.632
A	1	5.1745	5.17453	5.17453	4.31	0.106
B	1	0.2028	0.2028	0.2028	0.17	0.702
C	1	0.0341	0.03413	0.03413	0.03	0.874
S_1	1	0.7105	0.71053	0.71053	0.59	0.485
D	1	0.3136	0.31363	0.31363	0.26	0.636
E	1	0.2133	0.21333	0.21333	0.18	0.695
S_2	1	0	0.00003	0.00003	0	0.996
Residual	4	4.8009	4.8009	1.20022		
Total	11	11.4499				

Optimization by the path of the steepest ascent experiment

The method of the steepest ascent mainly focused on the high yielding region by moving through the experimental region along a path that yields increases in the response with the direction based on the sign of the coefficient (Feng *et al.*, 2010). The results of the path of the steepest ascent are shown in Table 4. The experiment run 4 showed the highest values of 5.953 mg/g among all other responses (phenol activity). All other runs exhibited decreased values. Therefore, it can be concluded that, (*A*) mass concentration of 2.0 g/l, (*B*) temperature of 55 °C and (*C*) at pH of 8, obtained in run 4 were close to the optimum point and hence, the combination of run 4 was further selected for BBD experiment.

Box-Behnken design and response-surface method

BBD is devised to evaluate the interaction influence of factors since this method has an added advantage of requiring minimum number of runs (Sohrabi and Akhlaghian, 2016). BBD is a class of rotatable second-order design based on the combination of two level factorial design and an incomplete block design. In every block, a few factors are conserved at the central value and other factors are put through each combination of factorial design. This method has been widely used in RSM to optimize various processes of various scientific origin (Sahoo and Gupta, 2012).

Three variables (mass concentration, temperature and pH) suggested from PBD and the method of the steepest ascent were used to determine the optimum levels of these parameters. The design matrix of the variables, including *a*, *b*, and *c* are the coded values of mass concentration, temperature and pH, respectively, as shown in Table 5, along with the experimental values of the response. The experiments conducted in 15 trials were used to construct a quadratic model. The results of the analysis of variance (ANOVA), goodness-of-fit and the adequacy of the models are summarized in Table 6. The phenol content varied from 5.932 to 7.145 mg/g. To determine the optimal levels of each variable for phenol activity, three-dimensional response surface plots were constructed by plotting the response (extracted phenol activity of mulberry fruit) of three independent variables (Pan *et al.*, 2010; Kong *et al.*, 2011), while maintaining other variables

Table 4: Experimental design and results of the steepest ascent experiment

Run	Mass concentration (g/l) (*a*)	Temperature (*b*)	pH value	Total phenol content (mg GAE/g)
1	0.5	40	5	4.583
2	1.0	45	6	5.858
3	1.5	50	7	5.647
4	2.0	55	8	5.953
5	2.5	60	9	5.110

Table 5: Levels and coded variables of Box-Behnken design

Variable	Coded variable	Level		
		-1	0	1
Mass concentration (g/l)	a	1.5	2.0	2.5
Temperature (°C)	b	50	55	60
pH value	c	7	8	9

Table 6: Box-Behnken design for three independent variables

Run	Mass concentration (g/l) (*a*)	Temperature (*b*)	pH value (*c*)	TPC (mg GAE/g)
1	1	0	1	6.157
2	1	-1	0	6.062
3	1	1	0	6.743
4	0	0	0	7.145
5	-1	0	1	5.932
6	1	0	-1	6.017
7	-1	-1	0	6.196
8	0	1	-1	6.487
9	0	-1	1	6.277
10	0	0	0	7.093
11	-1	1	0	6.321
12	0	-1	-1	6.077
13	0	0	0	7.024
14	0	1	1	6.382
15	-1	0	-1	6.064

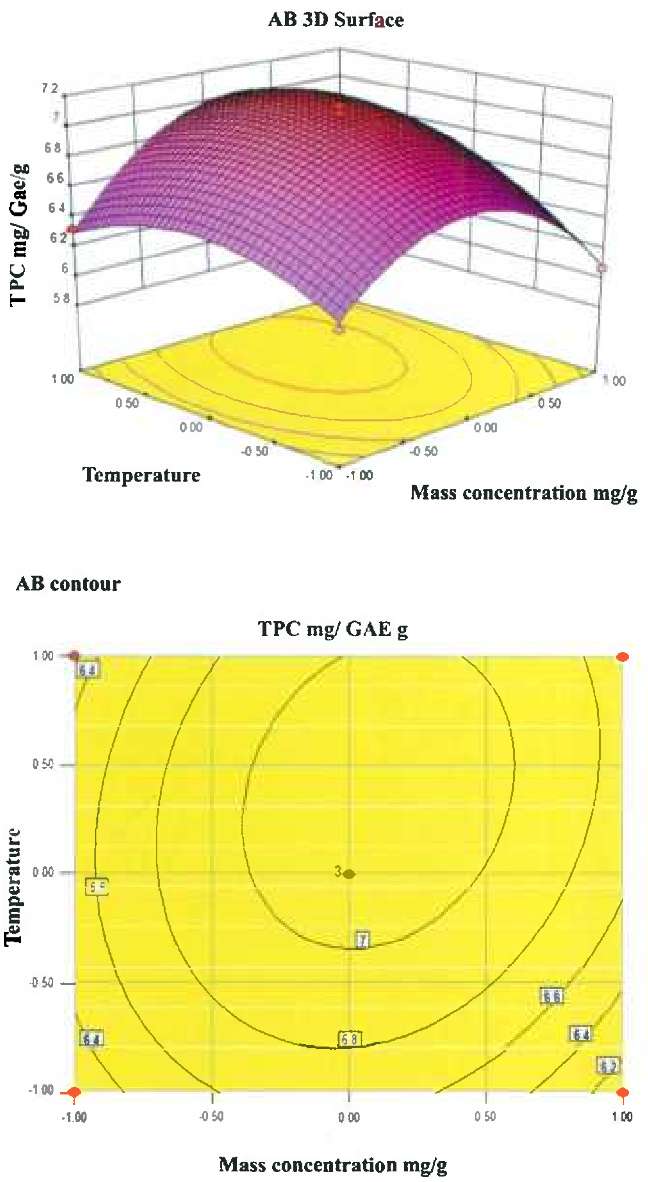

Figure 1: Three dimensional response and contour plot of TPC extraction by varying mass concentration and temperature

at their optimal levels (Figure 1). An empirical relationship between the response and the independent variables has been expressed as follows:

$$Y= 7.09 + 0.06a + 0.16b + 0.013c + 0.14ab + 0.07ac - 0.08bc - 0.51a^2 - 0.25b^2 - 0.33c^2 \quad (1)$$

Table 7: ANOVA of the response surface quadratic model

Source	SS	DF	MS	F Value	P Value Prob > F
Model	2.3489613	9	0.2609957	63.93062275	0.0001
A-A	0.0271445	1	0.0271445	6.649016734	0.0495
B-B	0.2181301	1	0.2181301	53.43074477	0.0008
C-C	0.0013261	1	0.0013261	0.324832924	0.5934
AB	0.077284	1	0.077284	18.93063454	0.0074
AC	0.018496	1	0.018496	4.530575753	0.0866
BC	0.0232563	1	0.0232563	5.696593985	0.0626
A2	0.9605262	1	0.9605262	235.279873	< 0.0001
B2	0.2248842	1	0.2248842	55.0851386	0.0007
C2	1.0560079	1	1.0560079	258.6680132	< 0.0001
Residual	0.0204124	5	0.0040825		
Lack of Fit	0.0130438	3	0.0043479	1.180109473	0.4892
Pure Error	0.0073687	2	0.0036843		
Cor Total	2.3693737	14			

Table 8: Experimental values of the responses at optimum conditions

Mass concentration (g/l)	Temperature	pH value	Total phenol content (mg GAE /g)
2.05	56.8	8.0	7.32

The regression coefficient values of Eq. (1) are listed in Table 7. The *F*- and *P*-values were used to examine the coefficient significance and the interaction strength between independent variables. The corresponding coefficient will be more significant if the absolute *F*-value becomes larger and the *P*-value becomes smaller (Guo *et al.*, 2010; Wang *et al.*, 2013). The results indicated that mass concentration, temperature and pH are found to be significantly correlated with the extraction of phenol. The ANOVA for response surface quadratic regression model showed that the model was highly significant ($P<0.001$) with a very high *F*-value (63.93). The best fitness of the regression equation was evaluated by the coefficient of correlation (R) and the coefficient of determination (R^2).

The P-value (0.0001) of the model and lack-of-fit (0.4892) represented utmost significant nature of the model, which adequately explains the existence of good relationship between the response and the significant variables. The *F*-value (1.18) and *P*-value (0.4892) of lack-of-fit indicated that there was a 23.02 % chance that

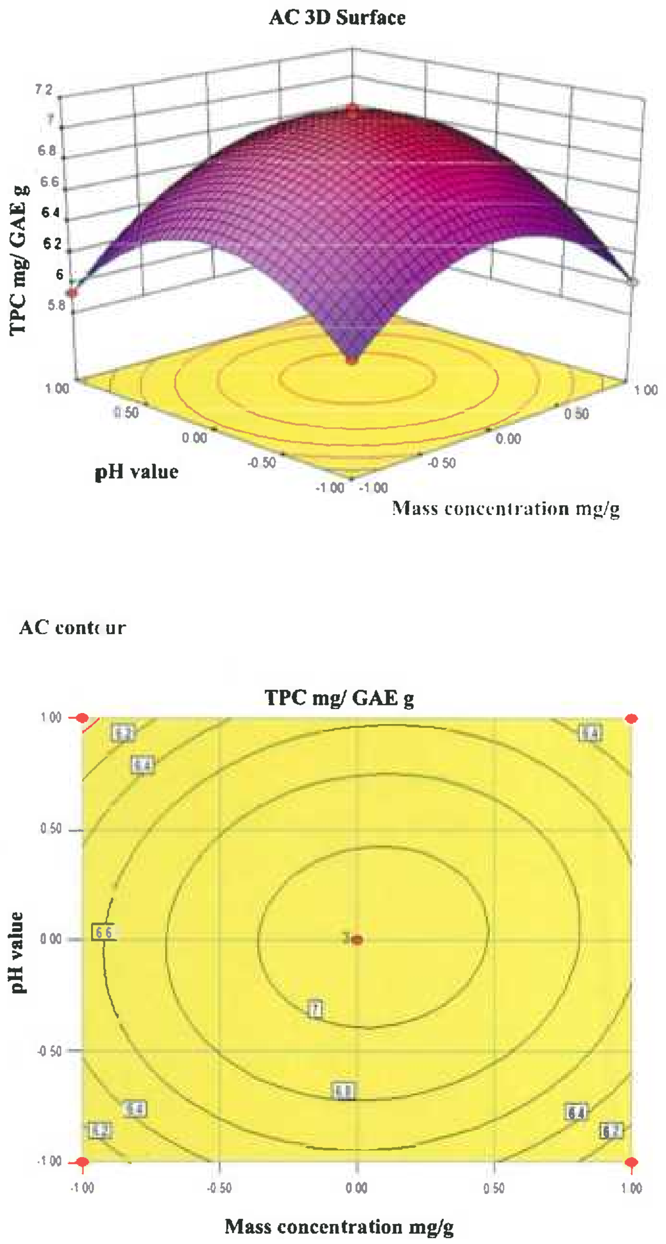

Figure 2: Three dimensional response and contour plot of TPC extraction by varying mass concentration and pH

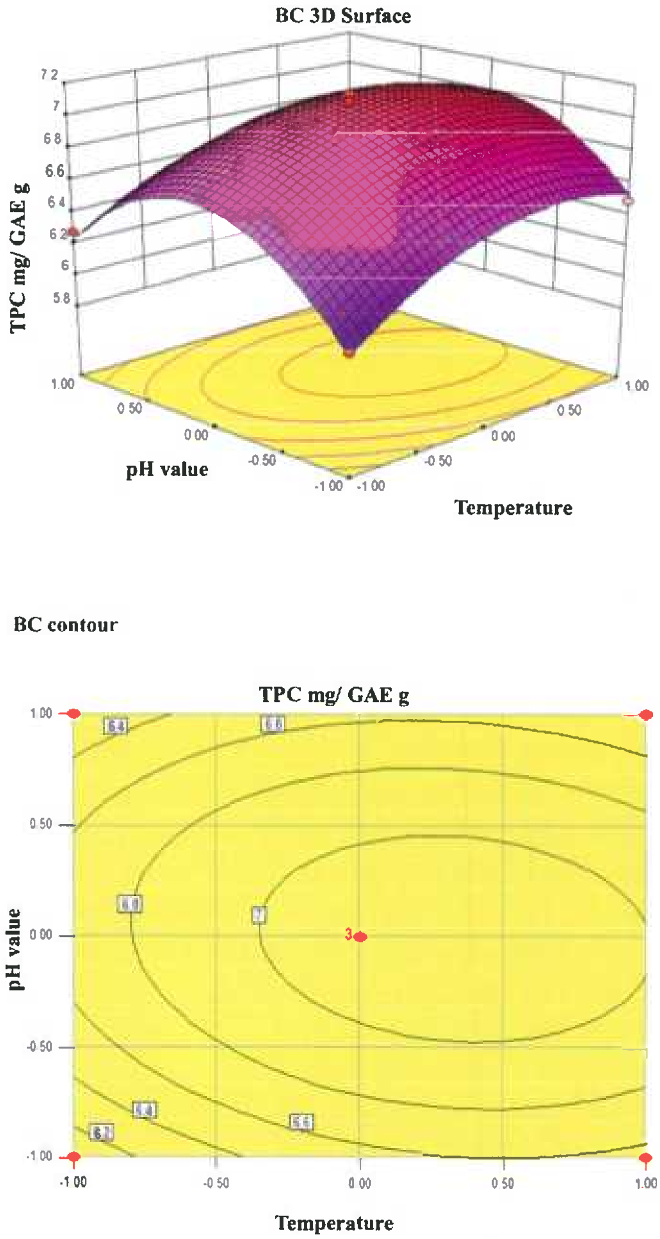

Figure 3: Three dimensional response and contour plot of TPC extraction by varying pH and temperature

the lack of fit F-value could occur due to noise (Dadzie *et al.*, 2013).

The model equation was thus appropriate to predict the yield of polyphenol extracted by any combination of values. The data showed that extracted total phenol content was found to be 7.32 (mg GAE/g) at mass concentration of 2.05 g/l, temperature of 56.8 °C and at pH, 8.0 as shown in Table 8.

Interpretation of response surface model and contour

The 3D response surface and contour plots are used to visualize the interactions between two variables with one variable kept at zero level and aid for the identification of the location of optimum experimental condition (Yolmeh *et al.*, 2014). The 3D response surfaces and contour plots (Figures 1-3) explained interactions of selected parameters on the chosen response. 3D response surface plots showed a clear peak, indicating that there is existence of optimum point inside the design boundary well (Sun *et al.*, 2010). Established by the above test regression equation (Eq. 1), the response surface opens downwards, hence there are extreme points, accessible through the extraction process of regression equations to determine the best conditions (Liu and Tang, 2010). Quadratic regression equations are first-order partial derivative score as follows:

$$0.06 + 0.14b + 0.07c - 1.02a = 0$$
$$0.16 + 0.14a - 0.08c - 0.5b = 0$$
$$0.013 + 0.07a - 0.08b - 1.06c = 0$$

Solving the above equation, the value of $a = 0.1065$, $b = 0.35097$ and $c = - 0.0072$. The maximum extraction of polyphenols from mulberry fruit sample was predicted to be 7.32 mg GAE/g at the mass concentration of 2.05 g/l, temperature of 56.8 °C and pH, 8.0. Under these optimum conditions, the predicted extraction of polyphenols in terms of its activity was reported to be 1000 %. These optimum values were verified experimentally, which resulted in 99 % feasibility of the model. The good correlation between these two results confirmed that the response model had adequately reflected the optimization process (Table 8).

DISCUSSION

For the extraction of mulberry fruit polyphenols, microwave assisted extraction greatly influenced the yield, which was reported to be of the order of 7.8 mg GAE/g in dry fruits. Varying parameters used for the same TPC extraction indicates that this was the maximum yield (Teng and Lee, 2013). Similar yield was observed in our study with UAE. Using the same RSM design, the UAE and microwave assisted extraction can complement each other with regard to the product. However, over exposure in microwave can lead to thermal degradation of polyphenol constituents (Hao *et al.*, 2002). Many efforts have been made to provide a highly sensitive and selective analytical method for the determination and characterisation of polyphenols.

Up to now, several conventional techniques have been reported for the extraction of phenols, such as hot water extraction, alkaline based extraction, resin based extraction, enzyme-assisted extraction, electron beam- and γ-irradiation-based extractions and supercritical fluid extraction. These conventional or more innovative extraction techniques may either cause the degradation of the targeted compounds due to high temperature and long duration of extraction as in solvent extractions, or pose some health-related risks due to the unawareness of safety criteria during irradiation. With the development of the "Green Chemistry" concept, environment friendly techniques are becoming increasingly attractive over the last few years.

The extraction of bioactive compounds under ultrasound irradiation is one of the upcoming extraction techniques that can offer high reproducibility in shorter times, simplified manipulation, reduced solvent consumption and temperature and lower energy input (Chemat *et al.*, 2008). During sonication, the cavitation process causes the swelling of cells or the breakdown of cell walls, which allow high diffusion rates across the cell wall. Besides maintaining the solvent, temperature and pressure, better recoveries of cell contents can be obtained by optimising ultrasound application factors including frequency, sonication power and time, as well as ultrasonic wave distribution (Wang and Weller, 2006). However, environmentally benign and non-toxic food grade organic solvents like ethanol, n-butanol and isopropanol are recommended by the US Food and Drug Administration for extraction purposes (Bimakr *et al.*, 2013).

Overall, ultrasound-assisted extraction of polyphenols from fruits, such as mulberry and extraction by use of food grade solvents has a strong potential of industrial development as an efficient and environment-friendly process for the preparation of extracts rich in natural antioxidants aimed at replacing synthetic antioxidants.

Conclusion

RSM was effective in estimating the effect of three independent variables on the extraction of total phenolic compounds from mulberry fruit. The chosen combination of the variables was reported to be effective for increasing the yield of total phenolic content. The UAE assisted extraction of polyphenols showed enhanced activity. The optimal conditions achieved in the current study could be useful for further research on mulberry phenolic compounds.

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REFERENCES

Abozed S. S., El-kalyoubi M., Abdelrashid A. and Salama M. F. (2014) Total phenolic contents and antioxidant activities of various solvent extracts from whole wheat and bran. *Ann. Agric. Sci.*, **59**: 63-67.

Alothman M., Bhat R. and Karim A. A. (2009) Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chem.*, **115 (3)**: 785-788.

Bimakr M., Abdul Rahman R., Taip F. S., Mohd Adzahan N., Sarker Z. I. and Ganjloo A. (2013) Ultrasound-assisted extraction of valuable compounds from winter melon (*Benincasa hispida*) seeds. *Int. Food Res. J.*, **20 (1)**: 331-338.

Chemat F., Tomao V. and Virot M. (2008) Ultrasound-assisted extraction in food analysis. *Handbook Food Anal. Instru.*, pp. 85-103.

Chen Z., Zhu C. and Han Z. (2011) Effects of aqueous chlorine dioxide treatment on nutritional components and shelf-life of mulberry fruit (*Morus alba* L.). *J. Biosci. Bioeng.*, **111 (6)**: 675-681.

Dadzie R. G., Ma H., Abano E. E., Qu W. and Mao S. (2013) Optimization of process conditions for production of angiotensin I-converting enzyme (ACE) inhibitory peptides from vital wheat gluten using response surface methodology. *Food Sci. Biotechnol.*, **22 (6)**: 1531-1537.

Do Q. D., Angkawijaya A. E., Tran-Nguyen P. L., Huynh L. H., Soetaredjo F. E., Ismadji S. and Ju Y. H. (2014) Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnophila aromatica. J. Food Drug Anal.*, **22**: 296-302.

Feng Y. L., Li W. Q., Wu X. Q., Cheng J. W. and Ma S. Y. (2010) Statistical optimization of media for mycelial growth and exo-polysaccharide production by *Lentinus edodes* and a kinetic model study of two growth morphologies. *Biochem. Engin. J.*, **49 (1)**: 104-112.

Gheshlaghi R., Scharer J. M., Moo-Young M. and Douglas P. L. (2005) Medium optimization for hen egg white lysozyme production by recombinant *Aspergillus niger* using statistical methods. *Biotechnol. Bioengin.*, **90 (6)**: 754-760.

Guo X., Zou X. and Sun M. (2010) Optimization of extraction process by response surface methodology and preliminary characterization of polysaccharides from *Phellinus igniarius. Carbohydr. Polym.*, **80 (2)**: 344-349.

Hao J. Y., Han W., Xue B. Y. and Deng X. (2002) Microwave-assisted extraction of artemisinin from *Artemisia annua* L. *Separat. Purif. Technol.*, **28 (3)**: 191-196.

Jiang D. Q., Guo Y., Xu D. H., Huang Y. S., Yuan K. and Lv Z. Q. (2013) Antioxidant and anti-fatigue effects of anthocyanins of mulberry juice purification (MJP) and mulberry marc purification (MMP) from different varieties mulberry fruit in China. *Food Chem. Toxicol.*, **59**: 1-7.

Kamiloglu S., Pasli A. A., Ozcelik B. and Capanoglu E. (2014) Evaluating the *in vitro* bioaccessibility of phenolics and antioxidant activity during consumption of dried fruits with nuts. *LWT-Food Sci. Technol.*, **56 (2)**: 284-289.

Khan M. K., Abert-Vian M., Fabiano-Tixier A. S., Dangles O. and Chemat F. (2010) Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus* *sinensis* L.) peel. *Food Chem.*, **119 (2)**: 851-858.

Kim S. B., Rho S. B., Rhyu D. Y. and Kim D. W. (2005) Effect of *Nelumbo nucifera* leaves on hyperlipidemic and atherosclerotic bio F1B hamster. *Kor. J. Pharmacogn.*, **36**: 229–234.

Kong Q., Chen F., Wang X., Li J., Guan B. and Lou X. (2011) Optimization of conditions for enzymatic production of ACE inhibitory peptides from collagen. *Food Bioproc. Tech.*, **4 (7)**: 1205-1211.

Lapornik B., Prošek M. and Wondra A. G. (2005) Comparison of extracts prepared from plant by-products using different solvents and extraction time. *J. Food Engin.*, **71 (2)**: 214-222.

Li X., Wang Z., Wang L., Walid E. and Zhang H. (2012) Ultrasonic-assisted extraction of polysaccharides from *Hohenbuehelia serotina* by response surface methodology. *Int. J. Biol. Macromolec.*, **51 (4)**: 523-530.

Liu J., Miao S., Wen X. and Sun Y. (2009) Optimization of polysaccharides (ABP) extraction from the fruiting bodies of *Agaricus blazei* Murill using response surface methodology (RSM). *Carbohydr. Polym.*, **78 (4)**: 704-709.

Liu R. S. and Tang Y. J. (2010) *Tuber melanosporum* fermentation medium optimization by Plackett–Burman design coupled with Draper-Lin small composite design and desirability function. *Biores. Technol.*, **101 (9)**: 3139-3146.

Ma T., Sun X., Tian C., Luo J., Zheng C. and Zhan J. (2016) Polysaccharide extraction from *Sphallerocarpus gracilis* roots by response surface methodology. *Int. J. Biol. Macromolec.*, **88**: 162-170.

Mohamad A. N., Abdullah N. and Aminudin N. (2013) Anti-angiotensin converting enzyme (ACE) proteins from mycelia of *Ganoderma lucidum* (Curtis) P. Karst. *BMC Complement. Altern. Med.*, **13**: 256.

Pan D. and Guo Y. (2010) Optimization of sour milk fermentation for the production of ACE-inhibitory peptides and purification of a novel peptide from whey protein hydrolysate. *Int. Dairy J.*, **20**: 472-479.

Park Y. S., Cvikrová M., Martincová O., Ham K. S., Kang S. G., Park Y. K. and Gorinstein S. (2015) *In vitro* antioxidative and binding properties of phenolics in traditional, citrus and exotic fruits. *Food Res. Int.*, **74**: 37-47.

Pinelo M., Arnous A. and Meyer A. S. (2006) Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends Food Sci. Technol.*, **17 (11)**: 579-590.

Sahoo C. and Gupta A. K. (2012) Optimization of photocatalytic degradation of methyl blue using silver ion doped titanium dioxide by combination of experimental design and response surface approach. *J. Hazard Mater.*, **215**: 302-310.

Sivakumar T. R., Ajaykrishna P. G. G., Fang Y., Ren Z. X., Chen C., Jin C., Jia J. Q. and Gui Z. Z. (2015) Comparative analysis of the chemical composition of different mulberry fruit varieties. *Sericologia*, **55 (1)**: 11-18.

Sohrabi S. and Akhlaghian F. (2016) Modeling and optimization of phenol degradation over copper-doped titanium dioxide photocatalyst using response surface methodology. *Process Saf. Environ. Prot.*, **99**: 120-128.

Spigno G., Tramelli L. and De Faveri D. M. (2007) Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Engin.*, **81 (1)**: 200-208.

Šumić Z., Vakula A., Tepić A., Čakarević J., Vitas J. and Pavlić B. (2016) Modeling and optimization of red currants vacuum drying process by response surface methodology (RSM). *Food Chem.*, **203**: 465-475.

Sun Y. X., Liu J. C. and Kennedy J. F. (2010) Extraction optimization of antioxidant polysaccharides from the fruiting bodies of *Chroogomphis rutilus* (Schaeff.: Fr.) O. K. Miller by Box-Behnken statistical design. *Carbohydr.*

Polym., **82**: 209-214.

Teng H. and Lee W. Y. (2013) Optimization of microwave-assisted extraction of polyphenols from mulberry fruits (*Morus alba* L.) using response surface methodology. *J. Korean Society Appl. Biolog. Chem.*, **56 (3)**: 317-324.

Thasma Raman Sivakumar, Palanigounder Ganeshan Ajay Krishna, Yin Fang, Zi-Xu Ren, Cheng Chen, Chao Jin, Jun-Qiang Jia and Zhong-Zheng Gui (2015) Process optimization for preparation of mulberry fruit extract to inhibit angiotensin converting enzyme activity. *Sericologia*, **55 (3)**: 134-143.

Thoo Y. Y., Ho S. K., Liang J. Y., Ho C. W. and Tan C. P. (2010) Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (*Morinda citrifolia*). *Food Chem.*, **120 (1)**: 290-295.

Uluko H., Li H., Cui W., Zhang S., Liu L., Chen J., Sun Y., Su Y. and Lv J. (2013) Response surface optimization of angiotensin converting enzyme inhibition of milk protein concentrate hydrolysates *in vitro* after ultrasound pretreatment. *Innov. Food Sci. Emerg. Technol.*, **20**: 133-139.

Vijayan K., Tikader A., Weiguo Z., Nair C. V., Ercisli S. and Tsou C. H. (2011) *Morus*. In: *Wild Crop Relatives: Genomic and Breeding Resources*, Springer Berlin Heidelberg, pp. 75-95.

Wang C. Y., Liu Y., Jia J., Sivakumar T. R., Fan T. and Gui Z. (2013) Optimization of fermentation process for preparation of mulberry fruit wine by response surface methodology. *Afr. J. Microbiol. Res.*, **7 (3)**: 227-236.

Wang L. and Weller C. L. (2006) Recent advances in extraction of nutraceuticals from plants. *Trends Food Sci. Technol.*, **17 (6)**: 300-312.

Waterhouse A. (1999) Folin-Ciocalteau micro method for total phenol in wine. *http://waterhouse. ucdavis. edu/phenol/folinmicro. htm* (Accessed: May, 2005).

Xiao J. H., Xiao D. M., Xiong Q., Liang Z. Q. and Zhong J. J. (2010) Nutritional requirements for the hyperproduction of bioactive exopolysaccharides by submerged fermentation of the edible medicinal fungus *Cordyceps taii*. *Biochem. Eng. J.*, **49**: 241-249.

Yolmeh M., Najafi M. B. H. and Farhoosh R. (2014) Optimization of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). *Food Chem.*, **155**: 319-324.

Yu Y., Xu Y., Wu J., Xiao G., Fu M. and Zhang Y. (2014) Effect of ultra-high pressure homogenisation processing on phenolic compounds, antioxidant capacity and anti-glucosidase of mulberry juice. *Food Chem.*, **153**: 114-120.

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Research Paper

LIPOPHORIN OF ERI SILKMOTH, *SAMIA RICINI* AND ITS IMMUNOLOGICAL PROPERTIES

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ABSTRACT

Samia ricini (Lepidoptera: Saturniidae), the Indian eri silkworm, contributes significantly to the production of commercial silk and is the only domesticated non-mulberry silkworm. In the present study, we demonstrated the immunological properties of the lipophorin (Lp) of eri silkworm and its cross-reactivity with Lps of other insects. Lp was purified by potassium bromide (KBr) density gradient ultracentrifugation and SDS-PAGE. The high density Lp (density: 1.1038 g/ml) of *S. ricini* consisted of two apolipophorins: apolipophorin I (apoLp-I) and apolipophorin II (apoLp-II) with an estimated molecular mass of 260 and 80 kDa, respectively. Specificity of the *S. ricini* HDLp polyclonal antiserum was demonstrated by western blotting as HDLp antiserum strongly reacted with apoLp-I and apoLp-II proteins. Further, apoLp-I and apoLp-II antiserums cross-reacted with apoLp-II and apoLp-I, respectively, albeit with less intensity. Cross-reactivity studies indicated that HDLp antibodies of *Bombyx mori* recognized *S. ricini* apoLp-I and apoLp-II, however, *Locusta migratoria* HDLp antibodies did not cross-react with either *S. ricini* or *B. mori* apoLp-I and apoLp-II. These results indicate that despite the sequence similarities of insect HDLps, there are immunological differences of lipophorins between distantly related species.

Key words: Cross-reactivity, high density lipophorin, *Samia ricini*.

INTRODUCTION

Lipoproteins function in the transport of lipids in vertebrates as well as invertebrates. In insects, two major lipoproteins exist in the hemolymph, lipophorin (Lp) and lipid transfer particle (LTP) in which Lp being the most abundant (Kawooya *et al*., 1984; Van der Horst, 1990; Ryan and Van der Horst, 2000). Lp delivers lipids to various organs via the hemolymph, functioning as a reusable shuttle, loading and unloading lipids into and from fat body cells (Kanost *et al*., 1990). Lp molecules are globular in shape having a lipid content of 40-50 % which comprises of diacylglycerol, cholesterol and phospholipid (Van der Horst, 1990). Based on its density, the Lp is classified as low-density Lp (LDLp), high-density Lp (HDLp) and very HDLp. The typical insect lipophorin is a high density lipophorin (HDLp, density = 1.063 to 1.210 g/ml) composed of two glycosylated apolipophorins, apolipophorin-I (apoLp-I) and II (apoLp-II), with molecular mass ranging from 212 to 275 kDa and 70 to 90 kDa, respectively (Kanost *et al*., 1990; Van der Horst *et al*., 1993; Soulages and Wells, 1994; Kim, 2005). There is also a third exchangeable protein, apolipophorin III (apoLp-III) with a molecular mass of approximately 17 kDa. During vitellogenesis, Lp delivers lipids and other yolk precursors to ovaries and in some species, Lp itself takes part of the egg yolk platelets (Van der Horst *et al*.,1993; Sun *et al*., 2000; Swevers *et al*., 2005). Lp incorporates into the cells through receptor-mediated endocytosis and the lipophorin receptor (LpR) is functionally characterized as members of the low-density lipoprotein receptor gene superfamily in a number of insects (Tufail and Takeda, 2009) including silkworm, *Bombyx mori* (Gopalapillai *et al*., 2006).

The eri silkworm, *Samia ricini*, is an economically important insect being commercially exploited for the silk production. It also offers a great scope for silk biomaterial studies. Since very little is known about the immunological properties of insect lipophorins, we undertook the present study to examine the biochemical and immunological properties of HDLp of eri silkworm.

MATERIALS AND METHODS

Silkworms

S. ricini and *B. mori* (Pure Mysore race) were maintained in the laboratory and fed on castor and mulberry leaves, respectively, under standard rearing conditions.

Lp collection

Lp was purified as described earlier (Shapiro *et al.*, 1984; Ravikumar *et al.*, 2011). Briefly, hemolymph from fifth instar larvae of *S. ricini* and *B. mori* (day 5) were collected in PBS (pH 7.4). Hemocytes were removed by centrifugation at 20,000 × g for five minutes. Potassium bromide (KBr, 0.44 g/ml) was added to the supernatant, overlaid with 0.9 % NaCl and centrifuged at 50,000 rpm (Beckman 70.1 TI for 16 hours at 4° C). HDLp, which formed a clear yellow band, was collected, desalted, and used for the assays. The density of Lp was determined from the refractive index of KBr following density gradient centrifugation. Protein estimation was performed with the BCA protein assay kit using BSA as standard. Total lipid was estimated by Sulfo-phospho-vanillin method (Frings *et al.*, 1972). Hemolymph samples were mixed with concentrated sulfuric acid, boiled and cooled. Phospho-vanillin reagent was added and after incubation, absorbance was measured at 540 nm using olive oil as standard. The percentage of lipid was then calculated by dividing the weight of the lipids by the weight of the lipids plus the weight of the protein and multiplying by 100.

Purification and production of polyclonal antibodies of HDLp

The purified Lp was confirmed by 7.5 % SDS-PAGE and polyclonal antibodies (apoLp-I and apoLp-II) were produced in rabbits by Bhat Biotech India Pvt. Ltd., Polyclonal antibodies of *B. mori* and *L. migratoria* HDLps were kindly gifted by Dr. Kozo Tsuchida, Japan and Professor Dick J. Vander Horst, The Netherlands.

Immunoblotting

Extracted proteins were separated on 7.5 % SDS-PAGE and electrophoretically transferred using a TE 77 Semi dry transfer unit (Amersham) to polyvinylidene difluoride membranes (Hybond, Amersham). Blots were probed with rabbit anti-Sr/Bm/Lm HDLps antibodies. Bound antibodies were detected with alkaline phosphatase conjugated goat anti-rabbit IgG and 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (BCIP/NBT, Roche).

RESULTS AND DISCUSSION

Lp was purified by KBr density gradient ultracentrifugation and confirmed by SDS-PAGE. The Lp formed a clear yellow band on the top after ultracentrifugation (Figure 1A). The density was found to be 1.1038 g/ml for SrLp categorising it as HDLp comprising of 49 % of lipids and 51 % of protein. Generally, the density of Lp ranges from 1.063 to 1.210g/ml, containingh up to 40 – 50 % lipids (Chino, 1985; Van der Horst *et al.*, 1993; Ryan and Van der Horst, 2000). Further, SrHDLp consisted of two apolipophorins, apoLp-I and apoLp-II with apparent molecular mass of 260 and 80 kDa, respectively (Figure 1B) which is in the same range as that of the molecular mass of HDLps of other insects. The molecular mass of apoLp-I ranges from 212 to 275 kDa and that of apoLp-II from 70 to 90 kDa; *B. mori* - 250 and 90 kDa (Kim and Kim, 1994), *Manduca sexta* - 245 and 78 kDa (Shapiro *et al.*,1984), *L. migratoria* - 220 and 72 kDa (Weers *et al.*, 1993), *Hyphantria cunea* - 230 and 80 kDa (Yun and Kim,1993), *Spodoptera litura* - 235 and 79 kDa (Jeon *et al.*, 1997), *Drosophila*

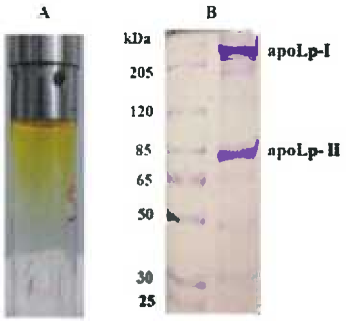

Figure 1: HDLp of *Samia ricini*

A: HDLp formed as a yellow band at the top after density gradient KBr ultracentrifugation; B: Purified HDLp protein (25 µg) analyzed on a 7.5 % SDS-PAGE showing apoLp-I and apoLp-II. Protein size markers are shown on the left.

melanogaster - 275 and 76 kDa (Fernando-Warnakulasuriya and Wells, 1988), *Aedes aegypti* - 240 and 70 kDa, (Capurro *et al.*, 1994) and *Blattella germanica* - 212 and 80 kDa, respectively (Sevala *et al.*, 1999). However, in *Lymantria dispar*, the molecular mass of apoLp-I and apoLp-II is 230 and 49 kDa, respectively (Ryu and Kim, 1991).

The specificity of the polyclonal antiserum raised against SrHDLp showed strong reactions with apoLp-I and apoLp-II whereas, no reaction was observed in the control where secondary antibody was omitted (Figure 2A and B). Further, anti-apoLp-I reacted with apoLp-II and anti-apoLp-II reacted with apoLp-I, albeit with lesser intensity, showing reactivity between apoLp-I and apoLp-II of eri silkworm (Figure 2C and D). Subsequently, we studied the immunological properties of SrHDLp as against a close and a distant species, *i. e.*, *B. mori* and *L. migratoria*, respectively. The cross-reactivity experiments indicated that anti-BmHDLp reacted with SrapoLp-I and apoLp-II and likewise, anti-SrHDLp reacted with BmapoLp-I and apoLp-II (Figure 2E and F). However, anti-LmHDLp did not recognize both *S.ricini* and *B. mori* apoLp-I and apoLp-II (Figure 2G and H). These observations indicate that SrLp has biochemical and immunological homogeneity with closely related species. This was further supported by the binding studies of the lipophorin receptor (LpR) of *S. ricini* in which SrLpR not only binds to SrLp but also to BmLp (Bala *et al.*, 2016).

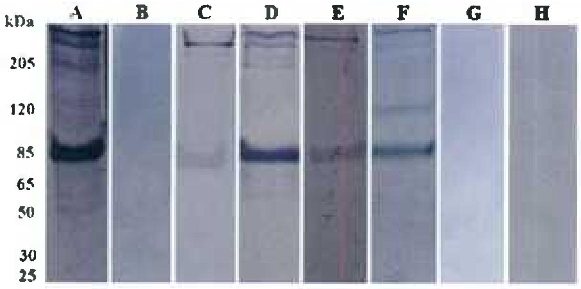

Figure 2: Immunoblot analysis of purified HDLp

Proteins were transferred electrophoretically to PVDF, incubated with A: anti-SrHDLp, B: anti-SrHDLp without secondary antibody as negative control, C: anti- SrapoLp-I, D: anti- SrapoLp-II, E: anti-BmHDLp, G: anti-LmHDLp, F: BmHDLp proteins incubated with anti- SrHDLp; and H: anti-LmHDLp. Protein size markers are shown on the left.

Our findings are in agreement with some studies showing antigenic cross-reactivity of HDLp within related species, but no cross-reactivity among species of different orders. The immunoblotting data showed that *B. mori* HDLp is related to *H. cunea* Lp, but not with those of *Periplaneta americana* (Blatteria) and *Lucilia illustris* (Diptera) Similarly, anti-*S. litura* HDLp antiserum recognized Lp of other lepidopteran insects (Yun *et al.*, 1996). Interestingly, anti-*M. sexta* apoLp-I showed no cross-reactivity with the honeybee apoLp-I and those of other six insect species of different orders, whereas, anti-*M. sexta* apoLp-II cross-reacted with apoLp-II of honeybee and those of other insects (Ryan *et al.*, 1984; Robbs *et al.*, 1985). Immunological similarity of *B. germanica* HDLp to lipophorins from other cockroaches showed cross-reactivity in the same family but not with different family of the same order, Blattodea (Sevala *et al.*, 1999). Anti-locust HDLp cross-reacts neither with cockroach, *P. Americana* nor with silkworm, *B. mori* HDLps, indicating high immunological species specificity (Chino and Kitazawa, 1981). These conflicting reports make it difficult to generalize the cross-reactivity of HDLps among insect species. Interestingly, apoLp-I and apoLp-II are two subunits of Lp and encoded by a single gene (Babin *et al.*, 1999). Unlike apoLp-III, which is a small protein, the amino acid sequences of apoLp-I and II are not available for comparison in most insects. The evolutionary conservation and immunological and biochemical properties of HDLp among insects need to be supported by amino acid sequence data of apolipophorins and their comparisons.

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REFERENCES

Babin P. J., Bogerd J., Kooiman F. P., VanMarrewijik W. J. andVanderHorst D. J. (1999) Apolipophorin II/I, apolipoprotein B, vitellogenin, and microsomal triglyceride transfer protein genes are derived from a common ancestor. *J. Mol. Evol.*, **49**:150–160.

Bala R., Saba U., Varma M., Thomas D. S., Sinha D. K., Rao G., Trivedy K., Kunjupillai V. and Gopalapillai R. (2016) Cloning and functional characterization of a vertebrate low-density lipoprotein receptor homolog from eri silkmoth, *Samia ricini. J. Mol. Biochem.* (Accepted).

Capurro Mde L., de Bianchi A. G. and Marinotti O. (1994) *Aedes aegypti* lipophorin. *Comp. Biochem. Physiol. Biochem. Mol. Biol.*, **108**: 35-39.

Chino H. (1985) Lipid transport: biochemistry of hemolymph lipophorin. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 10, Kerkut G. A. and Gilbert L. I. (Eds.), Pergamon Press, New York, pp. 115–135.

Chino H. and Kitazawa K. (1981) Diacylglycerol-carrying lipoprotein of hemolymph of the locust and some insects. *J. Lipid Res.*, **22**: 1042-1052.

Fernando-Warnakulasuriya G. J. P. and Wells M. A. (1988) Isolation and characterization of lipophorin from *Drosophila melanogaster* larvae. *Arch. Insect Biochem. Physiol.*, **8**: 243-248.

Frings C. S., Fendley T. W., Dunn R. T. and Queen C. A. (1972) Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction. *Clin. Chem.*, **18**: 673-674.

Gopalapillai R., Kadono-Okuda K., Tsuchida K., Yamamoto K., Nohata J., Ajimura M. and Mita K. (2006) Lipophorin receptor of *Bombyx mori*: cDNA cloning, genomic structure, alternative splicing, and isolation of a new isoform. *J. Lipid Res.*, **47**: 1005-1013.

Jeon B. H., Kim E. S., Choi C. S., Yun H. K. and Kim H. R. (1997) Identification and characterization of lipophorin receptor in ovarial membrane of the common cutworm, *Spodoptera litura. Korean J. Entomol.*, **27**: 153-162.

Kanost M. R., Kawooya J. K., Law J. H., Ryan R. O., Van Heusden M. C. and Zeigler R. (1990) Insect hemolymph proteins. *Adv. Insect Physiol.*, **22**: 299-396.

Kawooya J. K., Keim P. S., Ryan R. O., Shapiro J. P., Samaraweera P. and Law J. H. (1984) Insect apolipophorin: Purification and properties. *J. Biol. Chem.*, **259**: 10733-10737.

Kim B. S. and Kim H. R. (1994) Purification and characteristics of lipophorin in *Bombyx mori. Korean J. Zool.*, **7**: 76-87.

Kim H. R. (2005) Lipophorin and its Receptor in Lepidoptera. *Entomol. Res.*, **35**: 1-8.

Ravikumar G., Vardhana K. V. and Basavaraja H. K. (2011) Characterization of lipophorin receptor (LpR) mediating the binding of high density lipophorin (HDLp) in the silkworm, *Bombyx mori. J. Insect Sci.*, **11**: 150-158.

Robbs S. L., Ryan R. O., Schmidt J. O., Keim P. S. and Law J. H. (1985) Lipophorin of the larval honeybee, *Apis mellifera. J. Lipid Res.*, **26**: 241-247.

Ryan R. O., Schmidt J. O. and Law J. H. (1984) Chemical and immunological properties of lipophorins from seven insect orders. *Arch. Insect Biochem. Physiol.*, **1**: 375-383.

Ryan R. O. and Van der Horst D. J. (2000) Lipid transport biochemistry and its role in energy production. *Annu. Rev. Entomol.*, **45**: 233-260.

Ryu J. K. and Kim H. R. (1991) Isolation and characterization of lipophorin from *Lymantria dispar*. *Korean J. Zool.*, **34** 1-7.

Sevala V., Shu S., Ramaswamy S. B. and Schal C. (1999) Lipophorin of female *Blattella germanica*: characterization and relation to hemolymph titers of juvenile hormone and hydrocarbons. *J. Insect Physiol.*, **45**: 431-441.

Shapiro J. P., Keim P. S. and Law J. H. (1984) Structural studies on lipophorin, an insect lipoprotein. *J. Biol. Chem.*, **259**: 3680-3685.

Soulages J. L. and Wells M. A. (1994) Lipophorin: the structure of an insect lipoprotein and its role in lipid transport in insects. *Adv. Protein Chem.*, **45**: 371-415.

Sun J., Hiraoka T., Dittmer N. T., Cho K. H. and Raikhel A. S. (2000) Lipophorin as a yolk protein precursor in the mosquito, *Aedes aegypti*. *Insect Biochem. Mol. Biol.*, **30**: 1161–1171.

Swevers L., Raikhel A. S., Sappington T. W., Shirk P. and Iatrou K. (2005) Vitellogenesis and post-vitellogenic maturation of the insect ovarian follicle. In: *Comprehensive molecular insect science,* Volume 1, Gilbert L. I., Iatrou K. and Gill S. S., (Eds.), Elsevier, Amsterdam, pp. 87-156.

Tufail M. and Takeda M. (2009) Insect vitellogenin/lipophorin receptors: Molecular structures, role in oogenesis, and regulatory mechanisms. *J. Insect Physiol.*, **55**: 87-103.

Van der Horst D. J. (1990) Lipid transport function of lipoproteins in flying insects. *Biochimica et Biophysica Acta*, **1047**: 195-211.

Van der Horst D. J., Weers P. M. and Van Marrewijk W. J. A. (1993) Lipoproteins and lipid transport. In: *Insect lipids: Chemistry, Biochemsitry and Biology*, D. W. Stanley-Samuleson and D. R. Nelson (Eds.), University of Nebraska Press, Lincoln, pp. 1-24.

Weers P. M. M., Van Marrewijk W. J. A., Beenakkers A. M. T. and Van der Horst D. J. (1993) Biosynthesis of locust lipophorin: Apolipophorins I and II originate from a common precursor. *J. Biol. Chem.*, **268**: 4300-4303.

Yun H. K. and Kim H. R. (1993) Characterization of lipophorin from hemolymph of fall webworm, *Hyphantria cunea*. *Korean J. Zool.*, **36**: 231-237.

Yun H. K., Park C. H. and Kim H. R. (1996) Characterization and biosynthesis of lipophorin in *Hyphantria cunea*. *Korean J. Entomol.*, **26**: 385-392.

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Research Paper

A NOVEL METHOD FOR MEASUREMENT OF PROTEIN OF CASTOR LEAVES USING OPTOELECTRONIC SENSOR SYSTEM

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ABSTRACT

Castor leaves enriched with protein are the primary feed of eri silkworms. This protein resource forms the basis of secretion of superior quality silk. Proteins are normally analyzed by the classical Spectrophotometric method. In this paper, an alternative to Spectrophotometer is proposed by using optical sensing systems based on cheaper and faster sources and detectors for estimation of protein content in castor leaves. Castor leaves of four different stages collected from a flowering plant were used for estimation. The highest protein content was reported for the second leaf from the tip of the plant whereas, the least amount of protein was recorded for the youngest leaf which has the highest water content. The optical sensor system using both the coherent and non-coherent sources gave similar results as that of the Spectrophotometric method whereas, the system with the coherent source was more sensitive to variations compared to the non-coherent optical sensor system.

Key words: Castor, optical sensor system, protein estimation, Spectrophotometric method.

INTRODUCTION

There are numerous methods by which protein can be extracted, quantified and analyzed using Spectrophotometer (McDonagh *et al.*, 2008; Memon *et al.*, 2010; Talreja, 2011; Munoz-Huerta *et al.*, 2013). One such common photometric method for determination of protein concentration is the Lowry (1951)method. Apart from the Spectrophotometric analysis, there are other optical sensor based system based on the absorption spectrum of the sample and evanescent phenomenon (McDonagh *et al.*, 2008) and reflectance from the sample (Kaffka *et al.*, 1982; Long *et al.*, 2008; Xiao-li and Yong He, 2008). Different types of optical sources are used for this but being uneconomical, it increases the overall cost of the system. This necessitates the development of a cheaper sensor system which can analyze a particular type of analyte. In this work, two different sources have been chosen which are cheaper and easily available for determination of protein concentration in castor leaves.

Optical sensor based system is a preferred method for determination of chemical analyte in a solution as light does not directly come in contact with the molecules present in the chemical. One of the ways of optical sensing is by measuring the change in the optical properties, such as absorption, transmittance or reflectance of the sample solution when it interacts with light. The change in the optical property of the intermediary material is largely dependent on the wavelength of the light. In this paper, variation in the absorbance with variation of protein concentration in a solution was studied. This was carried out with different light sources for varying wavelengths using Spectrophotometer (660, 650 and 633 nm) and optical sensor system (650 and 633 nm).

Experimental set-up for determination of protein in castor leaves using optoelectronic sensor system

In this experiment, two optical modules were used to study the spectral interaction with the chemical analyte. The first optical module was set up by using a commercially available Laser source of 632.8 nm wavelength, a 10X lens and a pin photodiode as the detector as shown in Figure 1. The distance between the source and the detector was maintained at 13 cm and a 1cm quartz cuvette was placed in the middle. Since the distance between the source and detector is more, a lens was placed in front of the source so that the light is fully focused on the solution. The separation was kept more as laser light is coherent in nature and it may affect the molecular structure of the solution of concern if it is placed too near to the source.

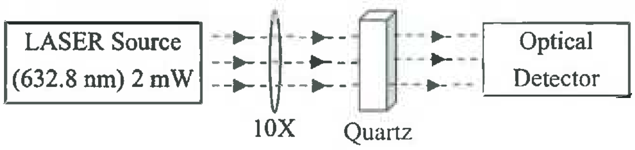


Figure1: Schematic diagram of the optical module-I

The other optical system was framed by using commercially available LED source of 650 nm wavelength and a phototransistor as the detector. In this case, the distance between the source and the detector was kept at 3 cm and a similar cuvette was used to hold the chemical whereas, the light was not focused on the cuvette using the lens as the detector already has an inbuilt lens in it for focusing. Since the LED is of non-coherent light source, the distance between the source and the detector is kept less so that the light does not get lost with larger distance. The detector output for both the systems was measured using a voltmeter. The phototransistor was used in this as the spectral line width of a LED is larger than a LASER source for which higher sensitivity is required in detection and this is fulfilled by a phototransistor rather than a photodiode. Moreover, a drive circuit to control the flow of current through the LED was implemented in this module. The output voltage was recorded across the collector-emitter junction of the phototransistor. The block diagram of optical sensor of module II is as shown in Figure 2. Figure 3 shows the circuit diagram of the optical sensor.

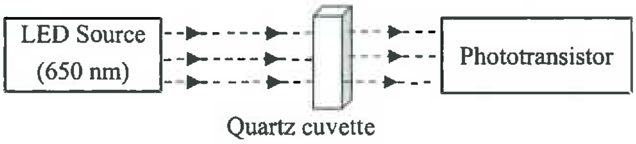


Figure 2: Schematic diagram of the optical module-II

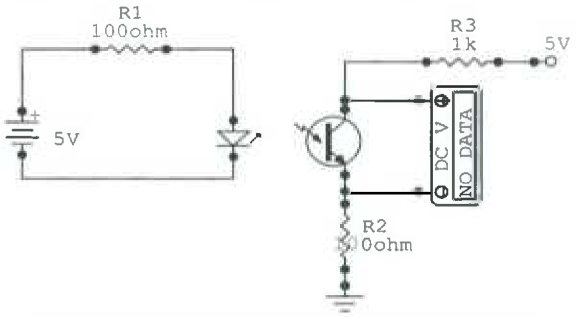


Figure 3: Circuit diagram of the optical sensor

The output of both the optical modules was revealed in a digital display using microcontroller 8051. Since the microcontroller cannot read the analog signal generated by the sensor system, the output was first amplified and then converted to digital signal using an analog-to-digital converter. The block diagram of the system is as shown in Figure 4. The exact value of the protein is not displayed but a qualitative status, such as less, moderate and high concentration is shown.

The absorbance of the solution was calculated from the transmittance of the solution in terms of volts and is given by:

T = Io/I, where Io is the transmittance of the sample solution and I is the transmittance of the reference solution. This value of transmittance was then used to estimate the absorbance using the expression, A = log (1/T).

Experimental procedure

The experiment was carried out in two stages – firstly the sample preparation following the steps of Folin-Lowry method and then photometric analysis using three different light sources.

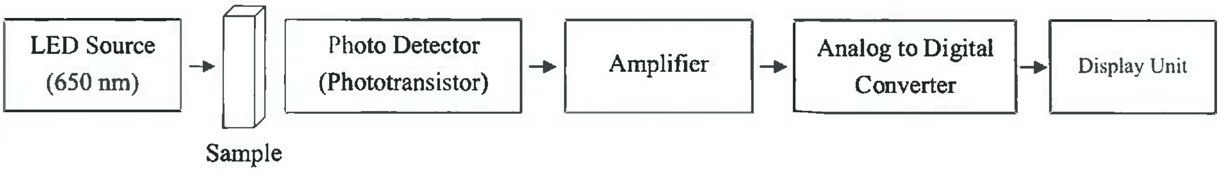


Figure 4: Schematic diagram of the complete optical module

a) Sample collection

Leaves from castor plant that grow on the roadside were gathered. Leaves of four different growth stages from the same plant *i.e.,* the topmost leaf (sample 1), second (sample 2), third (sample 3) and fifth (sample 4) leaf from top were collected and denoted as S1, S2, S3 and S4, respectively.

b) Sample preparation for protein extraction

The phosphate buffer (0.1 M, pH-7.6) used for extraction of protein was prepared by mixing 5.4 ml of acid solution with 44.6 ml of base solution. The 0.2 M monobasic sodium phosphate was prepared by dissolving 1.38 g of NaH_2PO_4 in distilled water, and the volume was adjusted to 50 ml in volumetric flask. The 0.2 M dibasic sodium phosphate was made by adding 1.42 g of Na_2HPO_4 (anhydrous) to distilled water in a volumetric flask and adjusting the volume to 50 ml. Alkaline reagent was prepared by dissolving 2 g of Na_2CO_3 and 0.4 g of NaOH in distilled water and the volume was adjusted to 100 ml. For preparation of copper sulphate reagent, 0.5 g of $CuSO_4$ was added to 1 g of sodium potassium tartarate, dissolved in distilled water and volume adjusted to 100 ml. The copper sulphate was made alkaline by mixing 1 ml of copper sulphate reagent with 50 ml of alkaline carbonate reagent.

For extraction of protein from the leaves, 3.052 g of sample 1, 3.11 g of sample 2, 2.95 g of sample 3 and 3.77 g of sample 4 were taken and ground in a mortar, separately using buffer solution corresponding to the weight of the samples (*i.e.,* 3.052, 3.11, 2.95 and 3.77 ml buffer solution, respectively). The prepared samples were kept overnight for complete extraction. The next day, the extract was separated from the paste using a very fine piece of cotton cloth. The pellet was discarded and the extract was used for chemical analysis.

Lowry method

For preparation of the experimental solution, 1 ml of sample was mixed with 5 ml of alkaline copper sulphate reagent. This mixture was allowed to stand for 10 minutes and then 0.5 ml of Folin's reagent was added to it. After this, the solution was kept in a dark chamber for 30 minutes for developing the colour. It was then used to record absorbance in Spectrophotometer at 660, 650 and 633 nm. The absorbance of the solution was also recorded by using the two optical sensor systems that used Laser (632.8 nm) and LED (650 nm) light sources. To make a comparative study with a standard solution, Bovine Serum Albumin (BSA) which is a standard protein concentrate was used. The standard protein solution was made by dissolving 50 mg of BSA in 50 ml of distilled water. Subsequently, five different samples were prepared by taking 2, 4, 6, 8 and 10 ml of the BSA solution and by following the same steps as mentioned above to make the desired solution for Lowry method by using copper sulphate and Folin reagents. The absorbance values were recorded for these solutions by using all three methods. 0.1 N NaOH was used as reference solution for the Spectrophotometer as well as both the sensor systems.

RESULTS AND DISCUSSION

The protein content of the leaf samples was determined using the methods described above. The differences in sensitivity at different wavelengths for different concentrations of BSA in Spectrophotometer were found very less. Figure 5 depicts the variations in absorbance for different concentrations of BSA solution at 660, 650 and 633 nm.

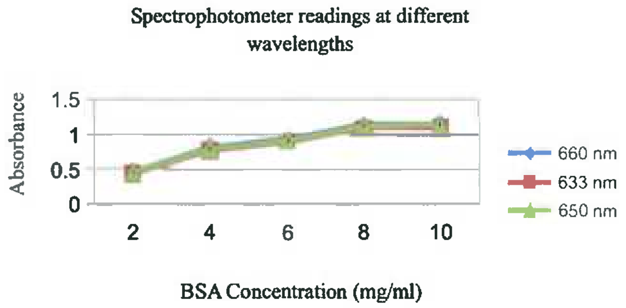


Figure 5: Absorbance for BSA solution at different wavelengths

Using first order curve fitting technique, the absorbance of the light wave (with different wave lengths) was related to the different concentrations of BSA (x) as following.

$F(x)\ 660\ \text{nm} = 0.17193x + 0.37925$
$F(x)\ 650\ \text{nm} = 0.16898x + 0.37196$
$F(x)\ 633\ \text{nm} = 0.1657x + 0.35943$

Figure 6 represents the variations in absorbance for different concentrations of BSA solution at 633 and 632.8 nm. The relationship between the absorbance of the light wave (633 and 632.8 nm) and the different concentrations of BSA (x) worked out using first order curve fitting technique are also plotted in the Figure.

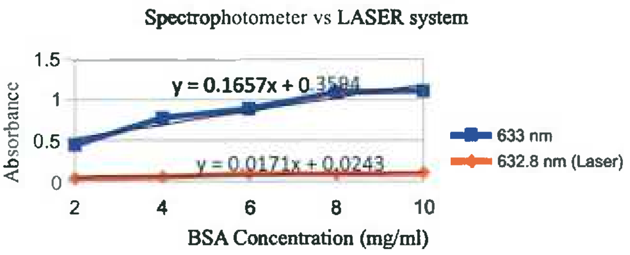


Figure 6: Absorbance for BSA solution at 633 nm (Spectrophotometer) and 632.8 nm (Optical module-I)

Figure 7 presents the variations in absorbance for different concentrations of BSA solution at 650 nm for spectrophotometer and LED. Using first order curve fitting technique, the absorbance of the light wave [650 nm (spectrophotometer) and 650 nm (LED)] was related to the different concentrations of BSA (x) and also shown in the Figure.

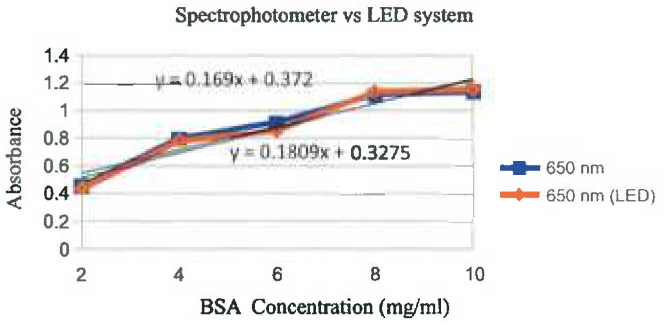


Figure 7: Absorbance for BSA solution at 650 nm (Spectrophotometer and Optical module-II)

It is seen that at these wavelengths, the absorbance increased with the increase in the protein concentration. Moreover, it is observed that the LED system gave better results when compared with the Spectrophotometric readings whereas, for LASER based system, the sensitivity was observed to be quite less. Based on this established fact, the same process was repeated for the leaf samples to estimate the protein content using Spectrophotometer and the data recorded at the three wavelengths are shown in Figures 8-10, respectively.

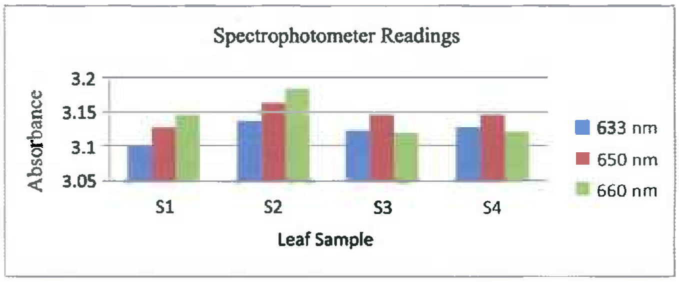
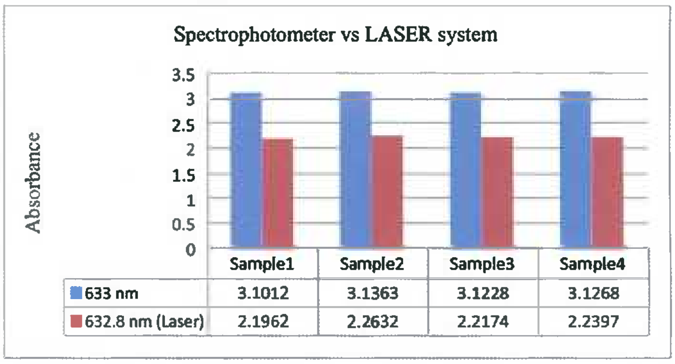


Figure 8: Absorbance for the four leaf samples at 660, 650 and 633 nm



	Sample1	Sample2	Sample3	Sample4
🟦 633 nm	3.1012	3.1363	3.1228	3.1268
🟥 632.8 nm (Laser)	2.1962	2.2632	2.2174	2.2397

Figure 9: Absorbance for four leaf samples at 633 nm (Spectrophotometer) and 632.8 nm (Optical module-I)

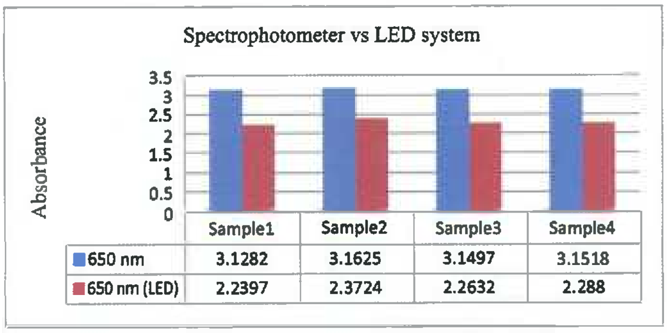


Figure 10: Absorbance for four leaf samples at 650 nm (Spectrophotometer and Optical module-II)

Sample 2 registered the maximum protein content as based on maximum absorbance at the three different wavelengths. The concentration decreased from sample 3 to sample 4 and sample 1 had the least protein concentration. The same was repeated using the two optical modules which showed the same trend as that of the Spectrophotometer. The process was carried out for both the BSA solution and castor leaf sample solutions and in all cases, it revealed the same trend of variations.

Conclusion

From the analysis of the results, it is quite evident that sensor systems can be used to quantify the protein concentration and hence can replace Spectrophotometer. These systems are simpler and cost effective, rather the LED system can be built by investing a few hundreds of rupees. The systems have the limitation that these can be used for specific analysis only whereas, the Spectrophotometer can be used for multiple purposes. This limitation can in fact be cashed on by opting for the optical sensor system where the purpose is single point such as estimation of protein rather than opting for the costlier, multipurpose Spectrophotometer which is more useful in the laboratory rather than in *in-situ* conditions.

REFERENCES

Kaffka K. J., Norris K. H., Kulcsár F. and Draskovits I. (1982) Attempts to determine fat, protein and carbohydrate content in cocoa powder by the Nir technique. *ActaAlimentaria*, **11 (3)**: 271-288.

Long D. S., Engel R. E. and Siemens M. C. (2008) Measuring grain protein concentration with In-line Near Infrared Reflectance spectroscopy. *Agron. J.*, **100 (2)**: 247-252.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193 (1)**: 265-275.

McDonagh C., Burke C. S. and MacCraith B. D. (2008) Optical chemical sensors. *Chem. Rev.*, **108**: 400-422.

Memon S., Memon A. and Arain B. A. (2010) Analysis of protein by spectrophotometric and computer colour based intensity method from stem pea (*Pisum sativum*) at different stages. *Pak. J. Anal. Environ. Chem.*, **11(2)**: 63 - 71.

Muñoz-Huerta Rafael F., Guevara-Gonzalez Ramon G., Contreras-Medina Luis M., Irineo Torres-Pacheco, Juan Prado-Olivarez and Ocampo-Velazquez Rosalia V. (2013) A review of methods for sensing the Nitrogen status in plants: Advantages, disadvantages and recent advances. *Sensors (Basel)*, **13 (8):** 10823–10843.

Talreja T. (2011) Biochemical estimation of three primary metabolites from medicinally important plant *Moringa oleifera. Int. J. Pharm. Sci. Rev. Res.*, **7 (2)**: 186-188.

Xiao-li Li and Yong He (2008) Chlorophyll assessment and sensitive wavelength exploration for tea (*Camellia sinensis*) based on Reflectance Spectral characteristics. *HORTSCIENCE*, **43(5)**: 1586–1591.

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Research Paper

LYSINIBACILLUS SPHAERICUS INFECTION IN MUGA SILKWORM (*ANTHERAEA ASSAMENSIS* HELFER, LEPIDOPTERA: SATURNIIDAE): A NEW REPORT

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ABSTRACT

In an experimental probe to detect the pathogenic bacteria affecting muga silkworm, a new strain was isolated and identified as *Lysinibacillus sphaericus* SIVAAB-13. It formed spores and the infection caused mortality of muga silkworm larvae. Identification was based on the biochemical characteristics and 16S rDNA sequences analysis which revealed similarity with the reference strain JUN-6. *L. sphaericus* was detected for the first time as pathogenic bacteria in the intestine of muga silkworm.

Key words: 16S rDNA, biochemical characteristics, *Lysinibacillus sphaericus,* muga silkworm.

INTRODUCTION

The muga silkworm, *Antheraea assamensis* Helfer, is a Lepidopteran insect with high economic value and has been domesticated for golden yellow silk production since long. In Southeast Asia, sericulture is an important component of agriculture (Gheysens *et al*., 2011). Due to rising worldwide demand of the vanya silk products, eco-friendly ways of production and processing, women participation, and promoted commercial exploitation, this craft has the potential for growth as an emerging industry. *A. assamensis* and its related *Antheraea* spp. are reported throughout Northeast India and the Sub-Himalayan foot hills, mostly confined to the Brahmaputra valley of Assam (Kakati and Chutia, 2009). Muga silkworm consists of a single race in nature, but shows larval colour polymorphism (Neog *et al*., 2010). Rearing is mostly practiced outdoor and hence, the silkworms are exposed to fluctuating environmental conditions with profound impact on production and productivity during different seasons. Silk production has been fluctuating every year due to insufficiency of quality seed (eggs) and other inherent constraints. Though, India is the second largest producer in silk sector, a wide gap exists in terms of quality and productivity of raw silk per unit area compared to China (TOI, 9th March, 2012). One of the constraints faced by us is the widespread occurrence of silkworm diseases which leave the silkworm lots to remain infected under the prevailing pathogenic environmental conditions (Priyadharshini *et al*., 2008). The loss of silkworms due to bacterial infection (>80 %) is the most critical aspect and considered as bottleneck in augmenting muga silk production. Flacherie disease is the most common and severe disease of mulberry silkworm, caused by *Bacillus* and a few other bacterial species (Savanurmath *et al*., 1992; Nataraju *et al*., 2007).

In mulberry silkworm, bacterial flacherie diseases are classified into three groups, namely septicemia, bacterial toxicosis and bacterial gastroenteritis. However, *Pseudomonas aeruginosa* (AC-03) has been reported as a pathogen of muga silkworm although a few other bacteria (*B. thuringiensis*, *Aeromonas salmonicida, Streptococcus bombycis)* are also known as pathogenic to silkworm (Choudhury *et al*., 2002; Das *et al.*, 2010 a). The virulence of these bacteria to the silkworms differ and therefore, the symptoms of septicemia and course of the disease may not be uniform (Chen and Huang, 2008).

In this study, we have reported a new pathogenic bacterium from the muga silkworm which has so far not been reported from any other silkworm. The pathogen was predominant in the silkworm rearing sites and was isolated from naturally infected larvae. The pathogenic bacterium was identified based on morphological and biochemical features, and by 16S rDNA sequence analysis.

MATERIALS AND METHODS

Collection of silkworms and isolation of the bacterium: The infected cadavers of *A. assamensis* were collected from different silkworm rearing farms (Dibrugarh, Sivasagar, Jorhat and Golaghat districts) during the regular monitoring surveys conducted as part of the disease forewarning and forecasting programme of the Central Silk Board. For experimental purpose, muga silkworms were reared in the farm of Central Muga Eri Research and Training Institute, Central Silk Board, Jorhat, Assam, India. Naturally infected silkworms were collected in sterile polythene bags separately. The larvae were disinfected with 70 % (v/v) ethanol for 1 to 2 min. and washed twice with sterile distilled water after it had just begun to soften. The larvae were then dissected with the help of sterilised scissors in PBS buffer and the midgut portion was collected separately in distilled water and homogenized. The bacterial pathogen was isolated using serial dilution agar plating method. The body fluid of the infected larvae was also collected separately with the help of sterilized insect needles. The diluted body fluid and midgut samples were then inoculated onto Nutrient and *Bacillus* Agar media (Himedia) and incubated at 35°C for 48 h (Tao *et al.*, 2011).

Each of the different bacterial colonies was purified and preserved at 4°C for further experiments. Further, the purified strain was fed to test silkworms and clinical symptoms were observed in the infected silkworm in order to confirm pathogenicity by the strains (Koch's laws). After confirmation of the pathogenicity, the isolated, pathogenic bacterium (strains) was purified and incubated on a nutrient agar plate at 30°C for 24 h. During this period, the colony characteristics, such as size, colour, transparency, shape and edge were observed. The bacterial colony that had been cultured for 24 h was subjected to Gram's staining and observed using an oil immersion objective under bright field of phase contrast microscope (Lica DM 750).

Pathogenicity of bacterial isolates

The purified bacteria (AAB-13) were washed with 5 ml of sterile phosphate-buffered saline (PBS: 2 mol/l, pH 7.4) by repeated blowing and suction. Bacterial suspension was centrifuged at 4,000 rpm for 15 min in a new sterile centrifuge tube. The supernatant was removed and re-suspended in 5 ml of PBS buffer and calculated bacterial population by plate colony-counting method for counting the bacteria in the solution (Luo *et al.*, 2007).The bacterial inoculums were diluted up to 10^{-7} in PBS. Fifty healthy 4th instar larvae were considered in one experimental group (*in vitro*) and treated with bacterial suspension *per os*, along with feed. The host plant leaves were grown indoor with appropriate precautionary measures to check contamination. Concurrently, the control sets were administered with PBS only. The infected larvae were observed at every 12 hours and the mortality was recorded. The isolates from gut portion of infected silkworms were again plated on the NA for the confirmation of the same bacteria. Statistical analysis was done using SPSS 16.0 and Origin Pro 8.0 (Jia, 2006).

Median lethal dose (LD50) determination

Fifty healthy 4th instar larvae were inoculated (*per os*) with 10^2–10^8 CFU of the pathogenic isolates in one experimental group and the mortality was recorded. Median lethal dose (LD50) and regression analysis were performed based on the data recorded.

Biochemical tests

The utilization of sugars under aerobic and anaerobic conditions was tested according to Bergey's manual through bacterial minimum biochemical reaction tubes (Dong and Cai, 2001). The tests were conducted for starch hydrolysis, citrate utilization, gelatin liquification, H_2S production, phenylalanine deamination, mannitol, lysin utilization and ornithine utilization in addition to Voges-Proskauer test, urease test, methyl red test, catalase test, oxidase test, sorbitol test, lactose test, glucose test, trehalose test, arabinose test and adonitol test.

Molecular identification of the pathogen

Bacterial genomic DNA was isolated from previously grown overnight culture on nutrient broth at 37°C. The phenol-chloroform method for extracting DNA was used as previously described (Cheng and Jiang, 2006). Universal primers for amplification of the bacterial 16S rDNA gene were used as previously reported (Weisburg *et al.*, 1991). Quality was evaluated on 0.8 % agarose gel, a single band of high-molecular weight DNA was observed. Fragment of 16SrDNA gene was amplified by PCR using 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primers. The PCR reaction was performed as follows: 95°C for 5min; 30 cycles of 95°C for 20 s, 50°C for 20 s and 72°C for 3 min; and a final extension at 72°C for 10 min. PCR product was visualized through 1% agarose gel electrophoresis. The PCR amplicon was purified and further processed for sequencing. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 704F and 907R primers using BDT v3.1 Cycle sequencing kit on Applied Bio-systems 3730xl DNA Analyzer. Consensus sequence of 1427 bp 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST alignment search tool of NCBI genbank database. Based on maximum identity score, first fifteen sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 5.

RESULTS

Disease symptoms

The infected larvae became lethargic and motionless, soft and flaccid, inactive and vomited gut juice. The larvae released chain type excreta and exhibited rectal protrusion as well. Larval head and thorax became translucent and paralyzed. After death, the larval body turned slightly hard and blackish in colour with foul smell (Figure 1).

The pathogenic bacterium

The isolated pathogen was a gram-positive, rod-shaped, non-motile spore forming bacterium with the active cell size of 2.4-3.2 x 0.5-0.8 µm. It was positive towards starch hydrolysis, methyl red test, phenylalanine deamination, ornithine utilization, lysin utilization and oxidase test while there were negative results for citrate utilization, gelatin liquefaction, H_2S production, arabinose test, lactose test, trehalose test, adonitol test, urease test, catalase test, glucose test, sorbitol test and Voges-Proskauer test (Table 2).These observations are in accordance with the reported biochemical characters of *Lysinibacillus* sp.

Molecular identification

Consensus sequence of 1427 bp 16S rDNA gene was used to carry out BLAST alignment search tool of NCBI genbank database and based on sequence data, the pathogenic bacterium was identified as *Lysinibacillus sphaericus* strain (SIVAAB-13) (similar to JUN-6 GenBank Accession Number: KF228925.1) based on nucleotide homology and phylogenetic analysis (Figure 2B). The nucleotide sequence data were submitted to the

Table 1: Pathogens and their inoculum concentrations challenged with 4th instar larvae (Relative humidity, 80 (±5) %; Temperature, 30 (±2) °C

Strain / inoculum	Inoculum dose CFU/ml	No. of larvae challenged	LD 50 value (x10^4)		
			24 h	48 h	72 h
L. sphaericus (SIVAAB-13)	10^2	50	0	9.4	8.2
	10^3	50			
	10^4	50			
	10^5	50			
	10^6	50			
	10^7	50			
	10^8	50			
Control	-	50	-	-	-
(PBS buffer)					

Table 2: The biochemical characteristics of isolated *Lysinibacillus sphaericus*

Characteristic	Result	Characteristic	Result
Starch hydrolysis	+ve	Vogesproskauer test	-ve
Citrate utilization	-ve	Urease test	-ve
Gelatin liquefaction	-ve	Catalase test	-ve
Methyl red test	+ve	Oxidase test	+ve
H_2S production	-ve	Glucose test	-ve
Phenylalanine deamination	+ve	Sorbitol test	-ve
Arabinose test	-ve	Adonitol test	-ve
Lactose test	-ve	Lysin utilization	+ve
Trehalose test	-ve	Ornithine utilization	+ve

NCBI with accession number KP888564.

Pathogenicity

The isolated *L. sphaericus* strain (SIVAAB-13) was confirmed to be pathogenic after re-inoculation in mature larvae which was administered *per os*. The pathogen was so virulent that the visual symptoms, such as cessation of feeding, vomiting, lethargic movement *etc.* were displayed within 6-12 h after inoculation. The larvae died within 2-3 days after infection. However, the degree of virulence may depend on the inoculum concentrations, host plants variability and quality, and prevailing environmental parameters (Das *et al.*, 2010 b). The inoculum load of $\geq 10^6$ CFU/ml (LD50, 8.2 x 10^4) was observed to be sufficient for causing mortality of the larvae.

Median lethal dose (LD50) of the isolates

To find out the virulence of SIVAAB-13, the LD50 was determined in 4th instar larvae. Larvae were inoculated orally for establishment in the gut, with different doses, and their deaths were recorded for 3 days after challenge (Table 1). The LD50 of the SIVAAB-13 isolates was 9.4 x 10^5 at 48 h, whereas, it was 8.2 x 10^4 at 72 h. The virulence was much higher and proportionate to the inoculum concentration and also differed at different time point.

Disease epidemiology

It was observed that the disease occurred throughout the year (2013-14) with the intensity varying in different months. This was because of the environmental factors particularly the temperature variations round the year. The season-wise variations of relative humidity (82 ± 10%) and precipitation (11.92 mm) were not significantly different (data not shown) in the sites. Extremely heavy rainfall and wind may affect the silkworm larvae during outdoor rearing if proper precaution (*e.g.*, use of nylon net) is not taken. The disease was recorded throughout the year in the rearing fields and the infection percentage varied from season to season. Temperature variations were not significantly different in the rearing sites (n=4, r^2=0.98, P<0.001), but varied season wise (Figure 3). The per cent disease infections (PDI) were negatively correlated with the temperature but were not dependent on the RH (r^2=0.16). The disease was more prevalent during summer months (June-August) when temperature rose up to 30-35°C. However, higher temperature and RH were the most important factors for disease development.

Figure 1: Disease symptoms of silkworms infected with *Lysinibacillus sphaericus* (SIVAAB-13)

DISCUSSION

Information on many silkworm diseases and the causative pathogens was previously reported by many workers (Lu-Yun-Lian, 1991; Nataraju *et al.*, 2007; Priyadharshini *et al.*, 2008; Tao *et al.*, 2011; Ayoade *et al.*, 2014). However, there was no report of *L. sphaericus* infection in silkworm till date even in mulberry or tasar silkworm. *L. sphaericus* is a naturally occurring, common environmental, soil borne bacterium. The bacterium produces an insecticidal toxin similar to that produced by *B. thuringiensis* and hence, is normally used as a biopesticide to kill mosquito larvae (Wirth *et al.*, 2014). The bacterium is not harmful to humans and other animals (Hu *et al*, 2008). It is not known whether it has been infecting silkworms since long or else, it became pathogenic to the silkworm recently, as it is a soil borne, naturally occurring bacterium. Probably, the bacterium became virulent recently due to many unknown evolutionary mechanisms (Tinsley *et al.*, 2006). A recent review reported that phage-like particles (gene transfer agents) are involved in this process and they can transfer bacterial genes, driving bacterial evolution and promoting the emergence as novel pathogens (Penadés *et al.*, 2015). Hence, the diversity of this bacterium in terms of virulence to the silkworm should be properly studied and understood. The bacterium can form dormant endospores that are resistant to heat, chemicals, and ultraviolet light and can remain viable for a long time (Wirth *et al.*, 2014).

So, it is necessary to take proper disinfection measures to check the disease in the rearing field. However, even the infected mosquitoes may disseminate and contaminate the sites. The cross infectivity of this bacterium has to be studied to understand the pathogen transmission patterns. An integrated approach is required for application of the bacterium as bio-pesticide to control the mosquito larvae and also to check cross contamination and infection to the beneficial silkworms. It is worthy to mention that muga silkworm is endemic to this region and not reported elsewhere for which it has a great significance in terms of ecology, ecosystem and culture. Previous study suggested that the silkworm is having single race from the evolutionary point of view and there is no significant genetic diversity within and between the populations (n=5) and the populations reported from the different areas of the region derived from a single population (Arunkumar *et al.*, 2012). This is very important from the conservation

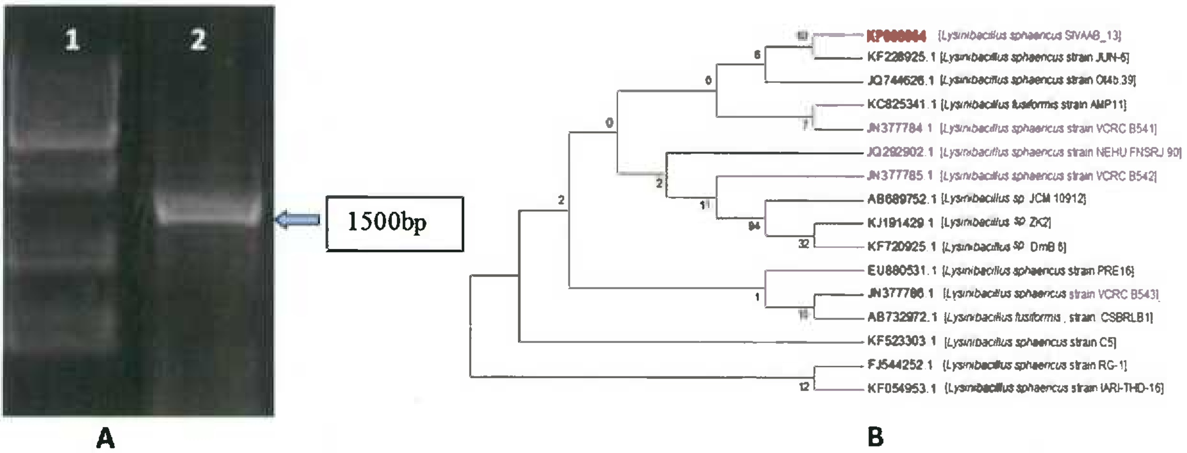

Figure 2: A - PCR amplicon of 16S rDNA gene in Agarose gel (1.2 %) of SIVAAB-13. [Lane 1: DNA marker (1kb ladder); Lane 2: 16S rDNA amplicon]. **B - Phylogenetic tree of the 16S rDNA sequences of the SIVAAB-13 strain.** The phylogenetic tree was constructed by the NJ method.

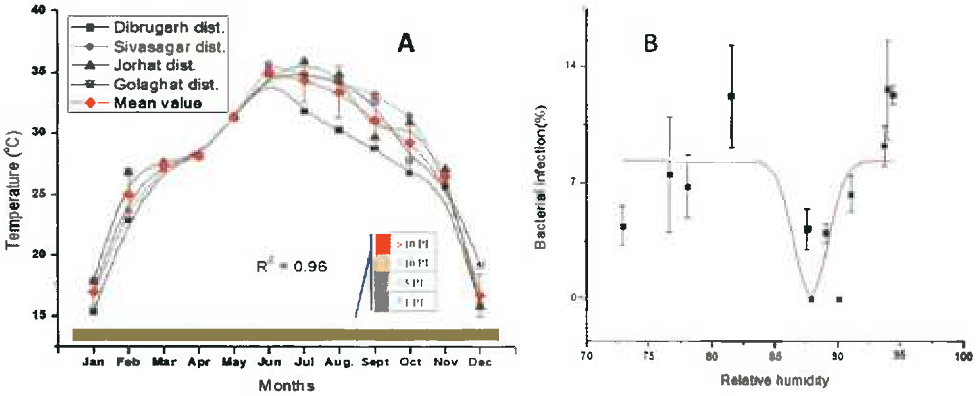

Figure 3: A - Temperature variation and disease occurrence (monthly forewarning) (PI percent disease infection, n =50), **B - Nonlinear regression curve between bacterial infection and relative humidity during 2013-14 in the four different regions**

point of view to preserve this unique genetic pool surviving in this particular niche of the globe. Only a few populations have been reported in the wild habitat in the surrounding area (Kakati and Chutia, 2009; Tikader *et al.*, 2013).

Further, disease monitoring and its prevention in wild and domesticated silkworms are utmost necessities to save their population and ultimately to conserve them. *L. sphaericus* (SIVAAB-13) is the new report as the bacterial pathogen of muga silkworm and needs further study of the disease biology as well as control measures.

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REFERENCES

Arunkumar K. P., Sahu A. K., Mohanty A. R., Awasthi A. K., Pradeep A. R., RajeUrs S. and Nagaraju J. (2012) Genetic diversity and population structure of Indian golden silkmoth (*Antheraea assama). PLoS One,* **7 (8)**: e43716.doi:10.1371/journal.pone.0043716.

Ayoade F., Oyejide N. E. and Fayemi S. O. (2014) Isolation, identification, antibiogram and characterization of bacterial pathogens of the silkworm, *Bombyx mori* in South-West Nigeria. *J. Biol. Sci.*, **14**: 425-430.

Chen Y. M. and Huang J. R. (2008) Occurrence and control of the silkworm bacterial septicemia. *Yun Nan Agricultural Science and Technology*, z3.

Cheng H. R. and Jiang N. (2006) Extremely rapid extraction of DNA from bacteria and yeasts. *Biotechnol. Lett.*, **28**: 55–59.

Choudhury A., Guha A., Yadav A., Unni B. G. and Roy M. K. (2002) Causal organism of flacherie in the silkworm, *Antheraea assama* Ww: isolation, characterization and its inhibition by garlic extract. *Phytother. Res.*, **16**: S89–S90.

Das K., Das R., Neog K. and Rahman S. A. S. (2010 a) Identification, characterization and diagnosis of some muga silkworm diseases. *Annual Report*, Central Muga Eri Research and Training Institute, Lahdoigarh, Jorhat, Assam, India, p. 31.

Das R., Chutia M., Das K. and Jha D. K. (2010 b) Factors affecting sporulation of *Pestalotiopsis disseminata* causing grey blight disease of *Persea bombycina* Kost., the primary food plant of muga silkworm. *Crop Prot.*, **29**: 963-968.

Dong X. Z. and Cai M. Y. (2001) Determination of biochemical properties. In: *Manual for the systematic identification of general bacteria.* Science Press, Beijing, pp. 370-398.

Gheysens T., Collins A., Raina S., Vollrath F. and Knight D. P. (2011) Demineralization enables reeling of wild silk moth cocoons. *Biomacromolecules,* **12** 2257–2266.

Hu X., Fan W., Han B., Liu H., Zheng D., Li Q., Dong W., Yan J., Gao M., Berry C. and Yuan Z. (2008) Complete genome sequence of the mosquitocidal bacterium *Bacillus sphaericus* C3-41 and comparison with those of closely related *Bacillus species. J. Bacteriol.*, **190**: 2892-2902.

Jia C. S. (2006) Calculating the LC50 of insecticides with software SPSS. *Chin. Bull. Entomol.*, **43 (3)**: 414-417.

Kakati L. N. and Chutia B. C. (2009) Diversity and ecology of wild sericigenous insects in Nagaland, India. *Trop. Ecol.*, **50 (1)**:137-146.

Luo T. L., Shen L. L., Bai Y. L. and Xue Y. (2007) Comparative study of *Mycobacterium* counting methods. *J. Fourth Military Med. Univ.*, **28 (4)**: 353-357.

Lu-Yun-Lian (1991) *Silkworm diseases*. Food and Agriculture Organization of United Nations, Rome, p.5.

Nataraju B., Balavenkatasubbaiah M., Sharma S. D., Rao P. S., Chandrasekharan K., Naik A. R. N. and Dandin S. B. (2007) Flacherie- causes and management. *Indian Silk,* **45(10)**: 4-6.

Neog K., Singh H. R., Unni B. G. and Sahu A. K. (2010) Analysis of genetic diversity of muga silkworm (*Antheraea assamensis* Helfer; Lepidoptera: Saturniidae) using RAPD-based molecular markers. *Afr. J. Biotechnol.*, **9**:1746-1752.

Penadés J. R., Chen J., Quiles-Puchalt N., Carpena N. and Novick R. P. (2015) Bacteriophage-mediated spread of bacterial virulence genes. *Curr. Opin. Microbiol.*, **23**: 171-178.

Priyadharshini P., Mahalingam C. A. and Shashidhar K. R. (2008) Identification and characterization of bacterial pathogens in silkworm, *Bombyx mori* L. *Curr. Biotica*, **2 (2)** :181-192.

Savanurmath C. J., Basavarajappa S., Hinchigeri S. B., Ingalhalli S. S., Singh K. K. and Sanakal R. D. (1992) Relative incidence of silkworm viral diseases in agro-climatic zones of Northern Karnataka, India. *Proceedings of*

National conference on Mulberry Sericulture Research, Cenral Sericultural Research and Training Institute, Mysore, India, p. 123.

Tao H. P., Shen Z. Y., Zhu F., Xu X. F., Tang X. D. and Xu L. (2011) Isolation and identification of a pathogen of silkworm *Bombyx mori. Curr. Microbiol.,* **62 (3)**:876-883.

Tikader A., Vijayan K. and Saratchandra B. (2013) Muga silkworm, *Antheraea assamensis* (Lepidoptera: Saturniidae) - an overview of distribution, biology and breeding. *Eur. J. Entomol.,* **110 (2)**: 293-300.

Tinsley C. R., Bille E. and Nassif X. (2006) Bacteriophages and pathogenicity: more than just providing a toxin? *Microb. Infect.,* **8**:1365-1371.

TOI (2012) Times of India, *English News Paper*, 9th March, 2012. The Times Group, Kolkata, India.

Weisburg W. G., Barns S. M., Pelletier D. A. and Lane D. J. (1991) 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.,* **173**: 697-703.

Wirth M. C., Berry C., Walton W. E. and Federici B. A. (2014) Mtx toxins from *Lysinibacillus sphaericus* enhance mosquitocidal cry-toxin activity and suppress cry-resistance in *Culex quinquefasciatus. J. Invertebr. Pathol.,* **115**: 62–67.

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Research Paper

IMPROVEMENT OF FECUNDITY IN COMMERCIALLY EXPLOITED DABA TROPICAL TASAR SILKWORM, *ANTHERAEA MYLITTA* DRURY THROUGH RECURRENT SELECTION

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ABSTRACT

Fecundity is one of the important quantitative characters of commercial importance in tasar silkworm. In the natural Daba ecorace of *A. mylitta,* fecundity varies from 86 to 400 and in the ruling Daba race, under seed multiplication system, it is in the range of 200 to 220. Due to continuous exploitation of Daba over many years to meet the demand of seed, the fecundity has been declining. In a breeding plan, the pooled population of ruling Daba was crossed with the natural Daba population in order to introgress the beneficial genes and in subsequent generations, recurrent selection was followed. Initially, in the F1 generation, 10 breeding lines were isolated and selected based on the fecundity range of 201-300. Subsequently, three breeding groups were selected based on fecundity range *i.e.*, BG1 (241-260), BG2 (261-280) and BG3 (281-300) with average female pupal weight of 11.00, 11.85 and 12.27 g, respectively. Concomitantly, the average male shell weight in these groups was 1.65, 1.75 and 1.85 g, respectively. Similarly, for parental stock, the lot with fecundity range, 200-222 with average value of 210 was selected and considered as control. After six generations, the lines with high fecundity (>260) were isolated and evaluated for the phenotypic expression of some of the quantitative traits.

Key words: *Antheraea mylitta,* Daba ecorace, fecundity, recurrent selection.

INTRODUCTION

Tasar culture is one of the important agro-forestry based activities in the Central, Northern and Eastern India. It involves the rearing of semi-domesticated tasar silkworm, *Antheraea mylitta* Drury on its food plants to produce silken cocoons. Also, a large quantity of tasar cocoons produced by wild tasar silkworms is being collected from the forest by the aboriginals. *A. mylitta* exhibits wide phenotypic variability, such as in biological/commercial and technological characters among the populations (Sengupta and Sengupta, 1982; Sengupta *et al.*, 1993; Singh and Srivastava, 1997; Srivastava *et al.*, 2003). Among the forty four ecoraces identified (Suryanarayana and Srivastava, 2005), Daba and Sukinda are of semi-domesticated nature and these two ecoraces are being continuously utilized for commercial tasar silkworm seed production. Due to continuous exploitation, without proper breeding plan and inadequate seed selection norms, there is an apprehension of deterioration of quality as well as quantity of seed. It is well-known that the success of sericulture is based on production of sound seed with high vigour. Hence, in the present situation, there is a need to improve the productivity through enhancing the fecundity in the commercially exploited Daba race.

For the better utilization of tasar silkworm breeds, several attempts were made for commercial exploitation of heterosis following hybridization method (Jolly *et al.*, 1968; Bardaiyar *et al.*, 1976; Sengupta *et al.*, 1987; Siddiqui *et al.*, 1988). The choice of breeding methodology depends upon the nature and magnitude of gene action. For exploitation of the additive gene effects, inter-crossing of the requisite segregates is desirable. The presence of significantly high proportions of dominance effect stresses the need for maintaining certain degree of heterozygosity. Under such a situation, in tasar silkworm, where additive and non-additive gene effects are important, it was suggested that some form of recurrent selection programme should be adopted for greater improvement (Comstock *et al.,* 1949). The genotype-environment interaction has significant role in expressing the commercial cocoon traits in tropical silkworms (Petkov *et al.*, 2000). Season specific performance of silkworm on commercial cocoon traits was studied (Kumar *et al.,* 2003; Malik and Reddy, 2007; Zhao *et al.,* 2007) and particularly in tasar silkworm (Ojha *et al.,* 2009; Reddy *et al.*, 2010 a).

Selection provides scope for the stock improvement in the desirable characters. Possibly, it can bring about an enviable change in the genetic structure of the population. The basic essential step for selection is the best possible identification of the quality of an individual silkworm population through estimation of average breeding values (Petkov, 1981).

The fecundity in the Daba ecorace, varies from 86 to 400 and at field level, it is 200 to 220. The egg laying potential or the realized fecundity could be improved through the adoption of appropriate breeding plan. In view of this, isolation of line (s) with high fecundity was attempted from commercially exploited Daba through introgression of beneficial genes available in wild Daba with an aim of amalgamation of alleles followed by recurrent selection. A line of Daba with improved fecundity may play an important role in filling the gap against demand of quality seed which have a direct bearing on productivity. The wide range of variability and higher GCA value for fecundity in Daba add the scope for selection. Hence, in the present work, isolation of an improved line of tropical tasar silkworm, *A. mylitta* for higher fecundity was accomplished through introgression of genes of wild relative followed by recurrent selection, which can be utilized by the farmers through seed channel after satisfactory performance in the multi-location trials.

MATERIALS AND METHODS

The present experiment was conducted at Silkworm Breeding and Genetics Laboratories, Central Tasar Research and Training Institute (CTRTI), Ranchi, India. The cocoon maintenance and selection was carried out at the grainages and rearing of the silkworm was conducted at CTRTI farm with block plantation of well-maintained host plants *viz.*, Arjun (*Terminalia arjuna*) and Asan (*Terminalia tomentosa*).

Preparation of base population with wider gene pool

Initially, a source population was developed for the accumulation of maximum genes in the experimental lot. For the purpose, Daba semi-domestic populations were collected from different agro-climatic conditions of Central, Southern and Eastern India [Basic Seed Multiplication and Training Centres *viz.*, Balaghat (Madhya Pradesh), Madhupur (Jharkhand), Dudhi (Uttar Pradesh), Chinoor (Andhra Pradesh) and Central Tasar Silkworm Seed Station, Kargi Kota (Chhattisgarh)]. Collected populations were pooled for composite grainage, cocoon selection was made keeping the bench mark of semi-domestic Daba having female pupal weight >11.5 g and male shell weight >1.5 g to develop the base population (recipient parent).

Cross breeding

For the introgression of beneficial genes and for the improvement of desired traits in the semi-domestic Daba used as recipient population, nature grown Daba ecorace was used as donor parent. This wild counterpart is supposed to harbour many beneficial alleles / genes. The cocoons were collected from original ecopockets situated in West Singhbhum (Jharkhand). Selection was carried out for higher male pupal weight (>12.5 g) with higher shell weight (>2.4 g). Cross combinations of Daba semi-domestic (recipient parent) and Daba natural (donor parent) were prepared and F1 population was developed during the seed crop. Further selection was carried out for higher fecundity in the F1 population.

Recurrent selection and maintenance of the stock

Rearing was carried out for the selected lots for further evaluation of the performance in cocoon yield, shell weight and shell percentage. Family selection for higher fecundity (Mean ± SD) was done and the lines (10 DFLs each) were reared. The process of selection was repeated for subsequent generations till the desired level of the trait (fecundity) was attained. Due to the directional selection, there was a positive shift in the original mean.

Evaluation of developed breed for high fecundity

Evaluation of the breed was carried out with the parameters, such as cocoon weight, shell weight, shell percentage, filament length, non-breakable filament length (NBFL), denier, silk recovery and reelability during each crop harvest. The data obtained were used for further estimation of selection index (Hazel, 1943).

RESULTS

Development of base population

Base population was developed by pooling cocoons of ruling Daba (BV) race obtained from different agro-climatic regions of the country. Similarly, nature grown cocoons of Daba ecorace were collected from original ecopockets situated in West Singhbhum (Jharkhand) (Table 1). Average fecundity of pooled population was 255±14 while it was 302±38 and 281±26 for nature grown Daba and the F1 progenies, respectively (Table 2). Hatching was nearly 90 % in all the three populations. Female cocoon weight ranged from 13.73±1.824 to 14.989±1.67 g in semi-domestic population (recipient parent) whereas, the average nature grown female cocoon weight was recorded as 15.529±1.27 g. Shell weight varied from 2.037±0.383 to 2.553±0.366 g. Similarly, in the male, the cocoon weight varied from 10.390±1.138 to 12.783±0.872 g and the shell weight, from 1.798±0.337 to 2.44±0.376 g.

Development of breeding lines

Ten breeding lines from the F1 population of Daba were isolated. Quantitative traits, such as fecundity, hatching %, larval weight, larval duration and cocoon yield in ten breeding lines were assessed and recorded for further selection. Accordingly, based on the performance, selection indices scores were derived for fecundity among the ten lines (Figure 1). The V instar larval weight ranged from 38 to 48 g, larval duration from 46 to 55 days and yield recorded was 78 to 100 cocoons /DFL (Table 3). Based on selection parameters *viz*., female cocoon weight, pupal weight and male shell weight, three breeding groups (BG) were selected out of the 10 breeding lines. Further selection was carried out for fecundity range 241-260, 261-280 and 281-300 during I crop (July-August) and for 251-270, 271-290 and 291-310 in II crop (September – October). The selected lines performed better than parental stock during both the 1st and 2nd crop seasons (Tables 4 and 5).

Table 1: Quantitative cocoon traits (Mean ± SD) of collected populations during diapausing grainage

Trait	Madhupur		Balaghat		Kargi Kota		Ranchi		Chakradharpur		Nature grown		F value	CD at 5 %
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female		
Pupal weight (g)	9.48 ±1.13	12.79 ±1.41	8.59 ±0.89	11.69 ±1.64	9.18 ±1.21	11.78 ±1.61	9.57 ±1.41	12.16 ±1.72	9.39 ±1.62	12.09 ±1.26	10.34 ±0.88	12.98 ±1.16	6.88**	0.930
Cocoon weight (g)	11.34 ±1.28	14.99 ±1.67	10.390 ±1.138	13.73 ±1.82	11.039 ±1.409	13.82 ±1.85	11.467 ±1.530	14.25 ±2.02	11.242 ±1.622	14.36 ±1.29	12.783 ±0.872	15.53 ±1.27	10.39**	1.257
Shell weight (g)	1.85 ±0.31	2.19 ±0.36	1.79 ±0.34	2.04 ±0.31	1.86 ±0.31	2.037 ±0.38	1.89 ±0.36	2.09 ±0.39	1.85 ±0.25	2.27 ±0.34	2.44 ±0.37	2.55 ±0.37	17.22**	0.374
Shell %	16.36 ±2.17	14.62 ±1.59	17.22 ±2.21	14.93 ±1.91	16.87 ±1.99	14.75 ±2.06	16.66 ±2.97	14.62 ±1.78	16.63 ±2.51	15.88 ±2.51	19.17 ±3.03	16.46 ±2.18	6.29**	1.531

** Significant at $p<0.05$

Table 2: Fecundity and hatching of Daba pooled, Daba natural and F1 populations

	Fecundity			Hatching %		
	Pooled population	Nature grown	F1 population	Pooled population	Nature grown	F1 Population
Mean	255	302	281	87.5	85	89
SD	14	28	26	5.4	5.7	4.5
Min	215	254	234	82	83	85
Max	289	388	345	90	91	93

Table 3: Quantitative trait analysis in the breeding lines

Breeding line	Fecundity	Hatching %	Larval weight (g)	Larval duration (days)	Yield (Cocoons /DFL)
BL-1	206	91.00	38.10	46.50	78.40
	±2.53	±0.94	±4.77	±1.78	±2.91
BL-2	214	91.70	36.90	46.20	93.00
	±3.31	±0.95	±4.61	±1.69	±4.81
BL-3	224	91.20	37.80	47.00	91.70
	±2.91	±0.92	±3.65	±2.45	±5.12
BL-4	233	92.00	39.30	46.90	85.20
	±2.39	±0.94	±2.91	±2.13	±3.05
BL-5	244	92.50	39.00	48.80	91.10
	±1.97	±1.27	±2.45	±1.75	±4.18
BL-6	254	91.40	39.40	46.10	89.40
	±2.57	±0.97	±1.90	±2.88	±3.72
BL-7	265	90.90	41.20	51.40	97.40
	±2.91	±0.88	±4.59	±4.74	±2.22
BL-8	273	92.30	40.00	46.60	94.60
	±1.62	±1.89	±3.56	±2.27	±4.99
BL-9	284	91.10	39.70	52.90	100.20
	±2.16	±1.10	±2.21	±3.84	±3.77
BL-10	295	91.50	40.40	55.20	99.60
	±2.63	±1.35	±2.41	±3.16	±3.10

Data are mean ± SD

Evaluation of isolated lines/breed for high fecundity

In Breeding Group (BG)-3, mean female cocoon weight, pupal weight and male shell weight were 14.68, 12.75 and 1.81 g, respectively. The values for BG-2 female were 13.52, 11.85 and 1.75 g, respectively. The male shell weight was maintained above bench mark *i.e.*, 1.543 g in all the groups (Table 6). Post cocoon characters in breeding groups (300 cocoons each) showed improvement over parental line (Daba) and also when compared to ruling Daba population. Filament length of Group-3 was the highest (885 m) followed by Group-1(847 m) and Group-2 (805 m) whereas, in parental line, it was 830 m (wild Daba) and 761 m (ruling Daba). Denier ranged from 9.29 to 11.07 in breeding groups and from 8.9 (parental line) to 10.66 (ruling Daba) in controls (Table 6). Selection index scores were derived based on the performance in different quantitative traits in each group and is represented in the graph based on the ranking such that the performance of group, BG-3 was comparatively better than other groups as well as control group (parental line-Daba) (Figure 2).

Figure1: Selection Indices scores of 10 breeding lines (descending order)

DISSCUSION

The inter-relationship between different quantitative characters of economic importance which determines silk productivity is of principal importance to the breeders and

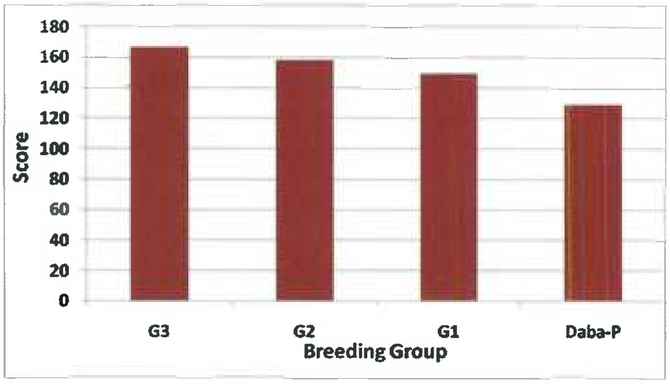

Figure 2: Selection Indices scores of breeding groups

Table 4: Quantitative traits (Mean ± SD) of breeding groups and parental stock (I crop season, July-August)

Ecorace	Fecundity (No.)	Hatching (%)	Female cocoon weight (g)	Female pupal weight (g)	Male shell weight (g)	Yield/ DFL
Breeding Group-1	258±11 (241-260)	92.1±3.3 (85-95)	12.14±0.33 (11.00-12.5)	10.54±0.38 (10.00-11.5)	1.65±0.11 (1.5–1.7)	78±9 (72-89)
Breeding Group-2	272±13 (261-280)	90.8±3.1 (83-94)	12.78±0.29 (12.51-13.5)	11.56±0.21 (11.51-12.5)	1.74±0.18 (1.71-1.8)	81±11 (76-93)
Breeding Group-3	287±19 (281-300)	91.1±3.8 (86-92)	13.88±0.59 (13.51-14.5)	12.71±0.33 (12.51-13.5)	1.86±0.21 (1.81-1.9)	82±8 (75-88)
Parental Stock Daba	215±14 (200-220)	85.1±4.2 (80-91)	11.04±0.23 (9.5-11.5)	10.08±0.25 (8.5-10.5)	1.52±0.08 (1.48-1.6)	86±15 (72-95)
CD at 5 %	13.45	2.51	0.55	0.33	0.14	14.57

Figures in parentheses indicate the range.

Table 5: Quantitative traits of breeding groups and parental stock (II crop season, September- October)

Ecorace	Fecundity (No.)	Hatching (%)	Female cocoon weight (g)	Female pupal weight (g)	Male shell weight (g)	Yield/ DFL
Breeding Group-1	263±13 (251-270)	90.2±3.3 (85-95)	12.51±0.38 (12.00-13.0)	11.23±0.37 (11.00-12.0)	1.73±0.12 (1.5-1.8)	65±5 (60-70)
Breeding Group-2	278±11 (271-290)	91.8±3.5 (83-94)	13.33±0.26 (13.01-14.0)	12.16±0.31 (12.01-13.0)	1.85±0.11 (1.81-2.0)	67±8 (60-70)
Breeding Group-3	298±19 (291-310)	88.3±3.7 (86-92)	14.25±0.69 (14.01-15.0)	13.56±0.35 (13.01-14.0)	2.11±0.31 (2.01-2.2)	63±6 (60-70)
Parental Stock Daba	225±11 (210-230)	87.1±4.2 (80-90)	11.59±0.56 (10-12.5)	10.87±0.31 (10.0-11.5)	1.58±0.38 (1.5-1.8)	58±4 (50-65)
CD at 5 %	13.45	3.51	0.55	0.83	0.13	11.57

Data are mean ± SD; Figures in parentheses indicate the range.

Table 6: Post cocoon parameters (Mean ± SD) of three breeding groups and parental stock

Group	Filament length (m)	NBFL (m)	Denier	Reelability (%)	Recovery (%)
BG-1	885 ± 55	158.16	10.59±1.3	17.06±2.5	56.2±3.5
BG-2	805 ± 35	133.08	9.29±1.05	17.06±1.8	54±5
BG-3	847 ± 38	124.66	11.07±0.8	15.34±1.5	54.78±6.5
Parent	830 ± 65	139.14	8.9±0.85	15.28±1.25	45.9±3.5
Daba Ruling	761 ± 30	104.21	10.66±1.25	16.67±1.55	46.85±5.5

other stake holders in sericulture industry (Singh and Saratchandra, 2004) wherein the grainuers (egg producers) favor strong, potential genotypes producing more number of eggs. However, selection of high egg yielding genotype as parent is vital for the improvement of silkworm from the commercial point of view (Petkov, 1981; Narasimharaju *et al.*, 1990). Cross-breeding signifies an efficient method for animal population amelioration. For silkworms, the cross-breeding is intended on one hand for the amelioration of the breeds and on the other hand, for immediate economical purposes consisting of development of commercial hybrids, with superiority against pure breeds. The introgression of traits present in both domesticated and wild populations of tasar silkworm for heterosis effect in F1 was reported (Moorthy *et al.*, 2007; Reddy *et al.*, 2010 b; Talebi and Subramanya, 2009) to avail a viable breed for sustainable cocoon production. The purpose of crossing the semi-domestic Daba with the wild Daba in the present work was to ameliorate and introgress beneficial genes into the semi-domestic Daba population and also to create wider variability in the population to bring in greater opportunity for the selection. This aspect is evident from the quantitative traits analysis *i.e.*, fecundity and hatching. Estimation of correlation and heritability of quantitative characters are of special significance for selection, as its magnitude indicates the accuracy with which a genotype can be recognized by its phenotypic expression (Singh *et al.*, 1994).The relationship between female pupal weight and fecundity indicated highly significant (P < 0.01) positive correlation between the two, irrespective of the weight (Singh *et al.*, 1994; Singh *et al.*, 2011). Fecundity has been found increasing along with pupal weight (Singh *et al.*, 2010). Similar observation was also made by Singh and Saratchandra, 2004 and Jayaswal *et al.*, 1990. Hence, selection based on female pupal weight, for fecundity improvement will lead to positive results (Basavaraja *et al.*, 1998).The cocoon as well as post-cocoon characters are important components, which determine the overall performance of the silkworm and also important for final out-put of the silk industry. Various factors such as genetic background of silkworm, food quality, environment *etc.*, influence the economic traits of the silkworm (Lokesh and Anantha Narayana, 2011). In the present work, the breeding lines developed through recurrent selection showed higher values for the quantitative characters, such as fecundity, hatching, cocoon weight and male shell percentage. Besides, higher values were also recorded for post-cocoon traits than that of the ruling Daba (control). This reiterates that the recurrent selection is an imperative means for the improvement of qualitative as well as quantitative characters in tasar silkworm. Further, the isolated, high fecundity breeding lines need to be evaluated for better validation of the expression of improved characters through multi-location trials at different agro-climatic regions of tasar growing areas before being recommended for commercial exploitation at farmers' level.

REFERENCES

Bardaiyar V. N., Jolly M. S., Benchamin K. V. and Sinha B. R. R. P. (1976) Heterosis in relation to single, three way and double cross in *Antheraea mylitta* Drury. *Indian J. Seric.*, **15**: 3-8.

Basavaraja H. K., Suresh K. N., Mal Reddy N. and Datta R. K. (1998) New approaches to bivoltine silkworm breeding. In: *Silkworm Breeding*, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 131-139.

Comstock R. E., Robinson H. F. and Harvey P. H. (1949) A breeding procedure designed to make maximum use of both general and specific combining ability. *Agron. J.*, **41**: 360-367.

Hazel L. N. (1943) The Genetic basis for constructing selection indexes. *GENETICS*, **28:** 476-490.

Jayaswal K. P., Singh T. and Sen S. K. (1990) Correlation between some economic parameters and their application in silkworm breeding. *Indian Silk*, **29**: 25-27.

Jolly M. S., Chaturvedi S. N. and Prasad S. (1968) A survey of tasar crops in India. *Indian J. Seric.*, **1**: 50-58.

Kumar N. S., Basavaraja H. K., Nanjegowda B., Joge P. G. and Kalpana G. V. (2003) Effect of high temperature and high humidity on the post cocoon parameters of parents, foundation crosses, single and double hybrids of bivoltine silkworm, *Bombyx mori* L. *Indian J. Seric.*, **42**: 162-168.

Lokesh G. and Anantha Narayana S. R. (2011) Changes in the protein profile of silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) in response to the chemical mutagen. *Int. J. Sci. Nat.*, **2 (3)**: 559-563.

Malik F. A. and Reddy Y. S. (2007) Role of mulberry nutrition on manifestation of post cocoon characters of selected races of the silkworm *Bombyx mori* L. *Sericologia*, **47**: 63-76.

Moorthy S. M., Das S. K., Kar N. B. and Urs S. R. (2007) Breeding of bivoltine breeds of *Bombyx mori* L. suitable for variable climatic conditions of tropics. *Int. J. Indust. Entomol.*, **14**: 99-105.

Narasimharaju R., Govindan R., Ashoka J. and Rayar S. G. (1990) Genetic variability for quantitative traits in silkworm *Bombyx mori* (L). *Entomon*, **15**:197-201.

Ojha N. G., Reddy R. M., Hansda G., Sinha M. K., Suryanarayana N. and Vijayaprakash N. B. (2009) Status and potential of Jata, a new race of Indian tropical tasar silkworm (*Antheraea mylitta* Drury). *Acad. J. Entomol.*, **2**: 80-84.

Petkov N. (1981) The possibility of forecasting the effectiveness of selecting lines of the silkworm, *Bombyx mori* based on weight and silk content of cocoons. *Selskotopanska Nauka*, **19**: 92-96.

Petkov N., Nacheva I., Tsenov P., Grekov D., Golovko V. and Braslavskii M. (2000) Hybridization schemes for developing new silkworm (*Bombyx mori* L.) races by using populations from different eco-geographical zones. *Acta Agric. Serbica.*, **5**: 3-9.

Reddy R. M., Sinha M. K. and Prasad B. C. (2010 a) Application of parental selection for productivity improvement in tropical tasar silkworm *Antheraea mylitta* Drury-A review. *J. Entomol.,* **7**:129-140.

Reddy R. M., Suresh Rai, Srivastava A. K., Kar P. K., Sinha M. K. and Prasad B. C. (2010 b) Heterosis pattern and commercial prospective of assorted F1 hybrids of Indian tropical tasar silkworm, *Antheraea mylitta* Drury. *J. Entomol.*, **7 (3)**: 160-167.

Sengupta A. K. and Sengupta K. (1982) Ecoraces of tasar and their potentialities. Base paper, *Workshop on tasar culture and tasar silk industry*, Central Tasar Research and Training Institute, Central Silk Board, Ranchi, India, **6**: 1-14.

Sengupta A. K., Siddiqui A. A., Mohapatra D. P., Kumar A. and Sengupta A. K. (1987) Studies on the potential of heterosis in tropical tasar, *Antheraea mylitta* D. *Sericologia*, **27**: 519-524.

Sengupta A. K., Sinha A. K. and Sengupta K. (1993) Genetic reserves of *Antheraea mylitta* Drury. *Indian Silk,* **32**: 39-46.

Siddiqui A. A., Sengupta A. K., Kumar A., Mohapatra D. P. and Sengupta K. (1988) Studies on the genetic architecture and gene action in yield and yield components in diallel population of tropical tasar, *Antheraea mylitta* D. *Sercologia,* **28**: 519-524.

Singh B. M. K. and Srivastava A. K. (1997) Ecoraces of *Antheraea mylitta* Drury and exploitation strategy through hybridization. Base paper 6, *Current Technology Seminar on Non-Mulberry Sericulture*, Central Tasar Research and Training Institute, Central Silk Board, Ranchi, India, pp. 1-39.

Singh T., Bhat M. M. and Khan M. A. (2010) *Silkworm egg science—Principles & protocols*. Daya Publishing House, New Delhi, India, p. 277.

Singh T., Bhat M. M. and Khan M. A. (2011) Critical analysis of correlation and heritability phenomenon in the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). *Adv. Biosci. Biotch.*, **2**: 347-353.

Singh T., Chandrashekaraiah A. and Samson M. V. (1994) Selection strategies in relation to correlation and heritability in the silkworm *Bombyx mori* (L). *Bull. Seric. Res.*, **5**: 37-41.

Singh T. and Saratchandra B. (2004) *Principles and techniques of silkworm seed production*. Discovery Publishing House, New Delhi, India, p. 342.

Srivastava A. K., Sinha A. K. and Sinha B. R. R. P. (2003) *Descriptor of tropical tasar silkworm, Antheraea mylitta Drury (Lepidoptera: Saturniidae)*. Central Tasar Research and Training Institute, Central Silk Board, Ranchi, India, pp.1-14.

Suryanarayana N. and Srivastava A. K. (2005) *Monograph on Tropical Tasar Silkworm*. Central Tasar Research and Training Institute, Ranchi, Central Silk Board, India.

Talebi E. and Subramanya G. (2009) Genetic distance and heterosis through evaluation index in the silkworm, *Bombyx mori* L. *World Appl. Sci. J.*, **7**: 1131-1137.

Zhao Y., Chen K. and He S. (2007) Key principles for breeding spring and autumn silkworm varieties; from our experience of breeding 873 x 874. *J. Environ. Sci.*, **5**: 57-61.

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Technical Report

DEVELOPMENT OF A MODEL REARING HOUSE OPERATING ON SOLAR PASSIVE ENERGY FOR SILKWORM SEED CROP UNDER TROPICAL CONDITIONS

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ABSTRACT

In India, about 7,96,000 families in 53,814 villages are involved in sericulture industry with about 6 million people having their livelihood out of it. One of the main activities in sericulture is rearing of silkworms for which the use of a rearing house is essential. Healthy and successful silkworm rearing needs optimum levels of temperature (23-28 °C) and relative humidity (70-85 %). Under the tropical climate, it is a difficult task to provide these desired conditions to silkworms. In conventional type of rearing houses, the room temperature and humidity are maintained by using electrical heater and humidifier. Hence, in an effort to develop a cost-effective, eco-friendly and solar energy based rearing house, the Silkworm Seed Technology Laboratory (SSTL), National Silkworm Seed Organization, Central Silk Board, Bengaluru, India and The Energy and Resources Institute (TERI), Bengaluru, India undertook a collaborative research project. TERI contributed in designing and building of the solar passive silkworm rearing house of first of its kind at SSTL in 2011. Later, SSTL test verified the efficacy of this model rearing house in three main seasons of the year during 2011-13. Popular silkworm breeds, CSR2 and CSR4 were reared in both the solar building and control rearing house to compare the results of seed crop rearing between the two houses. Efficacy of the solar rearing house in maintaining temperature and humidity was almost on par with that of control rearing room indicating its suitability for conducting silkworm seed crop rearing.

Key words: ERR, humidity, seed crop yield, solar passive energy rearing house, temperature.

INTRODUCTION

There are many contributing factors attributed towards cocoon yield, such as leaf quality, silkworm race, variations in temperature and humidity *etc*. The required temperature for optimal growth of silkworm ranges between 23 and 28 °C and the desired relative humidity (RH) is in the range of 70 to 85 % (Krishnaswami, 1978; Kawakami, 2001). Rearing house is an essential component of the infrastructure required to conduct silkworm rearing to produce quality cocoons. The structure, orientation, materials used for construction of rearing house and agro climatic conditions play a vital role in maintenance of temperature and humidity during silkworm rearing. Even though some traditional methods are being adopted by farmers for maintaining the temperature and humidity, they often realise very low cocoon yield mainly due to difficulty in maintaining optimum level of temperature and humidity in the rearing house. The main objective of the joint venture undertaken by the Silkworm Seed Technology Laboratory (SSTL), National Silkworm Seed Organization, Central Silk Board, Bengaluru and The Energy and Resources Institute (TERI), Bengaluru was to develop and demonstrate a solar passive building for silkworm rearing by utilizing solar passive energy for maintenance of optimum condition during silkworm rearing throughout the year for improving the quality of seed cocoon. TERI have extensive expertise on active as well as passive solar thermal systems and many energy and environmental technology development related issues. The SSTL has exclusively been involved in silkworm seed production research and development for more than 20 years and has expertise on seed crop rearing and seed production. Initially, a survey was conducted in the states of Karnataka, Andhra Pradesh and Tamilnadu by both the teams to explore the traditional techniques being adopted for maintaining the temperature and humidity in conventional rearing houses which were thought as to provide key scientific tips towards design and construction of solar passive house for silkworm rearing at SSTL campus, Bengaluru.

Design feature of the solar passive silkworm rearing house

The following are the solar passive design features integrated in the rearing house constructed at SSTL campus, Bengaluru (Figures 1 and 2)

Orientation

The house was designed with long façade of the house oriented towards North – South. Solar radiation analysis was carried out and it was observed that North-South is the favourable orientation. East and West walls are fully shaded to cut down on the direct solar heat gains.

Figure 1: A view of the solar passive silkworm rearing house in the campus of SSTL, Bengaluru

Building envelope

External walls of the house are insulated with 50 mm thermocol, roof of the house is also insulated through 75mm mineral wool. There are no windows provided in the house. For natural ventilation, ventilators and solar chimneys are provided. For minimum natural light that is required by silkworms, north light is provided in the house.

Figure 2: Shaded west façade of the solar passive silkworm rearing house

Natural ventilation

Natural ventilation was achieved inside the house through stack effect. Ventilators are provided with North and West orientation which act as inlet points. The rearing room has pitched roof sloping towards South orientation. Alternate section of this pitched roof is connected to a solar chimney which helps to enhance ventilation inside the room during summer due to stack effect (Figure 3).

Figure 3: Ventilators provided with shutters to operate appropriately in different seasons

Evaporative cooling

When the outside temperature is high and relative humidity (RH) is low, evaporative cooling is proposed in the house through khas khas pads which will be watered to keep them wet. The pads are custom made according to the size of the ventilators.

Radiant cooling and heating

One section of the roof of the rearing house is flat metal deck with water bags. Water is stored above the metal deck in black colour plastic bags. Movable insulation is provided above the water bags either to expose them to the sky or cover them. During summers, when cooling is required, the water bags will be exposed to the night sky to cool and will be insulated from sun during the day time. The cooled bags are in direct contact with the metal deck and thus the roof acts as a cooling element to the space below.

During winter, the water bags will be exposed to the sun during day time and covered by insulation panel during night time.

Trombe wall

The south wall of the house comprises of three trombe walls. Trombe wall consists of a glazing or clear polycarbonate sheet facing the sun and a brick wall which is painted black from outside. There is a gap of 100 mm provided between the wall and clear polycarbonate sheet, which act as a passage for air to move. The black colour helps in absorption of radiation. Solar radiation which penetrates through the glass is absorbed by the wall, raising its external temperature and at the same time, temperature of the air.

There are two openings provided in the wall, one at lower level and the second, at a higher level. During winter, both of these are kept open. Cool air enters through trombe wall from the lower opening, gets heated up and flows inside the room from the upper level opening. During summer season, trombe wall openings are closed and external shade is to be provided.

Strategies adopted for operation of solar passive building

Summer strategies

When the outside temperature is high and RH is low, evaporative cooling is provided through wet khas pads. These are custom made according to the size of the ventilators. During summer months, the pads will be fit in the ventilators on North and West facade, which are facing the leeward side.

The pitched roof of the rearing room has opening connected to the solar chimney. These alternate sections of the rearing room roof are similar to solar collectors, where the metal deck is painted black on the outer side, insulated from below, and after an air gap of 100 mm, clear polycarbonate sheets are fixed. This is to enhace stack effect and also allow ventilation of the roof. Provision of turbo ventilator is also made in the solar chimneys to enhance stack effect and natural ventilation inside the rearing room. North light is provided in the rearing room to allow natural lght integration. One fourth roof over the rearing room is flat with roof pond integrated to achieve comfort in summer as well as winter. The south wall of the rearing room has collapsible overhang, which shall provide shading during summer months but however, will be removed during the winter months. The trombe wall openings remain closed by use of shutters during summer.

In the summer months, the water in plastic bags on the roof will be exposed to night sky, where the sky will act as a sink and water will dissipate the heat to the sky. During the day time, by the use of movable insulation, the water in plastic bags will be covered by insulation.

Monsoon strategies

During the monsoon months, the wet khas khas pads are removed, and free air movement is allowed though the vents. If required, turbo ventilators are also used to increase the air changes per hour inside the rearing room, to maintain comfortable conditions inside the house. Further, the trombe wall openings remain closed by use of shutters.

Winter strategies

To maintain comfortable conditions inside the rearing house, when the outside air temperature is low, heating through trombe wall is proposed in the solar passive rearing house. Cool air enters through the lower opening of the trombe wall, gets heated up, rises and diffuses into the room. During the winter months, solar chimney opening in the roof is closed. The collapsible overhang on south orientation is removed.

The roof pond over the rearing room is open to solar radiation during day time in the winter months to keep the house warm, but in the night, the water bags are covered by movable insulation to retain the heat stored during the day.

Performance and monitoring (Methodology)

The suitability of solar passive building for carrying out silkworm seed crop rearing during different seasons was test verified. Six trials were conducted using CSR2 and CSR4 breeds. The environmental conditions, such as temperature and humidity were monitored through data loggers. Rearing was conducted both in solar passive building and control rearing house during winter, summer and rainy seasons of 2011, 2012 and 2013. In the control rearing house, the temperature and humidity were maintained by using electrical heaters and humidifiers, whereas, in the solar passive building, rearing was conducted under natural conditions.

RESULTS AND DISCUSSION

The temperature and humidity were monitored through data loggers (Dickson) installed inside the solar passive building, control rearing house and outside the rearing house (ambient condition) (Table 1). The frequency of data monitored was once in every hour for 15 days during late age rearing.

Maintenance of abiotic factors during different seasons

Winter

Temperature: When the ambient temperature was in the range of 18 – 25 °C, the solar passive building could effectively maintain it between 23 and 25 °C. This was almost at par with that of the control room, where the temperature was maintained between 23 and 27 °C.

Relative humidity: An optimum range of humidity was maintained within the solar passive building *i.e.,* between 72 and 89 % during rearing, when outside (ambient) humidity had a much wider range of 60 – 80 %. The average humidity recorded in the solar passive building was 78 %. The required humidity for adult rearing is in the range of 70 – 75 % which is very close to that being naturally maintained in solar passive building. In the control room, the humidity was maintained between 73 and 82 %.

Summer

Temperature: The high range of ambient temperature (30 – 35 °C) was tackled efficiently by the solar passive building by keeping it between 25 and 29 °C. The control room temperature was in the range of 26–28 °C during the rearing period. The average temperature in the solar passive building was 26.2 °C compared to the control of 27.0 °C.

Relative humidity: Humidity maintained in the solar passive building during rearing was between 47 and 77 % with an average of 65 %. The ideal range of humidity for adult rearing is 70 - 75 % which falls close to that maintained naturally in the solar passive building. It is evident that the humidity maintained in the control room (45 -78 %) was at par to that of the experimental room.

Monsoon

Temperature: Temperature was maintained in the solar passive building between 24and 28 °C during rearing with an average of 25.2 °C. In the control room, the temperature was maintained between 25 and 28 °C. The ambient

Table 1: Temperature and humidity recorded in the rearing rooms and ambience

Particulars	Winter		Summer		Rainy	
	Temperature (°C)	Humidity (%)	Temperature (°C)	Humidity (%)	Temperature (°C)	Humidity (%)
Solar rearing house	23-25	72-89	25-29	47-77	24-28	60-88
Ambient condition	18-25	60-80	30-35	26-56	24-32	58-82
Control rearing room	23-27	73-82	26-28	45-78	25-28	58-80

Table 2: Seed crop rearing performance in TERI model rearing house (race: CSR2)

Trial	DFLs (No.)	Fecundity (No.)	Hatching (%)	ERR No.	Cocoon weight (g)	Cocoon shell weight (g)	Cocoon shell (%)	Yield per 100 DFLs (kg)
1	50	491	93.24	9002	1.525	0.322	21.11	57.520
2	50	513	96.18	8596	1.609	0.357	19.75	64.000
3	50	518	94.32	9000	1.595	0.355	21.63	60.000
4	50	554	95.35	8847	1.662	0.365	21.96	62.350
5	50	516	92.00	8847	1.686	0.367	21.83	64.950
6	50	554	95.35	9124	1.680	0.368	21.90	63.750
	Avg.	524	94.04	9100	1.626	0.356	21.36	62.050
	SD ±	25	1.555	279	0.062	0.017	0.849	2.913

Table 3: Seed crop rearing performance in control rearing house (race: CSR2)

Trial	DFLs (No.)	Fecundity (No.)	Hatching (%)	ERR No.	Cocoon weight (g)	Cocoon shell weight (g)	Cocoon shell (%)	Yield per 100 DFLs (kg)
1	50	509	94.61	8231	1.515	0.302	19.93	52.880
2	50	562	92.67	7840	1.578	0.340	21.54	54.500
3	50	502	90.26	8256	1.612	0.346	21.46	55.820
4	50	545	95.59	8119	1.659	0.361	21.88	57.800
5	50	502	90.5	9524	1.763	0.378	21.45	70.163
6	50	545	95.59	8119	1.656	0.361	21.88	57.500
	Avg.	528	93.20	8348	1.631	0.350	21.36	58.11
	SD ±	26	2.43	595	0.084	0.026	0.73	6.19

temperature recorded was in the range of 24- 32 °C.

Relative humidity: Humidity in the range of 60 - 88 % was maintained in the solar passive building when outside (ambient) humidity was 58 – 82 %. The average humidity in the solar passive building was 72.4 % whereas, in the control room, the humidity was maintained between 58 and 80 %.

Silkworm rearing performance

Data pertaining to seed crop rearing in solar rearing house and control rearing room are depicted in Tables 2 – 5.The rearing parameters recorded in TERI model rearing house such as, hatching, ERR, cocoon weight, shell weight, shell percentage and cocoon yield per 100 DFLs were 94.04 %, 91.00 %, 1.626 g, 0.356 g, 21.36 % and 62.050 kg in the case of CSR2 whereas, for CSR4, the

Table 4: Seed crop rearing performance in TERI model rearing house (race: CSR4)

Trial	DFLs (No.)	Fecundity (No.)	Hatching (%)	ERR No.	Cocoon weight (g)	Cocoon shell weight (g)	Cocoon shell (%)	Yield per 100 DFLs (kg)
1	50	550	96.00	8880	1.528	0.325	21.26	56.275
2	50	510	92.00	8900	1.660	0.354	21.33	61.096
3	50	446	94.25	9833	1.549	0.333	21.49	62.925
4	50	489	92.25	8589	1.636	0.358	21.94	58.069
5	50	489	90.32	9200	1.739	0.395	22.71	65.995
6	50	489	93.25	8589	1.632	0.358	21.94	58.069
	Avg.	489	93.01	8999	1.623	0.354	21.78	60.40
	SD ±	49	1.97	468	0.077	0.024	0.544	3.634

Table 5: Seed crop rearing performance in control rearing house (race: CSR4)

Trial	DFLs (No.)	Fecundity (No.)	Hatching (%)	ERR No.	Cocoon weight (g)	Cocoon shell weight (g)	Cocoon shell (%)	Yield per 100 DFLs (kg)
1	50	581	89.92	8000	1.540	0.324	21.04	52.280
2	50	487	94.00	8300	1.506	0.312	20.72	51.880
3	50	456	93.67	9180	1.553	0.342	22.02	58.526
4	50	487	94.25	8039	1.614	0.355	22.00	53.900
5	50	479	91.63	9163	1.772	0.398	22.46	66.945
6	50	487	94.25	8039	1.614	0.355	22.00	54.000
	Avg.	496	92.95	8454	1.600	0.348	21.71	56.27
	SD ±	43	1.78	566	0.094	0.030	0.67	5.73

respective values were 93.01 %, 89.99 %, 1.623 g, 0.354g, 21.78 % and 60.40 kg. Under the conventional control rearing, the above said parameters were recorded as 93.20%, 83.48 %, 1.631 g 0.350 g, 21.357 % and 58.11 kg in CSR2 and in CSR4, it was, 92.95 %, 84.54 %, 1.600g,0.348 g, 21.71 % and 56.270 kg. The rearing performance in both solar passive building and control did not show a significant difference which is an indication of the suitability of this model rearing house for conducting bivotine seed crop rearing all through the year for tropical sericultural areas.

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REFERENCES

Kawakami K. (2001) *Illustrated working process of new biovoltine silkworm rearing technology*. Japan International Cooperation Agency, pp. 23-51.

Krishnaswami S. (1978) New technologies of silkworm rearing. *Bulletin, No 2,* Central Sericultural Reasearch and Training Institute, Mysuru, India.

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Technical Report

BIODIVERSITY EXPLORATION OF WILD SILKMOTHS' HOST PLANTS IN NAGALAND, NE INDIA

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ABSTRACT

The study is an attempt to explore the biodiversity of wild silkmoths' host plants in Nagaland (25°26′-27°40′ N-93°20′-95°15′ E), a small mountainous state of North East India. This biodiversity rich area is found to home 16 species of wild silkmoths under 9 genera which is reported to feed on 23 varieties of host plants belonging to 13 families. However, only three varieties of wild silkmoths, *viz.*, eri, muga and tasar are commercially exploited in Nagaland and their wild counterparts freely thrive in the wild state. Most of the wild silkmoths are polyphagous in nature, *i.e.*, *Antheraea assamensis* primarily feeds on 4 host plants, *Antheraea roylei* on 5, *Antheraea proylei* on 4, *Attacus atlas* on 3, *Actias selene* on 6, *Cricula trifenestrata* on 3, *Samia ricini* on 4 and *Samia canningi* on 9, while *Antherea* sp. novo. and *Theophila religiosa* are reported to feed on only one host plant each. Except for a few host plants, which are site specific, others are distributed throughout Nagaland.

Key words: Host plant, Nagaland, wild silkmoth.

INTRODUCTION

Seri-biodiversity refers to the variability in sericigenous or silk producing insects and their host plants (Srivastava and Thangavelu, 2005). Wild silk moths or the non-mulberry silk moths, also known as 'Vanya silks' are economically and ecologically important forest based insects which are in general, not reared in captivity. Nagaland, a small mountainous state of North East India, lies between 25°26′-27°40′ N and 93°20′-95°15′ E with a geographical area of 16,579 sq km and occupies an important position in the sericulture map of India due to its unique faunal and floral wealth. Congenial climatic conditions have made the state a natural abode for a number of wild sericigenous insects and their host plants; of these, three varieties *viz.*, oak tasar silkworm (*Antheraea proylei*), muga silkworm (*Antheraea assamensis*) and eri silkworm (*Samia ricini)* have been commercially exploited and are an endemic prerogative of North-East India (Suryanarayana *et al.*, 2002).

Fairly good number of references are on record about wild silkmoth diversity and their host plants in Indian subcontinent (Arora and Gupta, 1979; Thangavelu, 1991; Nassig *et al.*, 1996; Chinnaswamy, 2001; Thangavelu *et al.*, 2002; Srivastava and Thangavelu, 2005). Neog *et al.* (2005) recorded 15 species of muga silkworm host plants and their distribution throughout the world along with *ex-situ* and *in-situ* conservation strategies. Kumar *et al.* (2005) reported 10 host plants for tasar silkworm, of these, *Terminalia arjuna, T. tomentosa* and *Shorea robusta* as primary host plant for *Antheraea mylitta* and *T. belerica, T. chebula, T. paniculata, Lagerstroemia speciosa, L. parviflora* and *Anogeissus latifolia* as secondary host plants are maintained in the germplasm at Central Tasar Research and Training Institute (CTRTI), Ranchi, India. Singh *et al.* (2005) reported three species of *Quercus* as host plants for *A. pernyi* and four species of *Quercus* for rearing of *A. yamamai.* Utilization of oak species for rearing of saturniid moths has also been reported from Mexico, Columbia and Arizone (Peigler, 1999).

Srivastava *et al.* (1998 a, b) reported more than three dozen of tasar food plants, of which, *S. robusta, T. tomentosa* and *T. arjuna* are the primary food plants and abundantly available in tropical forest of India. Other major but secondary food plants are *T. belerica, T. chebula, T. paniculata, Lagerstroemia speciosa, L. parviflora* and *A. latifolia.* The primary food plants of oak tasar silkworm in North East India are *Quercus acutissima, Q. grifithii, Lithocarpus dealbata* while in North Western India, they are *Q. incana, Q. semecarpifolia* and *Q. himalayana* (Singh *et al.*, 2005). Negi and Naithani (1995) reported 35 species of oaks and out of which, 15 species belong to the genus *Quercus*, which are distributed in the Sub-Himalayan belt of India including Nagaland. Further, Singh and Misra (2003) estimated nearly 1.26 million hectares of natural oak flora with 8 varieties of primary and secondary food plants in temperate region of sub Himalayan belt. Sarmah *et al.* (2005) listed 10 eri silkworm host plants in North Eastern India, of which, *Ricinus communis* is considered to be the major food plant, while *Heteropanax fragrans, Manihot utilissima, Evodia fraxinifolia* and *Ailanthus excelsa* are the secondary food plants. Till date, a detailed and systematic account is lacking except for certain fragmentary notes on biodiversity of wild silkmoths' host plants. Hence, an attempt was undertaken with an aim to explore and document the wild silk moths' host plant diversity in the state of Nagaland.

MATERIALS AND METHODS

Extensive surveys were conducted throughout the state of Nagaland covering various seasons and forest types with an objective to document the diversity of wild silkmoths and their host plants. During the survey, officials of State and Central Sericulture departments as well as other private rearers were interviewed for information on existence of wild silk moths and their host plants. Natural habitats and undisturbed forests of 11 districts *viz.*, Mokokchung, Tuensang, Kiphire, Zunheboto, Kohima, Phek, Wokha, Mon, Dimapur, Peren and Longleng were surveyed. The specimens of host plants were collected following standard procedure (Jain, 1977; Singh and Subramanium, 2008) and identified based on relevant literature (Chaudhuri, 1993; Soerianegara and Lemmens, 1993; Wu and Raven, 1996, 1999, Wu *et al.*, 2003, 2007, 2008; Kanjilal, 2005; Orwa *et al.*, 2009) and with the help of subject experts. Collected specimens are preserved in the Department of Zoology, Nowgong College, Nagaon, Assam, India for further studies.

RESULTS AND DISCUSSION

The details of host plants and non-mulberry silkworms reported from Nagaland are enlisted in Table 1 and illustrated in Figures 1 and 2. Except for a few host plants which are site specific, others are found throughout Nagaland. A few species belonging to families Fagaceae, Lauraceae and Myrsinaceae are recorded in certain districts of Nagaland which may be due to characteristic ecological niches exhibited by hilly topography and altitudinal variation from 199 to 3841 m above sea level. Climate is humid sub-tropical to warm temperate exhibiting prolonged rainy season from May to October with a record of mean annual rainfall of 2600 mm. The temperature ranges from 12 to 32°C in summer and 5 to 20°C in winter and average relative humidity varies from 67 % (March) to 88 % (October). Despite being a small state, it possesses variety of forest types like tropical wet evergreen forests, tropical semi evergreen forests and sub-tropical broad-leaf wet hill forests distributed throughout the state (Kakati and Chutia, 2009). Most of the wild silkmoth species are found to be polyphagous in nature. *A. assamensis* primarily feeds on *Persea bombycina* and *Litsea polyantha* and also on two other secondary host plants. Temperate tasar silkworm or oak tasar silkworm feeds on five different *Quercus* species, of which, *Q. serrata* is mostly palatable and all the five *Quercus* species are abundantly distributed in Kohima, Phek, Kiphire, Tuensang and Mokokchung. *A. roylei*, the wild tasar silkworm feeds on *T. myriocarpa* and *Betula alnoides* which are distributed over the state. *Attacus atlas*, the giant silkworm was collected while feeding on *Maesa indica* and *Evodia fraxinifolia* and the plants are widely distributed in different districts of Nagaland. *Actias selene*, the Indian moon moth is also polyphagous in nature and feeds on six different host plants. *Cricula trifenestrata* is found to feed on three different food plants, which are distributed in five districts of Nagaland. *S. ricini*, the domesticated silkworm is reared on *Ricinus communis, Heteropanax fragrans, E. fraxinifolia* and

Table 1: Non-mulberry silkworm host plants in Nagaland

Family	Scientific name	Local name	Silkworm species	Distribution of host plants
Anacardiaceae	*Rhus javanica* L. var. *chinensis* Miller (syn. *R. semialata* Murray)	Tangmo	*Actias selene*	Throughout Nagaland
Araliaceae	*Heteropanax fragrans* (Roxb) Seemann	Kesseru	*Samia ricini/ Samia canningi*	
Betulaceae	*Betula alnoides* Buch-Ham. ex D. Don (syn. *B. acuminata* Wall.)	Meriamtong	*Actias selene/ Cricula trifenestrata / Antheraea roylei*	
	Alnus nepalensis D. Don	Alder	*Actias selene*	
Combretaceae	*Terminalia myriocarpa* V. Huerck *et* Muell.-Arg.	Hollock	*Antheraea roylei*	
Euphorbiaceae	*Manihot esculenta* Crantz (syn. *M. utillissima* Pohl.)	Tapioca	*Samia ricini/ Samia canningi*	
	Ricinus communis L.	Castor	*Samia ricini/ Samia canningi*	
	Hevea brasiliensis (Willd. ex A. Juss.) Mull.-Arg.	Rubber	*Theophila religiosa*	
Fagaceae	*Quercus serrata* Murray	Oak	*Antheraea proylei*	Kohima, Phek, Kiphire, Tuensang, Mokokchung
	Quercus incana W. Bartram.		*Antheraea proylei*	
	Quercus semiserrata Roxb.		*Antheraea proylei*	
	Quercus acutissima Carruthers		*Antheraea* sp. *Novo/ Antheraea proylei*	
	Quercus grifithii Hook. f & Thomson ex. Miq.		*Antheraea proylei*	
Lauraceae	*Persea bombycina* King ex Hook. f. Kost (syn. *Machilus bombycina* King)	Som	*Actias selene/ Cricula trifenestrata / Antheraea assamensis*	Mokokchung, Wokha, Tuensung, Dimapur
	Litsea polyantha Juss. (syn. *Litsea monopetala* Roxb.)	Soalu	*Antheraea assamensis /Attacus atlas*	
	Litsea salicifolia Roxb. ex Nees	Dighloti	*Antheraea assamensis/ Samia canningi*	Throughout Nagaland
	Litsea cubeba (Lour.) Pers.	Mejankaori	*Antheraea assamensis / Cricula trifenestrata / Samia canningi*	Mokokchung, Wokha, Tuensung, Dimapur
Lythraceae	*Duabanga grandiflora* (Roxb. ex DC.) Walp. (syn. *D. sonneratoides* Buch-Hum)	Khokon	*Samia canningi*	Throughout Nagaland
Myrsinaceae	*Maesa indica* (Roxb.) A. DC.		*Attacus atlas*	Mokokchung, Wokha, Phek, Zunheboto
Myrtaceae	*Psidium guajava* L.	Guava	*Samia canningi*	Throughout Nagaland
Rosaceae	*Prunus cerasoides* D. Don (syn. *P. puddum* Roxb. ex Brandis Non-Miq)	Cherry	*Actias selene*	
Rutaceae	*Evodia fraxinifolia* (D. Don) Hook. f.	Payam	*Actias selene/ Attacus atlas / Samia ricini / Samia canningi*	Throughout Nagaland
Rubiaceae	*Anthocephalus cadamba* (Roxb.) Miq. [Syn. *A. chinensis* (Lam.) Rich. ex. Walp.]	Kadam	*Samia canningi*	

Figure 1: Seribiodiversity in Nagaland: Host plants of wild silkworms

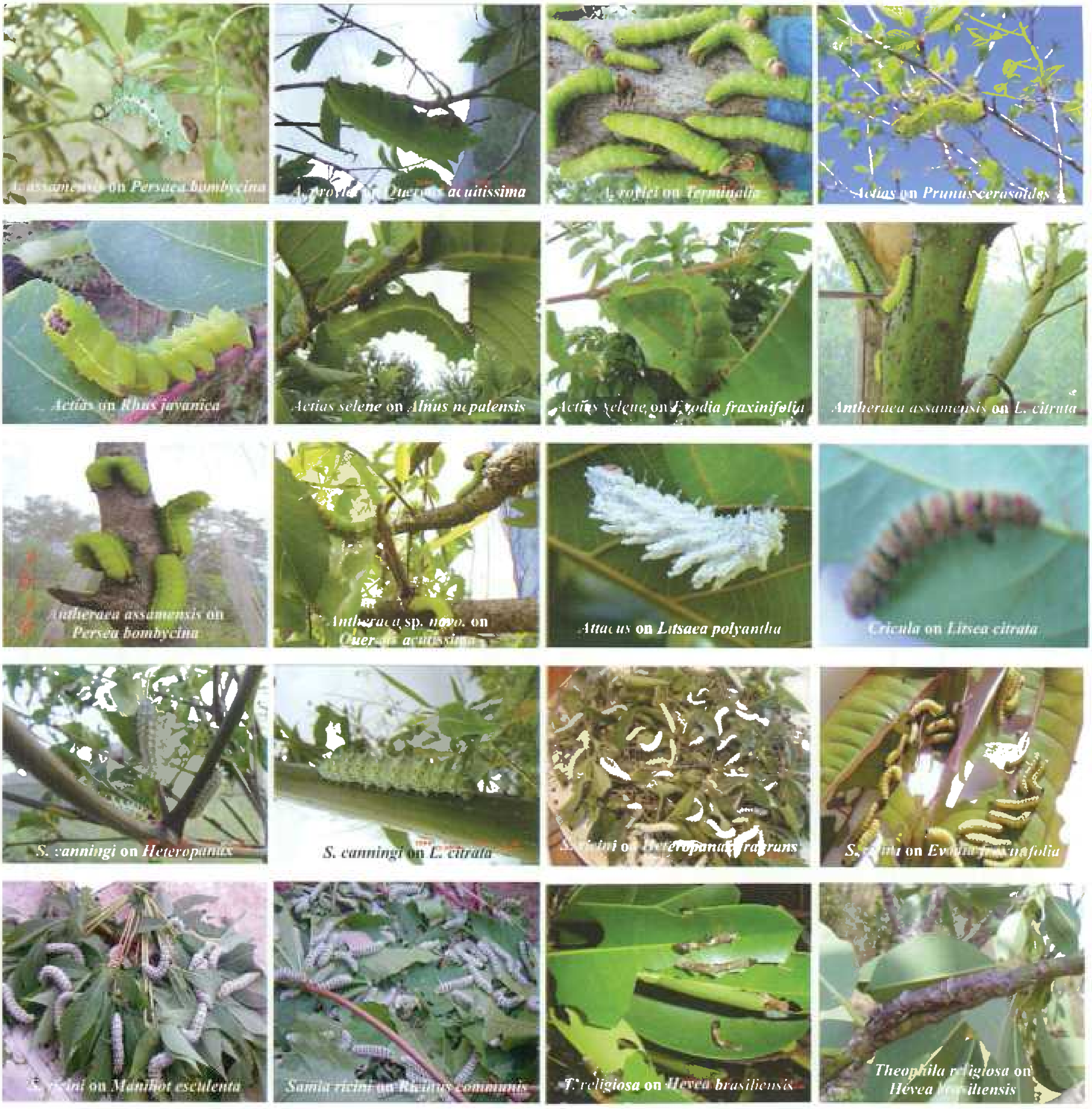

Figure 2: Seribiodiversity in Nagaland: Wild silkworms and host plants

Manihot esculanta, whereas. the wild counterpart, *S. canningi* is reported to feed on nine different host plants which are uniformly distributed throughout Nagaland. *Theophila religiosa* is reported to feed on *Hevea brasiliensis* which has uniform distribution throughout Nagaland. All the plants listed in Table 1 are not commercially exploited. Details of the host plants are enumerated as follows:

***Rhus javanica* L. var. *chinensis* Miller (syn. *R. semialata* Murray):** Deciduous shrub, height 3-7 m, Branches pubescent with lenticels. Leaves compound (20-

25 cm long), imparipinnate, leaflets (6-12 cm x 3-7 cm), 8-6 pairs, elliptical, apex acute, base rounded, terminal leaflet base cuneate, margin dentate, Lateral veins 10-15 pairs. Inflorescence compound spike. Flower white. Fruit drupe, globose, red when ripe and soured in taste The plant is uniformly distributed throughout Nagaland and *Actias selene*, the Indian moon moth is found to thrive on this plant.

***Heteropanax fragrans* (Roxb.) Seemann:** Evergreen tree, height 5-30 m. Leaves 3-5 pinnate. Rachis often with a pair of leaflets at the nodes. Leaflets ovate, elliptical or lanceolate, (5.5-13 cm x 3.5-6 cm), apex mucronate or acuminate, base cuneate, margin loosely toothed. Lateral veins 6-10 pairs. Inflorescence terminal panicle of umbels; Flowers polygamous. Fruit spherical, slightly flat on side with persistent style, soft, black and with two hetero seeds. *H. fragrans* enjoys a uniform distribution in the state of Nagaland and the silkworm spp. *viz*., *Samia ricini* and *S. canningi* live on this plant.

***Betula alnoides* Buch-Ham. ex D. Don (syn. *B. acuminata* Wall.):** Evergreen tree, height 30 m. Leaves opposite, sometimes alternate, oval, elliptical or lanceolate, (5-8 cm x 2.5- 3.5 cm), margin dentate, petiole long hairy and stipulate. Female inflorescence is raceme and pendulous while male inflorescence is catkin. Fruit is a nut. *B. alnoides* is locally known as 'Meriamtong', where wild silkworms *viz*., *Actias selene*, *Cricula trifenestrata* and *Antheraea roylei* successfully complete their lives on this host plant. The plant has a uniform distribution in Nagaland.

Alnus nepalensis D. Don: Semi deciduous tree, height 30m. Bark silver grey, wood soft. Leaves alternate, obovate or obovate oblong (4-16 cm x 2.5-10 cm), apex acute, base cuneate, margin entire or shallowly toothed. Lateral veins 8-16 pairs. Male catkins 10-25 cm long, drooping in terminal panicles. Female catkins 1-2 cm long, 3-8 together in axillary racemes. Empty catkins persist on tree. Fruit nut, compressed, dark brown and similarity with pine cone. The plant is commonly known as 'Alder' which grows uniformly in Nagaland and *Actias selene*, the Indian moon moth feeds on this plant.

***Terminalia myriocarpa* V. Huerck *et* Muell.-Arg.:** Evergreen tree, height 25-35 m with large plate root. Twig terete. Leaves opposite, oblong or lanceolate, (10-18 cm x 5-8 cm), apex short-deflected, base obtuse. Lateral veins 15-25 pairs, Inflorescence compound spike with smooth bristles. Fruit achene, triangular and pale yellow. *Terminalia myriocarpa*, is also uniformly distributed throughout Nagaland and the wild tasar silkworm, *Antheraea roylei* exists on this plant.

***Manihot esculenta* Crantz (syn. *M. utillissima* Pohl.):** Perennial shrub, height 1.5-3 m. Leaves palmatipartite, nearly circular and stipulate. Lobes 3-7, elliptic or oblanceolate, (8- 18 cm x 1.5-4 cm), apex acuminate and margin entire. Terminal or axillary receme with unisexual flowers. Fruit capsule with 6 narrow crests. The plant is seen as uniformly distributed in Nagaland and the silkworm species, *Samia ricini* and *S. canningi* are reported to thrive on this plant.

***Ricinus communis* L.:** Evergreen shrub, height 5 m. Usually twig, leaves and inflorescence are glaucous. Leaves palmate, 30-45 cm in diameter, palmate 7-11 bifida, cleft almost central. Lobes ovate-oblong or lanceolate, apex acute or acuminate with serrated edged. Venation multicostate reticulate. Inflorescence receme or panicle. Fruit capsule either smooth or with soft spines. With uniform distribution in Nagaland, the plant is reported as the host plant of silkworm species, *Samia ricini* and *S. canningi*.

***Hevea brasiliensis* (Willd. ex A. Juss.) Mull.-Arg.:** Evergreen tree, height 20-40 m. Bark smooth, hoop marked, gray with abundant white latex, crown conical, branches slender. Leaves palmate and with 3 leaflets. Leaflets alternate, elliptical or lanceolate, (10-15-50 cm x 3-6-15 cm), apex acuminate, base acute, stipulate and margin entire. Venation pinnate. Inflorescence pyramidal-shaped axillary panicles produced along with new leaves and arranged in cymose form. Flowers small, greenish-white. Fruit schizocarpic capsule and dehisce explosively. *H. brasiliensis* has a uniform presence throughout Nagaland and *Theophila religiosa is* found to feed on this plant.

***Quercus serrata* Murray:** Deciduous tree, height 25 m, bark deeply splitting longitudinally. Leaves subsessile or petiolate, glabrous, obovate, ovate-elliptic, obovate-lanceolate, (7-17 cm x 3-9 cm), apex acuminate to acute, base cuneate to nearly rounded, margin glandular serrate and spiral. Lateral veins 7-12 pairs. Female inflorescence 1.5-3 cm. Acorn globose, 1.7 cm in diameter.

***Quercus incana* W. Bartram:** Evergreen tree, height 6 m. Bark strongly furrowed, broken into square plates. Leaves petiolated, glabrous, obovaI to elliptical, (5-10 cm x 1.2-2.5 cm), apex rounded with short tooth, base rounded to attenuate, margin entire while toothed on young twigs, glossy green above, woolly haired grey green beneath and spiral. Acorn globose, 1.5 cm in diameter.

***Quercus semiserrata* Roxb.** Evergreen tree, height 10 m. Bark rough, hard with numerous white lenticels. Leaves petiolate, glabrous, oval-oblong to oboval-lanceolate, (13-25 cm x 3-7 cm), apex acuminate to obtuse, base cuneate, margin entire while apically minutely serrate, shiny above, waxy beneath and spiral. Lateral veins 9-12 pairs. Female inflorescence 2-6 cm. Acorn oblong-ellipsoid, 3.5 cm in diameter.

***Quercus acutissima* Carruthers:** Deciduous tree, height 30 m. Bark furrowed with yellowish brown lenticels. Leaves petiolate, glabrescent, narrowly elliptic-lanceolate, (8- 19 cm x 2-6 cm), apex long acuminate, base rounded to broadly cuneate, margin with spiniform teeth and spiral. Lateral veins 13-18 pairs, Acorn ovoid to ellipsoid, 2-4 cm in diameter.

***Quercus grifithii* Hook. f & Thomas ex. Miq.:** Deciduous tree, height 25 m. Leaves petiolate, obovate to obovate-elliptic, (10-20-30 cm x 4-10 cm), apex shortly acuminate to acuminate, base rounded to narrowly cuneate, margin serrulate, lateral veins 12-18 pairs. Acorn ellipsoid to ovoid ellipsoid, 1.2-1.5 cm in diameter. All the five *Quercus* species are mostly distributed in Kohima, Phek, Kiphire, Tuensang and Mokokchung districts of Nagaland and temperate tasar silkworm or oak tasar silkworm are reported to be thriving on these plants.

***Persea bombycina* King ex Hook. f. Kost (syn. *Machilus bombycina* King):** Evergreen tree, height 25m, straight hairs in young shoot, bark warty. Leaves simple, alternate, obovate or oblanceolate (7-15 cm x 2.5-5 cm); glabrous or finely pubescent and silky beneath; apex abruptly short to acuminate; base cuneate to attenuate and lateral veins 6-10. Inflorescence cymose panicle. Flowers white, green or yellow. Fruit drupe and globose with persistent tepals; purplish black when ripe. *P. bombycina* is mostly distributed in Mokokchung, Wokha, Tuensung and Dimapur and three wild silkworm species, namely *Antheraea assamensis*, *Actias selene* and *Cricula trifenestrata* feed on this plant to complete their life cycle.

***Litsea polyantha* Juss. (syn. *Litsea monopetala* Roxb.):** Semi-deciduous tree, height 18 m; brown bark longitudinally fissured. Leaves alternate, simple, elliptical (4.5-17 cm x 2.5-10 cm), apex rounded, base acute, glabrous above and pubescent beneath, lateral veins 6-13 pairs, leaf size varies. Inflorescence umbel, flowers unisexual and yellow. Fruit ellipsoid on small flat perianth cup. *L. polyantha* is abundantly distributed in Mokokchung, Wokha, Tuensung and Dimapur and two wild silkworm species, namely *Antheraea assamensis* and *Attacus atlas* are reported to live on this plant.

***Litsea salicifolia* Roxb. ex Nees:** Evergreen tree, height 10 m, branchlets are glabrous or minutely hairy. Leaves alternate and variable in shapes from elliptical to linear lanceolate (9-19 cm x 3.5-5 cm), apex acuminate or acute, base acute. Lateral veins 10-15 pairs. Inflorescence umbel. Fruit oblong. The plant has uniform distribution in Nagaland and three wild silkworm species *viz.*, *Antheraea assamensis*, *Samia canningi* and *Attacus atlas* are reported to feed on this plant.

***Litsea cubeba* (Lour.) Pers.:** Deciduous tree, height 8-10m, sapling bark yellow-green and smooth. Leaves alternate, fragrant, oblong or lanceolate (4-11 cm x 1.1-2.4 cm), apex acuminate, base cuneate. Lateral veins 6-10 pairs. Inflorescence umbel. Fruit nearly spherical; green in young and black when ripe. The plant is reported as the host of three wild silkworm species *viz.*, *Antheraea assamensis*, *Samia canningi* and *Cricula trifenstrata* and is found uniformly throughout Nagaland.

***Duabanga grandiflora* (Roxb. ex DC.) Walp. (syn. *D. sonneratoides* Buch-Hum):** Evergreen tree, height 20-30 m with large buttresses. Bark brown gray with wrinkled crack. Leaves oblong or ovate oblong (18-30 cm x 6-10 cm), apex short acuminate, base heart-shaped, lateral veins 20-24 pairs. Flowers in clusters, white, perigynous and arranged in cup shaped calyx. Fruit capsule, splits into 6-9 valves on maturity. The plant is uniformly distributed throughout Nagaland and *Samia canningi* is reported to thrive on this plant.

***Maesa indica* (Roxb.) A. DC.:** Evergreen shrub, height 1-3 m. Leaves alternate, ovate or oblong ovate, (7.5 -17 cm x 5-7 cm), apex acute, base cuneate or oblong, leathery, margin wavy with loosely dentate and glabrous. Lateral veins 7-12 pairs. Inflorescence spike. Fruit berry spherical with longitudinal ribbed and persistent calyx, whitish pink when ripe. *M. indica* is mostly distributed in Mokokchung, Wokha, Phek and Zuhneboto and *Attacus atlas* is the insect that feeds on this plant.

***Psidium guajava* L.:** Evergreen shrub, height 4-10 m. Shining bark, very smooth, gray and flaking. Twigs quadangled. Leaves oblong to elliptical, (6-12 cm x 3.5-6 cm), apex acute or obtuse, base nearly circular, lateral veins 12-15 pairs. Inflorescence axillary solitary or 2-3 flowers arranged in a cyme. Flower white, epigynous. Fruit pear shaped fleshy berry. The plant is uniformly distributed throughout Nagaland and *Samia canningi* is reported to survive on this plant.

***Prunus cerasoides* D. Don (syn. *P. puddum* Roxb. ex Brandis Non-Miq) :** Deciduous tree, height 10-30 m. Stem with glossy ringed bark in vegetative phase. Leaves alternate, elliptical with long dentate stipules (3-6 cm x 2-4 cm), apex acute, base cuneate or rounded, margin irregularly toothed. Flower scented, pinkish white. Fruit globose drupe, unripe yellow fruit turns red when ripe. *P. cerasoides* shows uniform distribution in Nagaland and *Actias selene*, the Indian moon moth use it as host plant.

***Evodia fraxinifolia* (D. Don) Hook. f.:** A medium-sized tree, height 10-20 m with aromatic glossy green leaves and clusters of small round edible purple fruits. Bark smooth and light grey; wood soft white. Leaves opposite, imparipinnate; leaflets ovate oval or oblong (4-20 cm x 2-8 cm), symmetrical or a few sides slightly oblique, dorsal midrib pubescent, petiole glabrous. Inflorescence corymb. Flower white. Fruit drupe and purple. Silkworm species, namely *Samia ricini*, *Samia canningi*, *Attacus atlas* and *Actias selene* feed on *E. fraxinifolia* which shows uniform distribution in Nagaland.

***Anthocephalus cadamba* (Roxb.) Miq. [Syn. *A. chinensis* (Lam.) Rich. ex. Walp.]:** Deciduous tree, height 20-45 m with a broad crown. Bark smooth in young, longitudinally fissured in old stem. Wood soft and white. Leaves opposite with interpetiolar stipules, ovate or somewhat elliptical (15-45 cm x 8-25 cm), apex acute, leathery and margin entire. Inflorescence terminal globose cyme. Flower epigynous. Fruit, fleshy capsules together form a fleshy yellow-orange globose structure. With uniform distribution in Nagaland, *A. cadamba* is reported as the host plant of wild silkworm, *Samia canningi*.

Conclusion

The sericultural flora and fauna of vanya silk are restricted to a particular geographical pocket and as such, more prone to extinction. These are endemic to their areas, so if the local habitats are eliminated, these species will suffer extinction. Loss of habitats for host plants due to anthropogenic activities endowed with adverse impact on loss of diversity of sericigenous insects pose as a major issue in sericulture resources of this region. India having the distinction of producing all the three types of vanya silk, *i.e.*, eri, muga and tasar, germplasm collection of these silkworm and their host plants need to be much more extensively maintained and documented so that breeders/biotechnologists can have a ready access to what they need for future and also to counteract the genetic vulnerability.

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REFERENCES

Arora G. S. and Gupta I. J. (1979) Taxonomic studies of some of the Indian non-mulberry silk moths (Lepidoptera:

Saturniidae). *Memoirs Zoological Survey, India,* **16**:1-63.

Chaudhuri A. K. (1993) *Forest Plants of Eastern India*. APH Publishing, India, ISBN-81-7024-537-0.

Chinnaswamy K. P. (2001) Sericulture Biodiversity in India. In: *Participatory Biodiversity Conservation in the South Asia Region, Proceedings of Regional Networking Seminar and Second General Assembly*, Forum of Natural Resource Managers (FONAREM)/ WWF Nepal Program, pp. 54-61.

Jain S. K. (1977) *A Handbook of Field and Herbarium Methods*. Today and Tomorrow Printers and Publication, New Delhi, India.

Kakati L. N. and Chutia B. C. (2009) Diversity and ecology of wild sericigenous insects in Nagaland. *J. Trop. Ecol.,* **50 (1):** 137-147.

Kanjilal U. N. (2005) *Flora of Assam*, Vol. IV (Revised), Omsons Publication, New Delhi, India, p. 328.

Kumar R., Bardaiyar V. N., Beck S., Hembrom Gargi I., Joshi M. C. and Sinhadeo S. N. (2005) Germplasm conservation and management of Tasar silkworm host plants. In: *Proceedings of National workshop on 'Strategies for Non-mulberry Germplasm Maintenance*, R. Chakravorty, K. Neog, K. C. Singh, S. A. S. Rahman and A. Barah (Eds.), Central Muga Eri Research and Training Institute, Central Silk Board, Lahdoigarh, Jorhat, Assam, India, pp. 18-27.

Nassig W. A., Lempe R. E. J. and Kager S. (1996) Saturniidae of Sumatra (Lepidoptera). *Heterocera Sumatrana,* **10 (3)**:1-110.

Negi S. S. and Naithani H. B. (1995) *Oaks of India, Nepal and Bhutan*. M/s International Book Distributor, Dehradun, India.

Neog K., Gogoi S. N. and Chakravorty R. (2005) Present status and constraints of muga silkworm host plant germplasm conservation. In: *Proceedings of National workshop on 'Strategies for Non-mulberry Germplasm Maintenance*, R. Chakravorty, K. Neog, K. C. Singh, S. A. S. Rahman and A. Barah (Eds.), Central Muga Eri Research and Training Institute, Central Silk Board, Lahdoigarh, Jorhat, Assam, India, pp. 1-10.

Orwa C., Mutua A., Kindt R., Jamnadass R. and Simons A. (2009) Agroforestry Database: a tree reference and selection guide version 4.0 (http://www.worldagroforestry.org/af/treedb/). Accessed on 03/08/2014.

Peigler R. S. (1999) Taxonomy, distribution and sericultural potential of the American species of *Antheraea* (Saturniidae). *Bull. Indian Acad. Seric.*, **3 (1)**:1-9.

Sarmah M. C., Rahman S. A. S. and Chakravorty R. (2005) Biodiversity of eri silkworm host plants. In: *Proceedings of National workshop on Strategies for Non-mulberry Germplasm Maintenance*, R. Chakravorty, K. Neog, K. C. Singh, S. A. S. Rahman and A. Barah (Eds.), Central Muga Eri Research and Training Institute, Central Silk Board, Lahdoigarh, Jorhat, Assam, India, pp. 11-17.

Singh B. D. and Mishra P. N. (2003) Culture of Vanya Silk *vis-à-vis* forestry with relevance to North-Western Himalayan Ecosystem. *Proceedings of the National Workshop on Vanya Silk Culture and Forestry*, Bangalore, India, pp. 76-80.

Singh H. B. and Subramanium B. (2008) *Field Manual on Herbarium Techniques*. National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India.

Singh N., Ibotombi L., Singh, Somen, Singh N. Ibohal and Suryanarayana N. (2005) Biodiversity of oak Tasar host plant germplasm and its maintenance. In: *Proceedings of National workshop on Strategies for Non-mulberry Germplasm Maintenance*, R. Chakravorty, K. Neog, K. C. Singh, S. A. S. Rahman and A. Barah (Eds.), Central Muga Eri Research and Training Institute, Central Silk Board, Lahdoigarh, Jorhat, Assam, India, pp. 28-36.

Soerianegara I. and Lemmens R. H. M. J. (1993) Plant

Resources of South-East Asia. In: *Timber trees: major commercial timbers*, Backhuys Publishers, Leiden, No. **5 (1).**

Srivastava A. K., Geetika P. D., Singh R., Siddiqui A. A., Das P. K. and Raghuvanshi S. S. (1998 a) Nutritional status of muga food plants-*Litsaea polyantha. Sericologia,* **38 (4):** 693-695.

Srivastava P. K., Sinha U. S. P. and Thangavelu K. (1998 b) Foliar characters and constituents in spontaneous hybrid genotypes of *Terminalia* (Pentoptera). *Indian J. Seric.,* **37 (1)**: 76-78.

Srivastava P. K. and Thangavelu K. (2005) *Sericulture and Seri-biodiversity.* Associated publishing Company, New Delhi, India, pp. 1-254.

Suryanarayana N., Sarmah M. C. and Phukan J. D. (2002) Seri-Biodiversity conservation for sustainable development in North-East India. Abstract, *Regional seminar on "Role of Biodiversity and Environmental Strategies in North East India for Sustainable Development*, 27-28th November 2002, Shillong, Meghalaya, India.

Thangavelu K. (1991) Wild sericigenous insect of India- a need for conservation. *Int. J. Wild Silkmoth Silk*, pp. 71-77.

Thangavelu K., Rao K. V. S. and Pandey V. K. (2002) Wild silk moths biodiversity and Conservation. *Int. J. Wild Silkmoth Silk,* **7**: 87-93.

Wu Z. Y. and Raven P. H. (1996) *Flora of China,* Vol. 15, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, USA.

Wu Z. Y. and Raven P. H. (1999) *Flora of China.* Vol. 4, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, USA.

Wu Z. Y., Raven P. H. and Hong D. Y. (2003) *Flora of China,* Vol. 9, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, USA.

Wu Z. Y., Raven P. H. and Hong D. Y. (2007) *Flora of China,* Vol. 13, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, USA.

Wu Z. Y., RavenP. H. and Hong D. Y. (2008) *Flora of China,* Vol. 7, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, USA.

Wu Z. Y., Raven P. H. and Hong D. Y. (2008) *Flora of China,* Vol. 11, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, USA.

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Brief Note

ANTIOXIDANTS DEFENCE IN THE DIAPAUSING PUPAE OF DABA AND MODAL ECORACES OF TASAR SILK MOTH, *ANTHERAEA* SP.

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ABSTRACT

In India, tropical tasar is produced by the silkworm, *Antheraea* sp. where, Modal is one of the wild ecoraces and Daba is one of the semi-domesticated ecoraces of this species. The present investigation accounts for the status of some of the antioxidants in the haemolymph and fat body tissues of diapausing pupa of the above two ecoraces with emphasis on the contents of protein, ascorbic acid (Vitamin C), reduced glutathione (GSH) and level of lipid peroxidation (LPX), *i.e.*, by assessing the malondialdehyde (MDA) content formed. Results of the present study indicate that the pupae of Modal are superior to those of Daba based on their antioxidant status.

Key words: *Antheraea*, antioxidant, fat body, haemolymph, silkworm.

INTRODUCTION

Reactive oxygen species (ROS) generated as part of normal physiological processes or by exogenous factors, are known to induce oxidative damage to biological macromolecules, such as membrane lipids, proteins and DNA (Khoobchandani *et al.*, 2009). All aerobic organisms are endowed with mutually supportive teams of defense against ROS or the pro-oxidant forces, commonly called as antioxidant defences. Insects, like other animals, possess a suite of antioxidant systems for the removal of damaging ROS (Felton, 1995). Ascorbic acid is a redox catalyst which can reduce and neutralise ROS, such as hydrogen peroxide (Padayatty *et al.*, 2003). Glutathione (GSH) is one of the most important cellular antioxidants (Meister and Anderson, 1983). Acute or chronic oxidative stress may result in uncontrolled lipid peroxidation (LPX) and protein oxidation (Levine *et al.*, 1981) as proteins are vulnerable to oxidative damage (Dean, 1991). This may occur either due to the interaction between LPX products and proteins or by site-specific reactions of protein to ROS. In phytophagous insects, antioxidant defence mechanisms are of paramount importance because they are exposed to ROS promotive environments as well as allelochemicals present in the host plants (Patra *et al.*, 2013).

Modal ecorace of *Antheraea paphia* is exclusively wild, univoltine in nature and multiplies on Sal (*Shorea robusta*) plant during rainy season (Dey *et al.*, 2010). The quality of yarn produced out of Modal cocoon is the best of wild silk in the world. The antioxidant status of the pupae of commercial seed cocoons of this wild tasar silk moth especially, Modal ecorace has not been studied. Daba is one of the semi-domesticated ecoraces of *A. mylitta* which is bivoltine or trivoltine based on altitudinal gradient. The objective of this study is to compare the antioxidant status of these two ecoraces of *Antheraea* sp. in the pupal stage. Moreover, an attempt has been made to screen the antioxidant potential and the oxidative stress indices in their body tissues *viz.*, fat body (FB) and haemolymph (HL).

The live pupae of Daba ecorace of tasar silkworm, *A. mylitta* were collected from the host plants, arjun (*Terminalia arjuna*) and asan (*Terminalia tomentosa*) maintained in the local sericulture rearing fields of State Government, Baripada, Mayurbhanj, Odisha. The live pupae of Modal ecorace of tasar silkworm, *A. paphia* were collected from the host plant, sal (*Shorea robusta*) from the Similipal Biosphere Reserve, Mayurbhanj, Odisha.

Tissue preparation: The body segment of the pupa was cut and the haemolymph was collected in prechilled eppendorf tube coated with 3 % phenylthiourea to prevent denaturing or blackening of the haemolymph (Mishra *et al.*, 2009) and centrifuged at 7,000 x g for 10 minutes at 4°C to settle the haemocytes. Pupae were dissected to collect the fat body. After washing in ice-cold physiological saline (0.67 % NaCl), the tissues were retained in ice separately. The tissues were weighed in monopan digital balance and homogenised (10 %) in 50mM phosphate buffer of pH 7.4 with 1mM EDTA using hand homogeniser under ice (Patra *et al.,* 2013). Homogenates were centrifuged at 10,000 x g for 20 minutes at 4°C. The supernatants were collected for further chemical analyses.

Biochemical estimation: The amount of proteins was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Ascorbic acid (ASA) content was measured according to the method of Jagota and Dani (1982). The reduced glutathione (GSH) content in the tissue samples was determined according to the method of Ellman (1959). The sample after centrifugation was used for the estimation of lipid peroxidation (LPX) by monitoring the formation of malondialdehyde (MDA) by the method of Ohkawa *et al.* (1979). The amount of MDA formed was calculated from the extinction coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$ as per Wills (1969).

Statistics: To know the difference between means of two dependent samples, paired t-test was employed as per Chainy *et al.* (2008).

To compare the antioxidant contents between two ecoraces for the same tissue, paired t-test was employed. It was revealed that, the protein concentration in Daba was significantly higher ($P < 0.001$) than that of the Modal in the fat body. The opposite pattern was observed in the haemolymph protein content, wherein the Modal had it significantly higher ($P < 0.001$) than that of the Daba (Figure 1). The ascorbic acid (ASA) content was found significantly higher ($P < 0.01$; $P < 0.05$) in Modal than that of the Daba in the FB and HL tissues, respectively (Figure 2). The concentration of reduced glutathione (GSH) content was high in Modal than that of the Daba in FB and HL tissues but the differences were not significant (Figure 3). The level of lipid peroxidation (MDA content formed) was significantly higher ($P < 0.01$ and $P < 0.001$) in Modal than that of the Daba for FB and HL tissues, respectively (Figure 4).

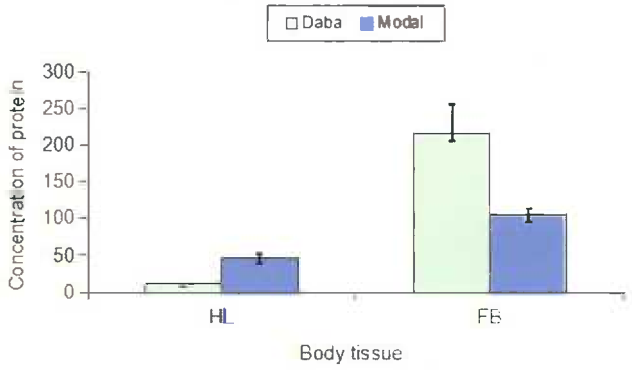

Figure 1: Concentration of protein in haemolymph (mg/ml) and fat body (mg/g) of Daba and Modal pupae of *Antheraea* sp. Data are mean± SD (n=6 each). HL-Haemolymph; FB-Fat body.

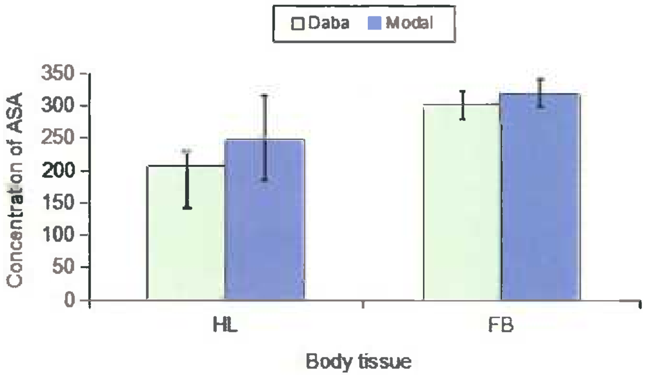

Figure 2: Concentration of ascorbic acid (ASA) in haemolymph (µg/ml) and fat body (µg/g) of Daba and Modal pupae of *Antheraea* sp. Data are mean ± SD (n=6 each). HL-Haemolymph; FB-Fat body.

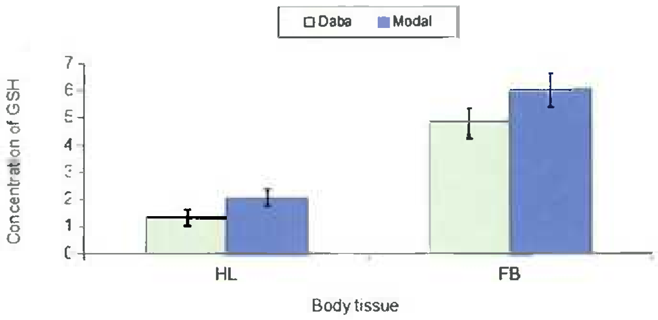

Figure 3: Concentration of glutathione in haemolymph (µmol/ml) and fat body (µmol/g) of Daba and Modal pupae of *Antheraea* sp. Data are mean± SD (n=6 each). HL-Haemolymph; FB-Fat body.

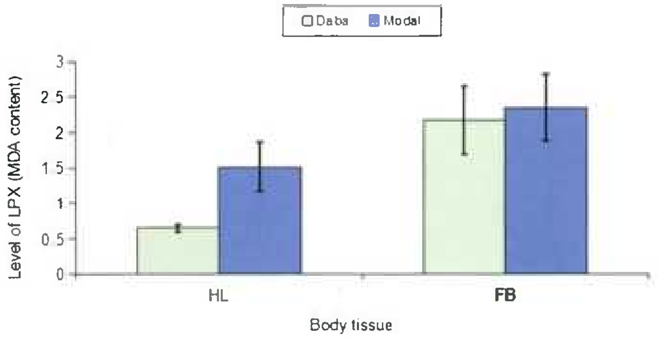

Figure 4: Concentration of MDA (malondialdehyde) in haemolymph (µg/ml) and fat body (µg/g) of Daba and Modal pupae of *Antheraea* sp. Data are mean± SD (n=6 each). HL-Haemolymph; FB-Fat body.

From the present results, it is evident that Modal pupae have high protein concentration than Daba in their haemolymph which may work as a preventive measure against oxidative damage in the tissues as reported by Dean (1991). On the other hand, in Daba, it is the FB that carries high protein content than that in Modal which takes care of the oxidative damage. The high concentration of ASA content in the HL and FB tissues of Modal is an indication of reduction and neutralisation of ROS, such as hydrogen peroxide as described by Padayatty *et al.* (2003). The higher concentration of GSH content in the FB and HL tissues of Modal is the indication of higher cellular antioxidant activity to protect the cell from oxidative damage. Comparisons of levels of LPX in both the tissues of pupae indicate that FB tissue is challenged with relatively higher level of oxidative threat than HL. Considerably higher level of MDA in the FB than that of HL reflects a relatively high level of activated oxygen species which could be due to their increased production and/or decreased neutralisation.

From the present findings, it is concluded that the oxidative stress was comparatively high concomitant with better antioxidant protection in the fat body of both the groups. It was further observed that between the two ecoraces, Daba and Modal, the latter experiences more oxidative assault with simultaneous induction of non-enzymatic antioxidants (GSH and ASA) probably as an adaptive cellular response due to higher rate of oxygen consumption.

REFERENCES

Chainy G. B. N., Mishra R. and Mohanty P. K. (2008) *Basic Biostatistics*. Kalyani Publishers, New Delhi, India, p. 353.

Dean R. T. (1991) Protein damage and repair: An overview. In: *Oxidative Damage and Repair: Chemical, Biological and Medical Aspects,* Davies K. J. A. (Ed.), Pergamon Press, New York, pp. 341-347.

Dey D. G., Mohanty N., Guru B. C. and Nayak B. K. (2010) *Tasar Silk moth of Similipal.* Indian Academy of Sericulture, Bhubaneswar, Orissa, India, p. 97.

Ellman G. L. (1959) Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*, **82**: 70-77.

Felton G. W. (1995) Antioxidant defence of vertebrates and invertebrates. In: *Oxidative Stress and Antioxidant Defences in Biology,* Ahmad S. (Ed.), Chapman and Hall, Springer, New York, pp. 356-434.

Jagota S. K. and Dani H. M. (1982) A new colorimetric technique for the estimation of vitamin-C using folin phenol reagent. *Clin. Biochem.*, **127**: 178-182.

Khoobchandani M., Ojeswi B. K., Hazra D. K. and Srivastava M. M. (2009) *Brassica oleracea* extracts: Potential for antioxidative and anticancer activities for $B_{16}F_{10}$ and $B_{16}F_1$ mice melanoma cell lines. *Natl. Acad. Sci. Lett.*, **32 (9-10)**: 297-302.

Levine R. L., Oliver C. N., Fulks R. M. and Stadtman E. R. (1981) Turnover of bacterial glutamine synthase: Oxidative inactivation proceeds proteolysis. *Proc. Natl. Acad. Sci.*, USA, **78**: 2120- 2124.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **19**: 265-275.

Meister A. and Anderson M. (1983) Glutathione. *Annu. Rev. Biochem.*, **52**: 711-760.

Mishra P. K., Kumar D., Jaiswal L., Kumar A., Singh B. M. K., Sharan S. K., Pandey J. P. and Prasad B. C. (2009) Role of haemolymph biochemical constituents and brain proteins in maintenance of pupal diapause in Daba bivoltine ecorace of tropical tasar silkworm *Antheraea mylitta* Drury. *J. Ecophysiol. Occup. Hlth.*, **9**: 243-252.

Ohkawa H., Ohishi N. and Yagi K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Annu. Rev. Biochem.*, **95**: 351 - 358.

Padayatty S., Katz A., Wang Y., Eck P., Kwon O., Lee J., Chen S., Corpe C., Dutta A., Dutta S. and Levine M. (2003) Vitamin C as an antioxidant: Evaluation of its role in disease prevention. *J. Am. Coll. Nutr.*, **22 (1)**: 18-35.

Patra G. C., Mohanty N. and Dey D. G. (2013) Oxidative stress indices in the larva and pupa of wild tasar silk moth, *Antheraea paphia* Linn. *Sericologia*, **53 (3&4)**:176-181.

Wills E. D. (1969) Lipid peroxide formation in microsomes: General considerations. *Biochem. J.*, **113**: 315 - 324.

NEWS CORNER

24th International Congress of ISC on Sericulture and Silk Industry held at Bangkok, Thailand during 10-14 August 2014

The 24th ISC Congress was organized at Queen Sirikit Convention Centre, Bangkok during 10-14 August 2016. The Congress was formally inaugurated on the 2nd day by the Hon'ble Deputy Minister of Agriculture and Cooperatives, Govt. of Thailand. While inaugurating the Congress, the Hon'ble Minister appealed to the International Sericultural Commission to support the developing countries to introduce and develop sericulture as an economically viable occupation for employment generation and poverty alleviation. The Minister appreciated the various initiates of ISC after shifting its Office from Lyon, France to Bangalore, India and hoped that this change would facilitate to replicate the successful model of sericulture practice developed in India to other ISC Member Countries including Thailand. The Minister has also advised the Department of Sericulture, Thailand to enter close collaboration with India to learn on the intricate aspects of commercial sericulture practice.

The Congress witnessed the participation of 313 delegates from 13 Member Countries. 152 scientific papers were presented in oral and poster sessions from 10th to 13th August 2016.

The Congress commenced with the keynote speeches on two issues which are related to the theme of the Congress "Silk for the green world and sustainable development". The keynote speeches were delivered by (1) Mr. Jitti Mungkalasiri, National Metal and Materials Technology Center, National Science and Technology Development Agency, Thailand on "Sericulture for Decent Work life Balance" and (2) Mr. Surindr Supasavasdebhandu, Deputy Managing Director, Thai Silk Company Limited, Thailand on "Sericulture Development for Farmers with Sustainable Agriculture". Both the subjects were concentrated on the contemporary issues and emphasised the need for innovative practice of sericulture and silk industry in the changed scenario. The issues found greater resonance among the delegates on a variety of areas prevailing in their respective countries.

NEWS CORNER

The Plenary Session of the Congress was conducted on 13th August 2015. The Chairpersons of eight Sessions have presented the highlights of the papers from their respective Sessions for final adoption by the Congress. The Session ended with formal thanksgiving speech delivered by Dr. H. Nagesh Prabhu, Secretary General, ISC. The SG expressed his profound thanks to the Govt. of Thailand, the organizing Committee Members of the Queen Sirikit Department of Sericulture, delegates from different countries and his colleagues from ISC for organizing a highly successful event.

LOUIS PASTEUR AWARD - 2016

ISC has been conferring the prestigious Louis Pasteur Award to persons who have outstanding contributed to the development of silk industry in their country or across the world. The award is given away once in three years for three persons selected from across the globe. The awardee need not be a researcher, but anyone who had outstandingly contributed for the development of sericulture and silk industry. The Louis Pasteur Award consists of a Citation and a Medal. Nominations received from different countries were evaluated and selected by an International Committee comprising 5 renowned scientists/technocrats from Japan, France, Romania, Thailand, and India. The unique distinction of the award 2016 is that an outstanding researcher from Japan, a brilliant bureaucrat from India, and an enduring silk industrialist from Thailand have been chosen for this prestigious award. The Louis Pasteur Awardees -2016 are:-

1	Dr. Masahiro Tomita, Japan
2	Dr. Sanjay Kumar Panda, India
3	Mr. Surindr Supasavasdebhandu, Thailand

Louis Pasteur Awardees with the Hon'ble Deputy Minister of Agriculture and Cooperatives, Govt. of Thailand

GHANA, THE 18TH MEMBER COUNTRY OF ISC

The Republic of Ghana has become the 18th Member Country of ISC with effect from 1st August, 2016. His Excellency, Mr. Ahmed Yakubu Al Hassan, Deputy Minister, Ministry of Food and Agriculture, has signed the Instrument of Accession signalling Ghana's entry in ISC.

On behalf of the ISC Member Countries, the ISC Secretariat cordially welcomes Republic of Ghana Kenya as the 18th Member Country of ISC.

REPUBLIC OF GHANA

NEWS CORNER

GLOBAL SILK PRODUCTION STATISTICS (METRIC TONNE)

Country	2011	2012	2013	2014	2015
Bangladesh	38	42.50	43	44.5	44
Brazil	558	614	550	560	600
Bulgaria	6	8.5	8.5	8	8
China	104000	126000	130000	146000	170000
Colombia	0.6	0.6	0.6	0.5	0.5
Egypt	0.7	0.7	0.7	0.82	0.83
India	23060	23679	26480	28708	28523
Indonesia	20	20	16	10	8
Iran	120	123	123	110	120
Japan	42	30	30	30	30
North Korea	300	300	300	320	350
South Korea	3	1.5	1.6	1.2	1
Philippines	1	0.89	1	1.1	1.2
Syria	0.5	0.5	0.7	0.5	0.3
Thailand	655	655	680	692	698
Tunisia	3	3.95	4	4	3
Turkey	22	22	25	32	30
Uzbekistan	940	940	980	1100	1200
Vietnam	500	450	475	420	450
Madagascar	16	18	18	15	5
Total	**129661.80**	**152845.64**	**159737.10**	**178057.62**	**202072.83**

24th ISC Congress held at Bangkok during 10th to 14th August 2016

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INFORMATION TO CONTRIBUTORS

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- **Key words** should follow the abstract, subject to a maximum of five, arranged in alphabetic order.
- **Main text** should have serially arranged sections such as Introduction, Materials and Methods, Results and Discussion for an effective and systematic presentation of the contents. Acknowledgements may be included if relevant. Only standard abbreviations should be used. Whenever specialized abbreviations are used, it should firstly be given in full with the abbreviation indicated in parenthesis Scientific names should be given for all species used in the investigation.
- **Tables** should be simple, centered, separately numbered & self explained, and titles must be above the tables. Sources of data if any, should be mentioned below the table. All tables and figures should be referred to in the text.
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Dollo M , Samal P. K., Sundriyal R. C. and Kumar K. (2009) Environmentally sustainable traditional natural resource management and conservation in Ziro Valley, Arunachal Himalaya, India. *J. Am. Sci.*, **5:** 41–52.

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

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

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

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

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