

ISSN 0250-3980



Volume 53 Number 2 2013

SERICOLOGIA

Journal of Silkworms
A Quarterly Publication of International Sericultural Commission



SERICOLOGIA

Journal of Silkworms

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Fax: +91 80 26681663, E-mail: sericologia@gmail.com, www.insarco.org

Printed at Creative Graphics, #149, 1st Floor, Somanna Complex, Sultanpet Main Road, Bengaluru - 560 053, INDIA. Tel: +91 80 22370262

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SCREENING AND EXPRESSION PROFILING OF *HAL3A*, DEHYDRIN, ABC TRANSPORTER AND *NHX1* IN TEN GENOTYPES OF MULBERRY, *MORUS* SP., FOR ABIOTIC STRESS TOLERANCE

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ABSTRACT

Drought and salinity stress are two major abiotic factors that limit plant growth and productivity. By using an efficient *in vitro* screening method, ten economically important mulberry genotypes were screened for tolerance against drought and salinity stress. Leaf discs from field-grown plants were subjected to three hours of simulated drought and salinity stress by aerial drying and 200 mM sodium chloride treatment, respectively to study their physiological characteristics as indicators of tolerance index in mulberry. Important parameters such as relative water content (RWC), proline accumulation, nitrate reductase activity, cell membrane stability (CMS) and photosynthetic yield were estimated to evaluate the tolerance of genotypes against drought and salt stress. Expression analysis of stress responsive genes *i.e.*, *Hal3A*, *NHX1*, *dehydrin* and *ABC transporter* was undertaken to quantify the levels of expression under simulated drought and salt stress. S13 and AR-12 varieties exhibited drought resistance whereas, S34 and K2 appeared to be salt tolerant genotypes. Distinguishing salt and drought tolerant genotypes would be an added advantage to expand mulberry cultivation to salt and drought affected lands, thereby increasing the availability of leaf material for sericulture industry.

Key words: Cell membrane stability, drought, genotype, mulberry, proline, salinity.

INTRODUCTION

The silkworm, *Bombyx mori* feeds exclusively on leaves of mulberry. Mulberry being a deep rooted perennial tree, grows in tropics and temperate regions. The advancement of sericulture industry in India depends upon the quantitative and qualitative improvement of mulberry varieties, thereby to suit various agroclimatic zones. Mulberry leaf metabolism is severely affected by high temperature, water and salinity stresses (Khurana and Checker, 2011). The degree of stress tolerance varies greatly between species and also amongst varieties of same species. Therefore, a thorough understanding of the physiological basis of such differences in stressed conditions would be a tool for selection or creation of new, promising varieties under challenged conditions. Drought and salinity are

particularly widespread in many regions of the world and may cause serious salinization of more than 50 % of all arable lands by the year 2050 (Ashraf and Harris, 2004). In the present study, attempts have been made to screen the mulberry germplasm for drought and salinity stress using simulated stress conditions. Additionally, expression of stress induced genes of mulberry *i.e.*, *Hal3A*, *Dehydrin*, *ABC transporter* and *NHX1*, which were earlier characterized from cDNA library of mature mulberry leaves (Lal *et al.*, 2009) and are reported to be involved in acquiring stress tolerance in other crops, was investigated in ten genotypes of mulberry. Dehydrin belongs to the group II of LEA proteins and is known to be one of the most abundant plant proteins produced during late embryogenesis or in response to drought, low-temperature, salinity, and

ABA (Close, 1997). Expression of *Oryza sativa* dehydrin 1 (*OsDhn1*), was strongly induced by low temperature as well as drought (Lee *et al.*, 2005). Over expression of barley and wheat LEA genes, *HVA1* and PMA1959, respectively increased tolerance to drought and salt stress in transgenic rice (Xu *et al.*, 1996; Cheng *et al.*, 2002). In mulberry, expression of LEA group protein *HVA1* also conferred tolerance to drought and salt stress (Lal *et al.*, 2008). Cellular homeostasis in salinity-stressed plants mainly depends on maintenance of the cytoplasmic K^+/Na^+ ratio with discrimination of Na^+ over K^+ . NHX-type transporters have a key function in maintenance of cytoplasmic Na^+ homeostasis by mediating a secondary active Na^+/H^+ antiporter at the tonoplast for vacuolar Na^+ sequestration (Apse *et al.*, 1999; Gaxiola *et al.*, 1999; Quintero *et al.*, 2000). Two *Hal3* genes were isolated and characterized from *Arabidopsis thaliana* and expression of these genes seemed to increase under salt and drought conditions. Also over expression of these genes showed tolerance against salt and drought (Espinosa-Ruiz *et al.*, 1999). Overexpression of *Nicotiana Hal3A* showed resistance against salt and osmotic stress (Yonamine *et al.*, 2004). ABC transporters form a large family of proteins involved in the transport of substances across the cell membrane in plants and other lower organisms and conferred resistance to various biotic and abiotic stresses (Martinoia *et al.*, 2002). Cloning of several genes encoded by members of this gene family in *Arabidopsis* and other species established that this protein is not only involved in ion regulation and detoxification but also in growth and development of plants. Hence, in this study, the trend of physiological parameters under drought and salinity stress as well as degree of expression of stress responsive genes were analysed in ten mulberry genotypes as indicators of their tolerance index. We hope that the information generated from this study would support the identification and development of mulberry genotypes well adapted to stress conditions and their exploitation under different agroclimatic zones.

MATERIALS AND METHODS

Plant Material

Leaf discs of uniform size (1.3 cm diameter) of ten mulberry genotypes *i.e.*, K2, DD, RFS-175, S13,

S1, C176, C776, Goshorami, AR12 and S34 were subjected to dehydration and salt stress (200 mM) for 3 hours and biochemical analysis was undertaken for relative water content (RWC), proline estimation, membrane stability index (MSI), nitrate reductase activity and chlorophyll fluorescence to screen them. The experiments were repeated thrice and the data presented are the mean of three independent values.

Relative water content (RWC)

Leaves from each genotype were weighed immediately after plucking to record fresh weight (FW), followed by immersing them in distilled water for 12 h. The leaves were blotted and weighed to record fully turgid leaf weight (TW), and subjected to oven drying at 70 °C for 24 h to record dry weight (DW). The RWC was determined as follows: $RWC = (FW - DW) \times 100 / (TW - DW)$, according to Lal *et al.* (2008).

Proline estimation

Free proline content was determined according to Bates *et al.* (1973). Absorbance was recorded at 520 nm in a UV-visible spectrophotometer (Beckman DUTM 640B Beckman instruments Inc., USA) against toluene blank and total proline content was calculated as: $\mu\text{moles of proline/gFW} = (\mu\text{g protein/mL} \times \text{mL toluene} \times 5) / 115.5 \mu\text{g}/\mu\text{moles} \times \text{g sample}$

Nitrate reductase activity

Nitrate reductase (NR) activity of leaf discs was estimated according to Sairam *et al.* (1997) Absorbance was recorded at 540 nm in a spectrophotometer (Beckman DUTM 640B Beckman instruments Inc., USA).

Cell membrane stability

Cell membrane stability (CMS) was determined according to the method of Sullivan and Ross (1979). Cellular injury was determined using the equation: $CMS = \{1 - (C1/C2)\}$ where C1 = conductivity measured 30 minutes after incubation and C2 = conductivity after autoclaving.

Photosynthetic yield

Chlorophyll fluorescence was measured with a portable fluorometer (PAM-210, Walz, Germany) after

the imposition of specific stress treatment. The initial fluorescence level (F_o) with all PSII reaction centers open was recorded by measuring the modulated light which was sufficiently low. The maximal fluorescence level (F_m) with all PSII reaction centers closed was determined by a 0.8 s saturating pulse at $8000 \mu\text{mol s}^{-1}\text{m}^{-2}$ in dark adapted leaves. Photosynthetic yield was measured as F_v/F_m where $F_v = F_m - F_o$. The leaves were illuminated by an actinic light of $500 \mu\text{mol m}^{-2}\text{s}^{-1}$. When the leaf reached steady state photo-synthesis, the steady state fluorescence (F_s) was recorded and a second 0.8 s saturating pulse at $8000 \mu\text{mol m}^{-2}\text{s}^{-1}$ was given to determine the maximal fluorescence (F_m') in the light adapted state. The actinic light was then turned off, the minimal fluorescence in the light adapted (F_o') state was determined by illumination with far-red light. Actual photosynthetic yield is measured as $\Phi \text{ PSII} = (F_m' - F_o')/F_m'$.

Gene expression analysis

Mature leaves of various cultivars of mulberry plants maintained in the Departmental garden were used for the stress treatments. Second or third leaf from the shoot apex was employed to study expression profiles of stress inducible genes under various kinds of treatments/simulations. For salinity stress, mature leaves were submerged in water purified through Reverse Osmosis system and supplemented with 200 mM and 400 mM NaCl for 3 h. At the end of three hours, tissue was frozen in liquid nitrogen. Similarly, dehydration stress was imposed by air drying the leaves in the laminar flow hood for three hours. Total RNA was isolated from leaf tissues using modified GITC (guanidium isothiocyanate) protocol. RNA quality was checked by loading equal concentration of RNA on 1.2 % formaldehyde agarose gel. Presence of intact 28 s and 18 s RNA in the ratio of 2:1 was taken as an indication of good quality RNA. Full length mulberry *NHX1* (1600bp) was amplified by gene specific primers NHX F (AAG GAT CCA TGA CTG GAG TTT TGA G) and NHX R (CGA GCT CTC ATT CCC ATT GAG GAG GAG C) whereas *Hal3A* (700bp), *dehydrin* (500bp) and *ABC transporter* (1000bp) were amplified from cDNA clones using M13F (CCC AGT CAC GAC

GTT GTA AAA CG) and M13R (GC GGA TAA CAA TTT CAC ACA GG) universal primers. Fragments obtained by amplification were purified and used as probes for expression analysis.

Northern blot analysis - A total of 10-20 μg of RNA was electrophoresed on 1 % (w/v) formaldehyde-agarose gels, and transferred to Hybond N⁺ membranes and hybridized with denatured $\mu\text{-}^{32}\text{P}$ -labelled DNA probe (Sambrook *et al.*, 1989) for 16-24 h at 60°C. The membrane washing and development of blot was done according to the protocol described. PCR purified products of *NHX1*, *Hal3A*, *dehydrin* and *ABC transporter* were used as probes.

RESULTS

Physiological / biochemical screening under simulated conditions of drought and salinity stress

Relative water content

Since mulberry leaves are harvested and stored for a minimum span of one day to meet the daily feeding schedule of silkworm, the water content of the leaf tissue is an important parameter in determining leaf quality. Relative water content of the genotype was immediately affected by simulated drought and salinity stress. Drought stress reduced the RWC of AR-12 by only 26 % but in the case of RFS-175, S13 and Goshorami, RWC had fallen to the levels of 67, 65 and 64 %, respectively, which indicates their overall sensitivity to drought. Other genotypes showed a moderate response to drought (Figure 1A). It was observed that salt stress also affected RWC. AR-12 was the least affected, followed by S13 having 13% and 28% RWC, respectively. At the same duration of stress, cv. Goshorami showed 41% reduction in RWC, followed by S1 and S34 (Figure 2A). Under both the conditions of salt and drought stress, RWC of AR-12 was the least affected, suggesting that it might be better tolerant to stress.

Proline accumulation

Proline serves as an indicator of cellular stress and acts like an osmoprotectant. In this study, the leaf discs were aerial dried for different time periods and it was observed that proline levels increased in all the genotypes

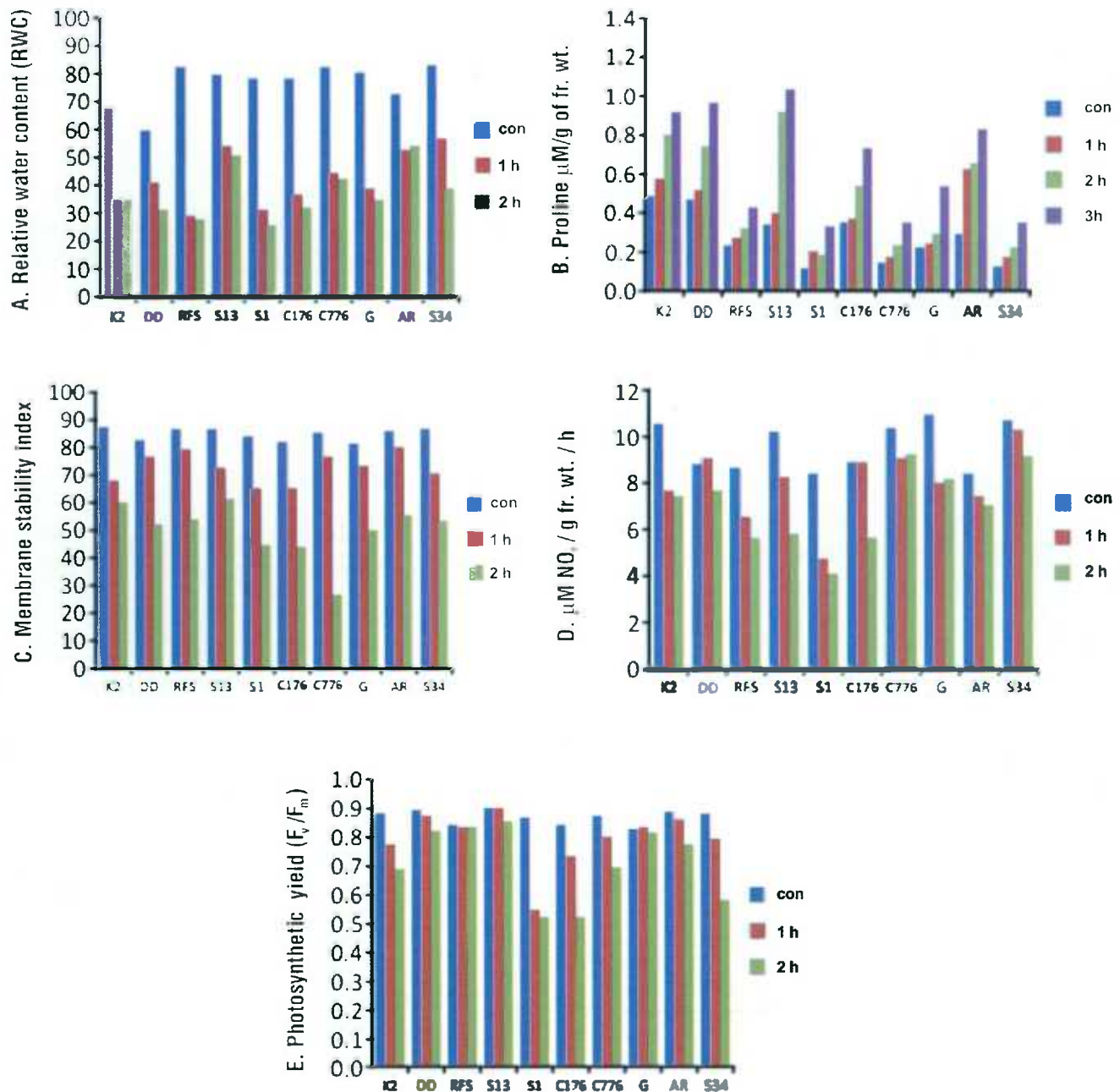


Figure 1: A: Relative water content, B: proline accumulation, C: membrane stability index, D: nitrate reductase activity and E: photosynthetic yield of leaf discs of mulberry genotypes subjected to 3 hours of dehydration stress.

with increasing duration of stress with prominent varietal difference in terms of amount of proline accumulated. In spite of continuous accumulation of proline after 2 h, per cent proline increase was remarkable in DD, S13 and AR12. S13 accumulated more proline than other genotypes, hence appears to be more tolerant to drought stress (Figure 1B). Proline accumulation was less in the case of K2 and RFS-175 after two hours of dehydration

stress, and therefore, these genotypes appear to be sensitive to drought stress (Figure 1B). The leaf discs were also given 200 mM NaCl stress for different time periods and free proline content in the leaves of control and NaCl-stressed plants of 10 varieties of mulberry was estimated. There was significant variability in free proline content amongst the cultivars and its levels increased with increasing duration of stress. After 3 hours, S34

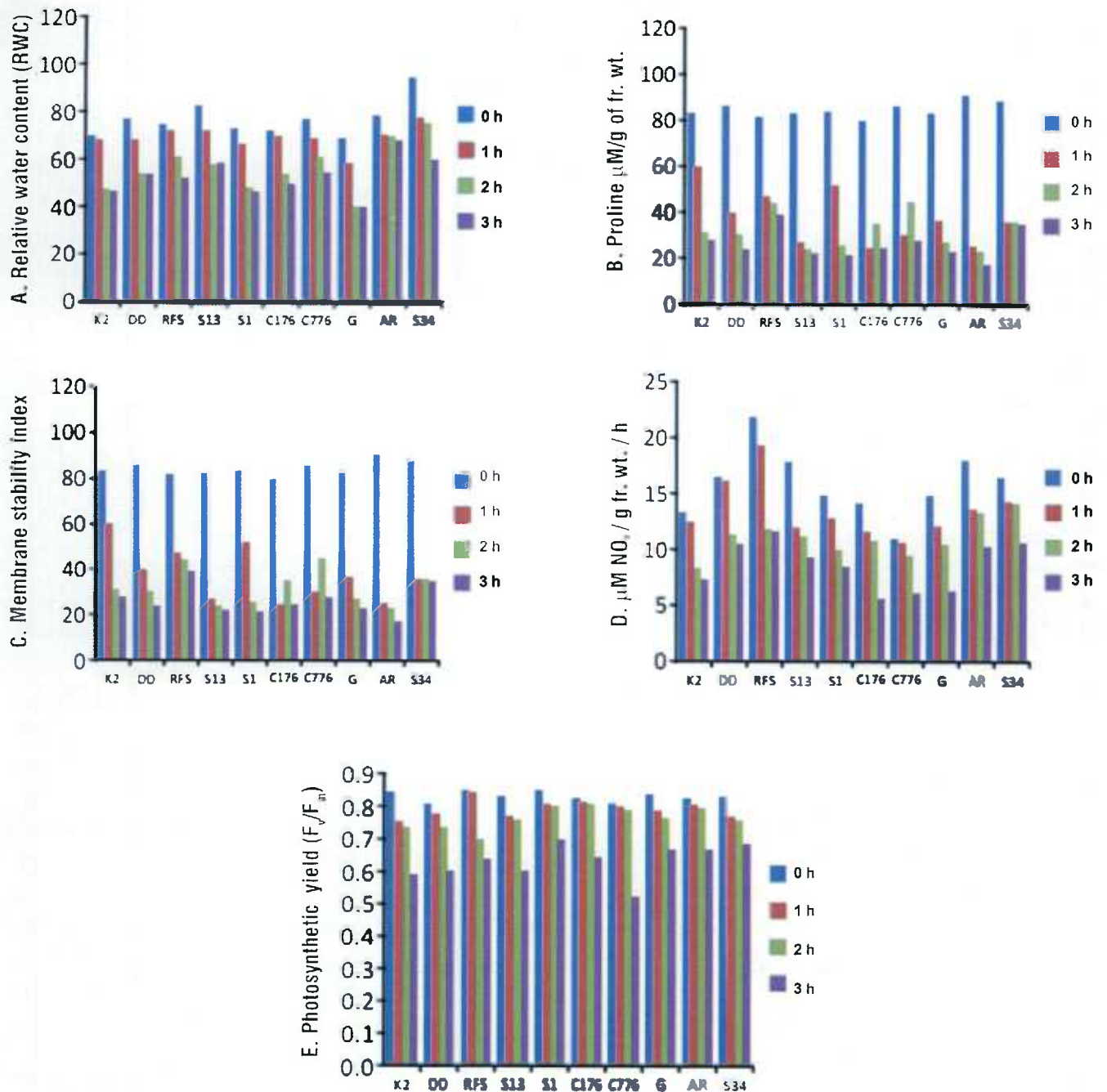


Figure 2: A: Relative water content, B: proline accumulation, C: membrane stability index, D: nitrate reductase activity and E: photosynthetic yield of leaf discs of mulberry genotypes subjected to 3 hours of 200 mM NaCl.

exhibited the highest proline accumulation that is approximately 5-6 folds than the basal proline level. A steady increase in proline accumulation was also observed in DD, K2 and RFS 175. S1 and AR-12 accumulated the least proline after 3 h of stress (Figure 2B)

Cellular membrane stability

Cellular membrane dysfunction due to stress is well expressed as increased permeability and leakage of ions, which can be readily measured by the efflux of electrolytes. In all the cultivars of mulberry tested, the

cell membrane stability decreased with increasing duration of aerial drying stress. After 2 hours of stress treatment, S13 showed 29 % reduction in membrane stability index (MSI), while C776 showed the maximum of 70 % reduction in MSI followed by S1 and C176, *i.e.*, 47 %, thus establishing the fact that these are drought sensitive cultivars. S13 showed the least reduction which indicates that its cellular integrity is not much affected due to drought stress (Figure 1C). In all these genotypes, with increasing duration of salt stress, MSI also decreased. The decrease in MSI in all the lines tested was very much prominent after 3 hours of salt stress. Amongst the ten cultivars tested, AR12 which recorded 81 % reduction in cellular membrane stability appears to be the most sensitive to salt stress. RFS 175 showed more resistance to salinity stress where membrane stability reduced by the least of 52 % followed by S34 after 3 hours of salt stress (Figure 2C).

Nitrate assimilation

Nitrate reductase localized in the cytoplasm, is the first enzyme in the pathway of nitrate assimilation and catalyses the reduction of nitrate to nitrite. NR activity is the main limiting step in the nitrate assimilation and amino acid production. In the present study, it was observed that simulated drought stress decreased NR activity with increasing duration of stress. It was noticed that drought stress causes a sharp decline in nitrate accumulation in S1 and C176, whereas DD, C776 and S34 showed a better response (Figure 1D). There was a 52 % decrease in NR activity after 2 hours of stress in the case of S1, indicating its sensitivity to drought stress (Figure 1D). With increasing duration of salt stress, NR activity decreased in all the genotypes tested. S34 showed the least reduction, *i.e.*, 36 % followed by DD and seems to be salt tolerant, while C176 showed the highest reduction percentage *i.e.*, 60 %, followed by Goshwerami (Figure 2D).

Photosynthetic Yield

Not much variation in the F_v/F_m ratio was noticed when the leaf discs were subjected to both simulated drought and salt stress. After 2 hours of drought stress, amongst all the genotypes, S1 and C176 showed 40 %

and 38 % reduction in photosynthetic yield, respectively. Other genotypes did not show any significant variation (Figure 1E). In the case of salt stress, C776 showed 35 % reduction and K2 showed 30 % reduction in photosynthetic yield and other genotypes were not much affected. This suggests that the efficiency of the quantum yield of PSII was not much lowered. The unaffected F_v/F_m implies that there is no loss in the yield of PSII photochemistry and confirms the resistance of the photosynthetic machinery to water deficit stress (Figure 2E).

Gene expression studies

To study the expression of halotolerance gene in different genotypes under control and drought stressed conditions, the PCR purified full length product of the *Hal3A* gene was used as a probe. It was observed that *Hal3A* is expressed in all the genotypes in both non-stressed and stressed conditions, but more accumulation of *Hal3A* was observed in stressed condition. In the case of S13, C-176, C-776, Goshwerami and AR-12, more transcripts accumulated after three hours of drought like conditions than the basal level of *Hal3A* transcript. (Figure 3A I). Expression of *Hal3A* is known to increase under salt stress too. The expression of *HAL3a* was up-regulated in all the genotypes when subjected to salinity stress (Figure 3B I). In the case of RFS-175 and S1, more expression was observed than other genotypes. DD, C776 and S34 also indicated quite high degree of expression levels under salt stress. Under both salt and drought stress, more *Hal3A* transcript accumulated in C-776 and AR-12 (Figure 3A I and B I).

Similarly, under both drought and salt stress, the expression of *NHX1* was also studied. After exposure to three hours of drought stress, it was noticed that there was not much difference in expression levels under drought stressed conditions in comparison to the basal levels of expression. However, more expression was noticed in K2 and C-776 (Figure 3A III). Contrary to this, it was noticed that three hours of salt stress up-regulated the expression of *NHX1* than the basal levels in all the genotypes. *NHX1* was seen to be up-regulated specifically in the case of RFS-175. Additionally, *NHX1*

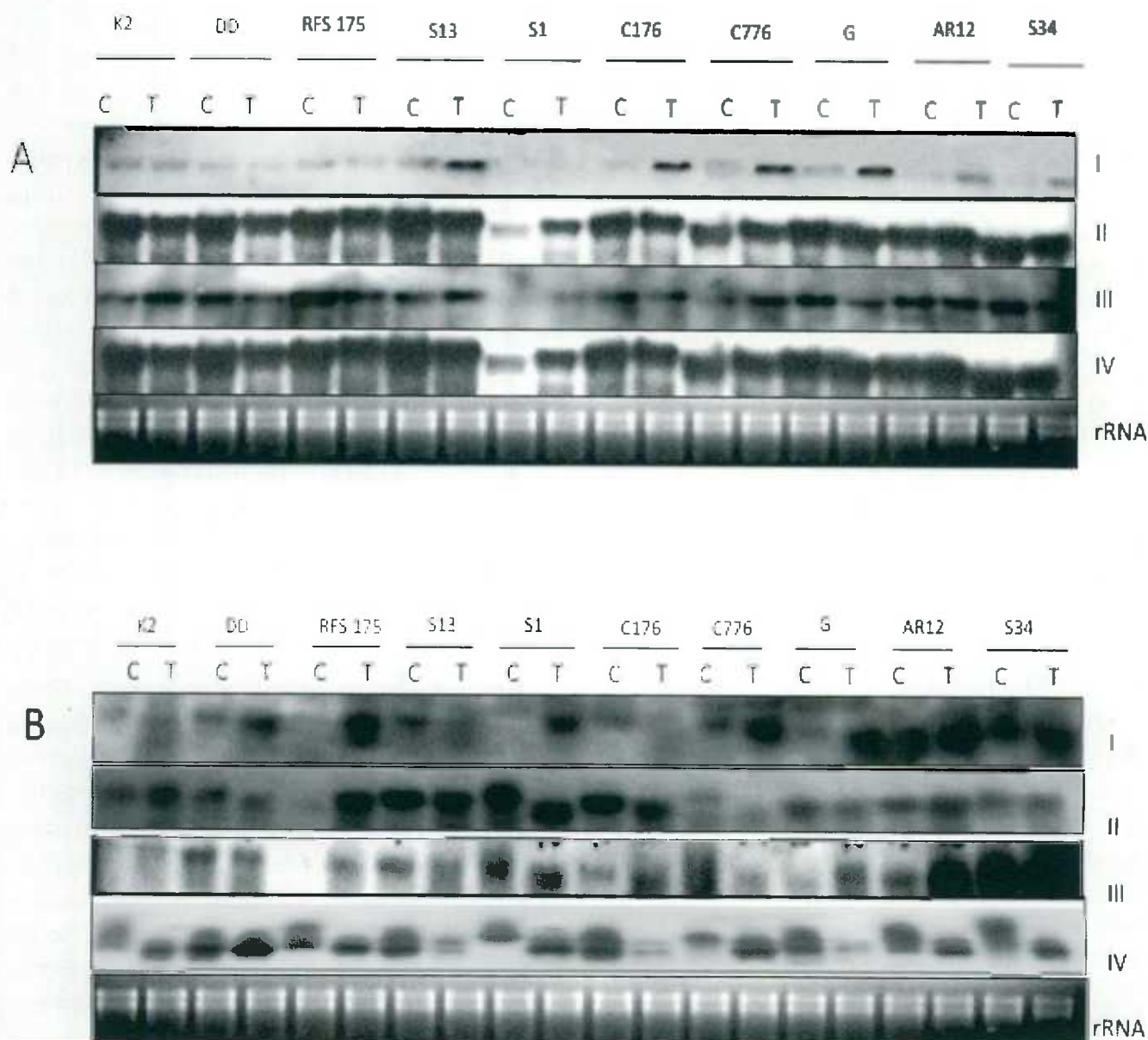


Figure 3 : Expression analysis of (I) Hal 3A, (II) Dehydrin, (III) NHX1 and (IV) ABC transporter after (A) three hours of aerial drying and (B) 200mM salt stress in mulberry leaves. C = control; T = treatment

transcripts accumulated at very high levels in AR-12 and S34 (Figure 3B III). Dehydrin is also known to be up-regulated by salt, drought and cold stress. In the present study, after three hours of dehydration stress, the expression of dehydrin remained as similar to the basal levels of expression (Figure 3A II). But three hours of salt stress resulted in up-regulation of dehydrin in some genotypes. In RFS175, it was observed that expression was inducible as compared to the basal level transcripts whereas, in other genotypes, no difference

was visible between normal and stressed leaves (Figure 3B II). It may be possible that the mature leaves encountered a mild stressful condition in the field and had higher basal levels of expression. After three hours of aerial drying stress, it was observed that there was no significant difference in ABC transporter transcript accumulation between non-stressed and stressed leaves (Figure 3A IV). However, under salt stress, significantly high levels of ABC transporter accumulation was observed in some genotypes. Interestingly, more than

one hybridization signals were present which may be due to the presence of more than one homolog of ABC transporters in mulberry (Figure 3B IV).

DISCUSSION

Abiotic stresses such as drought, cold and salt cause diverse physiological and molecular responses in plants including changes of gene expression. Drought stress is a major factor limiting crop productivity with nearly 48 % of the Indian cultivars of mulberry falling under rain-fed and water stress conditions. Therefore, understanding the response of mulberry towards drought stress is of paramount importance. Screening of drought tolerant genotypes of mulberry have been undertaken earlier (Bartels, 2001; Bajji *et al.*, 2002). Moreover, several studies have been initiated to identify the salt tolerant mulberry genotypes by screening under induced salinity conditions (Giridara *et al.*, 2000; Raghunath *et al.*, 2000; Kumar *et al.*, 2000, 2003). Since improvement of germplasm is a constant ongoing effort, it becomes important to identify and develop genotypes that are better adapted to stress conditions.

Screening for physiological traits

Relative water content (RWC) is a major factor in determining the plant's capability to survive adverse water conditions. Our results indicated that varieties such as AR12 and S13 which accumulated more proline under simulated drought stress consequently had more RWC. Similarly, Goshwami and S1, exhibited less RWC and accumulated less proline as well. So, there is a direct indication of a positive correlation between proline accumulation and relative water content under water deficit conditions. Al-hamdani and Barger (2002) examined three sorghum cultivars under drought conditions by estimating RWC as an evaluation tool. Salt tolerant plants have some degree of tolerance even for water deficiency (Munns, 2002). It has been observed in water stressed pea leaves that proline accumulation leads to decrease in relative water content (Fedina and Popova, 1996). Proline content enables the plants to maintain osmotic homeostasis when grown under low water potential. Proline may also act as a storage compound and nitrogen source for rapid growth

after stress. Proline is known to accumulate in large amounts under drought stress and plays an important role as a compatible solute to protect protein and membrane structures as well as scavenge reactive oxygen radicals species to regulate cellular redox status. In the present study, it was observed that proline content increased in all the varieties subjected to three hours of dehydration stress, the maximum being in S13 and AR12. These results are in corroboration with that of Chaitanya *et al.* (2009). Similarly, salt stress is also known to induce proline accumulation in plants as seen in all the genotypes tested in this study. S34 was observed to accumulate maximum proline under salt stress followed by RFS-175 (4 fold of the basal levels), AR-12 accumulated the least amounts of proline indicative of its sensitiveness to salt stress. Giridara *et al.* (2000) tested two high yielding mulberry genotypes and found that genotype S1 is more tolerant than ATP by accumulating more proline under salinity stress.

Measurement of cell membrane stability (CMS) has often been used for screening drought tolerance in various crops. In the present study, it was observed that after 3 hours of dehydration stress, the electrolyte leakage increased in all the genotypes. S13 was the least affected whereas, C776 seemed to be the most sensitive to dehydration stress. The ability of plants to maintain their membrane integrity under drought or salt stress, determines their resistance towards these stresses. Thus, this method is used widely for stress resistance evaluation. Electrolyte leakage also increased with increasing durations of salt stress in all the genotypes. After three hours of salt stress, the MSI of RFS175 was reduced by 52 %, which was the lowest in all the genotypes tested whereas, AR-12 was severely affected with 80 % reduction in membrane stability. Farooq and Azam (2006) have used membrane stability index (MSI) as one of the parameters to differentiate two wheat genotypes grown at various salinity levels. It was seen in two of the three peanut cultivars examined for drought stress that membrane disruption also decreases photosynthetic activity (Lauriano *et al.*, 2004). But, Meyer *et al.* (1992) suggested that lowering of photosynthetic activity was not a consequence of

membrane damage caused by changes in polar lipid content and composition but rather as a result of destruction or inactivation of ATPase.

Nitrate reductase catalyzes the first enzymatic step in nitrate assimilation in higher plants involving reduction of nitrate to nitrite and is a highly regulated enzyme. Kaiser *et al.* (1999) suggested that several environmental signals regulate NR activity as Nitrate reductase activity is reported to decrease during abiotic stress. Present study also suggests that NR activity is affected in the mulberry genotypes subjected to dehydration stress. NR activity of S1 was reduced by 52 %, making it the most affected by the dehydration stress. In contrast, the NR activity of C776, S34 and DD was very little affected. Several studies have reported that nitrate uptake and NR activity decreases in plants under salt stress (Rao and Gnaniham 1990; Gouia *et al.* 1994). NR activity is known to decrease with increasing concentration of PEG (Poly Ethylene Glycol) in rice seedlings (Sharma and Dubey, 2005). In this study, salt stress reduced the NR activity of C176 to a very low amount, whereas, S34 showed better NR activity. Lal *et al.* (2006) had observed reduced NR activity due to salt stress in four mulberry genotypes.

Estimate of chlorophyll fluorescence was used as a measure to evaluate the abiotic stress tolerance in crop plants. The F_v/F_m ratio measures the efficiency of excitation energy capture by open PSII reaction centers representing the maximum capacity of light dependent charge separation (Krause and Weis, 1991). In this study, it was observed that photochemical activity of all the genotypes was not much affected by both the stresses.

Expression analysis

Hal1 and *Hal3* are two genes involved in the regulation of K^+ and Na^+ transport, respectively, and enhance salt tolerance in yeast (Serrano, 1996). Transgenic melon (Borda *et al.*, 1997) and tomato (Gisbert *et al.*, 2000) over expressing *Hal1* gene were tolerant to salt to a certain level. Expression analysis in mulberry revealed that in all the genotypes tested, there was not much difference in *Hal3A* expression before and after dehydration stress. However, S13, C176, C776

and Goshierami accumulated noticeable amount of *Hal3A* transcript after dehydration stress, so it can be suggested that *Hal3A* is regulated by dehydration stress. There is a remarkable increase in expression of *Hal3A* after three hours of salt stress treatment in all the genotypes tested higher being in RFS-175, AR-12 and S34 indicating their higher stress tolerance efficiency. S34 was observed better in terms of proline accumulation, NR activity and membrane stability index after three hours of salt stress, suggesting that accumulation of salt responsive genes like *Hal3A* protect plants from salt stress injury. Supporting this result, over-expression of *Nicotiana Hal3A* gene in cultured tobacco cells was seen to increase intracellular levels of proline (Yonamine *et al.*, 2004) and over-expression of *AtHal3A* showed improved salt and osmotic tolerance (Espinosa-Ruiz *et al.*, 1999).

Dehydrin proteins are known to accumulate in vegetative plant tissues under stress conditions, such as low temperature, drought, or hyper salinity (Ingram and Bartels, 1996; Close, 1997; Allagulova *et al.*, 2003). In the present study, it was observed that expression of dehydrin was almost similar after three hours of dehydration stress and under control conditions except for S1 and AR12, wherein the expression levels were higher under stress. After salt stress, it was observed that dehydrin accumulation was up-regulated in RFS-175. Supporting this observation, Pulla *et al.* (2008) suggested that expression of dehydrin from *Codonopsis lanceolata* gets up-regulated by salt, drought, cold and wounding. Dehydrins have been found in many plants including *Arabidopsis*, wheat, barley, and rice (Close, 1997). Improved salt tolerance has been identified in many transgenic plants by sequestering of salts into the vacuole or root apoplast (Apse *et al.*, 1999). NHX plays an important role in vacuolar transport with the help of ATPase which mediates electrogenic H^+ ion translocation at the endomembrane of plant cells and energize secondary Na^+ -activated transport by NHX type Na^+/H^+ antiporters at the tonoplast (Ratajczak 2000; Dietz *et al.*, 2001). This study also suggests that mulberry NHX gets up-regulated under salt stress, whereas dehydration stress does not have any effect on the

activity of NHX1. Consistent with these results, over expression of a vacuolar Na⁺/H⁺ antiporter in *Arabidopsis thaliana* promotes sustained growth and development in soil watered with up to 200 mM sodium chloride (Apse *et al.*, 1999). Expression of *Oryza sativa* NHX1 is up-regulated by salt stress thereby playing an important role in salt tolerance of rice (Fukuda *et al.* 1999). Over expression of the vacuolar Na⁺/H⁺ antiporter and H⁺-pyrophosphatase pump (H⁺-PPase) has resulted in enhanced plant tolerance to salinity (Apse *et al.*, 1999; Zhang and Blumwald, 2001) and drought stress (Gaxiola *et al.*, 2001; Park *et al.*, 2005) and to both salt and drought stress (Brini *et al.*, 2007). ABC transporters form a large family of proteins involved in the transport of substances across the membrane in plants and other lower organisms and confers resistance to various biotic and abiotic stress (Martinoia *et al.*, 2002). In the present study, salt stress caused accumulation of ABC transporter transcript in all the genotypes analyzed. More than one hybridization signal was present after three hours of salt stress suggesting the existence of more than one ABC transporter gene in the mulberry genome. Interestingly, an additional third transcript accumulates under salt stress. Its specificity however needs to be investigated in future.

Conclusion

The results of this study indicate that drought or salt tolerance cannot be attributed to a single genotype because of its superiority in a single factor examined. Different parameters should be evaluated in order to observe as many physiological features in terms of response as possible. In this study, four parameters *viz.*, proline content, cell membrane stability, photosynthetic yield and relative water content were estimated as they are well documented for their use to screen crops for stress tolerance. In mulberry, there are very few reports using these parameters to screen different genotypes for their tolerance against stresses. Our results infer S13 and AR12 as tolerant to drought stress whereas, S34 appeared better than other genotypes under salt stress. Further, expression of four stress responsive genes *viz.*, *Hal3A*, *NHX1*, ABC transporters and dehydrin of mulberry were studied under drought and salt stress. *Hal3A* and *NHX1* were up-regulated more by salt stress

than drought stress. It was also observed that dehydrin was up-regulated by both drought and salt stress as reported earlier. It is therefore suggested that *Hal3A* and dehydrin accumulation is involved in cell membrane integrity under abiotic stress. These genes can be further used to transform mulberry to confer diverse abiotic stress tolerance.

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CRIBLAGE DU PROFIL D'EXPRESSION DE *HAL3A*, DE LA DEHYDRINE, DU TRANSPORTEUR ABC ET DE *NHX1* CHEZ DIX GENOTYPES DE MÛRIER, *MORUS* SP., POUR LA TOLERANCE A DES STRESS ABIOTIQUES

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RESUME

La sécheresse et la salinité sont les deux principaux facteurs abiotiques responsables de la réduction de la croissance et de la productivité des plantes. En utilisant une méthode de tri efficace, dix génotypes de mûrier économiquement importants ont été sélectionnés pour leur tolérance à la sécheresse et à la salinité. Des disques foliaires provenant de plants cultivés ont été soumis à un stress de sécheresse et de salinité pendant trois heures par un traitement de séchage aérien et au chlorure de sodium à 200mM. Des paramètres importants tels que le contenu relatif en eau (RWC), l'accumulation de proline, l'activité de la nitrate-réductase, la stabilité de la membrane cellulaire (CMS) et le rendement photosynthétique ont été estimés afin d'évaluer la tolérance des génotypes vis à vis de la sécheresse et de la salinité. Une analyse de l'expression des gènes répondant au stress, c'est à dire *Hal3A*, *NHX1*, *déhydrine* et le *transporteur ABC*, a été entreprise pour quantifier le niveau d'expression sous simulation de sécheresse et de salinité. Les variétés S13 et AR-12 ont montré une résistance à la sécheresse alors que S34 et K2 sont apparus comme des génotypes tolérant la salinité. Distinguer les génotypes tolérant le sel et la sécheresse doit permettre d'étendre la culture du mûrier à des terrains affectés par le sel ou la sécheresse et ainsi d'accroître la disponibilité en feuilles pour la sériciculture.



Research Paper

POSSIBILITY OF UTILIZING SILKWORMS FOR MULTIPLICATION OF *DENDROLIMUS SPECTABILIS* CYTOPOLYHEDROSISVIRUS

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ABSTRACT

Dendrolimus spectabilis is the major pest of Pinus plants in East Asia, and *D. spectabilis* cytopolyhedrosis virus (DsCPV) has been used to control the pest in China and Japan. Large scale multiplication of DsCPV on its primary host *i.e.*, *D. spectabilis* works out expensive. Possibility of utilizing silkworms as an alternative host for multiplying DsCPV was explored as a cost effective measure. Thirty five races of silkworms were per orally inoculated with 1×10^8 DsCPV polyhedra / g artificial diet. On examination after ten days, 29 races revealed the presence of DsCPV with the highest incidence of 26.7 % in Oh 1 Gou race. Hence, progenies of susceptible populations of Oh 1 Gou race were raised by subjecting to DsCPV inoculation followed by line breeding and directional selection for higher susceptibility for three generations. Resultant lines revealed a maximum DsCPV infection level of 90.9 %. DsCPV obtained from the silkworms were confirmed by reverse transcription polymerase chain reaction method, and the presence of BmCPV if any, could also be differentiated.

Key words: *Dendrolimus spectabilis* cytopolyhedrosisvirus, DsCPV, infection, silkworm.

INTRODUCTION

Dendrolimus spectabilis (the pine moth) is a major pest of Pinus plants and causes serious damage in East Asia (Furuno, 1972; Kuranaga *et al.*, 1975; Habu, 1976; Togashi and Takahashi, 1977; Bin-Cheng, 1994; Satomi *et al.*, 1997; Li *et al.*, 1998). *D. spectabilis* cytopolyhedrosisvirus (DsCPV), has been used as a biological means to control this pest in Japan (Koyama, 1961; Iwata *et al.*, 1967; Katagiri *et al.*, 1977). However, there are some practical difficulties associated with rearing of *D. spectabilis* in terms of its diapause nature, diets, rearing space and so on. Therefore, an attempt was undertaken to multiply and harvest DsCPV utilizing silkworm as host. On the other hand, *Bombyx mori* cytoplasmic polyhedrosisvirus (BmCPV) is a dreaded pathogen of silkworms (Hagiwara *et al.*, 2001; Kotani *et al.*, 2005), causing serious loss in sericulture (Samson *et al.*, 1990). As there are chances of silkworms getting infected with

BmCPV too, specific molecular techniques were relied on to differentiate between DsCPV and BmCPV prior to the purification process.

MATERIALS AND METHODS

DsCPV inoculum : DsCPV polyhedra were procured from National Institute of Forestry and Forest Products Research, and propagated by inoculating *D. spectabilis*. Infected and dead *D. spectabilis* larvae were collected and homogenized in a blender. The homogenate was filtered by double gauzes, and DsCPV was purified with 45 – 85 % (w/v) sucrose density-gradient centrifugation. The viral band zone of DsCPV was collected and washed with distilled water.

Inoculation of DsCPV : Fifteen larvae of each race at second stage were fed with 1×10^6 and 1×10^8 polyhedral inclusion bodies (PIBs) / g artificial food (Shirukumeito 2S, Nosan, Yokohama) for two days after

which the larvae were fed on the diet without DsCPV. The larvae were dissected ten days after the inoculation. The presence of polyhedra detected microscopically in the dissected larvae was a confirmation of pathogenic infection.

Primers for differentiation of DsCPV and BmCPV: Purified DsCPV was incubated with 0.2 M carbonate buffer (pH 10.5) for 1 h and was neutralized with 1M-Tris HCl (pH 8.0). The solution was treated with 1% (w/v) of protease K and 0.25 % (w/v) of SDS for 2 h at 37 °C. Viral RNA was purified using Isogen (Nippon Gene, Tokyo) and was precipitated using ethanol (Hagiwara *et al.*, 2001). Extracted RNA was denatured at 98 °C for 5 min. and was chilled on ice, quickly. cDNA of the RNA was reverse transcribed at 60 °C in the reaction mixture containing 5F-1 (5'-GAATTCGGCTAATTTCCCCTTACCTTA-3') primer and 5R-2852 (GAATTCGGCTAACCATCTCCCCGTGAT-3') primer using AMV Reverse Transcriptase First-strand cDNA Synthesis Kit (Life Sciences, INC, Florida). The synthesized cDNA was amplified by polymerase chain reaction (PCR) using 5F-1 and 5R-2852 primers under the following conditions: denaturation 94 °C for 1 min., annealing 52 °C for 1 min. and extension 74 °C for 2 min. The reverse transcription (RT-PCR) reactions were run for 20 cycles. The resulting RT-PCR products were cloned using pGEM-T Easy Vector System I (Promega, Tokyo). Sequence of the RT-PCR products was performed using the ABI PRISM TM 3700 DNA analyzer and the Big Dye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Tokyo).

Multiplex RT-PCR method: Hemolymph of silkworms was collected in carbonate buffer, and RNA was extracted using Isogen. RT-PCR was performed using the primers for detection of DsCPV or BmCPV (Table 1) in a total volume of 20 µl, and the cycling

parameters for PCR were as follows: 1 cycle, at 94 °C for 5 min; 25 cycles, at 94 °C for 1 min, at 50 °C for 1 min and at 72 °C for 1 min; 1 cycle, at 72 °C for 7 min using the DNA Thermal Cycler (Perkin Elmer, USA). Reaction solutions (5 µl) were electrophoresed using 1.5 % of agarose at 100 V for 30 min and the agarose was stained with ethidium bromide. We could confirm the pathogen as either DsCPV or BmCPV by detection of RT-PCR products from hemolymph of the silkworm.

RESULTS

Polyhedra were detected by microscopic method from seven races when inoculated per orally with 1×10^6 PIBs / g of DsCPV, whereas from 29 races out of 35 tested when done with 1×10^8 PIBs / g of DsCPV (Table 2). The Oh 1 Gou race was selected as suitable for multiplication of DsCPV because Maiera zebura race and Oh 1 Gou race had the highest percentage of infection with DsCPV when inoculated under the above two doses and it was difficult to rear Maiera zebura race. The infection percentage of the race was only 26.7 % by per oral infection with 1×10^8 PIBs / g. Therefore, we resorted to breeding and selection techniques to increase the susceptibility of Oh 1 Gou race to recover more DsCPVs.

In the first generation, fifteen larvae each from fifteen lines (A – O line) of the Oh 1 Gou race were used (Table 3). The infection percentage varied from 0 (N line) to 86.7 % (C line) with an average of 32 %. M, H and O lines recorded 53.3, 46.7 and 40.0 % infection, respectively. Proceeding further with only one line (C x C) posed the risk of inbreeding depression leading to its non-existence. Therefore, we selected C, H, M and O lines as breeding lines. Moreover, the N line was selected as control because of 0 % infection. Since the H line could not produce offspring, we continued with C, M, N and O lines in following experiments.

Seven lines were continued in the second generation. The highest percentage of infection (55.6 %) was detected in C x C line and the lowest (6.3 %) in O x O line (Table 3). Out of these, two lines were utilized in the third generation resulting as high as 90.9 % of infection percentage in (C x C) x (C x C) line (Table 3).

Table 1: Primers for differentiation of DsCPV and BmCPV

Primer	Sequence	Size of product
DsCPV-F	CCCATACACTAATCAGAGAGGG	663 bp
DsCPV-R	ATACTGGTCGTATGTTGTCATC	
BmCPV-R	GTGGCTACACTGGCTAAC	462 bp
BmCPV-F	CTTGAGCGACACTGAGTGATT	

Table 2: Percentage of DsCPV infection detected in silkworm races after per oral inoculation

Race	DsCPV infection %	
	DsCPV (polyhedra / g)	
	1 x 10 ⁸	1 x 10 ⁶
Agunisan	13.3	0.0
Ajiakohen	0.0	0.0
Asurikoken	20.0	6.7
Bunrihaku	26.7	0.0
C-Kyuen	20.0	0.0
Daikojo	6.7	0.0
Datenishiki	0.0	0.0
Gubbio	20.0	13.3
H5(Takei)	6.7	0.0
Hakuyoen	0.0	0.0
Hinode (Odaka)	13.3	0.0
J137 x C146	13.0	0.0
Koishimaru (Sanshi)	6.7	0.0
Maiera Zebura	26.7	6.7
Maisohru	6.7	0.0
Murasakiko	13.3	6.7
Nanko	26.7	0.0
Nishiki (49)	13.3	0.0
Oh1Gou	26.7	6.7
Oha	6.7	6.7
Ohfu	20.0	0.0
Okusa	13.3	0.0
Renshin	13.3	0.0
Ringetsu	0.0	0.0
Ryukyutasanken (Shizuoka)	0.0	0.0
S No.1	13.3	0.0
Sbuennushiro	26.7	0.0
Seihaku (Yamagata)	6.7	0.0
Seikyo	6.7	0.0
Sekko	6.7	0.0
Shi7Gou	20.0	6.7
y35	13.3	0.0
Y4	20.0	0.0
Yoshi (N)	20.0	0.0
Yuhaku	0.0	0.0

Table 3: Percentage of DsCPV infection in different lines of Oh 1 Gou race through generations

Line	Percentage	Line	Percentage
First generation			
A	13.3	I	13.3
B	20.2	J	13.3
C	86.7	K	26.7
D	6.7	L	6.7
E	6.7	M	53.3
F	33.3	N	0
G	20.0	O	40.0
H	46.7		
Second generation			
C x C	55.6 ± 7.6	N x N	11.3 ± 2.5
C x M	18.8 ± 2.2	O x C	17.2 ± 3.8
M x C	28.4 ± 8.0	O x O	6.3 ± 2.5
M x M	29.2 ± 6.0		
Third generation			
(C x C) x (C x C)	90.9 ± 7.6		
(O x O) x (O x O)	62.5 ± 2.9		

Fifteen larvae each of each line were ingested with 1 x 10⁸ PIBs of DsCPV / g artificial diet for two days, and the larvae were dissected ten days after the ingestion. The percentage values are average of each line.

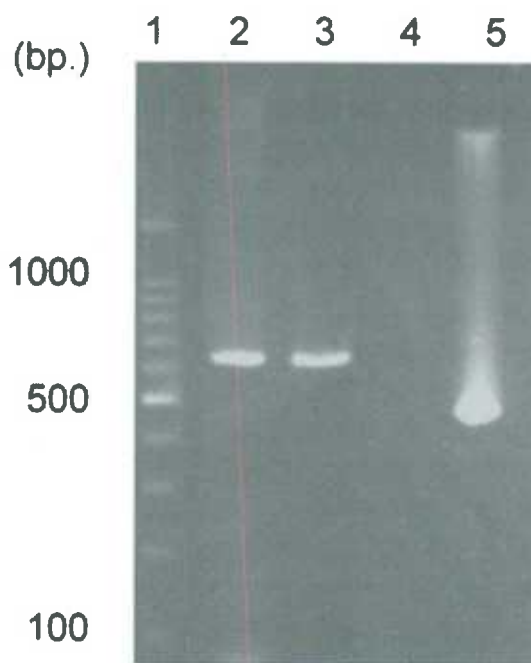


Figure 1: RT-PCR amplified products of DsCPV and BmCPV using primers (Table 1). Lane 1: 100 bp ladder marker (Takara); Lanes 2 and 3: RT-PCR product from hemolymph of silkworm infected with DsCPV using DsCPV-F and DsCPV-R primers; Lane 4: blank; Lane 5: RT-PCR products from hemolymph of silkworm infected with BmCPV using BmCPV-F and BmCPV-R primers.

There are all possibilities for the silkworms to get susceptible and infected with BmCPV during the process of multiplication of DsCPV. PIBs of DsCPV in the homogenized samples of silkworms cannot be distinguished from that of BmCPV by light microscopic method. Therefore, to differentiate these, RT-PCR products were relied on. cDNAs of RNA from segment 1 of DsCPV and of BmCPV were sequenced and primers designed for RT-PCR products. Sizes of RT-PCR products (Table 1) of DsCPV and of BmCPV were 663 bp and 462 bp, respectively (Figure 1).

DISCUSSION

Entomopathogenic viruses are used as microbial control agents in integrated pest management programmes (IPM). NPVs and CPVs are employed in IPM and some viruses are produced on industrial scale. In Japan, DsCPV is used to control *D. spectabilis* (Katagiri 1981). However, production of viruses using its own host larvae is expensive because of its time consuming and labour intensive nature, requirement of rearing space and diet and complications of harvesting the diseased larvae. On the other hand, since there exists a well standardized rearing procedure for silkworms, we tried to multiply DsCPV by utilizing them.

The results confirm that DsCPV infects silkworms and the infection levels could be increased in silkworm larvae by selection and mating of susceptible parents as observed in Oh 1 Gou race. The pathogenicity of DsCPV multiplied on Oh 1 Gou was tested by inoculating on *D. spectabilis* larvae. However, we consider that DsCPV must be multiplied on *D. spectabilis* at intervals to use as microbial control agents because it is possible that the pathogenicity of DsCPV multiplied on silkworms may decrease against *D. spectabilis*.

Of late, in the field of agriculture, NPV, granulovirus (GV) and CPV have been used as natural enemies for control of pests, but it is difficult to multiply these viruses in large scale because of the difficulty to rear their host insects. Rearing of insects for this purpose proved less economic too (Goto, 1999). Moreover, since the host specificities of NPV and of GV are high, these viruses

infect only limited number of host insects. However, DsCPV could be successfully multiplied in silkworm.

The presence and the percentage of DsCPV in infected silkworms could be detected from a sample of hemolymph without killing them. This facilitated selection of highly susceptible, infected parents for raising further generations. These results point towards the fact that silkworms may be useful for increase not only DsCPV but also other CPVs as CPVs have broad host range than NPV and GV. This is the first report on utilization of silkworm for multiplication of biological control agent, CPV.

ACKNOWLEDGEMENT

We thank Dr. M. Shimazu for providing DsCPV inoculum.

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POSSIBILITE D'UTILISER DES VERS A SOIE POUR LA MULTIPLICATION DU VIRUS DE LA POLYEDROSE CYTOPLASMIQUE *DENDROLIMUS SPECTABILIS*

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RESUME

Dendrolimus spectabilis représente la peste principale affectant les plants de *Pinus* dans l'Est asiatique, et le virus de la polyèdrose de *D. spectabilis* (DsCPV) a été utilisé pour contrôler la peste en Chine et au Japon. La multiplication à grande échelle de DsCPV sur son hôte primaire, *D. Spectabilis*, est honoreuse. La possibilité d'utiliser les vers à soie comme hôte alternatif pour multiplier DsCPV a été explorée comme mesure moins coûteuse. Trente cinq races de vers à soie ont été inoculées *per os* avec 1×10^8 polyèdres/g d'aliment artificiel. Dix jours après, 29 races présentaient une infestation avec l'incidence maximale de 26.7 % pour la race Oh 1 Gou. Les progénitures des populations sensibles de Oh 1 Gou ont été élevées en les soumettant à l'inoculation de DsCPV puis à un breeding en ligne et à une sélection en direction de la plus forte sensibilité pendant trois génération. Les lignées résultantes présentent un niveau d'infection maximum de 90.9 %. Les DsCPV obtenus des vers à soie ont été caractérisés par PCR.

EFFECT OF LETTUCE SEED OIL (LSO) ON SOME ECONOMIC TRAITS AND HAEMOLYMPH PROTEIN PROFILE OF THE MULBERRY SILKWORM, *BOMBYX MORI* L.

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ABSTRACT

The effects of lettuce seed oil (LSO) on the economic traits of the mulberry silkworm, *Bombyx mori* L. and its impact on total haemolymph protein profile of the fifth instar larvae were studied. Fourth and fifth instar larvae of *B. mori* were fed on mulberry leaves supplemented with different concentrations (0.01 to 1.00 %) of LSO. Treatment of 0.10 % LSO proved to be the most effective since the larval, cocoon and shell weight and the number of deposited eggs were significantly higher when compared with the control. Changes in haemolymph proteins of 5th instar larvae of *B. mori* fed with LSO were monitored quantitatively and qualitatively using SDS-PAGE technique. Haemolymph proteins exhibited a gradual increase, subsequently to reach the maximum level by the end of 5th instar. The SDS-PAGE electrophoretic analysis of haemolymph proteins revealed the existence of 16 - 26 bands. Feeding of *B. mori* larvae on mulberry leaves treated with 0.10 % LSO resulted in a significant enhancement in quantity and quality of protein.

Key words: *Bombyx mori* L., haemolymph proteins, lettuce seed oil, silkworm.

INTRODUCTION

Egypt has always been promoting the production of natural silk of the mulberry silkworm, *Bombyx mori* L. for many socio-economic reasons. During the spring, *B. mori* larvae are reared solely on seasonal mulberry leaves (*Morus* sp.) which play an important role in productivity of the silk and fecundity of moth. In order to enhance the quality of the feed, mulberry leaves can be treated with nutritional supplements.

Lettuce (*Lactuca sativa*) was first cultivated in ancient Egypt for the production of oil from its seeds. Its nutritional contents were reported to contain high quantities of vitamins (A, C, K and B group), antioxidants, beta-carotene, lutein-zeaxanthin, flavonols and minerals (calcium, magnesium, iron, iodine, zinc, copper, manganese and selenium) (Mou, 2005; David, 2007). Lettuce seed oil (LSO) also contains

folic acid, which regulates digestion, strengthens the nervous system, and is necessary for normal functioning of female hormonal system and body metabolism. The present study is an attempt to evaluate the effect of LSO, as a food additive, on economic parameters of the mulberry silkworm *B. mori* and its impact on the quantitative and qualitative profiles of total haemolymph proteins during the fifth larval instar. Haemolymph proteins are the most important constituents that ultimately determine the quality and quantity of the silk (Horie and Watanabe, 1971). There are reports of variations in number of protein bands in the haemolymph of *B. mori* either due to the differences in electrophoretic techniques used for analysis or racial differences in origin and geographic distribution of the experimental samples (Gamo and Ohtsuka, 1980; Lee *et al.*, 1985; Banno *et al.*, 1994; Staykova, 2007).

MATERIALS AND METHODS

Eggs of univoltine local hybrid (Dch) were obtained from the Sericulture Research Department, Plant Protection Research Institute, Agricultural Research Centre, Giza, Egypt. Larvae were reared under the laboratory conditions of 25 ± 2 °C and 75 ± 5 % RH. Fresh mulberry leaves were fed to *B. mori* larvae four times a day. Recommended practices of maintenance of hygiene such as bed cleaning, disposal of silkworm litter and diseased larvae were followed regularly.

Lettuce seed oil of *Lactuca sativa* var. longifolia was purchased from EL Captain Company (manufactured by the company), Al Obour City, Cairo, Egypt. It was dissolved in 1.0 % Triton X-100 and diluted using distilled water to reach the experimental concentrations (0.01, 0.05, 0.10, 0.50, and 1.00 %). The concentrations of LSO were chosen based on the results of preliminary experiments (Idriss, 2002, unpublished data). Mulberry leaves treated with different concentrations of LSO were fed to the 4th and 5th instar larvae 4 times a day, while the control larvae were offered mulberry leaves dipped in 1.0 % Triton X-100 solution. Each treatment was replicated thrice, and each replicate contained 500 larvae.

Haemolymph was collected into sterile tubes containing a few crystals of phenylthiourea to prevent oxidation process and then centrifuged at 10,000 rpm for 10 min to remove haemocytes and cell debris. Cell-free haemolymph samples prepared from fourth and fifth instar larvae were either immediately used or stored at -20 °C until being used as described by Morishima *et al.* (1988). Total haemolymph proteins (THP) were estimated according the method of Bradford (1976) using bovine serum albumin as a standard.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a discontinuous buffer system (Laemmli, 1970). To determine the molecular weights (MW) of separated proteins, Precision Plus Protein Standards (Bio-Rad) were used according to the method described by Weber and Osborn (1969).

The data were subjected to analysis of variance (ANOVA) of Duncan's multiple range function in SAS.

The intensity of the resolved protein bands was determined using Total Lab Software Analysis (Microsoft version 11.0).

RESULTS

Economic parameters

LSO treatment significantly ($P \leq 0.05$) increased the mean weight of mature *B. mori* larvae at 0.05, 0.10 and 0.50 % concentrations (Figure 1 A). A similar trend of results was obtained when the effect of LSO on silk yield was studied. Significant increase in the mean weight of cocoon shells were recorded for 0.05, 0.10 and 0.50 % LSO treatments (Figure 1 B). The maximum and significantly higher number of eggs were laid when treated with 0.05 and 0.10 % LSO. (Figure 1 C).

Total haemolymph protein concentration

The quantitative changes in haemolymph protein profiles were examined (Figure 2). The total proteins during the 5th larval instar gradually increased (from 19.27 to 120 mg/ml) to reach the maximum on the 10th day (mature larva). The highest protein content was detected when larvae were fed with mulberry leaves supplemented with 0.10 % LSO followed by 0.05 % (120 mg/ml and 116.70 mg/ml, respectively). Other LSO treatments led to comparatively less increase, however, still higher than that of the control.

Electrophoretic separation of haemolymph proteins

Figure 3A depicts the SDS-PAGE electrophoretic patterns of haemolymph proteins at 48 h of 5th larval instar. Patterns showed the presence of a few bands with molecular weight of 5, 11, 16 and 21 kDa with a total of 19 protein bands. Their molecular weight ranged between 5 and 346 kDa. Of these bands, the major polypeptide bands (70-80 kDa) were more characterized in control and 0.10 % LSO treatment.

A steady increase in the protein subunit bands was noticed in haemolymph of the 5th instar larvae at day 4 (Figure 3B). About 23 subunit bands with molecular mass ranging between 25 and 346 kDa and six major subunit bands with molecular weight of 30, 70, 75, 80, 260 and 300 kDa were observed.

The haemolymph of 5th instar larva was characterized by the steep increase in proteins especially storage and 30 kDa proteins. The increase of total haemolymph protein subunit bands was detected during the 6th day of 5th instar (Figure 3C). A total of 26 subunit bands with molecular weight ranging between 5 and 346 kDa and the major 6 subunit bands with molecular mass of 30, 70, 75, 80, 260 and 300 kDa were detected.

A considerable decrease in the number of haemolymph protein subunit bands was recorded on day 8 of the 5th larval instar (Figure 3D). The molecular mass of the 18 protein subunit bands ranged from 26 to 346 kDa. Some protein subunit bands with molecular mass of 21, 25, 62, 65, 68, 218, 260 and 321 kDa disappeared. The 5 major subunit bands detected on day 8 were with molecular mass of 30, 70, 75, 80 and 300 kDa.

Another decrease in the subunit bands was observed on day 10 (Figure 3E). A total of 16 protein subunit bands with molecular mass ranging from 30 to 346 kDa were detected.

At the spinning stage, 5 major bands were present in both control and LSO treated samples with molecular masses 30, 70, 75, 80 and 300 kDa. The results reveal that the characteristics of haemolymph proteins in terms of both concentration and constitution readily increased and components with molecular mass 30, 70, 75, 80 and 300 kDa became strong in intensity. The presence of 3 – 5 major peptides in the haemolymph with molecular mass ranging from 260 to 346 kDa were detected. The results obtained clearly determined the well-defined and characterized protein fractions with some undetermined protein fractions of molecular mass ranging from 5 to 176 kDa.

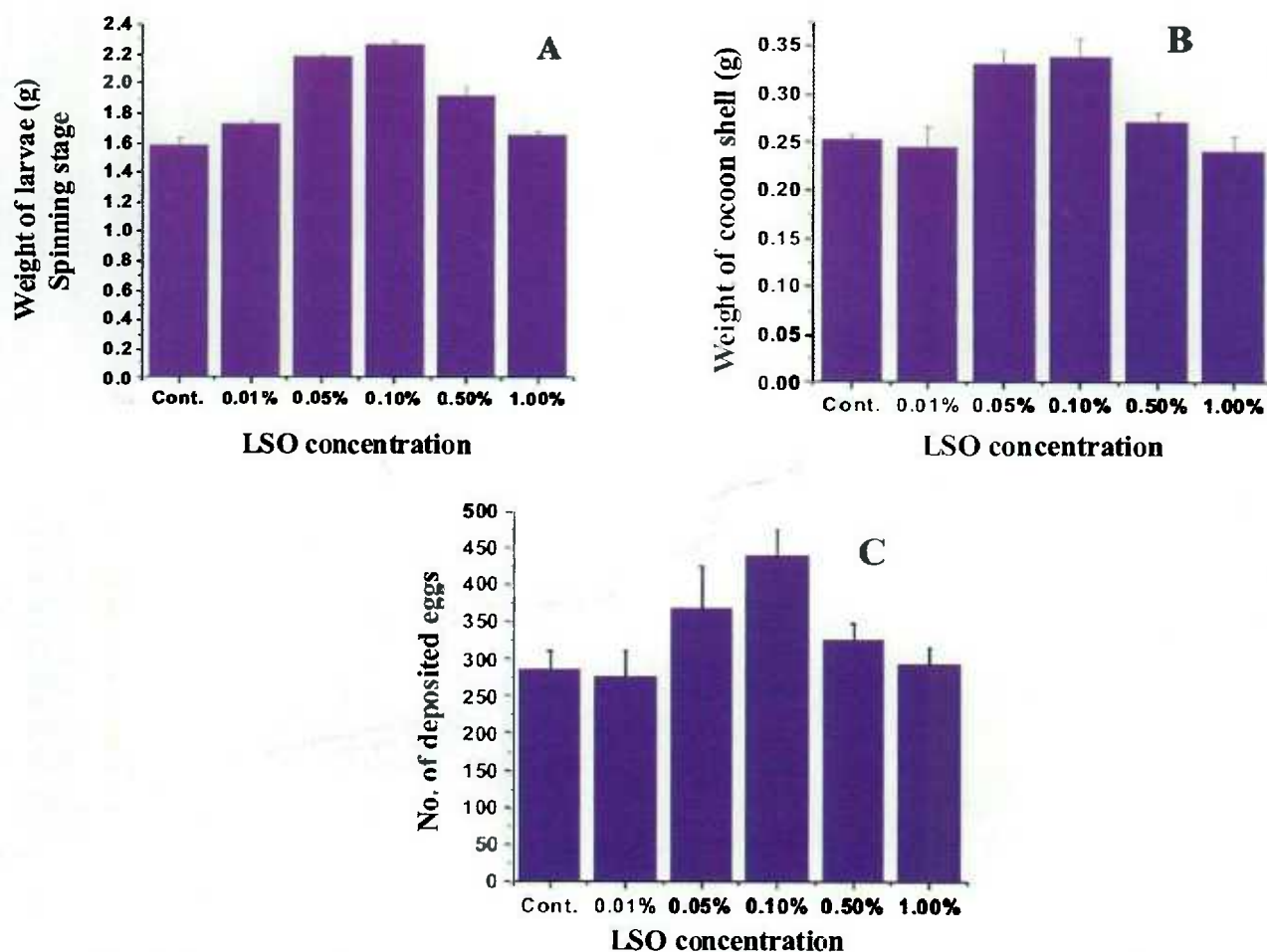


Figure 1: The effects of LSO on some biological and economic aspects of the mulberry silkworm, *Bombyx mori*:

Each column represents the mean of three replicates (each replicate consists of 10 records) and the vertical line is the standard error.

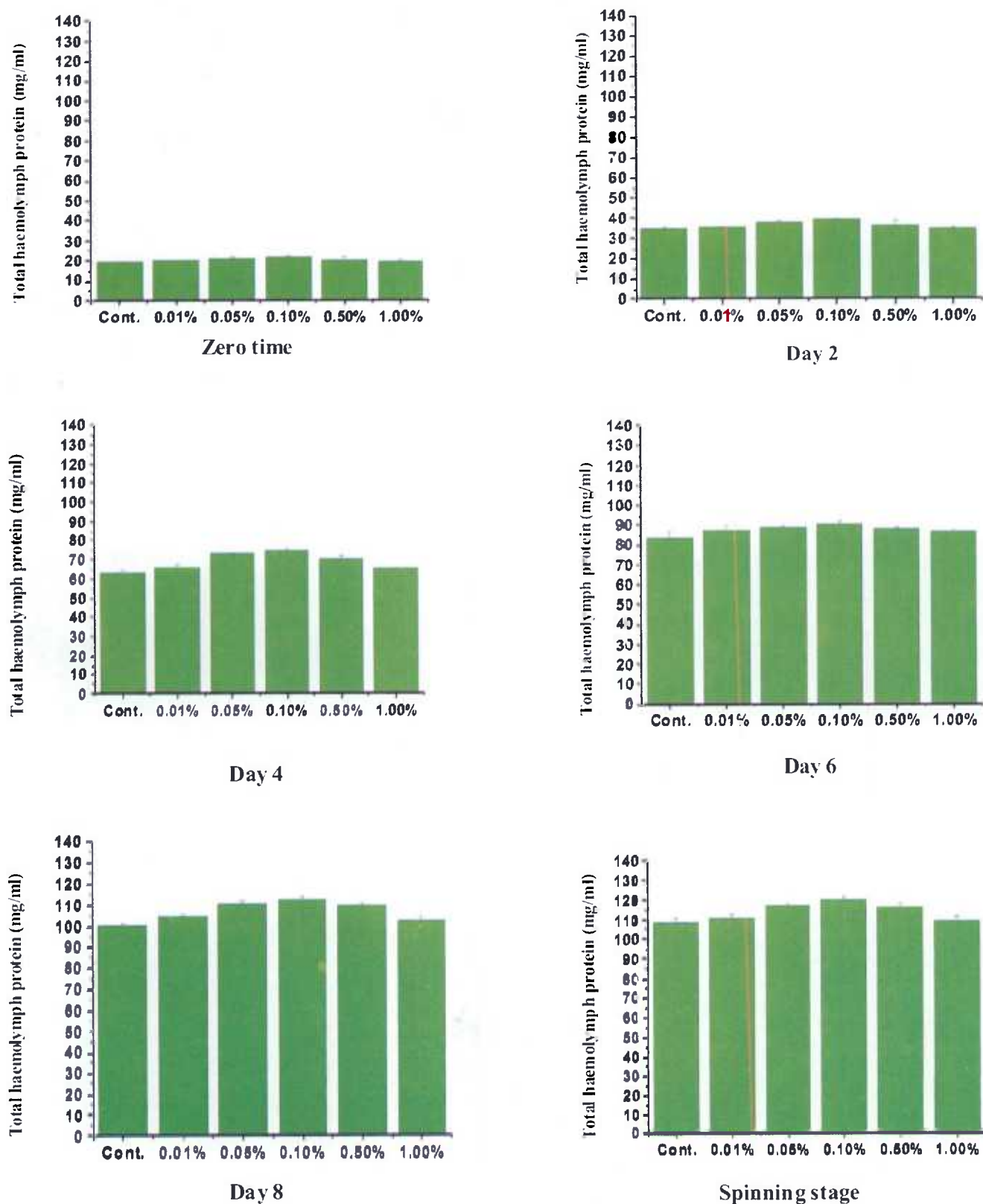


Figure 2: The effect of lettuce seed oil on the haemolymph proteins of *B. mori* L. on 0th, 2nd, 4th, 6th, 8th and 10th day (spinning stage) of fifth instar:

Each column represents the mean of three replicates (each replicate consists of 10 records) and the vertical line is the standard error.

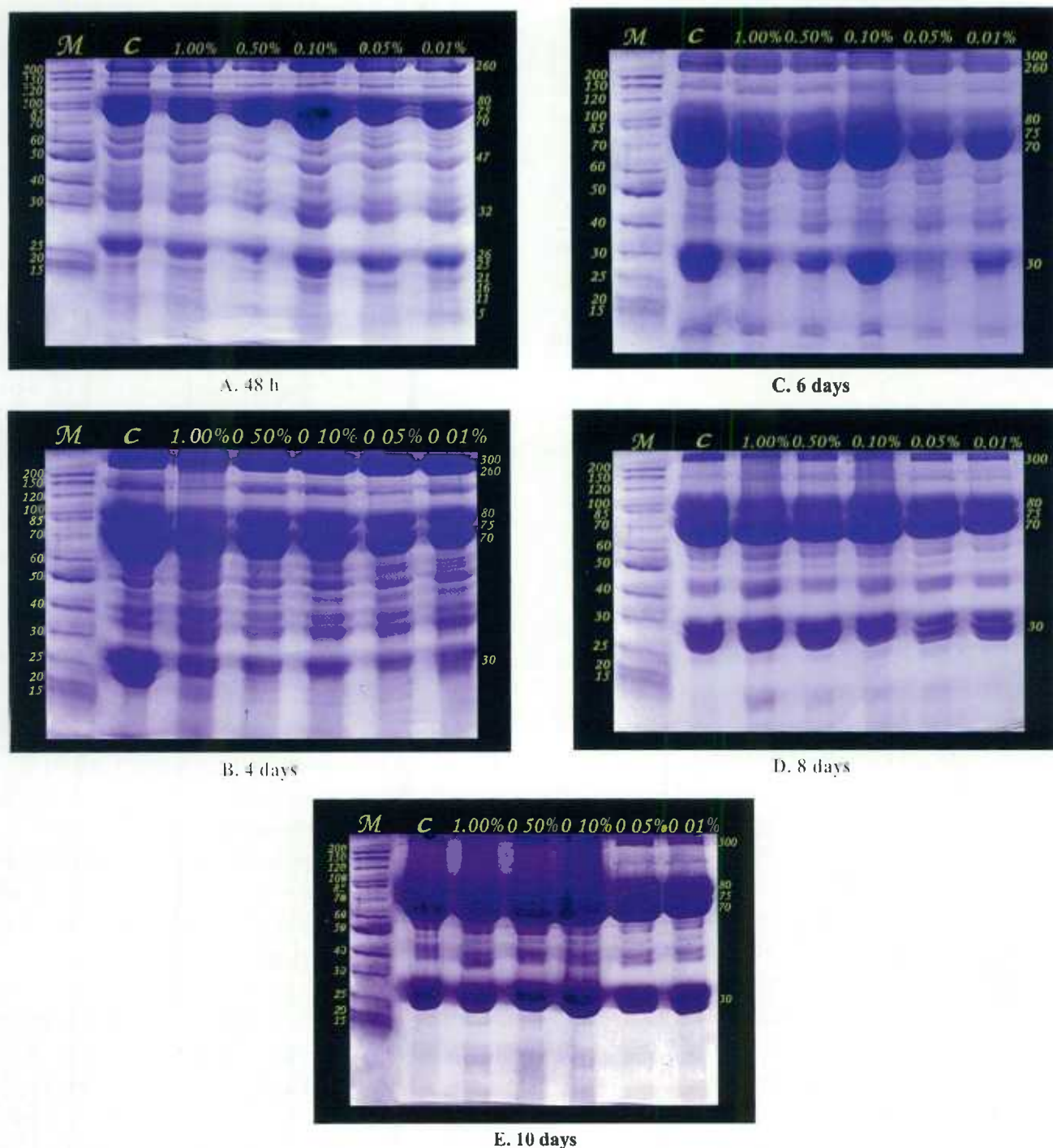


Figure 3: SDS-PAGE patterns of the haemolymph protein of *B. mori* L. 5th instar larvae: M - Standard protein marker, Lane C: Control, Lanes 2-6: Treatments with LSO; 1.00, 0.50, 0.10, 0.05 and 0.01 %, respectively.

DISCUSSION

Nutrition plays an important role in the growth and development of the silkworm. Silk production is in turn dependent on the larval nutrition and hence ultimately it is the nutritive value of the mulberry leaves which determine the quality of cocoons produced. Our results

reveal significant improvement in the economic characters of the silkworm in all LSO treatments compared to the control. These results are supported by Sengupta *et al.* (1972) who reported that silkworm requires specific essential sugars, amino acids, proteins and vitamins for silk gland development, overall normal

growth and survival. Haemolymph proteins may also provide the needed amino acids for the tissue formation of internal organs (Nagata and Kobayashi, 1990). Nirwani and Kaliwal (1998) in their experiments on thiamine supplementation observed that larval duration, cocoon weight, shell weight and fecundity significantly increased in all the treated groups though weight of larvae and silk gland did not show any significant change. Vitamins and mineral salts play an important role in the nutrition of silkworm (Nirwani and Kaliwal, 1998; Etebari and Matindoost, 2004; Faruki, 2005). Vitamin E (alpha tocopherol) is reported to have a positive effect on fecundity and beta - carotene as a growth promoter. El-Karakasy and Idriss (1990) and Chauhan and Singh (1992) reported the favourable effect of ascorbic acid on the fecundity of emerged females.

The concentration of protein in haemolymph increases progressively and reaches maximum on the last day of the 5th larval instar. Similar observations were made by Horie and Watanabe (1971) who found that haemolymph proteins increased from 35.00 mg/ml on day 2 to reach 87.00 mg/ml on day 6 of *B. mori* 5th larval instar. Kajiura and Yamashita (1989) and Ravikumar and Sarangi (2004) could also record an increase in haemolymph protein contents during the 5th larval instar. They attained a peak value on the last day of 5th larval instar of both *B. mori* L. and *Philosamia ricini* silkworms. This increase apparently was due to the storage of more nutrients for further production of amino acids and other materials necessary for silk formation as the maximum concentration was reached at the 5th instar as suggested by Mahmoud (1973). According to Fujiwara and Yamashita (1990), the increase in haemolymph protein concentration was due to an increase of a major protein (30 kDa) and storage proteins. The increase in haemolymph proteins is also attributed to the development of reproductive organs (Kawaguchi *et al.*, 1993; Sinha and Sinha, 1994). Further, Nagata and Yoshitake (1989) could demonstrate that the total protein concentration in the haemolymph increased during the intermoult period and decreased during each moulting period. Fujiwara and Yamashita (1998) noticed that total haemolymph protein concentration of fourth instar larvae was 20 mg/

ml, it decreased temporarily at the beginning of the fifth instar, and then increased again to reach 45 mg/ml in male larvae and 115 mg/ml in female larvae. Uranil *et al.* (2010) also reported that the protein concentration in the haemolymph remained low at the beginning of the final larval instar to increase slowly until day 8 in both the sexes. A peak occurred just before pupation.

The present results indicated remarkable increase in total protein concentrations when LSO treated mulberry leaves were fed to 4th and 5th instar larvae with significant differences ($P \leq 0.05$) between treatments (120, 116.7 and 115.83 mg/ml for 0.10, 0.05 and 0.50 % LSO, respectively). Though other LSO treatments led to less increase, they were still higher than that of the control. The results obtained are in agreement with the findings of Horie and Watanabe (1983) that haemolymph protein content increased with the larval growth when pyridoxine was present in the diet of *B. mori* L. Thus, it is assumed that the presence of pyridoxine in lettuce might have triggered an enhancement of haemolymph proteins in this study.

Our results are in close conformation with the findings of Kovalevskaya and Filipovich (1970) that haemolymph protein concentration fluctuates during the development of *B. mori* causing variation in the number and specificity of protein bands during different days of the fifth larval instar. Kajiura and Yamashita (1989) noticed the presence of two peptides (SP-1 and SP-2) of 80 kDa which increased through intermoult period and decreased through the moulting period. Staykova (2007) also observed an increase in concentrations of major haemolymph proteins during the 5th larval instar of *B. mori*. The presence of a protein band near 30 kDa is assumed to be *B. mori* serum protein (BmLSP), which was detected as a thick band by Sakurai (1984). Later, BmLSP was shown to be a different protein by immunological studies (Fujiwara and Yamashita, 1990). A remarkable decrease in the number of haemolymph protein subunit bands was recorded on day 8 of the 5th larval instar. These results are in agreement with that of Lakshmi and Kumari (1991), that the haemolymph proteins of silkworm fluctuate during the fifth larval instar and the highest number of protein bands were detected in the

middle phase of the instar. Disappearance of protein bands indicates either the non-production, utilization or the degradation of haemolymph proteins to maintain amino acid concentration in the haemolymph as suggested by Lokesh *et al.* (2006). The appearance of the common bands through 5th instar observed in the present study is in conformity with earlier work of Mahmoud (1988) who detected 75, 63, 60 and 34.50 kDa protein bands through the 5th instar of *B. mori*. Miura and Shimizu (1989) recognized a peptide localized at around the 200 kDa region and identified it as the heavy subunit of lipophorin. Subsequently, Hyrsi and Simek (2005) found that the most expressive protein groups in the haemolymph are lipophorins with a molecular mass of 200 - 217 kDa, storage proteins in the range of 70 - 80 kDa and polypeptides of 30 kDa. In addition, some undetermined protein fractions ranging from 18 to 123 kDa were also detected. The results obtained clearly determined the well-defined and characterized protein fractions and some undetermined protein fractions of molecular masses ranging from 5 to 176 kDa.

The electrophoretic pattern obtained from LSO treatments shows that there were marked variations in the intensity as well as number of protein bands which is supported by Horie and Watanabe (1983) who stated that haemolymph protein contents increased with larval growth when pyridoxine was present in the diet of *B. mori* L., whereas it remained unchanged or at low levels when pyridoxine was omitted from the diet. Thus the presence of pyridoxine in LSO might have resulted in enhancement of haemolymph protein in the present study.

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EFFETS DE L'HUILE DE GRAINES DE LAITUE (LSO) SUR QUELQUES CARACTERES ECONOMIQUES ET SUR LE PROFIL DES PROTEINES DE L'HEMOLYMPHE CHEZ LE VER A SOIE, *BOMBYX MORI* L.

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RESUME

Nous avons étudié les effets de l'huile de graines de laitue (LSO) sur les caractères économiques du ver à soie mûrier, *Bombyx mori* L. et son impact sur le profil des protéines hémolymphatiques totales au cinquième âge larvaire. Des larves de *B. mori* du quatrième et du cinquième âge ont été nourries avec des feuilles de mûrier additionnées de LSO à différentes concentrations (0.01 à 1.00 %). Le traitement à 0.10 % est le plus efficace puisque les poids larvaire, du cocon et de la coque ainsi que le nombre d'oeufs pondus sont significativement plus élevés que ceux des témoins. Les changements qualitatifs et quantitatifs des protéines de l'hémolymph des larves du 5^{ème} âge ont été analysés par électrophorèse sur gel de polyacrylamide avec SDS (SDS-PAGE). La quantité de protéines hémolymphatiques croît graduellement pour atteindre un maximum à la fin du 5^{ème} âge. L'analyse SDS-PAGE révèle l'existence de 16 à 26 bandes. La quantité et la qualité des protéines sont significativement augmentées chez les larves de *B. Mori* nourries avec de la LSO à 0.10 %.



SCANNING ELECTRON MICROSCOPIC STUDIES ON THE MOUTH PARTS OF SILKWORM, *BOMBYX MORI* L. DEVELOPED ON ARTIFICIAL DIET

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ABSTRACT

Silkworms fed on artificial diet with well balanced ingredients can meet the specific requirements of early instar growth leading to a stable and healthy population of late instar larvae. But rearing young instar silkworms on artificial diet may pose a challenge on their behavioural and physiological aspects because any deviation from the natural course of events may act as a stress factor. Under a very systematically planned experiment, efforts were made to improve the feeding preference of selective bivoltine and multivoltine pure strains to artificial diet so that their hybrids can be reared commercially utilizing the technology. The feeding response of the races was improved over the generations through directional selection and stabilized along with other traits of economic importance. The present investigation was undertaken to ascertain whether the adaptation in the feeding behaviour of the silkworms over the generations impacted the mouth parts development triggering a transformation to suit feeding on artificial diet. In order to visualize the morphological differences if any in the sensory organs in the mouth parts, the head capsules of artificial diet fed and the regular leaf fed silkworm larvae were observed under Scanning Electron Microscope. A close examination of the antennae, maxillae and the epipharynx which are the crucial organs of gustatory and olfactory senses contributing to host discrimination and food acceptance, revealed no structural changes between diet fed and leaf fed larvae. It is inferred that since the artificial diet contained mulberry leaf powder as an ingredient of natural attractants and as its texture was also taken care to be on par with that of the mulberry leaf, it does not demand any structural alteration in the feeding organs.

Key words: Artificial diet, feeding response, mouth parts, silkworm.

INTRODUCTION

Silkworm, *Bombyx mori* is a typical monophagous insect and do not respond to any feed other than the leaf of its host plant, mulberry (*Morus* sp.). Since silkworms are reared for commercial gain as a major activity of fast expanding silk industry, the quality of cocoon produced, assumes significance in its pricing. Cocoon productivity also contributes to the viability of the enterprise and profitability of the avocation. One of

the most important contributing factors to the quality and quantity of the cocoon is the quality of the mulberry leaf fed to the silkworm. Although leaf fed during fifth instar decides the quality and quantity of the cocoon produced, proper rearing of young instar silkworm leads to healthy fifth instar larvae. Therefore, maximum care is taken during young instar rearing for the stability of cocoon crops. The mulberry leaf grown particularly in tropical conditions does not always contain the required

moisture and balanced nutrients for rearing young-instar silkworms throughout the year. The nutritional requirement of young instar silkworm is specific and to meet such requirement, exclusive mulberry gardens are to be maintained. To overcome these problems to an extent, artificial diet is advocated for young instar rearing.

During the process of technology development, it was thought that the popular and productive silkworm hybrids available in India and their parental strains would accept the artificial diet for young instar rearing. Upon testing, it was realized that the feeding response (FR) of these strains to the artificial diet was indeed very low. Therefore, efforts were initiated to gradually convert the normal silkworm breeds to the ones which would feed on artificial diet, through directional selection and continuous inbreeding until the trait is stabilized. This would ultimately facilitate developing a common artificial diet for all the evolved silkworm breeds and hybrids. A few multivoltine and bivoltine strains were thus evolved and the hybrids were developed successfully from these improved strains which accepted the diet and performed well with respect to their economic traits (Trivedy *et al.*, 2001, 2003). Two hybrids which were evolved for rearing on artificial diet, performed at par on artificial diet during the young instar compared to their counterparts reared on mulberry leaf (Shantala *et al.*, 2003). Nair *et al.* (2010 a, b) already reported the results of an in depth study on stabilizing the feeding response of selected bivoltine and multivoltine silkworm strains for feeding on artificial diet. In continuation, the present study was undertaken to ascertain whether the adaptation in the feeding behaviour of the silkworms over the generations impacted the mouth parts development as an adaptation to suit feeding on artificial diet.

MATERIALS AND METHODS

Rearing of silkworm

In the present study, the long listed 19 bivoltine and 12 multivoltine silkworm strains were screened out for their feeding preference to artificial diet and selected strains were short-listed (6 bivoltine and 4 multivoltine

species). The feeding response of the short-listed strains was improved over generations through directional selection and the trait stabilized along with other traits of economic importance. The artificial diet required for feeding the silkworm was prepared and supplied by Silkworm Physiology Laboratory of CSRTI, Mysore, India. The feeding deterrents or anti-nutritional factors if any in the ingredients were removed before the diet preparation. The premixed dry diet powder was cooked under 15 lbs pressure for 30 minutes with required quantity of water for the diet to hold 70-75 % moisture and instantly cooled. It was further refrigerated at 5 °C until the day of brushing and subsequently brought to the room temperature of 25 °C by the time brushing was to take place.

Breeding procedure for evolution of artificial diet strains

The layings of the selected bivoltine and multivoltine strains which exhibited feeding response for artificial diet (>20 % in bivoltine and >40 % in multivoltine) were incubated and brushed on artificial diet. Twelve replications of the larvae of each strain were brushed as cellular batches. The standard procedure of rearing young instar larvae on artificial diet was followed. On completion of second instar, after careful examination of all the rearing beds, six beds with healthy and uniform larvae were retained and further reared on mulberry leaves until cocoon formation.

Based on FR and the performance of the batches, cocoons conforming to the original breed characters were selected and the moths were allowed to emerge followed by inter-bed crossing or half sib-mating. The eggs laid by the selected moths were allowed to incubate and hatch for the next generation. Following this, the bivoltine parental strains were continuously raised for 12 generations and that of multivoltine strains for 11 generations until the FR of the strains stabilized above 85 %.

Scanning Electron Microscopic studies

Since the intent of the study was to ascertain whether any changes appeared in the morphological and

electrophysiological characters in the mouth parts of young instar silkworm, a few bivoltine and multivoltine larvae were picked up from the diet fed and leaf fed batches after the second ecdysis. The larvae were first fixed in 70 % ethyl alcohol. The head capsules bearing antennae and maxillae were dissected from the larvae. The dissected head capsules were fixed in FAA (10 formalin: 50 ethyl alcohol: 5 acetic acid: 35 water) and dehydrated in a graded ethanol-acetone series. The dehydration started in 50 % ethanol, passed through 70 % and 90 % ethanol; then two changes each through 100 % ethanol, 3:1, 1:1 and 1:3 ethanol-acetone combination and thereafter through pure acetone, keeping the tissues for 15 minutes at each stage. The specimens were then critically dried in a critical point drier (EMS-850) using CO₂ as transition fluid. The dried samples were mounted over copper stubs using double-stick adhesive and coated with about 20 nm gold particles in a sputter coater (EMS-550). Specimens were observed with a JEOL 100 CX-II electron microscope fitted with a scanning attachment (ASID-4D) at 20 kV.

RESULTS

We have already reported in our earlier studies that through the breeding strategy employed in pursuit of developing bivoltine and multivoltine strains which would exclusively feed on artificial diet, we have succeeded to a great extent by improving the feeding response from a low level of about 20 % in bivoltine and 40 % in multivoltine in the initial generations to more than 85 % after 12 and 11 generations, respectively (Nair *et al.*, 2010 a, b). This is amply clear from Tables 1 and 2.

Table 1 shows that the feed response in the first generation ranged from 20.27 % in CSR3 to 70.65 % in 5 HT which indicated clear race dependency. But as the generation progressed to 12th coupled with directional selection procedure, the FR came to a level of about 90 %. The statistical analysis also shows no significant variation among the generation between generation 9 and 12 suggesting stability in feeding response. Similar was the case with the FR of multivoltine strains. Multivoltine strains had high FR in the beginning itself but was not stabilized as seen in the second generation.

Table 1: Feeding response of bivoltine strains over generations

Strain	5HT	GEN4	8HT	B71	CSR3	JPN8
Generation						
1	70.65	48.56	30.80	34.86	20.27	22.80
2	62.26	50.84	29.68	40.59	34.95	44.57
3	66.23	49.56	42.90	56.07	58.12	49.90
4	66.99	54.68	32.52	31.33	42.39	58.37
5	77.26	67.43	75.22	61.08	55.05	58.88
6	79.15	57.83	49.22	59.32	54.75	81.74
7	92.86	92.60	50.46	74.12	76.69	84.44
8	92.54	88.97	76.09	59.36	81.09	81.57
9	90.79	86.84	78.97	67.69	85.82	90.34
10	92.24	91.21	84.05	81.17	82.03	94.72
11	89.88	89.11	82.19	79.31	84.91	90.66
12	90.10	87.50	88.46	89.32	89.72	90.39
*Mean	90.75	88.67	83.42	80.32	85.62	91.53
*SE±	0.649	0.838	1.323	1.316	1.275	1.024
*CV(%)	2.48	3.273	5.50	5.68	5.16	3.876
Significance	NS	NS	NS	NS	NS	NS

* Refers to generations 9 to 12

But by 11th generation, the trait was well stabilized. The analysis also shows that the difference in FR between generation 8 and generation 11 was non-significant to prove that the trait was stabilized by that time.

Table 2: Feeding response of multivoltine strains over generations

Strain	MORIA	MAD	2000K	LMO
Generation				
1	88.89	64.67	59.38	49.88
2	41.07	63.73	34.36	14.95
3	68.01	62.31	64.83	44.27
4	58.95	76.52	82.50	77.88
5	71.59	67.62	78.92	63.14
6	76.74	76.01	83.95	74.54
7	79.43	85.13	79.09	84.75
8	88.01	90.19	90.15	89.09
9	87.11	88.51	89.92	91.35
10	89.00	89.40	89.71	89.72
11	89.37	93.35	88.21	88.18
*Mean	88.37	90.36	89.50	89.59
*SE±	0.894	0.930	0.808	0.599
*CV(%)	3.50	3.56	3.13	2.32
Significance	NS	NS	NS	NS

* Refers to generations 8 to 11

The mouth parts of the young larvae developed for rearing on artificial diet were studied in comparison with that of the normal leaf fed larvae to determine if any structural changes appeared after getting adapted to feed on artificial diet, compared to regular leaf feeding silkworm larvae. The most important entities in the mouth parts of the silkworm larvae with regard to feed selection are maxillae and antennae. The comparative account of the head capsule with maxillae and antennae of the larvae which were reared on mulberry or on the artificial diet (Figures 1 A and B) did not show any structural changes. The antennae, labrum and maxillae appear identical in larvae reared both on mulberry leaf and diet. In the SEM pictures as seen in Figures 1 C and D, the maxillae, labium antennae and spinneret are visible with no structural variations. Figures 2 A and B present the larval maxillary palpus of the leaf and diet strains and Figures 2 C and D show eight apical sensilla on the maxillary palpus of both the leaf and diet strains. In these cases as well, no structural variations were observed between the mouth parts of the larvae fed on mulberry leaf and artificial diet.

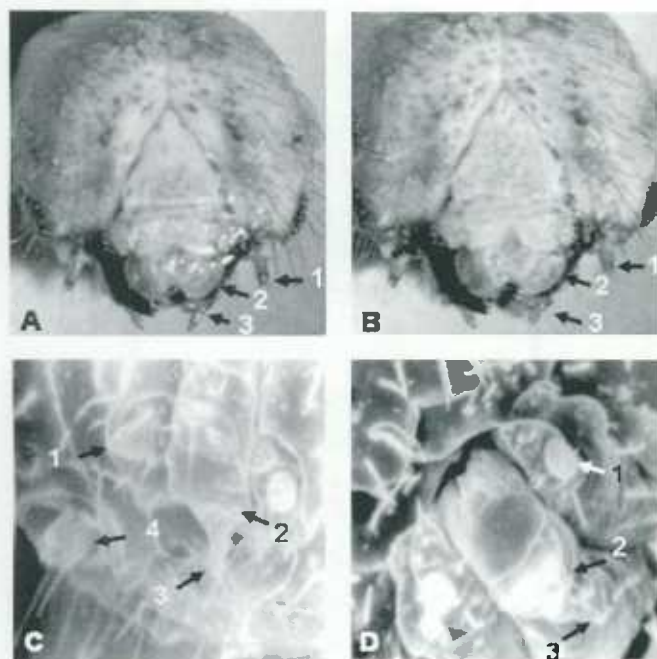


Figure 1 A and B: Anterio-dorsal view of the larval head capsule of leaf (A) and diet (B) fed strains of *Bombyx mori*: 1. Antenna, 2. Labrum, 3. Maxilla.

Figure 1 C and D: Anterio-ventral view of the larval head capsule of leaf (C) and diet (D) fed strains of *Bombyx mori*: 1. Maxillary palpus, 2. Labium, 3. Spinneret, 4. Antenna.

DISCUSSION

Feeding response of normal silkworm strains to artificial diet is low because various obligatory and facultative factors besides the genetical factors are responsible for feeding behaviour *vis-à-vis* its nutritional requirements. The feeding behaviour of silkworms on artificial diet is influenced by environmental conditions, food ingredients and genetic background suggesting that it is controlled by some major genes (Ohnuma and Tazima, 1996) and a few modifiers (Tazima *et al.*, 1984). But it is obvious from the results of this study that the FR can be improved over generation through directional breeding strategy.

Chemosensory systems play a key role in the survival and reproductive success in insects. In silkworm larvae, antenna, maxilla and epipharynx are considered

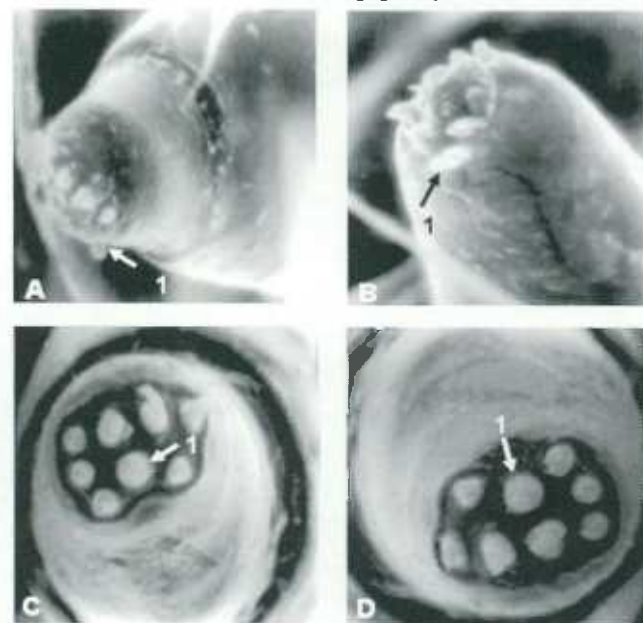


Figure 2 A and B: Maxillary palpus of leaf (A) and diet (B) fed silkworm showing sensilla styloconica (1).

Figure 2 C and D : Eight apical sensilla on the maxillary palpus of leaf (C) and diet (D) fed larva: 1. Sensilla basiconica.

to be organs of chemoreception (taste and olfaction) involved in feeding behaviour (Schoonhoven and Dethier, 1966). It has been demonstrated through various experiments that maxillary sensilla and epipharyngeal sensilla are crucial for host discrimination (Glendinning *et al.*, 1990; Kanda, 1992). As an appendage with primarily gustatory sensilla which also possess olfactory function, the maxillary palpus play an important role in

host selection behaviour in phytophagous insect (Albert, 1980; Glendinning *et al.*, 1998).

The feeding behaviour in *B. mori* consists of three steps *i.e.*, attracting, biting and swallowing, performed in coordination of the structural components and the chemical stimuli available in the feed (Hamamura, 1959). The attracting behaviour is controlled by the sensilla basiconica in the antennae and the biting behaviour by those in the maxillae (Hirao *et al.*, 1972). The feeding activity of silkworm larvae is stimulated by β -sitosterol (Hamamura *et al.*, 1961) and flavanoid (Horie, 1962), whereas it is inhibited by bitter phenolic compounds such as salicin (Ishikawa, 1966). The larvae to become polyphagous, the maxillary deterrent cells need to electrophysiologically respond poorly to salicin (Yazawa *et al.*, 1991). Asaoka (2000) also speculated that the low sensitivity of polyphagous silkworm strains to phenolic compounds is caused by abnormalities at the o-glycosidic receptor site on deterrent cells or an altered signal transduction system. These information do not indicate a prominent structural modification in the food selection and consumption process, instead, point towards a genetic modification.

In the present work, to visualize the morphological differences if any in the sensory organs in the mouth parts of silkworm larvae which have adapted to feed on artificial diet and regular leaf feeding ones, these organs were observed under scanning electron microscope. The dissected head capsules of both the groups were observed under SEM so that the mouth parts particularly maxillae, sensilla basiconica and sensilla styloconica in the maxillae could be seen. There was no prominent difference in the structural details between these groups with reference to the mouth parts which are involved in feed selection and feed consumption. In a similar exercise, Kanda (1992) investigated the possible morphological difference in sensory organs of silkworm races with high and low affinity to artificial diet feeding through scanning electron microscope. However, no difference between the two types could be detected.

Therefore, we reiterate that there is no much chance of a structural reorientation in the antennae and maxillae because the artificial diet contained mulberry leaf powder as a major ingredient and thus contained

the natural attractants. The feeding deterrents or anti-nutritional factors supposed to be there in the other ingredients are effectively removed before the diet preparation. Thus, in effect, constitution-wise, the artificial diet is not too different from mulberry leaf and do not demand a structural modification. It is also not too prudent to believe that just because the larvae which adapted to feed on the diet during the young instar would have undergone a structural change. This is because, after the second ecdysis, the larvae need to feed on mulberry leaf and the transition from diet feeding to leaf feeding was also smooth.

The gustatory sensilla on the epipharynx of the *B. mori* larvae and the morphological and electrophysiological characteristics of the epipharyngeal sensilla as investigated by Asaoka and Shibuya (1995) indicated that the gustatory sensilla were sensilla coeloconica composed of a small papilla with a pore at the tip and a swelling of cuticle encircling the papilla. Three bipolar neurons innervate each sensillum. One neuron is an inositol receptor which responds to inositol only. Another cell responds with action potentials of relatively large amplitude to some feeding deterrent substances, such as strychnine nitrate. At least two kinds of spikes could be observed when these sensilla were stimulated with some salts and acids. A study by Seok-Jo *et al.* (1996) indicated that the silkworm feeding behaviour revealed two types of FR *i.e.*, energetic and inert FR to artificial diet. The structures of functional organs of mouth part and antenna under energetic and inert FR to diet when observed under scanning electron microscope showed variations in the ultrastructures of different sensilla. The maxillary palpi or sensilla basiconica at the top of maxillary palpus were well developed in the energetic larva while in the inert larva, these organs were deformed. This gives a clear indication that the main structure responsible for artificial feed preference is the sensilla basiconica and when the larvae are in the active feeding form, these structures remain fully developed. In the present study, the larvae both on artificial diet and mulberry leaf were in the active feeding form and in both cases, the maxillary palpi and the sensilla basiconica appeared well developed with no difference whatsoever in the morphology.

While feeding, silkworm larvae bring maxillae in close contact with food. Consequently, each sensillum on the maxillae plays a crucial role in feeding behaviour. The medial trichoid sensillum on the maxillary lobe is mechanosensory (Ishikawa and Hirao, 1961). They serve to convey information regarding food structure and thereby control mandibular movements (Grimes and Neunzing, 1986). The two styloconic sensilla are called lateral and medial styloconic sensilla, respectively. Their gustatory function in *B. mori* was demonstrated by behavioural and electrophysiological studies (Hirao and Arai, 1991). Efforts were rife to determine whether there was structural difference in the maxillae and maxillary sensilla. The strains *viz.*, N601 x C601, Sawa-J and N01 are known as polyphagous (Asaoka and Mano, 1992; Kanda, 1992). The strain S2 with *Nps* gene is termed non-preference mutant (Tazima, 1989). Such strains feed on several kinds of low cost artificial diet and plant leaves which most normal strains reject. Underlying mechanism of feeding behaviour of such polyphagous strains or non-preference mutants of silkworm were areas of interest to Asaoka (2003). From the morphological aspects, abnormal cuticular structure of the maxilla was observed in some non-preference *Np* mutant larvae (Tazima *et al.*, 1984) while Kanda (1992) found no morphological aberration in a polyphagous strain, Sawa-J. Although Asaoka (2003) observed some characteristic cuticular structures of the galeal styloconic sensilla, it was not related to feed preference but to strain difference. In addition, probability in occurrence of the different number of basiconic sensilla on the maxillary palpus can also be attributed to strain difference and not the feeding habits of the strains. These observations support the results of the present study and explains that the mere adaptation of the young instar silkworm larvae to feed on the artificial diet do not bring about any structural change that decides feed selection and feed consumption.

The results of the study indicate that the feeding response varied within the strains, between the strains and between the generations and also clearly demonstrate the possibility of changes in a physiologically varying character by continuous selection from a rather small foundation stock by changes in the frequency of

many genes, none of which could have had much effect by itself. Further, the environmental conditions prevailed during the young instar and the persistence of association with artificial diet has allowed the strains to perform significantly better and achieve growth rates illustrating the adaptive significance in silkworms. Physiological adaptations permit the larvae to perform the special function (consumption of artificial diet) and more general functions such as growth and development. It is interesting to note that the response to selection was almost steady and progressive in multivoltine strains despite the fact that the initial FR regressed in the second generation as compared to the bivoltine strains wherein the progress was fluctuating for sometime. This behavioural adaptation of multivoltine strains can be attributed to the innate physiological mechanisms, direct acceptability and ingestion of artificial food modulated by changes in taste receptors which allows feeding more efficiently. Further, the quality of the diet plays a significant role in the relative acceptability of alternatives and the growth depending on nutrient need – a flexibility that is dependent on variation in the strength of inputs from different chemoreceptors.

It is summed up that the above account of the mouth parts of the larvae which were reared continuously on artificial diet for more than 10 generations did not reveal any structural change under scanning electron microscope when compared to that of the ones reared on mulberry leaves.

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MICROSCOPIE ELECTRONIQUE A BALAYAGE DES PIECES BUCCALES DE VER A SOIE, *BOMBYX MORI* L., NOURRIES SUR ALIMENT ARTIFICIEL

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RESUME

Les vers à soie nourris sur aliment artificiel contenant des ingrédients en proportion équilibrée trouvent les éléments spécifiques nécessaires à la croissance des premiers âges larvaires ce qui conduit à une population stable et saine aux derniers âges larvaires. Mais l'élevage sur aliment artificiel des jeunes vers à soie peut représenter un défi quant à leur comportement et à leur physiologie à cause de la modification du cours naturel des événements qui peut constituer un facteur de stress. Par des expériences systématiquement planifiées, des efforts ont été faits pour améliorer la préférence alimentaire vis-à-vis de l'aliment artificiel de races bivoltines et polyvoltines pures de sorte que leurs hybrides puissent être élevés commercialement en utilisant cette technologie. La réponse alimentaire des races a été améliorée par sélection au cours des générations et stabilisée avec d'autres caractères d'importance économique. Nous avons entrepris ici des investigations afin de savoir si l'adaptation comportementale à l'alimentation artificielle au cours des générations a un impact sur le développement des pièces buccales entraînant une transformation pour manger l'aliment artificiel. Afin de visualiser les différences morphologiques, si elles existent, entre les organes sensoriels des pièces buccales des larves nourries avec des feuilles et ceux des larves nourries avec l'aliment artificiel, nous avons observé les capsules céphaliques par microscopie électronique à balayage. L'examen précis des antennes, maxilles et épipharynx qui sont les organes cruciaux gustatifs et olfactifs contribuant à la discrimination de l'hôte et à l'acceptation de la nourriture, montre qu'il n'y a pas de différences structurelles entre les larves nourries avec l'aliment artificiel et celles nourries avec des feuilles. On en déduit que, puisque l'aliment artificiel contient de la poudre de feuilles de mûrier possédant des attractants naturels et que sa texture est maintenue pour être proche de celle des feuilles de mûrier, il n'y a pas nécessité de modifications structurelles des organes liés à l'alimentation.



Research Paper

ASSESSMENT OF GENETIC DIVERSITY AMONG DIFFERENT GENOTYPES OF *TERMINALIA ARJUNA* AND *T. TOMENTOSA*

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ABSTRACT

Genus *Terminalia* L. belongs to family combretaceae and consists of trees, shrubs and woody climbers. The genus is of immense economic importance in various industries viz., pharmaceutical, timber, paper, soap, match, food, fodder and fuel. Besides, the importance of *Terminalia arjuna* and *T. tomentosa* has been well recognized in tropical tasar silk industry as the two most important primary food plants. A total of thirty - four accessions of *Terminalia arjuna* (18) and *T. tomentosa* (16) of diverse origin were selected for studying genetic diversity. In *T. arjuna*, out of 50 decamer random primers, only 35 primers have given reproducible polymorphic products. In all, 641 amplified bands were obtained of which 589 bands were polymorphic and 52 were monomorphic. In *T. tomentosa*, out of 50 primers, only 37 primers have given reproducible polymorphic products. Thirty - seven random primers generated a total of 719 bands; in which 693 were polymorphic (96.38 %) and 26 were monomorphic. On an average, 18.73 polymorphic bands were generated per primer ranging between 11 and 35 bands. Dendrogram based on RAPD data grouped both *T. arjuna* (18 accessions) and *T. tomentosa* (16 accessions) in three major clusters. High level of heterozygosity was noticed in both the species. The Shannon's index was also very high (0.562 - 0.578) indicating the extent of genetic diversity available for these two species. The percentage of polymorphism generated by decamer primers indicates that RAPD is the most suitable marker to assess genetic diversity in *Terminalia*. The present study is the first report on molecular analysis of different accessions of divergent origin of *T. arjuna* and *T. tomentosa* which can be used in hybridization programme to evolve superior genotypes of this important multipurpose tree.

Key words: Genetic diversity, RAPD, *Terminalia arjuna*, *Terminalia tomentosa*.

INTRODUCTION

Genus *Terminalia* L. belongs to family combretaceae and consists of trees, shrubs and woody climbers. Hooker (1878) described 80 species of the genus while Brandis (1906) reported 105 species distributed throughout the humid, sub humid, tropical and sub tropical regions of both the hemispheres. In India, *Terminalia* is distributed widely and found almost in all the humid and sub humid forests.

The plants of this genus assume immense economic importance as they are utilized in various industries such as pharmaceutical, timber, paper, soap, match, food,

fodder and fuel. Besides, the importance of *Terminalia arjuna* and *Terminalia tomentosa* has well been recognized in tropical tasar industry as the two most important primary food plants. It is estimated that about 9 million hectares of forest land has been occupied by these species.

The over exploitation and anthropogenic pressure on these species lead to habitat fragmentation and loss of the species. Natural population is also decreasing especially in highly settled area.

The attempts initiated in the line of conservation of plant genetic diversity has recently generated a lot of

interest in the tropics because many years of mismanagement, adverse environment as well as socio-economic changes have already been contributing towards devastation. Plant species especially the perennials such as trees, rely on the available genetic diversity for stability and survival under the ever-changing environments.

Availability of genetic diversity is a prerequisite for improvement of all crops. Recently, the development of polymerase chain reaction (PCR) technique has revolutionised the field of molecular biology. RAPD is simple polymerase chain reaction (PCR) based technique and used extensively in studying genetic relationship among accessions of various plant species. Studies on assessment of genetic diversity between different species of *Terminalia* using RAPD markers have been reported by Tewary and Suryanarayana (2007) and Deshmukh *et al.* (2009). So far, a study on genotypic diversity in germplasm collections has not been reported. Keeping this fact in view, the present study was undertaken to evaluate genetic diversity among different accessions of *T.arjuna* and *T.tomentosa* established in field gene bank under *ex-situ* condition at Central Tasar Research and Training Institute, Ranchi, India. The result will help in understanding the extent of genetic diversity among different accessions of *T.arjuna* and *T.tomentosa* which will be useful for its genetic improvement.

MATERIALS AND METHODS

A total of thirty - four accessions of both *Terminalia arjuna* (18) and *T. tomentosa* (16) of diverse origin formed the experimental material for the study (Table 1). All the trees were maintained by adopting uniform management practices. At the start of the study, the trees were 6 years old. The cultural operations were implemented as per recommended practices by the Institute.

Total DNA of each genotype was isolated using tender and juvenile leaves of new flush by following Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (Doyle and Doyle, 1990, Deshmukh *et al.*, 2007) with minor modifications. The DNA was quantified by using 100 fluorometer (Hoefer, San Francisco, CA)

Table 1: Details of experimental *Terminalia* accessions

S. No.	Acc No.	IC No.	Place of collection
<i>T. arjuna</i>			
1	123	IC 355553	Jharkhand
2	135	ACC.135	Orissa
3	209	ACC.209	Jharkhand
4	211	ACC.211	Jharkhand
5	302	IC 326384	Maharashtra
6	307	IC 326385	Maharashtra
7	332	IC 382302	Andhra Pradesh
8	333	IC 410866	Chhattisgarh
9	430	IC 381635	Jharkhand
10	504	IC 410749	Andhra Pradesh
11	508	IC 410783	Jharkhand
12	512	IC 410829	Jharkhand
13	533	IC 560460	Uttar Pradesh
14	622	IC 382333	Uttaranchal
15	624	IC 382334	Uttaranchal
16	701	IC 410794	Maharashtra
17	702	IC 410796	Maharashtra
18	703	IC 410797	Maharashtra
<i>T. tomentosa</i>			
19	128	ACC.128	Jharkhand
20	216	IC 326377	Maharashtra
21	229	IC 331855	Chhattisgarh
22	309	IC 381648	Jharkhand
23	310	IC 331856	Chhattisgarh
24	313	IC 410868	Chhattisgarh
25	408	ACC.408	Jharkhand
26	409	IC 410808	Maharashtra
27	438	IC 381637	Jharkhand
28	443	IC 410869	Chhattisgarh
29	501	ACC.501	Jharkhand
30	522	ACC.522	Jharkhand
31	531	IC 410818	Maharashtra
32	535	IC 410787	Jharkhand
33	610	IC 355555	Jharkhand
34	612	IC 410764	Andhra Pradesh

PCR Amplification of DNA with RAPD primer

Fifty different 10 - mer primers, corresponding to kit M (OPM 1 to OPM 20), kit W (OPW 1 to OPW 20) from Operon Technologies, Alameda, California, USA and 10 of Bangalore Genei™, Bangalore, India (AM 750044 to AM 773771) were initially screened for their ability to amplify the DNA of *T. arjuna* and *T. tomentosa*.

DNA amplification was carried out by making final reaction volume to 25 μ l containing 1 X Taq buffer A, 0.2 mM dNTP mix, 1U of Taq DNA polymerase enzyme, 50 ng DNA templates, 0.8 μ M RAPD primer in thin walled PCR tubes. PCR amplification was carried out in a thermal cycler programmed for initial denaturation at 94 °C for 5 min followed by 40 cycles denaturation at 94 °C for 1 min, annealing at 36 °C for 1 min and extending primer at 72 °C for 2 min and a final extension step at 72 °C for 10 min.

The amplified PCR products were fractionated on 1.8 % agarose gel using 1x TAE buffer containing 5 μ g/ml ethidium bromide. The gel was run at a constant voltage of 120 volts for 2 hours. The resolved PCR products were visualized under UV light in Gel Documentation System (UV tec U.K.).

RAPD analysis

RAPD profiles were analyzed by scoring the presence (1) and absence (0) of DNA band of each accession of *T. arjuna* and *T. tomentosa*. A binary data matrix, 1 indicating the presence of a particular band and 0 indicating its absence was constructed. The genetic variability in the population was calculated using Nei's (1987) coefficient in POPGENE version 1.32 (Yeh and Boyle, 1997). A dendrogram was generated from the above matrix using Unweighted Pair Group Method with Arithmetical Averages (UPGMA) using NTSYS 2.1 (Rohlf, 1999). Other genetic parameters viz., number of alleles, heterozygosity and Shannon's information index were calculated using POPGENE.

RESULTS AND DISCUSSION

***T. arjuna*:** Out of 50 decamer random primers, only 35 primers (28 primers of Operon technologies and 7 of Bangalore Genei™) had given reproducible polymorphic products. (Table 2). In all, 641 amplified bands were obtained of which 589 were polymorphic and 52, monomorphic. The maximum and minimum numbers of bands were produced by the primer OPW 08 (30) and primer AM773310 (07), respectively. On an average, 16.83 polymorphic bands were generated per primer ranging from 7 to 30 bands. Only 10 primers (OPM-03,

OPM-04, OPM-05, OPM-07, OPM-11, OPM-16, OPW-08, OPW-12, OPW-18 and AM773315) generated 100% polymorphic products (Figure 1). Dendrogram

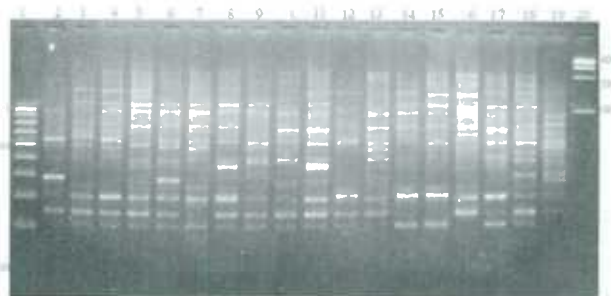


Figure 1: RAPD profiles of 18 *T. arjuna* accessions with primer OPM-3: Lane 1 - 100 bp DNA Ladder; Lane 20 - 1 Kb DNA Ladder; Lanes 2-19 - eighteen *T. arjuna* accessions.

generated from average similarity coefficient of 31 primers grouped 18 accessions in three major clusters (Figure 3). In cluster-I, accessions, 209, 123 and 135 formed one sub-cluster. Out of them, two are from Jharkhand and one from Orissa. The other sub-cluster comprised accessions 307, 211 and 302, where two of them were from Maharashtra and one from Jharkhand. In Cluster-II, accession 512 was placed as an isolate and this was from Jharkhand. One sub-cluster comprised of accessions 332 and 333, one from Andhra Pradesh and other from Chhattisgarh. The other sub-cluster comprised of three accessions, two (508, 430) from



Figure 2: RAPD profiles of 16 *T. tomentosa* accessions with primer OPW-05: Lane 1 - 100 bp DNA Ladder; Lane 18 - 1 Kb DNA Ladder; Lanes 2-17 - sixteen *T. tomentosa* accessions.

Jharkhand and one (504) from Andhra Pradesh. The third cluster had two sub-clusters; first one from Uttar Pradesh (Acc. 533) and second from Uttaranchal (Acc.622). Accessions 701, 702 and 703 (Maharashtra) and 624 (Uttarakhand) formed the second sub-cluster.

Table 2: RAPD primers, sequence and polymorphism per cent in *T. arjuna*.

S. No.	Primer Code	Sequence 5' - 3'	Total bands	Polymorphic bands	Monomorphic bands	Polymorphism (%)
1	OPM 01	GTTGGTGGCT	16	14	2	87.50
2	OPM 02	ACAACGCCTC	15	14	1	93.33
3	OPM 03	GGGGGATGAG	25	25	0	100.00
4	OPM 04	GGCGGTTGTC	20	20	0	100.00
5	OPM 05	GGGAACGTGT	22	22	0	100.00
6	OPM 07	CCGTGACTCA	18	18	0	100.00
7	OPM 09	GTCTTGCGGA	15	14	1	93.33
8	OPM 11	GTCCACTGTG	19	19	0	100.00
9	OPM 13	GGTGGTCAAG	14	11	3	78.57
10	OPM 14	AGGGTCGTTC	21	20	1	95.24
11	OPM 15	GACCTACCAC	14	13	1	92.86
12	OPM 16	GTAACCAGCC	19	19	0	100.00
13	OPM 19	CCTTCAGGCA	11	9	2	81.82
14	OPW 01	CTCAGTGTCC	19	15	4	78.95
15	OPW 02	ACCCCGCCAA	22	21	1	95.45
16	OPW 03	GTCCGGAGTG	21	19	2	90.48
17	OPW 04	CAGAAGCGGA	21	20	1	95.24
18	OPW 06	AGGCCCGATG	13	11	2	84.62
19	OPW 07	CTGGACGTCA	16	15	1	93.75
20	OPW 08	GACTGCCTCT	30	30	0	100.00
21	OPW 09	GTGACCGAGT	26	21	5	80.77
22	OPW 11	CTGATGCGTG	19	18	1	94.74
23	OPW 12	TGGGCAGAAG	21	21	0	100.00
24	OPW 13	CACAGCGACA	18	16	2	88.89
25	OPW 16	CAGCCTACCA	25	23	2	92.00
26	OPW 18	TTCAGGGCAC	26	26	0	100.00
27	OPW 19	CAAAGCGCTC	15	14	1	93.33
28	OPW 20	TGTGGCAGCA	18	16	2	88.89
29	AM765819	AAAGCTGCGG	17	16	1	94.12
30	AM750044	AACGCGTCGG	19	17	2	89.47
31	AM773310	AAGCGACCTG	7	3	4	42.86
32	AM773770	AATCGGGCTG	9	5	4	55.56
33	AM773771	ACACACGCTG	20	17	3	85.00
34	AM773312	ACATCGCCCA	8	5	3	62.50
35	AM773315	ACCGCCTATG	22	22	0	100.00
Total			641	589	52	

The bootstrap values were significant for all clusters except sub cluster comprising accessions 123, 135 and 209, therefore, they were kept in one cluster.

***T. tomentosa*:** Out of 50 primers, only 37 primers (31 of Operon technologies and 6 of Bangalore Genei™) had given reproducible polymorphic products (Table 3). Thirty - seven random primers generated a total of 719 bands; in which 693 were polymorphic (96.38 %)

and 26 were monomorphic. On an average, 18.73 polymorphic bands were generated per primer ranging between 11 and 35 bands. Among these, primer OPW 05 generated maximum number of bands (35). Primers, OPM-03, OPM-07, OPM-09, OPM-11, OPM-13, OPM-14, OPM-17, OPM-18, OPM-19, OPW-01, OPW-02, OPW-10, OPW-11, OPW-12, OPW-13, OPW-15, OPW-18, OPW-19, AM773371 and AM750045 generated

Table 3: RAPD primers, sequence and polymorphism per cent in *T. tomentosa*.

Sl. No.	Primer Code	Sequence	Total bands	Polymorphic bands	Monomorphic bands	Polymorphism (%)
1	OPM 01	GTTGGTGGCT	11	10	1	90.91
2	OPM 02	ACAACGCCTC	14	13	1	92.86
3	OPM 03	GGGGGATGAG	27	27	0	100.00
4	OPM 05	GGGAACGTGT	16	15	1	93.75
5	OPM 07	CCGTGACTCA	16	16	0	100.00
6	OPM 09	GTCTTGCGGA	23	23	0	100.00
7	OPM 10	TCTGGCGCAC	16	15	1	93.75
8	OPM 11	GTCCACTGTG	15	15	0	100.00
9	OPM 12	GGGACGTTGG	21	20	1	95.24
10	OPM 13	GGTGGTCAAG	22	22	0	100.00
11	OPM 14	AGGGTCGTTC	25	25	0	100.00
12	OPM 17	TCAGTCCGGG	15	15	0	100.00
13	OPM 18	CACCATCCGT	10	10	0	100.00
14	OPM 19	CCTTCAGGCA	20	20	0	100.00
15	OPM 20	AGGTCCTGGG	11	9	2	81.82
16	OPW 01	CTCAGTGTCC	14	14	0	100.00
17	OPW 02	ACCCCGCCAA	24	24	0	100.00
18	OPW 03	GTCCGGAGTG	23	20	3	86.96
19	OPW05	GGCGGATAAG	35	34	1	97.14
20	OPW 06	AGGCCCGATG	19	18	1	94.74
21	OPW 07	CTGGACGTCA	23	22	1	95.65
22	OPW 08	GA CTGCCTCT	28	27	1	96.43
23	OPW 09	GTGACCGAGT	26	23	3	88.46
24	OPW 10	TCGCATCCCT	19	19	0	100.00
25	OPW 11	CTGATGCGTG	17	17	0	100.00
26	OPW 12	TGGGCAGAAG	21	21	0	100.00
27	OPW 13	CACAGCGACA	24	24	0	100.00
28	OPW 15	ACACCGGAAC	20	20	0	100.00
29	OPW 16	CAGCCTACCA	24	23	1	95.83
30	OPW 18	TTCAGGGCAC	24	24	0	100.00
31	OPW 19	CAAAGCGCTC	16	16	0	100.00
32	AM750044	AACGCGTCGG	20	19	1	95.00
33	AM773310	AAGCGACCTG	12	8	4	66.67
34	AM773771	ACACACGCTG	14	14	0	100.00
35	AM773312	ACATCGCCCA	13	11	2	84.62
36	AM773315	ACCGCCTATG	24	23	1	95.83
37	AM750045	ACGATGAGCG	17	17	0	100.00
Total			719	693	26	

100 % polymorphic products (Figure 2). All the sixteen accessions were grouped in three major clusters (Figure 4). Cluster I was composed of seven accessions, where accession 501 (Jharkhand) remained as an isolate. Accessions 309, 128 (Jharkhand), 216 (Maharashtra) and 229 (Chhattisgarh) comprised one sub-cluster. On

the other hand, the other sub-cluster had accessions 310 (Chhattisgarh) and 408 (Jharkhand). The second major cluster had accessions, 313 and 443 in the same sub-cluster and both are from Chhattisgarh. Accessions 409 (Maharashtra) and 438 (Jharkhand) formed another sub-cluster. Major cluster-III comprised five accessions, 522,

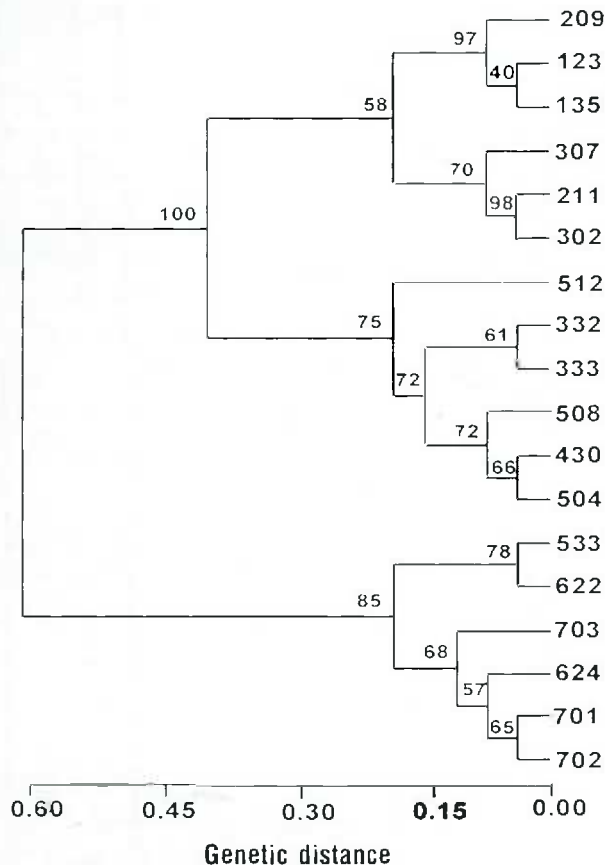


Figure 3:. Dendrogram of 18 accessions of *T. arjuna* based on RAPD data

535, 610 (Jharkhand), 531 (Maharashtra) and 612 (Andhra Pradesh). The bootstrap values were more than 50 except for accessions 216 and 229.

The results indicate that geographical distribution and genetic divergence did not follow the same pattern in both species. This shows that the geographical diversity may not necessarily be related to genetic diversity in this species. Similar observations have been recorded by Sharma *et al.* (2010) in 216 genotypes of walnut. The clustering pattern of walnut genotypes belonging to same eco geographical region revealed their distribution in more than one cluster showing non parallelism between geographic and genetic diversity. The present results are also in conformity with the findings of Srivastava *et al.* (2007), Katiyar *et al.* (1998) and Dwivedi and Mitra (1996) in apricot, forage maize and litchi, respectively.

Terminalia is a highly heterozygous, cross pollinated plant evolved on the lines of polyploidy which

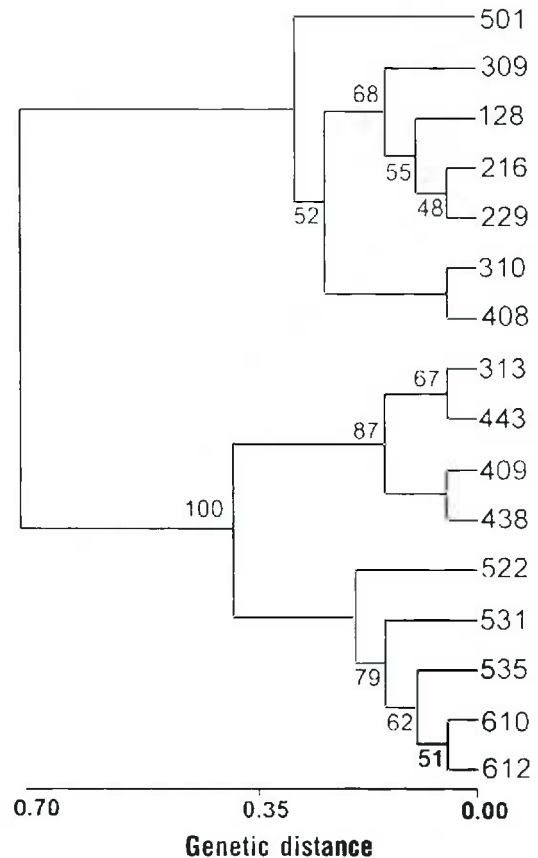


Figure 4: Dendrogram of 16 accessions of *T. tomentosa* based on RAPD data

increases its complexity. Therefore, assessment of genetic diversity in this genus is very difficult. Further, it showed a wide variation in morphological and quantitative traits. These variations are greatly affected by environment and do not reveal any genetic relationship (Rao and Ramaya, 1984; Tilney and Van Wyk, 2004; Kumar *et al.*, 2009, 2010). In the present study, dendrogram based on RAPD data grouped both *T. arjuna* (18 accessions) and *T. tomentosa* (16 accessions) in three major clusters. High level of heterozygosity (0.381 ± 0.112) was noticed in both the species (Table 4). The Shannon's index was also high indicating the extent of genetic diversity available for these two species (0.562 - 0.578). The percentage of polymorphism generated by decamer primers indicates that RAPD is the most suitable marker to assess genetic diversity in *Terminalia* as very little information is available on this aspect in this genus except for the studies by Deshmukh *et al.* (2009). The present study is the first report on molecular analysis of different

Table 4: Population genetic parameters analysed through RAPD data

Parameter		<i>T. arjuna</i>	<i>T. tomentosa</i>
Observed number of alleles	<i>na</i>	2.00 ± 0.00	2.00 ± 0.00
Effective number of alleles	<i>ne</i>	1.691 ± 0.252	1.658 ± 0.265
Nei's (1973) gene diversity	<i>h</i>	0.394 ± 0.098	0.380 ± 0.105
Shannon's information index	<i>I</i>	0.578 ± 0.110	0.562 ± 0.120
Total heterozygosity	<i>Ht</i>	0.381 ± 0.112	0.381 ± 0.112
Per cent polymorphism		91.89	96.38

accessions of divergent origin of *T. arjuna* and *T. tomentosa* which may serve as an additional information for hybridization programme to evolve superior genotypes of this important multipurpose tree.

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EVALUATION DE LA DIVERSITE GENETIQUE PARMi DIFFERENTS GENOTYPES DE *TERMINALIA ARJUNA* ET *T. TOMENTOSA*

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RESUME

Le genre *Terminalia* L. appartient à la famille des combretaceae et est composé d'arbres, d'arbustes et de plantes grimpantes ligneuses. Le genre est d'une importance économique immense dans plusieurs industries telles que la pharmacie, la construction, la papèterie, la savonnerie, les allumettes, l'alimentation et le combustible. D'autre part, *Terminalia arjuna* et *T. Tomentosa* sont essentiels pour l'industrie du Tasar tropical puisqu'ils représentent les deux plus importants plants alimentaires primaires. Un total de trente quatre acquisitions de *Terminalia arjuna* (18) et de *T. Tomentosa* (16) de diverses origines ont été étudiées. Chez *T. Arjuna*, sur 50 amorces décarnériques aléatoires, seules 35 amorces ont donné des produits polymorphiques reproductibles. Au total, 641 bandes d'amplification ont été obtenues dont 589 sont polymorphiques et 52 monomorphiques. Chez *T. Tomentosa*, sur 50 amorces, 37 seulement ont donné des produits polymorphiques reproductibles. Les trente sept amorces aléatoires ont généré un total de 719 bandes, parmi lesquelles 693 sont polymorphiques (96.38 %) et 28 monomorphiques. En moyenne, les amorces ont généré 18.73 bandes polymorphiques, la fourchette allant de 11 à 35 bandes. Le dendrogramme obtenu à partir des données RAPD rassemble *T. Arjuna* (18 acquisitions) et *T. Tomentosa* (16 acquisitions) en trois groupes. On note un fort niveau d'hétérozygotie chez les deux espèces. L'indice Shanon est également très élevé (0.562 – 0.578) ce qui indique l'étendue de la diversité génétique chez ces deux espèces. Le pourcentage de polymorphisme généré par des amorces décarnériques montre que la RAPD est le marqueur le plus approprié pour évaluer la diversité génétique chez *Terminalia*. La présente étude est la première publication sur l'analyse moléculaire de différentes acquisitions d'origines divergentes de *T. arjuna* et *T. Tomentosa*, ce qui peut être utilisé dans les programmes d'hybridation pour obtenir des génotypes supérieurs de cet arbre aux multiples usages.



IMPACT OF PUPAL WEIGHT ON REALIZED FECUNDITY AND REARING PERFORMANCE OF M.Con.4 (*BOMBYX MORI* L.)

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ABSTRACT

Impact of differential weight of both male and female pupae on realized fecundity and rearing performance was studied in the newly evolved multivoltine breed, M.Con.4 (*Bombyx mori* L.). Both male and female pupae categorized into 4 groups based on pupal weight were intercrossed. Results revealed that both the male and female pupal weight have significant impact on realized fecundity, hatching %, mature larval weight, single cocoon weight and single shell weight. The number of eggs laid by each group of female was correlated with the pupal weight. The results registered positive and significant correlation at 1% level.

Key words: *Bombyx mori*, correlation, differential pupal weight, realized fecundity.

INTRODUCTION

West Bengal, in India, is a state of highly fluctuating weather ranging from high temperature, high humidity to low temperature, low humidity conditions. Since multivoltine breeds only can sustain the wide variations in temperature and humidity, those are exploited here as female component for preparation of disease free layings (DFLs) of hybrids (multivoltine x multivoltine and multivoltine x bivoltine). This situation necessitates to evolve superior quality multivoltine breeds having improved quantitative traits *i.e.*, fecundity, survivability, yield potentiality *etc.* for rearing at seed cocoon production level. M.Con.4 is a new multivoltine breed of *Bombyx mori* L. evolved by silkworm breeders of Central Sericultural Research and Training Institute, Berhampur, West Bengal, India. Positive correlation between female pupal weight and fecundity have been established (Ahsan and Khannajain, 1976; Shamachary and Krishnaswami, 1980; Singh and Prasad, 1987; Rajanna and Puttaraju, 1998) in different insects. Though the female pupal weight is one of the main criteria for obtaining high fecundity, the potentiality of maximum number of realized eggs depends upon some crucial factors other than agro-climatic conditions. The present

study was planned to explicate the role of male pupal weight on recovery of maximum number of eggs/realized fecundity and fertility with an impact on other rearing parameters.

MATERIALS AND METHODS

Twenty per cent cocoons each of male and female were randomly picked from each of 3 replications of M.Con.4 reared under the same agro-climatic condition (temperature: 18-27 °C; relative humidity: 54-85 %) following standard rearing technique (Krishnaswami *et al.*, 1986). Individual cocoon assessment was carried out on the 6th day of spinning using digital electronic balance. Depending upon pupal weight, both male and female pupae were categorized into four groups *viz.*, A: heavy, B: moderate, C: Low and D: Lighter/control (Table 1). The moths emerged from these 4 groups were crossed by following diallele crossing method (Griffing, 1956) and 16 combinations were obtained. Silkworms of each such combination were reared in 3 replications with 300 larvae each after 3rd moult to observe the performance concerning hatching %, weight of mature larva, effective rate of rearing (ERR), both by number and weight, single cocoon weight, single shell weight

and shell %. Reproductive index for each group was evaluated by the method of Hough and Pimental (1978). Analysis of variance and correlation between pupal weight and fecundity as well as other rearing parameters were worked out following standard statistical methods.

RESULTS AND DISCUSSION

It is evident from Figure 1 that in a random population of M.Con.4, the percentage of moderate and light weighed pupae was the highest. Percentage of females in groups

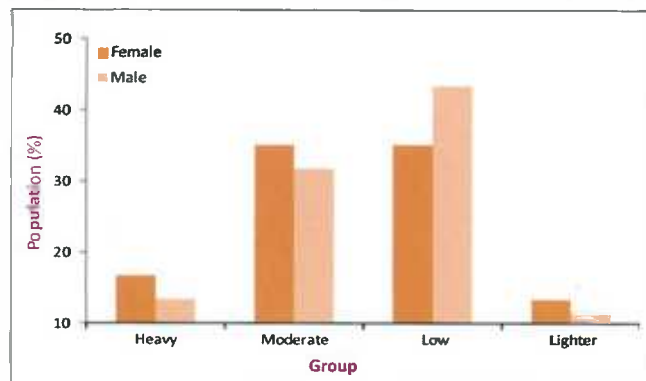


Figure 1: Percentage of female and male pupae in a population of M.Con.4.

A, B, C and D was recorded as 16.67, 35.00, 35.00 and 13.33 while among males, it was 13.33, 31.67, 43.33 and 11.67, respectively (Table 1). Average weight of female pupae was 1.643, 1.540, 1.429 and 1.283 g, and that of male ones was 1.260, 1.110, 1.021 and 0.923 g for 4 groups, respectively. It was also observed that females of each group were heavier than males of the respective group by 30.58, 38.84, 40.02 and 39.27 %, in 4 groups. Differential pupal weight both in female and male

Table 1: Status of pupal weight in the population of M.Con.4

Group	% of population		Average pupal weight: (g)		Weight increase in female (%)
	Female	Male	Female	Male	
A (Heavy)	16.67	13.33	1.643	1.260	30.58
B (Moderate)	35.00	31.67	1.540	1.110	38.84
C (Low)	35.00	43.33	1.429	1.021	40.02
D (Lighter)	13.33	11.67	1.283	0.923	39.27
Mean	—	—	1.474	1.078	37.18
CD at 5 %	—	—	0.081	0.085	—
CV %	—	—	2.91	4.19	—

corroborates with the findings of Enfielb *et al.* (1975) that “a class of genes exists associated with autosomes that differ in their dominance effect on pupal weight in the two sexes”.

The same group of females crossed with different group of males showed significant variation in fecundity. Results indicate that fecundity was significantly lower in the case of adults emerged from extreme heavy females when crossed with male moths of extreme heavy group *i.e.*, the females of group A and B when crossed with males of group A. This is in conformity with the findings of Narasimhanna (1988). The highest fecundity was realized when adults emerging from heavy females were crossed with medium weighed males indicating impact of physical fitness of male pupae. This observation is in agreement with the views of Singh *et al.* (1994), Calvo and Molina (2005) and Reddy *et al.* (2010) that males of medium to low pupal weight when crossed with females of medium to high pupal weight enhance the egg laying capability and hatching %. But no significant variation was observed in fecundity realized from crossing of females of group A with males of group B and C. This was true in other groups of females also. Females of group C and D showed significant variation while crossing with males of group A, B and C in comparison to crossing with males of group D. However, in all cases, significantly lowest fecundity was observed when females of any group were crossed with males of group D. This is in agreement with the view of Reddy *et al.* (2010) that the optimal reproductive success can be attained with availability of appropriate male counterpart. These observations establish the fact that fecundity of M.Con.4 breed of *Bombyx mori* L. depends upon pupal weight of both female and male. In the present study, group A female showed maximum fecundity of 674 (Table 2). This corroborates with the findings of Singh and Prasad (1987) and Barah and Sengupta (1991) that adult females having more weight have more fecundity. This view is also in agreement with the observation of Rahman *et al.* (1978), Gilbert (1984), Singh *et al.* (1994), Calvo and Molina (2005), Heisswolf *et al.* (2009), Reddy *et al.* (2009) and Reddy *et al.* (2010). Variation in fecundity

Table 2: Impact of pupal weight on fecundity and rearing performance in M.Con.4

Group of pupae	Cocoon wt.(g)			No. of cocoons / kg	Pupal weight (g)		Eggs laid (No.)	Reproductive index
	Female	Male	Av.		Female	Male		
A x A	1.947	1.504	1.726	580	1.707	1.246	599	2.85
A x B	1.901	1.404	1.652	605	1.555	1.125	674	2.46
A x C	1.944	1.278	1.611	621	1.645	1.038	651	2.5
A x D	1.953	1.171	1.562	640	1.663	0.941	505	3.29
B x A	1.857	1.494	1.676	597	1.571	1.230	556	2.83
B x B	1.821	1.393	1.607	623	1.570	1.121	613	2.56
B x C	1.824	1.286	1.555	643	1.549	1.059	603	2.57
B x D	1.814	1.138	1.476	678	1.538	0.957	481	3.20
C x A	1.713	1.445	1.579	633	1.472	1.182	614	2.40
C x B	1.713	1.365	1.539	650	1.445	1.126	596	2.42
C x C	1.699	1.280	1.490	671	1.426	1.055	576	2.48
C x D	1.706	1.177	1.442	694	1.437	0.978	509	2.82
D x A	1.478	1.419	1.448	691	1.270	1.175	549	2.31
D x B	1.452	1.349	1.400	716	1.221	1.127	442	2.76
D x C	1.518	1.275	1.396	717	1.251	1.059	412	3.03
D x D	1.483	1.152	1.318	759	1.228	0.964	375	3.27
CD at 5 %	0.084	0.051	0.055	26	0.078	0.037	31	-
CV %	2.90	2.30	2.16	2.39	3.19	2.07	3.36	-

within same group of females with different groups of males confirm the observation of Leopold (1976), Yamaoka and Hirao (1977), Setty and Ramaiah (1980), Brady (1983) and Raabe (1986) that oviposition has a strong correlation with male factors. Variation in same group of females crossed with different groups of males is also attributed to efficiency of utilization of male derived nutrients received by females during mating to increase their fecundity. This is in accordance with Wiklund *et al.* (1998). Present study reveals that the males emerged from medium to low weighed pupae are capable to fertilize more number of eggs which coincides with the opinion of Reddy *et al.* (2009) that this is due to capability of transferring ovulation stimulating substance (OSS) to the females during copulation by the males owing to better physical fitness.

Hatching percentage presented in Table 3 was significantly influenced by differential weight of male pupae within same group of females. Males of medium to low pupal weight contributed for more hatching % when crossed with high to medium group of females indicating the higher potency of male of specific group

in transferring sperms along with secretions of accessory glands. This is in accordance with Singh *et al.* (1994), Calvo and Molina (2005) and Reddy *et al.* (2010). But in group C and D of females, gradual decrease of fecundity and hatching % was observed with decrease in male pupal weight indicating imperative role of both female and male pupal weight in realized fecundity and hatching %. This view is in conformity with that of Gowda *et al.* (1988). The males of medium pupal weight were active enough to release male factors during copulation which showed a positive role in augmentation of fecundity and fertility status of the females of higher weight. Reddy *et al.* (2009) also recorded the same observation. It is well established that the better rearing conditions, environment and nutrition during larval period produce bigger females having potentiality of laying more eggs and the males in 1st mating transfer sufficient spermatid fluid resulting in higher realized fecundity and better hatchability (Reddy *et al.*, 2009).

Same trend was observed in other rearing parameters also. ERR by number and weight were found the highest in progenies when the females of group A

Table 3: Rearing performance of DFLs of different group of pupal weight

Group of pupae	Hatching %	Weight of 10 matured larvae (g)	ERR		Cocoon weight (g)	Shell weight (g)	Shell %
			By number	By weight (kg)			
A x A	92.36	30.450	7200	10.340	1.547	0.247	15.97
A x B	94.52	33.030	8233	12.500	1.596	0.249	15.60
A x C	92.90	32.230	7833	11.700	1.553	0.262	16.87
A x D	90.42	30.290	7467	10.825	1.515	0.241	15.91
B x A	93.37	26.720	7400	11.178	1.495	0.236	15.79
B x B	94.70	31.130	7744	11.733	1.511	0.256	16.94
B x C	91.69	29.800	7489	10.800	1.499	0.259	17.28
B x D	85.48	23.467	7178	9.760	1.465	0.227	15.49
C x A	89.12	34.200	8411	11.156	1.462	0.249	17.03
C x B	85.13	31.560	7811	10.011	1.442	0.238	16.50
C x C	82.51	30.750	7389	10.456	1.435	0.230	16.03
C x D	81.07	26.860	6156	7.444	1.400	0.218	15.57
D x A	93.42	32.250	8056	10.233	1.285	0.210	16.34
D x B	92.49	30.050	7589	9.000	1.248	0.202	16.19
D x C	82.85	28.500	7067	8.067	1.225	0.196	16.00
D x D	75.87	26.800	6089	6.056	1.195	0.183	15.31
CD at 5 %	1.62	0.223	97	0.104	0.030	0.013	0.34
CV %	1.10	0.45	0.79	0.64	1.33	3.43	1.25

ERR – Effective rate of rearing.

and B were crossed with males of group B. Shell % was recorded the highest in AxC and BxC combinations. Decrease in shell % with decrease in male pupal weight was observed in offsprings of females of groups C and D. Variations in rearing parameters as depicted in Table 3 is in accordance with the view of Wedell *et al.* (2002).

Number of cocoons per kg is one of the important parameters for procurement of seed cocoons in grainages. It was observed from Table 2 that 600 to 643 numbers of cocoon in 1 kg registered the highest number of fecundity *i.e.*, more than 600.

Correlation study (Table 4) reveals that weight of

Table 4: Simple correlation between pairs of characters

	Male pupal weight	Fecundity	Hatching %	Larval weight	ERR / No.	ERR / weight	Cocoon weight	Shell weight	Shell %
Female pupal weight	0.116	0.706**	0.516*	0.165	0.295	0.545*	0.950**	0.923**	0.734**
Male pupal weight		0.460	0.582*	0.465	0.534*	0.554*	0.221	0.283	0.373
Fecundity			0.634**	0.649**	0.687**	0.834**	0.843**	0.886**	0.855**
Hatching %				0.489	0.736**	0.783**	0.602*	0.695**	0.781**
Larval weight					0.759**	0.624**	0.289	0.437	0.663**
ERR / No.						0.865**	0.473	0.563*	0.682**
ERR / weight							0.723**	0.776**	0.791**
Cocoon weight								0.974**	0.792**
Shell weight									0.909**

* Significant at P<0.05, ** Significant at P<0.01.

female pupae has significant ($P < 0.01$) impact on realized fecundity, cocoon weight, shell weight and shell %. Male pupal weight had significant effect ($P < 0.05$) on important economic characters viz., hatching % and ERR by number and weight. Hatching % was significantly correlated with ERR by number and weight, cocoon weight, shell weight and shell % establishing the impact of male pupal weight on improvement of rearing performance of M.Con.4. The results of correlation study also show that quality of cocoons i.e., ERR by number is highly correlated ($P < 0.01$) with ERR by weight indicating higher yield of cocoons. This advocates that both the female and male pupal weight bear significant importance in realized fecundity and rearing performance of M.Con.4.

Present study infers that both female and male pupal weight bear significant impact on the realized fecundity, hatching % and other economically viable rearing parameters (Table 4). Hence, due importance should be given to the weight of both female and male pupae for selection of quality cocoons for production of disease free layings (DFLs). In the case of M.Con.4, number of cocoons in 1 kg in the range between 600 and 650 can be considered for seed production to get higher fecundity i.e., more than 600 with substantially high hatching %, that can contribute to a consistent quantum of brushed larvae which in turn attributes towards better return in terms of cocoon yield regardless of loss of larvae at initial stage of rearing.

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IMPACT DU POIDS DE LA CHRYSALIDE SUR LA FECONDITE ET LES PERFORMANCES D'ELEVAGE CHEZ LA RACE M.Con.4 DE *BOMBYX MORI* L.

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RESUME

Nous avons étudié, chez la lignée nouvellement élaborée M.Con.4 de *Bombyx mori*, l'impact des poids des chrysalides mâles et femelles sur la fécondité et les performances d'élevage. Les chrysalides mâles et les chrysalides femelles, réparties en 4 groupes selon leur poids, ont été croisées entre elles. Les résultats montrent que le poids des chrysalides mâles aussi bien que celui des femelles ont un impact significatif sur la fécondité, le pourcentage d'éclosion, le poids des larves mûres, le poids du cocon et le poids de la coque. Le nombre d'œufs pondus par chaque groupe de femelles est corrélé au poids de la chrysalide. On a enregistré une corrélation positive et significative au seuil de 1 %.



Research Paper

EFFECTIVENESS OF COMPOST PREPARED FROM SERI RESIDUE ENRICHED WITH BENEFICIAL AND ANTAGONISTIC MICROBES ON MANAGEMENT OF ROOT ROT DISEASE IN MULBERRY

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ABSTRACT

Studies were conducted to find out the effect of compost prepared from seri residue (SR: silkworm litter + rearing waste) enriched with rock phosphate (RP), beneficial microbes [Phosphate Solubilizing Bacteria (PSB), *Azotobacter* (Az)] and antagonistic microbes [*Trichoderma harzianum* (Th) and *T. pseudokoningii* (Tp)] for management of root rot disease caused by a group of fungi such as *Fusarium solani*, *F. oxysporum*, *Botryodiplodia theobromae* and *Macrophomina phaseolina* in mulberry. The trial was conducted in sick soil where mixed population of these pathogens was present @ 3.2×10^7 /g soil. The experiment was conducted following RBD with nine treatments. Results revealed the maximum disease control (74.3 %) recorded for treatment where SR was enriched with RP+Az+PSB+Th+Tp+ neem oil cake (NOC) followed by treatment, SR+RP+poultry manure (Pm)+Az+PSB+Th+Tp+NOC (72.6 %) which prevented 37.0 and 36.3 % leaf yield loss, respectively over control. There was no significant difference between these two treatments and interestingly, the results were on par with that of the recommended method [Indofil M-45 (Mancozeb 75 % WP) + Raksha (*T. harzianum*)] which could control the disease up to an extent of 77.6 %. Hence, compost prepared from seri residue enriched with beneficial/antagonistic microbes after mixing with neem oil cake in the ratio 2:1 @ 250 g/plant is recommended as an eco-friendly package for management of root rot disease as well as to improve the soil health.

Key words: Enriched compost, mulberry, root rot disease, seri-residue.

INTRODUCTION

Mulberry (*Morus alba* L.), the sole host plant of silkworm (*Bombyx mori* L.), is susceptible to different types of soilborne diseases viz., stem-canker (*Botryodiplodia theobromae*), cutting rot (*Fusarium solani*), root knot (*Meloidogyne incognita*) and root rot (*Fusarium solani*, *F. oxysporum*, *B. theobromae* and *Macrophomina phaseolina*). Among them, root rot is the most dangerous disease due to its epidemic nature, involvement of more than one pathogen (microbial complexity) and potentiality to kill the plants (Sharma *et al.*, 2003; Mala and Chowdary, 2005). The leaf yield loss due to root rot is estimated to be about 14 %. Further, the leaf quality which is a

prerequisite for production of quality cocoons is also deteriorated. Presently, an IDM package using soil application of Indofil M-45 (Mancozeb 75 % WP; @ 10 g/plant) and talc-based biofungicide named as Raksha (*Trichoderma harzianum*) has been recommended for the control of root rot disease (Sharma *et al.*, 2003). The continuous application of chemical fertilizers and use of fungicides for the control of diseases are resulting in environmental pollution and deterioration of soil health. During the course of mulberry cultivation and rearing of silkworms, enormous amount of farm and rearing residue is generated which is converted into seri compost using suitable technologies for maintaining the soil health (Bhogesha *et al.*, 2005 b). This compost after

enrichment with beneficial (phosphate solubilizing bacteria and *Azotobacter*) and antagonistic (*T. harzianum* and *T. pseudokoningii*) microbes is recommended for reducing the chemical fertilizer and other organic inputs like farm yard manure during mulberry cultivation (Bhogesha *et al.*, 2005 a). There are reports stating that compost enriched with PSB, *Azotobacter*, *T. harzianum*, *T. viride*, *Paecilomyces* spp., *Verticillium tenuipis*, etc. contribute greatly for management of various soilborne diseases in certain horticultural and agricultural crops besides improving the soil fertility (Hoitink and Kuter, 1984; Subbarao, 1999; Khan and Khan, 2001). In view of integrating the disease management technology with agronomical package for reducing labour & cost of cultivation and also to improve the soil health, the present study was undertaken in mulberry wherein the effect of compost prepared from seri residue enriched with rock phosphate, poultry manure, beneficial/antagonistic microbes *viz.*, phosphate solubilizing bacteria, *Azotobacter*, *T. harzianum* and *T. pseudokoningii* was assessed on management of root rot disease.

MATERIALS AND METHODS

The effect of compost prepared from seri residue (SR: silkworm litter + rearing waste) enriched with rock phosphate (RP), poultry manure (Pm), beneficial microbes [Phosphate Solubilizing Bacteria (PSB) & *Azotobacter* (Az)] and antagonistic microbes [*T. harzianum* (Th) and *T. pseudokoningii* (Tp)] was tested on management of root rot disease in mulberry. RP @ 40 kg/MT was added to SR before decomposition. The poultry manure was mixed with SR @ 1:1 ratio before composting. After 4 months, the carrier based product of *Azotobacter*, PSB and antagonists were mixed @ 2 kg/MT of SR (Bhogesha *et al.*, 2005 a, b). One month after, the different enriched composts were used for conducting the trial laid out in RBD with 9 treatments in a sick plot measuring 1944 m² having the mixed population of pathogens causing root rot disease *viz.*, *F. solani*, *F. oxysporum*, *B. theobromae* and *M. phaseolina* [3.2×10^7 colony forming unit (CFU)/g; Table 3], which was higher than that of economic

threshold level (ETL; $2.1 - 2.6 \times 10^6$ CFU/g soil; Naik, 2006). The mulberry plantation was 3 years old (V-1 variety) with Indo-Japanese spacing [(90 + 150) cm × 60 cm] maintained under irrigated conditions. The experimental plot was subdivided into 27 sub-plots of 72 m² each with 100 plants to accommodate nine treatments and 3 replications. Each sub plot was demarcated by bunds to minimize inter plot interference. Among the 9 treatments, 6 treatments belonged to enriched composts and remaining 3 treatments comprised of individual application of neem oil cake, control (soil with pathogen load only) and recommended method [Indofil M-45 (Mancozeb 75 % WP) + Raksha (*T. harzianum*)] (Table 1). The neem oil cake [obtained from seeds of neem (*Azadirachta indica* L.) after extraction of oil] was selected for the study because of its effectiveness in controlling the various other soilborne diseases in mulberry (Sharma *et al.*, 2003). Recommended method was selected as one of the treatments to know the effectiveness of enriched composts on management of root rot disease for selecting the best enriched compost treatment(s) to achieve the target on par with or more than the presently recommended method.

Method of application: Since mulberry is a perennial crop, the different treatments were imposed three times/year at an interval of 4 months followed by irrigation during intercultural operation. Enriched composts were applied individually (T-2, T-3 & T-4) and after mixing with neem oil cake (2:1) @ 250 g/plant (T-5, T-6 & T-7) around the root zone. For recommended method (T-8), 10 g Indofil M-45/plant (one time only) was applied to root zone and after 15-20 days, the bioformulation of Raksha (*T. harzianum*; 2.5×10^8 CFU/g) was applied after mixing with farm yard manure (FYM; 1: 50) @ 500g/plant in the root zone and control plot (T-0) was applied with only FYM @ 250 g/plant.

Observations: After imposition of different treatments, the leaf yield data were recorded on 70th day and 5 crops' data were recorded in a year. The prevention in leaf yield loss (%) over control was calculated. The severity of root rot disease was recorded based on the mortality of plants; percentage of wilting/yellowing of leaves and rotting of roots.

Table 1: Effect of microbially enriched compost prepared out of seri residue (SR) on management of root rot disease

Treatment	Disease severity					Leaf yield	
	Mortality of plants (%)	Wilting of leaves (%)	Decrease over control (%)	Rotting of root (%)	Decrease over control (%)	kg/ha/crop	Increase over control (%)
T-0 Control (pathogen load only)	66.8 ± 1.19	73.8 ± 0.41	-	70.0 ± 0.49	-	5801 ± 4.92	-
T-1 Neem oil cake (NOC)	31.3 ^a ± 0.36	27.3 ^a ± 0.30	63.0	27.0 ^a ± 0.25	61.4	7338 ^a ± 3.06	26.5
T-2 SR + RP + Th + Tp	34.6 ^b ± 0.72	28.5 ^a ± 0.25	61.3	27.7 ^a ± 0.30	60.4	6990 ± 4.67	20.5
T-3 SR+RP+Pm+Az+PSB+Th+Tp	32.9 ^b ± 0.06	27.9 ^a ± 0.35	62.2	26.7 ^a ± 0.59	61.9	7349 ^a ± 4.84	26.7
T-4 SR+RP+Az+PSB+Th+Tp	30.8 ^b ± 0.72	27.3 ^a ± 0.40	63.0	26.3 ^a ± 0.35	62.4	7541 ± 2.61	30.0
T-5 SR+RP+Th+Tp+NOC	33.6 ^b ± 0.32	22.7 ± 0.65	69.2	22.2 ± 0.21	68.3	7686 ^b ± 3.06	32.5
T-6 SR+RP+Pm+Az+PSB+Th+Tp+NOC	22.5 ^c ± 0.27	19.3 ^b ± 0.15	73.8	19.2 ^b ± 0.25	72.6	7906 ^a ± 3.05	36.3
T-7 SR+RP+Az+PSB+Th+Tp+NOC	21.8 ^c ± 0.47	18.3 ^b ± 0.35	75.2	18.0 ^b ± 0.25	74.3	7947 ^a ± 3.59	37.0
T-8 Recommended method	19.9 ^c ± 0.56	16.2 ^b ± 0.31	78.0	15.7 ^b ± 0.33	77.6	7999 ^a ± 3.76	37.9
SEM ±	0.89	1.12	-	1.20	-	33.01	-
CD (P ≤ 0.05)	2.68	3.37	-	3.61	-	99.35	-

Means in the column, followed by the same letter are not significantly different according to the Tukey's HSD test (P ≤ 0.05); The values preceded by ± are SE; RP = Rock Phosphate; Az = Azotobacter; PSB = Phosphate solubilizing bacteria; Th = *Trichoderma harzianum*; Tp = *T. pseudokoningii*; Pm = poultry manure; Recommended method = Indofil M-45 (Mancozeb 75 % WP) + Raksha (*T. harzianum*)

Table 2: Soil fertility status before and after application of different treatments

Soil parameter	Before treatment	Control plots	Treated plots (After imposing the III & final dose of different treatments)	Ideal range for mulberry cultivation (Source: Dandin <i>et al.</i> , 2007)
Physical / Biological				
Soil type	Red sandy loam	Red sandy loam	Red sandy loam	Black cotton and red sandy/ loamy soils
pH	7.2 - 7.8	7.2 - 7.8	6.9 - 7.5	6.5 - 7.5
Electric conductivity (ds/cm)	1.15	1.09	0.92	<1.0
Organic carbon (%)	0.28 - 5.9	0.30 - 6.0	0.76 - 9.9	0.65 - 1.00
Nitrogen (kg/ha)	190 - 260	192 - 264	280 - 560	250 - 500
Phosphorus (kg/ha)	10.9 - 15.5	11.0 - 16.0	15.2 - 26.4	15.0 - 25.0
Potash (kg/ha)	105 - 160	107 - 160	156 - 253	120 - 240
Microbial load (CFU/g soil × 10⁵)				
Beneficial microflora	0.49	0.62	171.0 - 290.0	10 - 100
Harmful microflora	490.0 (including root rot pathogens = 320 OR 3.2 × 10 ⁷)	460.0	0.24 - 0.71	0.30 - 0.80
Saprophytic microflora	0.39	0.16	81.0 - 148.0	0.6 - 0.8

For assessing the soil health before and after imposing (third and final dose) the treatments, the physical & biological properties of soil and microbial load in terms of CFU/g soil (Table 2) were estimated by following the

standard procedure (Waksman, 1927; Walkley and Black, 1934; Jackson, 1973). The data recorded on parameters viz., leaf yield, mortality of plants, wilting of leaves and rotting of roots were subjected to test of significance

(ANOVA) using INDOSTAT statistical software. In addition, post-hoc test viz., Tukey's Honestly Significantly Different (HSD) test was also done in order to calculate the significant differences among means belonging to different treatments.

RESULTS

Data (Table 1) recorded after imposing the third and final dose of different treatments indicated that among the treatments of enriched compost, the maximum

disease control of 74.3 % (based on rotting of root) was recorded in T-7 (SR enriched with RP+Az+PSB+Th+Tp+neem oil cake) followed by T-6 (SR+RP+ poultry manure+Az + PSB + Th+Tp+neem oil cake; 72.6 %). Similarly, the maximum reduction in wilting of leaves was also noticed in these two treatments to an extent of 75.2 and 73.8 % and prevention of leaf yield loss up to 37.0 and 36.3 %, respectively over the control (soil with pathogen load). The mortality of plants in these two treatments was 21.8 and 22.5 %, respectively. No

Table 3: Microbial profile in rhizosphere habitat of mulberry in different treatments

Microflora	Colony forming units (CFU)/g x 10 ⁵ (mean of 6 replications)									
	Before treatment	T-0	After imposing the III & final dose of different treatments							
			T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8
Beneficial										
<i>Trichoderma harzianum</i>	0.0	0.15	25.3	42.5	39.9	43.6	44.0	42.6	49.5	44.7
<i>T. pseudokoningii</i>	0.0	0.0	16.3	33.8	37.5	39.8	42.3	39.2	42.9	33.9
<i>T. viride</i>	0.10	0.15	19.4	32.9	30.8	40.5	38.9	34.8	39.7	28.7
<i>Bacillus subtilis</i>	0.18	0.12	50.5	48.8	43.9	43.6	40.3	55.8	59.7	49.6
<i>Azotobacter</i> spp.	0.0	0.0	10.2	26.4	30.3	33.7	36.8	31.7	34.9	29.9
<i>Pseudomonas fluorescens</i>	0.15	0.0	18.0	14.5	15.6	16.6	18.6	26.8	29.7	25.8
<i>Streptomyces</i> spp.	0.06	0.11	18.7	13.0	14.8	8.9	11.6	16.7	18.9	12.9
<i>Nocardia</i> spp.	0.0	0.09	12.6	7.8	11.8	7.0	8.7	12.8	14.7	10.7
Total	0.49	0.62	171.0	219.7	224.6	233.7	241.2	260.4	290.0	236.2
Harmful										
<i>Agrobacterium</i> spp.	84.8	38.9	0.06	0.08	0.06	0.04	0.03	0.02	0.01	0.03
<i>Botryodiplodia theobromae</i>	88.4	97.6	0.12	0.09	0.08	0.08	0.09	0.02	0.01	0.02
<i>Macrophomina phaseolina</i>	79.8	107.5	0.09	0.10	0.07	0.06	0.04	0.01	0.02	0.08
<i>Fusarium solani</i>	81.6	95.8	0.17	0.08	0.05	0.03	0.02	0.01	0.0	0.02
<i>F. oxysporum</i>	70.2	79.1	0.14	0.07	0.03	0.02	0.0	0.0	0.0	0.0
<i>Xanthomonas</i> spp.	85.2	41.1	0.13	0.14	0.06	0.03	0.01	0.01	0.009	0.09
Total	490.0	460.0	0.71	0.56	0.35	0.26	0.19	0.07	0.049	0.24
Saprophytic										
<i>Aspergillus niger</i>	0.12	0.07	12.3	24.8	22.5	26.8	23.9	29.7	31.9	28.3
<i>A. flavus</i>	0.15	0.03	10.9	20.5	23.6	25.2	27.8	20.6	22.4	19.4
<i>A. fumigatus</i>	0.08	0.0	15.8	17.8	18.8	19.0	21.0	23.8	19.7	18.6
<i>Penicillium</i> spp.	0.03	0.03	10.7	19.0	21.6	18.8	20.8	25.7	33.7	30.9
<i>P. oxalicum</i>	0.0	0.0	16.8	17.8	22.8	20.0	19.9	21.8	23.6	21.7
<i>Rhizopus</i> spp.	0.01	0.03	14.5	12.9	13.2	17.0	17.6	18.3	16.7	13.8
Total	0.39	0.16	81.0	112.8	122.5	126.8	131.0	139.9	148.0	132.7
Grand Total	490.9	460.8	252.7	333.1	347.5	360.8	372.4	400.4	438.0	369.1

T-0 = Control (pathogen load only); T-1= Neem oil cake (NOC); T-2 = SR + RP + Th +Tp;
 T-3 = SR+Pm+RP+Az+PSB+Th+Tp; T-4 = SR+RP+Az+PSB+Th+Tp; T-5 = SR+RP+Th+Tp+ NOC;
 T-6= SR+Pm+RP+Az+PSB+Th+Tp+NOC; T-7 = SR+RP+Az+PSB+Th+Tp+ NOC;
 T-8 = Recommended method [Indofil M-45 (Mancozeb 75 % WP) + Raksha (*T. harzianum*)].

significant difference was observed between the two treatments and values were on par with that of the presently recommended method [T-8; Indofil M-45 + Raksha (*T. harzianum*)] where mortality of plants and disease control was recorded up to 19.9 and 77.6 %, respectively with prevention of leaf yield loss to an extent of 37.9 % (Table I). However, enriched compost treatments without neem oil cake viz., T-4 (SR+RP+Az+PSB+Th+Tp), T-3 (SR+RP+ poultry manure+Az+PSB+Th+Tp) and T-2 (SR+RP+Th+Tp) as well as the individual application of neem oil cake (T-1) were found to possess significantly less impact than that of the presently recommended method in preventing the leaf yield loss (20.5-30.0 %) and reducing the disease severity of root rot (60.4 – 62.4 %).

The treatments of seri residue compost enriched with beneficial/antagonistic microbes, besides reducing the severity of root rot disease also improved the soil health by enhancing the physical and biological properties of soil viz., electric conductivity (ds/cm), organic carbon (%), nitrogen (kg/ha), phosphorus (kg/ha), potash (kg/ha) and microbial load (per gram of soil) especially by increasing the beneficial microflora and decreasing the harmful microflora (Tables 2 and 3). The beneficial forms such as *T. harzianum*, *T. pseudokoningii*, *Azotobacter* spp., *Bacillus subtilis* and *Pseudomonas fluorescens* were found more prevalent in plots treated either by enriched compost or enriched compost after mixing with neem oil cake while harmful forms viz., *F. solani*, *F. oxysporum*, *B. theobromae* and *M. phaseolina* were more prevalent in control plots. Fungi such as *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *Penicillium* spp. belonging to saprophytic group were also more prevalent in treated plots (Table 3).

DISCUSSION

In the present study, the maximum suppression of root rot disease recorded for the treatment of SR enriched with RP+Az+PSB+Th+Tp after mixing with neem oil cake may be attributed to the complementary effect of *Azotobacter*, PSB, *T. harzianum*, *T. pseudokoningii* and neem oil cake. The pathogens causing root rot disease viz., *F. solani*, *F. oxysporum*, *B. theobromae*

and *M. phaseolina* were suppressed thereby protecting the mulberry plants from the disease. Neem oil cake reduces the pathogen load in soil by production of toxic substances such as nimbidin, thionemone and phenolics (Alam, 1993) while the species of *Trichoderma* inhibit the growth of pathogens by hyper-parasitism, competition and antibiosis. During antibiosis, the *Trichoderma* is also known to produce hydrolytic enzymes and antifungal compounds viz., chitinases, trichodermin and gluconase, which dissolve the cell wall of the pathogenic fungi arresting their growth (Tronsmo, 1996). *Azotobacter* is able to produce growth hormones and vitamins viz., gibberellin, biotin, nicotinic acid, etc., inducing resistance to mulberry against certain pathogens (Sharma *et al.*, 1994; Subbarao, 1999). Phosphate solubilizing bacteria produce toxic metabolites such as bulbiformin, phenazin, organic acids, siderophores, etc., which help in decreasing the infection of soilborne pathogens (Dalla, 1986). The results of the present study are in conformity with the findings of Sharma *et al.* (2008) who reported that the enriched compost (PSB, *Azotobacter*, *T. harzianum* and *T. pseudokoningii*) after mixing with neem oil cake (2:1) at the rate of 2 kg/m² enhanced the sprouting and survival of cuttings in mulberry up to 45 % over the check by suppression of nursery diseases viz., stem canker (*B. theobromae*), cutting rot (*F. solani*), collar rot (*Phoma mororum*/*P. sorghina*) and die-back (*B. theobromae*) besides improving the soil health. Further, Hoitink and Kuter (1984) reported that compost prepared from tree barks enriched with *Trichoderma* spp. enhances the population levels of biocontrol agents and thus helps in suppressing the soilborne pathogens. Khan and Khan (2001) reported that application of PSB decreased the severity of tomato wilt caused by *F. oxysporum* f. sp. *lycopersici* by 23-49 %.

The beneficial microflora viz., *T. harzianum*, *T. pseudokoningii*, *P. fluorescens*, *B. subtilis* and *Azotobacter* spp. (Table 3) were observed more prevalent in rhizosphere of plots treated with compost prepared from seri residue enriched with beneficial/antagonistic microbes or after mixing with neem oil cake, which contributed to the growth of mulberry plant through microbial transformation of many complex plant

nutrients into simpler and available forms thereby enhancing the soil health. These beneficial/antagonistic microbes were also utilized as growth promoters to induce the systemic resistance in various agricultural and horticultural plants including mulberry (Pal and Jalali, 1998; Sharma and Gupta, 2005). Perusal of literature reveals that neem oil cake does not affect the survival and growth of beneficial microbes (Sharma *et al.*, 2009) but on the other hand, its application enhances the soil fertility (Khalis and Manoharachary, 1985; Goswami and Bhattacharya, 1989) due to its manurial nature containing nutrients *viz.*, nitrogen, phosphate and potash (Bhagawathi *et al.*, 2000; Gupta, 2003). Further, species of *Aspergillus* and *Penicillium* (saprophytic group) found more prevalent in treated plots help to enhance the soil health, as they are known for the degradation of many complex nutrients into simpler forms (Bolton *et al.*, 1993; Subbarao, 1999).

The present study brings out the advantages of compost prepared out of seri residue enriched with rock phosphate (40 kg/MT waste) + *Azotobacter* (2 kg/MT waste) + phosphate solubilizing bacteria (2 kg/MT waste) + species of *Trichoderma* (2 kg/MT waste) after mixing with neem oil cake (2:1) in mulberry cultivation and is recommended as a package @ 250 g/plant for the management of root rot disease. The recommended package is eco-friendly and effective in controlling the disease up to 74.3 % preventing the leaf yield loss to an extent of 37.0 % besides improving the soil health.

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EFFICACITE D'UN COMPOST PREPARE A PARTIR DE RESIDUS DE SERICICULTURE ENRICHI AVEC DES MICROBES BENEFIQUES ET ANTAGONISTES SUR LE CONTROLE DE LA MALADIE DE LA ROUILLE DE LA RACINE CHEZ LE MÛRIER

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RESUME

Nous avons mené des études afin de déterminer l'effet d'un compost préparé à partir de résidus de sériciculture (SR: litières de vers à soie + déchets d'élevage) enrichi de phosphate de roche (RP), de microbes bénéfiques [Bactéries solubilisant le phosphate (PSB), *Azotobacter* (Az)] et de microbes antagonistes [*Trichoderma harzianum* (Th) et *T pseudokoningii* (Tp)] pour gérer la maladie de la pourriture de la racine chez le mûrier provoquée par un groupe de champignons tels que *Fusarium solani*, *F. oxysporum*, *Botryodiplodia theobromae* et *Macrophomina phaseolina*. L'essai a été conduit sur un sol malade où un mélange de populations de ces pathogènes étaient présent à raison de 3.2×10^7 /g de sol. L'expérience a été conduite en suivant la méthode de distribution en blocs aléatoires (RBD) avec neuf traitements. Les résultats montrent que la réduction maximale de la maladie (74.3 %) est obtenue avec le traitement SR enrichi de RP+Az+PSB+Th+Tp+tourteau d'huile de rutabaga (NOC) puis le traitement SR+RP+fumier de poules(Pm)+Az+PSB+Th+Tp+NOC (76.6 %) qui évitent une perte de feuilles de 37.0 et de 36.3 % respectivement comparés au témoin. Il n'y a pas de différence significative entre les deux traitements et les résultats sont en accord avec la méthode recommandée [Indofil M-45 (Mancozeb 75 % poids par volume) + Raksha (*T. harzianum*)] qui peut contrôler la maladie jusqu'à un niveau de 77.6 %. Le compost préparé à partir de résidus de sériciculture enrichis par des microbes bénéfiques et antagonistes, mélangé avec du tourteau dans un rapport 2 :1, c'est-à-dire 250g/plant, est donc un paquet écologique recommandé pour gérer la maladie de la pourriture de la racine et pour améliorer la santé du sol.



COMPARATIVE PERFORMANCE OF MULBERRY AND TASAR SILK WASTE PROCESSING IN MILL SPINNING

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ABSTRACT

The new Italian technology of worsted system of spinning was adopted to produce international quality spun silk yarn from tasar silk waste. Degumming loss, yarn realization and economics were worked out and the yarns were characterized according to international standard testing methods. The results were compared with that of mulberry spun silk yarn. From the study, it is revealed that international quality tasar spun silk yarn can be produced through cheaper means from tasar silk waste thereby generating additional revenue to reelers and more employment opportunity as well.

Key words: Degumming, evenness, imperfections, spun silk, tasar waste, worsted spinning, yarn yield.

INTRODUCTION

Tasar culture is the main stay for many a tribal community in India, being specific to the states of Jharkhand, Chhattisgarh and Orissa, besides Maharashtra, West Bengal and Andhra Pradesh. The copperish coloured, coarse tasar (tussah) silk is used for making dress material, furnishings and interiors. It is less lustrous than mulberry silk, but has its own feel and appeal. It is obtained from semi-domesticated multivoltine silkworm, *Antheraea mylitta* which feed on the aromatic leaves of Assan and Arjun trees and build cocoons on the tree itself. The tasar raw silk (50 to 60 denier) is produced from tasar cocoons by the process of reeling. Tasar cocoon weight ranges from 8 to 15 g, shell weight from 1.5 to 3.5 g, filament length from 650 to 1450 m, raw silk recovery percentage from 50 to 55 % and 800 to 1000 cocoons are required to produce one kg raw silk of denier 50-60 (Kariyappa *et al.*, 2006 a).

Substantial amount of tasar silk waste is generated during the following stages of cocoon processing and production of fine quality raw silk:

- * Cocoon waste - Pierced, pin holed, rat cut, flimsy, double, urinated, stained and soiled cocoons.

- * Reeling waste - Deflossing, cooking, reeling, pelade and re-reeling waste.

After reeling of raw silk from the cocoons, the remaining entangled mass of filament referred to as reeling waste accounts for about 50 %. This silk waste was not having any commercial value until recently due to non-availability proper spinning technology. But the commercial value for tasar silk waste has improved of late, due to development of suitable spinning technology for processing it. It can be used to produce the hand spun as well as mill spun yarn. So far, tasar waste was being converted into spun silk through takali (a crude device) and CSTRI spinning machine (improved hand spinning device). Since these devices consume only small quantity of cocoons, the bulk reeling waste was rejected without proper utilization. Moreover, the spun yarn produced in hand spinning has limited scope of utilization, to produce only coarser material like cheddars, shawls and stoles and cannot be woven on power loom as well as shuttle less loom and knitting machines.

Hence, the present study was taken up to explore the possibility of utilizing tasar waste through mill spinning and thereby to generate additional income to the reelers.

The yarn characteristics were compared with mill spun mulberry silk yarn.

MATERIALS AND METHODS

100 kg each of tasar and mulberry waste were procured from Nagpur, DOS, Maharashtra and CSTRI, Bangalore, respectively.

Degumming for mill spinning

After several trials of tasar silk waste's degumming at CSTRI, following standardization degumming recipe, the mass degumming was done at M/s Afsal Khan degumming unit, Ramanagaram by using the following recipe (Kariyappa *et al.*, 2006 b).

Step 1: Soap and soda treatment

The silk waste was boiled with soap (10 grams / litre) and soda (5 grams / litre) solution for 1 h and then for half an hour with 5 grams / litre soap and 2.5 grams / litre soda solution followed by treatment with 13 cc or ml / litre H_2O_2 , 2 grams / litre sodium silicate and 2 grams / litre soda for 40 minutes at 80 °C.

Step 2: Carbonizing treatment

In this step, the material was treated with Sulfuric acid (0.5 %) in 1:30 material to liquor ratio in cold solution for 10 minutes and thoroughly washed in order to dissolve the cellulose and chrysalis material.

Step 3: Scrooping treatment

As the next step, the material was treated with Acetic acid (40 % concentrated), 0.5 % on weight of the material in 1:30 material to liquor ratio in luke - warm solution for 20 minutes and thoroughly washed to give scrooping effect to the silk.

Step 4: Drying and ammonia gas treatment

Finally it was hydro extracted and dried in steamed hot air oven for 6 h followed by drying under natural shade. Ammonia gas treatment was given for 10 minutes and kept it for 12 h in the same chamber to neutralize the acid, followed by spraying antistatic agent and conditioned for over night.

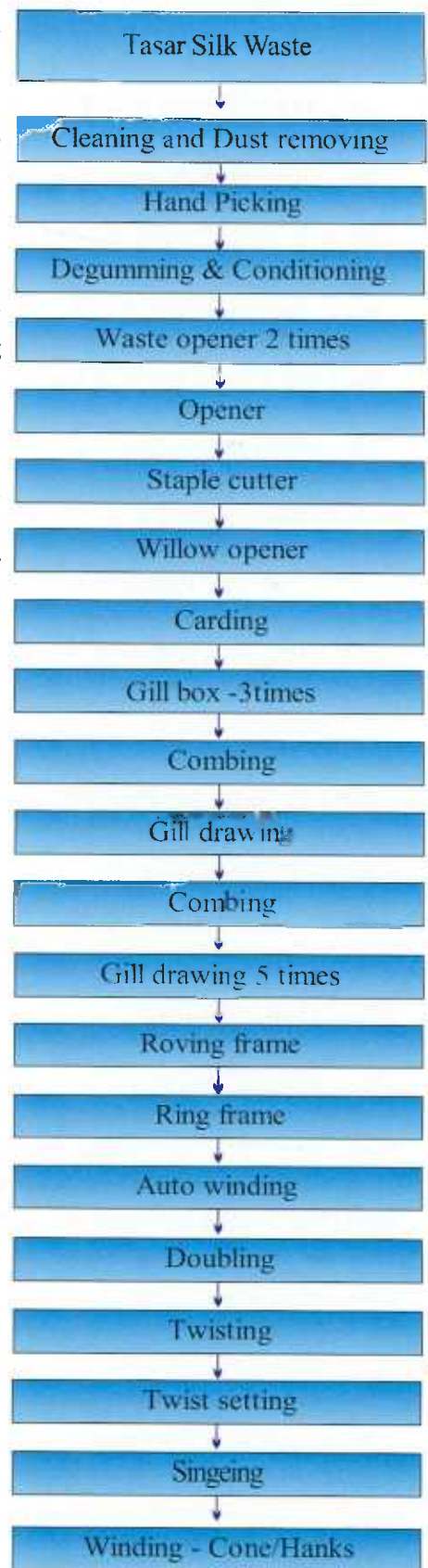
Spinning

Spinning was done on worsted system (Italian technology) at M/s Kareem silk as indicated in the flow chart (Kariyappa *et al.*, 2006 c).

Testing of yarn quality

The following instruments were used for testing fibre and yarn quality parameters:

1. Hauteur value and fibre length distribution were measured by using Texlab measuring instrument. This instrument has two devices, the



Flow Chart depicting the procedure of tasar silk waste spinning

first one, Uster FL100 collects the fiber from sliver, straightens and arranges the fibre according to its length by bear sorter principle. The collected fibres are analysed by another device, Uster AL 100. This instrument automatically scans the fibre and gives a diagram and value in printed form. Five replications were taken for each sample, each sample weighing about 15 g.

2. Yarn unevenness and imperfection were measured by using Uster 3 model, 5 replications were taken for each sample, keeping yarn speed at 400 m/min, recording time, 1 min and tension, 37.5 %.
3. Tensile and elongation properties of tasar spun silk yarn were measured by using Instron tensile tester model No. 5000R following IS 1670-1991 testing method - 500 mm Gauge length and 20 sec jaw traversing time. In this, 15 yarn samples were taken for each variety of spun silk yarn.

Statistical analysis

Data collected on yarn quality parameters were analyzed through one-way ANOVA using SPSS 11.5 and Windostat package.

RESULTS AND DISCUSSION

Hauteur analysis

The Hauteur distribution curve gives the detailed information about the fibre length distribution by number and other important fibre parameters. For good quality

spun yarn and good spinning performance, good fibre length distribution is very important.

Figure 1 (A and B) shows fibre length distribution histogram of tasar and mulberry silk fibre. This analysis has been made for measurement of fibre length distribution and short fibre percentage, which decides the spin ability and quality of spun silk yarn.

1. Arithmetic average of fibre length (H_{mm})

It is the Arithmetic average of fibre length. Spun silk yarn irregularity decreases with increase of fibre length H_{mm} up to 80 mm and increases afterwards due to breakage of fibre during spinning.

The average value of H_{mm} is given in Figure 2. Arithmetic average of fibre length (H_{mm}) of tasar silk is higher than mulberry silk by 19.39 %, this is due to the fact that during tasar silk reeling process, maximum good fibre goes as a waste and so it contributes to the higher fibre length.

2. Co-efficient of variation (C_{vh})

It is a measure of fibre length distribution. From the average values of C_{vh} given in Figure 2, it is observed that Co-efficient of variation (C_{vh}) of tasar silk fibre is higher than mulberry silk by 15.86 %. Since reeling of tasar silk is not as easy as mulberry silk reeling, due to hardness of tasar cocoons, tasar silk filaments are prone to more fibre breakages resulting in higher fibre length variation.

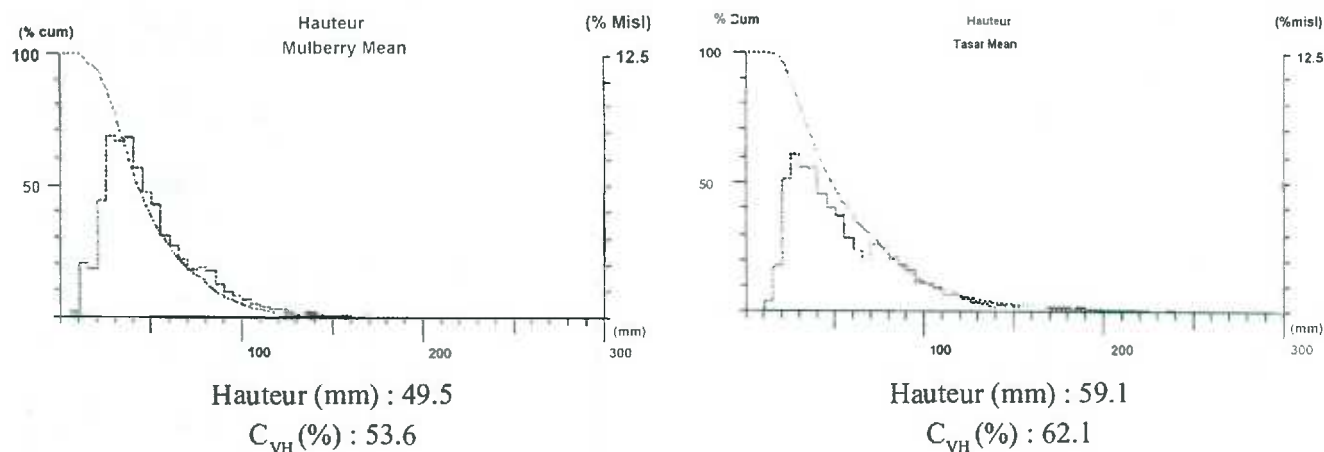


Figure 1: Histogram of mulberry and tasar fibre depicting fibre length distribution

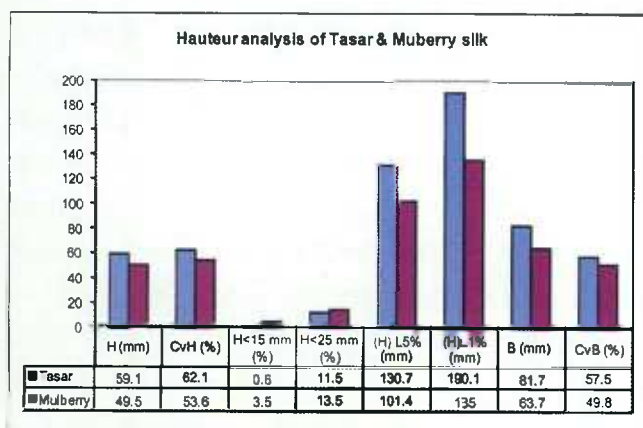


Figure 2: Hauteur analysis of tasar and mulberry silk

3. Short fibre % <15 mm

It indicates the percentage of fibre less than 15 mm fibre length. It is one of the parameters that influence the neps, thick and thin places in the spun silk yarn. The average value of short fibre % < 15 mm length of tasar silk is significantly less than mulberry silk by 82.85 % (Figure 2). This is because due to difficulty of tasar silk reeling process, maximum good fibre goes as waste, resulting in longer fibre.

4. Short fibre % <25 mm

It is a measure of percentage fibre less than 25 mm fibre length. Short fibre % < 25 mm of tasar silk (Figure 2) is significantly less than mulberry silk by 14.81 %. This is again due to the same reason that maximum good fibre goes as a waste during tasar silk reeling resulting in longer fibre.

5. Longer fibre L5 %

It is the length at which 5 % of the fibre exceeds the fibre length. It is a very important parameter to decide front and back roller setting. 5 % of fibre exceeds fibre length 130.7 mm in tasar while in mulberry, it is 101.4 mm. The average value of longer fibre L5 % of tasar silk is seen to be significantly higher than mulberry silk by 28.89 % (Figure 2).

6. Longest fibre L1 %

It is the length at which 1 % of the fibre exceeds the fibre length. 1 % of fibre exceeds fibre length 190.1 mm in tasar while in mulberry, it is 135 mm. Here

also, the average value of longest fibre L1 % (Figure 2) of tasar silk is significantly higher than mulberry silk by 40.81 %.

7. Weighted average of fibre length (B_{mm} %)

It is the average value of fibre length balanced by the weight. The average value of B_{mm} % of tasar silk is significantly higher than mulberry silk by 28.26 %.

8. Co-efficient of variation of weighed average of fibre length (CvB %)

It is the measure of fibre length dispersion by weight. It is observed that CvB% of tasar silk is significantly higher than mulberry silk by 13.39 % (Figure 2).

Tasar spun yarn realisation

1. Comparison of spun silk yarn yield (%) between mulberry and tasar silk waste spinning

From Table 1, it is observed that degumming loss in mulberry silk (35 %) is higher than that in tasar silk (19.80 %). Invisible loss is seen less in tasar silk (13.4 %) than in mulberry silk (17 %). Spun silk and noil yarn realisation were higher in tasar silk (41.9 and 24.9 %, respectively) than in mulberry silk (24 % each). On the other hand, mulberry spun silk yarn processing is characterized by higher degumming and invisible loss.

2. Economics of mulberry versus tasar spun silk yarn

A comparative account of the economics of mulberry and tasar spun silk yarn is presented in Table 2. While processing of any silk waste, two types of yarn are produced *viz.*, spun silk yarn and noil yarn (by - product of spun silk yarn). Twenty-four kg each of mulberry spun silk yarn and noil yarn were produced after processing of 100 kg mulberry waste. When the by - product *i.e.*, noil yarn revenue is accounted, the cost of conversion for one kg mulberry spun silk yarn amounts to ₹ 1009 generating a net profit of ₹ 491 per kg as against the selling price of ₹ 1500 / kg. In the case of tasar, 41.9 kg of spun silk yarn and 24.9 kg of noil yarn were produced after processing 100 kg tasar waste.

Table 1: Comparative statement of yarn realization in spinning

Particulars	Mulberry (%)	Tasar (%)
Degumming loss	35	19.8
Yarn yield	24	41.9
Noil yarn yield	24	24.9
Invisible /unusable loss	17	13.4
Total	100	100

The net profit after including the by - product revenue of noil yarn worked out to ₹ 767 per kg. Thus the revenue generated in the case of tasar spun silk yarn stands significantly higher than that of mulberry spun silk yarn.

Yarn parameters

The tasar and mulberry spun silk yarn were tested for the following evenness and imperfection parameters:

Table 2: Comparative statements of economics of spun silk yarn

Sl. No.	Particulars	Mulberry	Tasar
1	Cost of cocoons/waste @ ₹ 180 per kg for 100 kg each	18000	18000
2	Degumming charge @ ₹ 55 per kg for 100 kg each	5500	5500
3	Spinning charge @ ₹ 380 per kg for 24 kg mulberry and 41.9 kg tasar spun silk yarn	9120	15960
	A. Total (₹)	32620	39460
4	Realization from Noil yarn selling @ ₹ 350 per kg for 24 kg mulberry and 24.9 kg tasar noil yarn	8400	8750
	B. Total (A-4) (₹)	24220	30710
5	Production Cost (₹) per kg yarn (including Noil yarn realization) (Mulberry=24220/24=1009, Tasar =30710/41.9=733)	1009	733
6	Production Cost (₹) per kg yarn (excluding Noil yarn realization) (Mulberry=32620/24=1359, Tasar =39460/41.9=940)	1359	940
7	Net profit per kg spun silk yarn (selling price of spun silk yarn ₹ 1500 per kg) (₹ 1500-6)	141	560
8	Net profit per kg spun silk yarn (selling price of spun silk yarn ₹ 1500 per kg) (₹ 1500-5)	491	767

1. Uster evenness percentage ($U_m\%$)

It is the size of the yarn cross-section variation or a measure of percentage deviation of mass per unit length from the mean mass per unit length. Unevenness increases with increase of $U_m\%$. The Average values and CD at 5 % of $U_m\%$, coefficient of variation of mass with a cut length of approximately 1 cm ($C_{vm}\%$), coefficient of variation of mass with a cut length of 1 meter, [$C_{vm}(1m)\%$] are presented in Table 3, where it is observed that mulberry spun silk yarn in all these counts stands significantly lower than tasar spun silk yarn, and these values increase with increase in yarn count. The data indicate significant differences among all six varieties of spun silk yarns. This is due to the fact that the fibre diameter, fibre diameter variation and length distribution variation of tasar silk is higher than mulberry silk which influences $U_m\%$. The U_m , C_{vm} , $C_{vm}(1m)$ reduces with increasing number of fibre in the yarn cross section. Mulberry fibre is finer than tasar fibre and so will accommodate more number of fibers in the yarn compared to tasar spun silk yarn. As a result, U_m , C_{vm} and $C_{vm}(1m)$ are significantly less than that of tasar spun silk yarn.

2. Thin places

The Average values and CD at 5 % of thin places are given in Table 3. It is observed that thin places of mulberry spun silk yarn are significantly less than tasar spun silk yarn, and it increases with increase of yarn count in the case of tasar spun silk yarn. A significant difference in thin places could be observed between 2/60^SNm and 2/80^SNm tasar spun silk yarn.

3. Thick places

Thick places of mulberry spun silk yarn in all the counts are observed significantly less than tasar spun silk yarn (Table 3). These values increase with increase of yarn count in the case of tasar but reduces in the case of mulberry. This is due to the fact that tasar fibre diameter, diameter variation and fibre length distribution variation are significantly higher than that of mulberry

silk which results in thick places of mulberry spun silk being significantly less than tasar spun silk yarn.

4. Neps

Neps counts of mulberry spun silk yarn are significantly less than that of tasar spun silk yarn in all the counts (Table 3). In both the types of silk, neps decreases up to 2/80^s count and again increases with increases of yarn count. In both the spun silk yarns, Neps count has significant differences between the different counts of yarns.

5. Hairiness

This is the measure of length of the fibre protruding from the yarn. It is observed that hairiness of tasar spun silk yarn is significantly higher than that of mulberry spun silk yarn (Table 3). This is because fibre length distribution variation, fibre diameter variation and fibre fineness in tasar silk are higher than that of mulberry silk fibre, which influences the hairiness of the spun silk yarn.

Hairiness increases with increase of yarn count in the case of tasar but decreases with increase of yarn count in the case of mulberry. Data reveal that in both the types of spun silk yarn, hairiness has significant differences between different counts of yarn.

6. Standard deviation of hairiness Sh (-)

Standard deviation of hairiness of tasar spun silk yarn is higher than mulberry spun silk yarn. Sh(-) value increases with increase of spun silk yarn count in both mulberry and tasar spun silk yarn.

7. Index of irregularity (I)

The results indicate that Index of irregularity of mulberry spun silk yarn is significantly less than that of tasar spun silk yarn in all the counts, in both spun silk yarn (mulberry and tasar). Index of irregularity decreases with increase of yarn count but is not significant. This is due to the fact that the tasar fibre diameter, diameter variation and fibre length distribution variation are significantly higher than mulberry silk contributing to significantly higher index of irregularity.

Table 3: Evenness, imperfection and mechanical properties of tasar and mulberry spun silk yarn.

Property	Yarn Count						Standard	CD at
	2/60 ^s		2/80 ^s		2/120 ^s	2/140 ^s	Error	5 %
Evenness	Mulberry	Tasar	Mulberry	Tasar	Mulberry	Mulberry		
Pe ₂ / 100 km								
Um %	7.40	12.02	8.62	13.45	9.65	10.71	0.1667	0.4894
Cv m %	9.57	15.35	11.05	17.80	12.32	13.83	0.2527	0.7376
Cvm (1m) %	5.74	7.20	7.45	7.55	6.67	7.50	0.4584	1.3380 ^{ns}
Imperfection								
Thin Places (-50%)	0.00	30.00	0.00	74.00	0.00	2.00	1.5011	4.3814
Thick Places (+50%)	20.00	62.00	11.00	72.00	32.00	30.00	1.8184	5.3075
Thick Places (+100%)	3.00	12.00	2.00	8.00	1.00	4.00	0.7047	2.0570
Neps (+ 140 %)	35.00	156.00	25.00	138.00	84.00	190.00	4.6626	13.6091
Neps (+ 200 %)	18.00	91.00	12.00	59.00	36.00	55.00	3.1225	9.1138
Neps (+ 280 %)	12.00	56.00	9.00	32.00	22.00	26.00	2.0000	5.8375
Neps (+ 400 %)	7.00	38.00	20.00	6.00	4.00	10.00	2.0000	4.9933
Index (I)	1.40	2.25	1.40	2.16	1.27	1.32	0.0463	0.1352
Hairiness (-)	3.27	4.06	3.32	4.06	2.62	2.88	0.0688	0.2010
Sh (-)	0.83	1.40	0.92	1.74	0.77	0.83	0.0298	0.0870
Mechanical								
Breaking Force (gf)	1026.70	575.40	666.20	361.20	447.10	370.30	13.1204	36.7377
Elongation (%)	8.39	11.61	7.73	10.56	6.92	6.76	0.2904	0.8131
Tenacity (gf / den)	3.42	1.92	2.96	1.61	2.98	2.88	0.0580	0.1625
B-Work (gf.cm)	2697.20	2306.80	1625.80	1349.50	990.20	793.80	83.7425	234.4828
Young's modulus	58.00	23.80	71.50	43.96	80.80	69.96	0.9991	2.7819

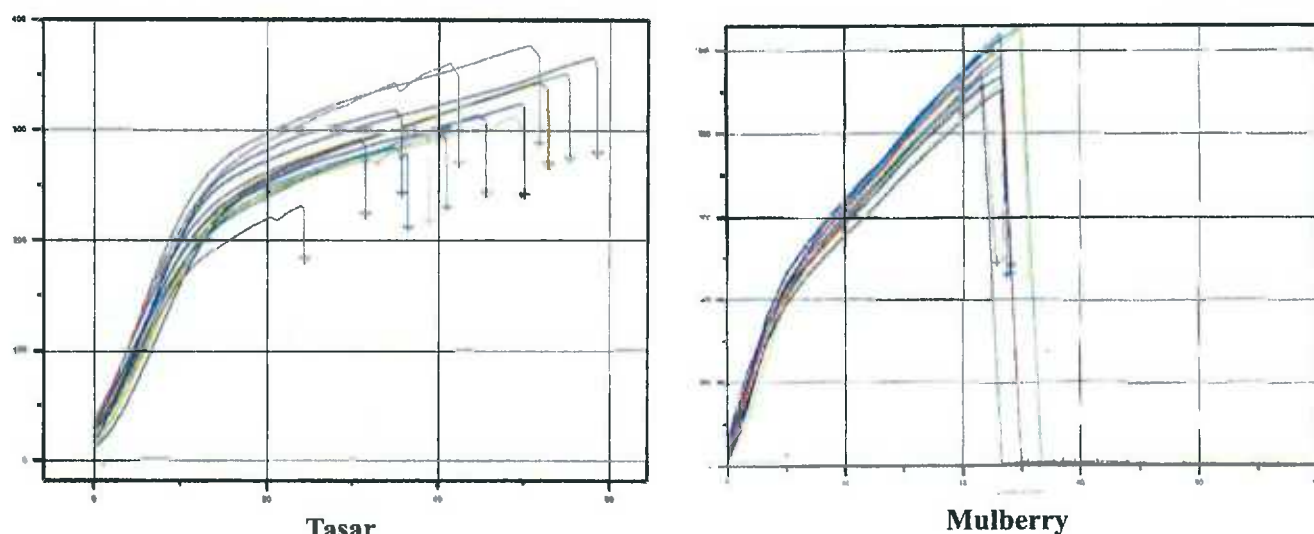


Figure 3: Load elongation curve of tasar and mulberry silk

Mechanical properties

Mechanical properties of tasar and mulberry spun silk yarn were tested on Instron machines, which gives the following important parameters to decide end uses of the yarn and fabric and also load elongation curve as shown in Figure 3. The load - elongation curve shows behavior of material during loading and shape of curve indicate molecular structure arrangement in the material. Load elongation curve of mulberry is catastrophic in nature and of non-catastrophic nature in the case of tasar.

1. Breaking force (gf)

The data on breaking force are shown in Table 3. Since the mulberry fibers are stronger than tasar silk, the breaking force (gf) of mulberry spun silk yarn is observed significantly higher than that of tasar spun silk yarn in all the counts.

The breaking force decreases with increase of yarn count in both the spun silk yarns. The results indicate that in both the spun silk yarns, there is a significant difference between different count yarns, but there is no significant difference between 2/80^s Nm tasar and 2/140^s Nm mulberry spun silk yarns.

2. Tenacity (g/d)

The data on tenacity of mulberry and tasar spun

silk yarn are presented in Table 3. The results show that tenacity (g/d) of mulberry spun silk yarn is significantly higher than tasar spun silk yarn in all the counts as mulberry fibre is stronger than tasar silk. The tenacity (g/d) decreases with increase of yarn count in both the spun silk yarns because of number of fibres in the yarn cross-section getting reduced with increase of yarn count. The data indicate significant differences between 2/60^s and 2/80^s spun silk yarns of both the categories, but mulberry spun silk yarn has no significant differences among 2/80^s, 2/120^s and 2/140^s counts.

3. Elongation %

The elongation percentage of mulberry spun silk yarn is observed significantly less than tasar spun silk yarn in all the counts (Table 3). The elongation percentage decreases with increase of yarn count. The present results indicate that there is no significant difference between 2/60^s and 2/80^s, 2/120^s and 2/140^s spun silk yarns of both mulberry and tasar. The silk fibre elongation percentage influences the spun silk yarn elongation percentage. Since the elongation percentage of tasar fibre is significantly higher than mulberry silk fibre (Kariyappa *et al.*, 2008), tasar spun silk yarn is characterised with higher elongation percentage.

4. B-work (gf-cm)

Our results (Table 3) show that B-work (gf-cm) of mulberry spun silk yarn is significantly higher than tasar spun silk yarn in all yarn counts. The B-work (gf-cm) decreases with increase of yarn count in both the spun silk yarns. Data indicate that there is a significant difference between different counts of yarns, but there are no significant differences between 2/120^s and 2/140^s counts of mulberry spun silk yarns. As mulberry fibre is stronger than tasar, B-work of mulberry spun silk yarn is on the higher side.

5. Young's modulus

The data presented in Table 3 shows that Young's modulus of mulberry spun silk yarn is significantly higher than tasar spun silk yarn in all the counts. The Young's modulus in both spun silk yarns increases with increase of yarn count. This is due to fact that Young's modulus is influenced by the elongation percentage, higher the elongation percentage, less the Young's modulus and *vice versa*.

CONCLUSION

From the study, it is observed that tasar fibers are coarser, has higher elongation percentage and variation in fibre diameter than mulberry silk fibre. Tasar waste can be easily degummed, spun into quality tasar spun silk yarn on worsted system of spinning like mulberry waste and can be produced up to 2/80^s Nm spun silk yarn, while in the case of mulberry, up to 2/140^s Nm spun silk yarn can be produced. From the Hauteur analysis, it is observed that tasar silk has good fibre length distribution than mulberry, which facilitates better spinning performance to produce quality spun silk yarn. Realization of spun silk yarn and noil yarn is higher from tasar silk waste than mulberry silk waste. Degumming and invisible loss percentage is less in tasar than in mulberry. The cost of production of tasar spun silk yarn is lower than that of mulberry spun silk yarn thereby making it cheaper than mulberry spun silk yarn. Yarn quality parameters *viz.*, Um%, imperfections such as thin places, thick places, Neps per kilometer, hairiness

and index of irregularity are more in tasar spun silk yarn compared to mulberry spun silk yarn. These parameters increase with increase of yarn count in both varieties of silk. Yarn properties such as breaking force, tenacity, B-work and Young's modulus are inferior in tasar spun silk yarn than mulberry spun silk yarn. These parameters decrease with increase of yarn count in both varieties of silk. Elongation percentage of tasar spun silk yarn is higher than mulberry spun silk yarn. As a result, fabric produced from tasar spun silk yarn has better crease resistance than mulberry fabric. Tasar spun silk produced on mill spinning can be woven on handloom, power loom and shuttle less loom. Hosiery products can also be produced on both hand and power operated flat and circular knitting machines. Tasar mill spun silk yarn can be used for manufacturing of lighter to heavy fabrics like innerwear, dress material, fashionable fabrics, ornamental fabrics, wall hangings, furnishings, cheddars and carpets. By adopting the new technology of spinning, tasar silk waste can be utilized effectively to generate additional revenue to tasar reelers and spinners besides creating more employment both directly and indirectly.

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PERFORMANCES COMPAREES DU TRATEMENT DES DECHETS DE SOIE MÛRIER ET TASAR EN FILATURE

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RESUME

Nous avons adopté le système développé de la nouvelle technologie italienne de filage pour produire du fil de soie filé à partir de déchets de soie tasar qui soit de qualité internationale. La perte au décreusage, la réalisation du fil et les paramètres économiques ont été étudiés et les fils obtenus ont été caractérisés par les méthodes de tests standards internationaux. Les résultats ont été comparés à ceux obtenus avec les filés de soie mûrier. Il ressort de cette étude que du fil de soie tasar filé de qualité internationale peut être obtenu avec des moyens moins coûteux à partir de déchets de soie tasar ce qui permet des revenus supplémentaires pour les filateurs et des possibilités accrues d'emplois.



Research Paper

IDENTIFICATION OF NEW DOUBLE HYBRID OF THE MULBERRY SILKWORM, TOLERANT TO NUCLEAR POLYHEDROSIS VIRUS (BmNPV)

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ABSTRACT

Double hybrids are known to have clear advantages of economic traits and yield on par or better than single hybrids. The rearing of double hybrids tolerant to diseases is much easier and polygenic expression of disease resistance in double hybrids is more stable than that in single hybrid in unfavorable environments because of their flexible genetic constitution within the population. Ten selected bivoltine parental breeds (CSR2, CSR3, CSR4, CSR6, CSR16, CSR17, CSR18, CSR50, CSR51 and 61N) were screened against Bm Nuclear polyhedrosis virus disease under inoculation and low temperature induction conditions. The breeds which are highly tolerant (CSR18 and 61N) were utilized for the preparation of 8 foundation crosses with other productive breeds *viz.*, (CSR18 x CSR2), (CSR18 x CSR3), (CSR18 x CSR17), (CSR18 x CSR50), (61N x CSR4), (61N x CSR6), (61N x CSR16) and (61N x CSR51). Six double hybrids prepared out of these were evaluated for ten metric traits such as fecundity, pupation rate, yield/10000 larvae, cocoon weight, cocoon shell weight, cocoon shell percentage, filament length, reelability, raw silk percentage and neatness. Based on the results under BmNPV inoculation and induction condition, the new double hybrid (CSR18 x CSR3) x (61N x CSR16) which recorded maximum average evaluation index value of 63.76 was selected for commercial exploitation being superior to the ruling double hybrid (CSR2 x CSR27) x (CSR6 x CSR26).

Key words: BmNPV, double hybrid, foundation crosses, low temperature, silkworm.

INTRODUCTION

Realisation of heterosis in silkworm promised a new era in sericulture which has become a reality through exploitation of heterotic effect expressed in F1 hybrids for economic traits. Many a study revealed the superiority of single, three way and double crosses over their parents (Pannegpet and Jaroonchai, 1975; Gamo, 1976; Datta, 1984). Due to the obvious advantages of double hybrids *i.e.*, easy rearing of foundation crosses, superiority to parental breeds in growth & vigor, 15 to 20 % improvement in egg productivity (Hirobe, 1985) and other economic characters, they could be popularized well. If the foundation crosses with high degree of heterosis are used for double hybrid preparation, the hybrid vigor may decline. Hence, to have more vigour in double hybrids, the foundation crosses which show low

heterosis should be used as parents. The susceptibility to disease is influenced by external factors such as agro-climatic conditions and other natural factors. The double hybrids involving four different parental materials possess higher degree of disease resistance / tolerance than the individual breeds and single hybrids. The polygenic expression in double hybrids is more stable than that in single hybrid in unfavorable environments because of their flexibility in gene constitution within the population (Watanabe, 2002). On the breeding and utilization of double hybrids, only scanty information (Nirmal Kumar *et al.*, 1998, 1999; Joge *et al.*, 2003; Naseema Begum *et al.*, 2005) is available in India. Mal Reddy *et al.* (2005) developed Indian bivoltine double hybrid, (CSR6 x CSR26) x (CSR2 x CSR27) for the first time and authorized it for commercial exploitation.

Recently, Suresh Kumar *et al.* (2010) also attempted in this direction and could develop one single hybrid, D13 x S5 and one double hybrid, (CSR52 x CSR50) x (CSR51x CSR53) which are relatively tolerant to silkworm diseases and high temperature situations. In spite of several advantages, double cross hybrid concept for commercial exploitation is yet to take momentum in India. Sericulturally advanced countries like China and Japan exploited the usage of double hybrids to the extent of 40 %. However, in the present study, an attempt was made to develop a suitable double hybrid resistant to Nuclear polyhedrosis virus induced under low temperature so as to exploit it commercially.

MATERIALS AND METHODS

Five productive bivoltine oval (CSR2, CSR3, CSR17, CSR18 and CSR50) and dumbbell breeds (CSR4, CSR6, CSR16, CSR51 and 61N) were procured and reared as per the standard rearing procedure. One hundred each of freshly moulted fourth instar larvae of each breed were fed with BmNPV (1×10^5) and LC_{50} values were determined. The cocoons harvested were utilized for the preparation of foundation crosses (FCs) between oval x oval and dumbbell x dumbbell breeds. The breeds observed as highly tolerant (CSR18 and 61N) were utilized as parents (Sowmyashree and Nataraju, 2007) for the preparation of 8 foundation crosses in combination with other breeds (CSR18 x CSR2), (CSR18 x CSR3), (CSR18 x CSR17), (CSR18 x CSR50), (61N x CSR4), (61N x CSR6), (61N x CSR16) and (61N x CSR51). Six double hybrids were prepared out of these and reared as per standard rearing procedure up to 3rd moult and freshly moult out fourth instar larvae in five replications of 100 each per hybrid were inoculated with one ml of BmNPV inoculum (LC_{50} : $1 \times 10^{6.003}$) *per os*. Each larva was examined daily for the visual symptoms of Grasserie disease since 72 h post inoculation up to 10 days. The cumulative mortality and the number of healthy larvae were recorded. The harvested cocoons were assessed for nine economic traits such as pupation rate, yield/ 10,000 larvae, cocoon weight, cocoon shell weight, cocoon shell percentage, filament length, raw silk percentage, reelability and neatness. The data were

collected for each replicate and averages of five replications in inoculated batches of double hybrids were analyzed statistically.

Another set of larvae from these double hybrids were reared in parallel as per standard rearing procedure up to 3rd moult. Freshly moulted fourth instar larvae in five replications of 100 each per hybrid were inoculated with one ml of BmNPV inoculum (LC_{50} : $1 \times 10^{1.464}$). The rest of the rearing was continued on fresh untreated mulberry leaves till fourth moult. Fresh, fourth moult out larvae of fifth instar were subjected to low temperature (5°C) induction within 5 h of moulting in preset cold room for 24 h. These larvae after treatment were brought back to room temperature (25 °C) and reared following the standard rearing procedure till cocooning. Observations were recorded on daily basis on mortality / survival due to nuclear polyhedrosis after *per os* inoculation and induction to low temperature. The harvested cocoons were assessed for economic traits. The data were collected for each replication and an average of five replications was computed for statistical analysis. Control batches of each double hybrid in three replications of 100 larvae each were retained till cocooning under prevailing room temperature. The performance of double hybrids was evaluated through multiple traits evaluation index method (Mano *et al.*, 1992).

RESULTS

The overall performance of selected parental breeds (oval and dumbbell) was evaluated by estimating Evaluation Index (EI) for each trait and the compounded average index value for all the traits. The oval (CSR18 - 59.48) and dumbbell (61N - 53.60) breeds having the highest EI values were considered as BmNPV tolerant breeds and utilized for making different FCs. The performance of foundation crosses under BmNPV inoculation is presented in Table 1. The fecundity ranged from 502 to 514, the highest being in CSR18 x CSR50. The per cent survival was in the range of 36.67 ~ 70.00 (CSR18 x CSR3). The cocoon yield/ 10,000 larvae ranged from a minimum of 5.831 to a maximum of 12.183 kg in CSR18 x CSR50. The cocoon and cocoon

Table 1: Performance of foundation crosses under BmNPV inoculation

Foundation cross	Fecundity	Survival rate (%)	Yield/ 10,000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell %	Filament length (m)	Raw silk (%)	Reelability (%)	Neatness (p)	Average E.I.
CSR18 × CSR2	505	67.67	10.615	1.495	0.308	21.08	998	16.09	80.00	90	59.12
CSR18 × CSR3	503	70.00	10.763	1.491	0.316	21.17	983	15.33	75.00	90	60.00
CSR18 × CSR17	506	65.00	9.873	1.433	0.301	21.15	887	14.09	80.00	90	56.50
CSR18 × CSR50	514	62.67	12.183	1.450	0.303	20.89	889	14.33	80.00	92	58.30
61N × CSR4	507	68.67	8.998	1.314	0.283	21.54	801	13.44	80.00	91	55.22
61N × CSR16	508	64.33	10.072	1.339	0.294	22.01	865	13.00	79.00	91	55.74
61N × CSR6	502	63.00	9.275	1.726	0.366	20.620	866	13.00	75.33	91	51.39
61N × CSR51	508	36.67	5.831	1.386	0.263	19.07	853	13.67	72.00	90	47.58
Mean	505.37	61.23	9.491	1.450	0.305	20.96	912	14.76	77.33	90.70	
SD ±	5.01	13.70	2.17	0.17	0.04	0.85	75.90	1.56	3.41	1.06	

Table 2: Performance of foundation crosses under BmNPV inoculation and low temperature (5 ± 1°C) induction

Foundation cross	Fecundity	Survival rate (%)	Yield/ 10,000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell %	Filament length (m)	Raw silk (%)	Reelability (%)	Neatness (p)	Average E.I.
CSR18 × CSR2	494	60.00	8.667	1.709	0.345	20.20	849	11.60	71.00	90	55.00
CSR18 × CSR3	491	62.30	9.306	1.713	0.331	19.30	856	11.70	72.33	92	58.00
CSR18 × CSR17	472	58.30	8.864	1.714	0.341	19.90	872	12.30	73.70	92	58.81
CSR18 × CSR50	474	60.00	8.606	1.718	0.340	19.80	813	12.00	71.00	91	55.08
61N × CSR4	490	45.70	7.221	1.720	0.345	20.10	802	12.50	73.70	92	57.59
61N × CSR16	511	65.70	10.632	1.482	0.315	21.30	875	13.00	74.00	93	60.34
61N × CSR6	480	49.70	6.162	1.145	0.233	20.43	773	12.80	73.00	92	57.70
61N × CSR51	492	26.70	5.228	1.692	0.341	20.10	807	12.10	75.70	91	54.56
Mean	487	51.61	7.871	1.631	0.327	20.10	829	12.21	73.14	92	
SD ±	12.66	13.82	1.88	0.19	0.03	0.52	34.58	0.45	1.58	0.71	

shell weights were in the ranges of 1.339 ~ 1.726 g and 0.263 ~ 0.366 g, respectively, the highest for both being in 61N x CSR6. The cocoon shell percentage varied in the range of 19.07 ~ 22.01 with the maximum recorded in 61N x CSR16. The cocoon filament length and raw silk percentage have recorded ranges of 801 ~ 998 m and 13.00 ~ 16.09 with the highest in CSR18 x CSR2. The reelability per cent ranged from 72.00 to 80.00, the highest value being shared by CSR18 x CSR2, CSR18 x CSR17, CSR18 x CSR50 and 61N x CSR4. The filament neatness varied in the narrow range of 90 ~ 92 points with CSR18 x CSR50 registering the upper limit value. Among the foundation crosses tested for BmNPV inoculation, CSR18 x CSR3 performed the best with the highest EI value of 60.00 and also found to be highly tolerant to BmNPV.

The performance of foundation crosses under BmNPV inoculation followed by low temperature induction is presented in Table 2. The fecundity, per cent survival, cocoon yield / 10,000 larvae, cocoon weight, cocoon shell weight, cocoon shell percentage, filament length, raw silk per cent, reelability per cent and filament neatness of the foundation crosses varied in the ranges of 472 ~ 511, 26.70 ~ 65.70, 5.228 ~ 10.632 kg, 1.145 ~ 1.720 g, 0.233 ~ 0.345 g, 19.30 ~ 21.30, 773 ~ 875 m, 11.60 ~ 13.00, 71.00 ~ 74.00 and 90 ~ 93 points, respectively. 61N x CSR16 recorded the highest values for all the traits except cocoon weight and cocoon shell weight. Hence, among the tested foundation crosses, 61N x CSR16 topped the list in terms of average EI value (60.34) and tolerance to BmNPV.

Performance of double hybrids under BmNPV inoculation condition: The performance of six newly developed double hybrids along with EI values for each economic trait under the inoculation with BmNPV is presented in Table 3. The newly developed double hybrid (CSR18 x CSR3) x (61N x CSR16)

Table 3: Performance of double hybrids under BmNPV inoculation

Double Hybrid	Fecundity	Survival rate (%)	Yield/10,000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell %	Filament length (m)	Raw silk (%)	Reelability (%)	Neatness (p)	Average EI
(CSR18 x CSR2) x (61N x CSR4)	593 (49.34)	71.33 (50.72)	12.835 (52.73)	1.631 (43.50)	0.312 (46.38)	22.79 (54.62)	914 (53.28)	14.33 (45.51)	73.00 (37.89)	91 (43.64)	48.68
(CSR18 x CSR2) x (61N x CSR16)	594 (50.52)	75.33 (52.73)	12.489 (47.20)	1.631 (43.50)	0.370 (45.24)	22.68 (50.80)	902 (43.72)	14.01 (42.98)	74.67 (44.62)	91 (43.64)	46.98
(CSR18 x CSR2) x (61N x CSR6)	578 (32.42)	75.33 (52.73)	13.214 (58.48)	1.630 (43.36)	0.365 (41.81)	22.39 (40.16)	904 (44.84)	14.10 (43.71)	75.67 (48.65)	91 (43.64)	45.64
(CSR18 x CSR3) x (61N x CSR4)	592 (48.95)	72.00 (43.79)	12.344 (44.88)	1.632 (43.84)	0.365 (41.81)	22.35 (38.97)	904 (45.40)	14.00 (42.90)	75.00 (45.96)	91 (43.64)	42.75
(CSR18 x CSR3) x (61N x CSR16)	603 (61.94)	76.67 (61.68)	13.409 (61.89)	1.727 (63.49)	0.400 (65.81)	23.11 (66.09)	932 (69.04)	17.00 (66.36)	77.67 (56.73)	93 (65.44)	63.19
(CSR18 x CSR3) x (61N x CSR6)	599 (56.82)	66.67 (33.35)	11.694 (34.51)	1.722 (62.31)	0.390 (58.95)	22.64 (49.36)	902 (43.72)	16.00 (58.54)	81.67 (66.15)	93 (59.99)	52.76
Mean	593	73.05	12.63	1.669	0.378	22.63	909	15.02	76.9	92	
SD ±	9.32	3.95	0.69	0.05	0.02	0.3	13.01	1.4	2.91	1.1	
(CSR2 x CSR27) x (CSR6 x CSR26) (c)	554	53.41	5.720	1.144	0.242	25.15	863	10.0	74.0	92.0	50.00

Table 4: Performance of double hybrids against BmNPV inoculation followed by low temperature ($5 \pm 1^{\circ}\text{C}$) induction

Double Hybrid	Fecundity	Survival rate (%)	Yield/10,000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell %	Filament length (m)	Raw silk (%)	Reelability (%)	Neatness (p)	Average E.I.
(CSR18 x CSR2) x (61N x CSR4)	496 (39.17)	61.00 (45.02)	8.849 (39.69)	1.509 (49.19)	0.306 (44.28)	20.30 (37.21)	893 (65.02)	14.00 (61.04)	70.00 (33.70)	90 (43.55)	46.54
(CSR18 x CSR2) x (61N x CSR16)	498 (42.91)	59.70 (41.88)	9.737 (50.45)	1.465 (44.56)	0.301 (41.81)	20.50 (41.25)	883 (56.06)	13.80 (56.18)	76.00 (61.64)	90 (43.55)	48.58
(CSR18 x CSR2) x (61N x CSR6)	500 (45.72)	60.00 (42.66)	9.433 (46.76)	1.431 (40.91)	0.305 (43.66)	21.30 (54.65)	874 (49.07)	13.50 (47.43)	74.30 (53.88)	90 (43.55)	47.49
(CSR18 x CSR3) x (61N x CSR4)	502 (48.52)	67.70 (60.74)	9.775 (50.90)	1.430 (40.84)	0.307 (44.74)	21.50 (57.65)	860 (37.03)	13.60 (51.33)	74.00 (52.33)	91 (50.00)	49.83
(CSR18 x CSR3) x (61N x CSR16)	509 (57.87)	61.00 (45.02)	9.180 (43.70)	1.62 (61.01)	0.350 (64.61)	21.80 (62.90)	877 (51.03)	13.70 (52.11)	74.70 (55.43)	91 (50.00)	54.34
(CSR18 x CSR3) x (61N x CSR6)	515 (65.81)	68.30 (64.67)	11.229 (68.50)	1.643 (63.49)	0.342 (60.91)	20.80 (46.33)	866 (41.79)	12.90 (31.91)	72.00 (43.01)	93 (69.36)	53.22
Mean	505	63.34	9.871	1.518	0.321	21.18	872	13.5	24.5	91	
SD $\pm$	6.69	4.29	0.8	0.11	0.02	0.53	908	0.35	1.12	1.1	
(CSR2 x CSR27) x (CSR6 x CSR26) (c)	500	43.08	5.117	1.190	0.240	20.16	802	10.0	74.30	92.0	41.92

recorded maximum EI values for eight economic traits viz., fecundity, survival, yield/10,000 larvae by wt., cocoon weight, shell weight, cocoon shell percentage, filament length, raw silk %, reelability and neatness with an average EI value of 63.19 followed by (CSR18 x CSR3) x (61N x CSR6) (52.76).

Performance of double hybrids under BmNPV inoculation and induction: The performance of double hybrids inoculated with BmNPV followed by low temperature induction is presented in Table 4. The hybrids were evaluated for ten economic traits. The double hybrid (CSR18 x CSR3) x (61N x CSR16) has recorded maximum EI values for two traits viz., cocoon shell weight and cocoon shell percentage with an average value of 54.34 followed by (CSR18 x CSR3) x (61N x CSR6) with an average EI value of 53.22. These two double hybrids were having better EI value under inoculated and induction condition over the ruling control hybrid (CSR2 x CSR27) x (CSR6 x CSR26) with an average EI value of 41.92.

DISCUSSION

Various workers have demonstrated the superiority of single, three way and double crosses over their parents (Pannegpet and Jaroonchai, 1975; Gamo, 1976; Datta, 1984). Due to the obvious advantages like easy rearing of foundation crosses, superiority to parental breeds in growth & vigor and 15 to 20 % improvement in egg productivity (Hirobe, 1985), double hybrids are a better option. The results of the present investigation revealed that the fecundity can be increased if the mother moth is from hybrid and this corroborates the findings

of Yokoyama (1957). This is possible only in the case of double hybrids where more heterosis for fecundity is observed. Mal Reddy *et al.* (2005) while developing the double hybrid (CSR2 x CSR27) x (CSR6 x CSR26) observed higher fecundity of 667 as against 536 in a single hybrid (CSR2 x CSR4). Pallavi *et al.* (2003) evaluated the performance of parents, foundation crosses (parents of double hybrids), single crosses and found that it was easy for rearing double hybrids with higher pupation rate and higher egg recovery. The study also showed the prospects of using foundation crosses for higher fecundity at double hybrids level. The double hybrid rearing is being popularized in India due to the easiness in rearing, superiority over parents in growth & vigor, besides higher egg number than that of the single hybrid (Nirmal Kumar *et al.*, 1999). Joge *et al.* (2003) were of the opinion that the cocoon yield in double hybrid was stable. Since the bivoltine hybrids have the genetic plasticity to buffer against the adverse climatic conditions, relative tolerance to various diseases and inherent genetic potential to produce good quality raw silk than the existing hybrids, they can be reared throughout the year. In the present study, it was observed that the best performing newly developed hybrid is superior to control hybrid when screened against BmNPV inoculum and low temperature induction. The polygenic expression in double hybrids is more stable than that in single hybrids in unfavorable environments because of their flexibility in gene constitution within the population (Watanabe, 2002). By realizing the clear advantages such as easy rearing, better growth, vigor & economic characters and yield on par or better than single hybrids, the newly developed double hybrid (CSR18 x CSR3) x (61N x CSR16) with disease tolerance and environmental stability could be popularized after authorization, in India, where adverse climatic conditions and high pathogen load prevails in the rearing environment.

ACKNOWLEDGEMENT

The authors are thankful to the scientists of Silkworm Pathology, Silkworm Breeding & Genetics Laboratories of this institute for experimental assistance and supply of germplasm stocks, respectively for the study.

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IDENTIFICATION D'UN NOUVEL HYBRIDE DOUBLE DU VER A SOIE MÛRIER TOLERANT VIS-A-VIS DU VIRUS DE LA POLYEDROSE NUCLEAIRE (BmNPV)

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RESUME

Les hybrides doubles sont connus pour apporter des avantages économiques et de rendement égaux ou supérieurs aux hybrides simples. L'élevage d'hybrides doubles tolérants aux maladies est plus facile et l'expression polygénique de leur résistance aux maladies est plus stable que chez les hybrides simples dans des conditions défavorables en raison de la flexibilité de la constitution génétique de la population. Dix lignées parentales bivoltines sélectionnées (CSR2, CSR3, CSR4, CSR6, CSR16, CSR18, CSR50, CSR51 et 61N) ont été testées par inoculation du virus de la polyédrose Bm dans des conditions d'induction à basse température. Les lignées qui sont fortement tolérantes (CSR18 et 61N) sont utilisées pour préparer 8 croisements de fondation avec d'autres lignées, précisément (CSR18 x CSR2), (CSR18 x CSR3), (CSR18 x CSR17), (CSR18 x CSR50), (61N x CSR4), (61N x CSR6), (61N x CSR16) et (61N x CSR51). Six hybrides doubles préparés à partir de ces croisements ont été évalués pour dix caractères mesurables tels que la fécondité, le taux de pupaison, le rendement pour 10000 larves, le poids du cocon, le poids de la coque, la richesse soyeuse, la longueur du filament, la dévidabilité, le taux de soie grège et la netteté. Basé sur les résultats obtenus après inoculation du BmNPV en condition d'induction, le nouvel hybride double (CSR18 x CSR3) x (61N x CSR16) qui donne la moyenne maximale (63,76) de l'indice d'évaluation, a été sélectionné pour l'exploitation commerciale puisqu'il est supérieur à l'hybride double courant (CSR2 x CSR27) x (CSR6 x CSR26).



Research Paper

SECONDARY MINERAL NUTRIENT COMBINATION IMPROVES YIELD WITH QUALITY IN TASAR CROPS

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ABSTRACT

The role of secondary nutrients in improving the tasar crops was evaluated. Different combinations of secondary mineral nutrients were formulated and its effect on the quality and quantity of leaves of tasar food plant *Terminalia arjuna* Bedd were studied. Results indicated that secondary nutrient combinations have promotary effect in crop improvement by enhancing the quality and yield of leaves as well as the commercial characters of cocoons. Among different combinations of secondary mineral nutrients tried, SM₅ [Foliar application of Magnesium sulphate (2 %, w/v) and basal application of Calcium carbonate (3 quintal / ha)] was the best in terms of crop improvement. Under this combination, leaf yield increased by 27.45 % over control. Further, chemo assay revealed a significant improvement in the quality of leaves. Moisture, total mineral, crude protein and total carbohydrate increased by 3.53, 19.44, 18.50 and 16.66 %, respectively over control. The increase in leaf quality was further confirmed by bio assay as is reflected in cocoon weight, shell weight and shell percentage which increased to the tune of 20.17, 35.71 and 13.01 % as compared with control.

Key words: *Antheraea mylitta*, bio assay, chemo assay, secondary mineral nutrient combinations, tasar crops, *Terminalia arjuna*.

INTRODUCTION

Secondary nutrients are as important to plants as major nutrients (Pasricha and Sarkar, 2002). Deterioration in soil fertility is often observed in crops / cropping system, even with adequate use of NPK fertilizers. It has been found to be associated with the deficiency of micro and secondary nutrients. Several studies have been carried out on application of major and micronutrients (Sinha *et al.*, 1999, 2002, 2006) for improvement in tasar crops. But no effort has so far been undertaken for the improvement of tasar crops through application of secondary nutrients which are the key nutrients responsible for increasing productivity in acidic soils (Sarkar and Singh, 2003). Soils of tasar producing areas are generally acidic. Hence, the present study aims to improve tasar crops through application of secondary mineral nutrients combinations.

MATERIALS AND METHODS

The study was conducted in the experimental field of Central Tasar Research and Training Institute, Nagri, Ranchi on *T. arjuna* plants with 1.2 m x 1.2 m spacing under rain fed condition during first (July-August) and second (September- October) rearing seasons. Before the layout of the experiment, the fertility gradient of the field was studied following the method described by Hesse (2002). The plot was quite uniform having sandy loam laterite soil with pH, 5.4; organic carbon, 0.37 %; available phosphorus, 14.6 kg/ha and available potassium, 62.0 kg/ha. Secondary nutrient status of the soil is as given below:

Secondary nutrient	Status in soil
Available Calcium	2.90 Cmol (P ⁺) kg ⁻¹
Available Magnesium	0.95 Cmol (P ⁺) kg ⁻¹
Available Sulphur	8.70 ppm

The experiment was laid out in Randomized Block Design with ten treatments in three replications, each replication having 24 plants. The usual package of practices of major nutrients *i.e.*, Nitrogen (200 kg/ha/yr), Phosphorus (50 kg/ha/yr), Potassium (50 kg/ha/yr) and Farm Yard Manure (6 MT/ ha) was also followed.

Ten treatments including different combinations of secondary mineral nutrients and control are as follows:

Foliar application of MgSO_4	Basal application of CaCO_3
SM_1 - 1.5 %, w/v	2 q ha^{-1}
SM_2 - 1.5 %, w/v	3 q ha^{-1}
SM_3 - 1.5 %, w/v	4 q ha^{-1}
SM_4 - 2 %, w/v	2 q ha^{-1}
SM_5 - 2 %, w/v	3 q ha^{-1}
SM_6 - 2 %, w/v	4 q ha^{-1}
SM_7 - 2.5 %, w/v	2 q ha^{-1}
SM_8 - 2.5 %, w/v	3 q ha^{-1}
SM_9 - 2.5 %, w/v	4 q ha^{-1}
SM_{10} - Control (without secondary nutrients)	

Basal application of Calcium carbonate was done in single dose in the month of June. Magnesium sulphate was applied foliarly on *T. arjuna* plants in three split doses from May onwards at 15 days intervals. The effect of these treatments was studied during first (July-Aug.) and second (Sept.– Oct.) crops through leaf yield determination, bio assay and chemo assay. Leaf yield was determined 45 days after the third spray of Magnesium sulphate following simple random sampling from 20 % of the population during crop I & II. It was determined by plucking all the leaves from a tree and weighing immediately for preventing loss of moisture. Plants exhibiting abnormal growth were not considered for determination of leaf yield. For bio assay, disease free layings of *Antheraea mylitta* were brushed and reared in three replicated batches of 100 worms each following the rearing technique suggested by Jolly *et al.* (1974). For Chemo assay, Simple Random Sampling method was followed for collecting the leaf samples excluding too tender and over mature leaves from each

treatment in three replications. All the biochemical constituents of leaves except moisture were determined on oven dry basis. Moisture, total mineral, total carbohydrate and crude fibre were estimated by the method of AOAC (Anonymous, 1955). Kjeldahl's method as described by Vogel (1978) was followed for the determination of total nitrogen. Crude protein was calculated by multiplying the estimated value of nitrogen content by 6.25.

RESULTS AND DISCUSSION

The leaf yield and per cent increase in leaf yield of two crops are presented in Table 1. It is evident that there has been significant increase in leaf yield over control in the case of all the secondary mineral nutrient combinations, the highest being 27.45 % over control for treatment SM_5 (foliar application of Magnesium sulphate, 2%, w/v & basal application of Calcium carbonate, 3 q ha^{-1}) followed by SM_6 (foliar application of Magnesium sulphate, 2 %, w/v & basal application of Calcium carbonate, 4 q ha^{-1}) wherein the leaf yield increased by 17.64 % over control. It has been reported that Magnesium sulphate is involved in the formation of Chlorophyll and activation of enzymes (Gunther, 1981; Tandon, 2002) thereby contributing to increase in leaf yield. Similar trends of increased yield of potato (21.00 to 41.60 %) and tea (20.20 %) have been reported by

Table 1: Effect of different combinations of secondary mineral nutrients on the leaf yield of *T. arjuna*

Treatment	Leaf yield (kg/ha)			% increase over control
	Crop I	Crop II	Average	
SM_1	27248.40	31285.20	29266.80	6.62
SM_2	28257.60	30948.80	29603.20	7.84
SM_3	29603.20	32428.96	31016.08	12.99
SM_4	29603.20	31217.92	30410.56	10.78
SM_5	32967.20	37004.00	34985.60	27.45
SM_6	30948.80	33640.00	32294.40	17.64
SM_7	28930.40	30948.80	29939.60	9.07
SM_8	30276.00	32967.20	31621.60	15.20
SM_9	28930.40	30545.12	29737.76	8.33
SM_{10} (control)	25566.40	29334.08	27450.24	-
C.D. at 5 %	1782.80	1703.71	1743.26	-

Sarkar and Singh (2003) by the application of Magnesium sulphate and Sulphur, respectively. Calcium carbonate provides Calcium in available form to plants. Calcium is an extremely important mineral in plant nutrition. It increases the uptake of nutrients. It is required for the growth of the meristematic tissues and for the functioning of the root tip. It also maintains the shape of the cells. Our findings also corroborate with that of Sarkar and Singh (2003) who reported that basal application of 2 - 4 q ha⁻¹ of Calcium carbonate increased the yield of Soya bean to the tune of 26.70 %.

Effect of different treatments on the commercial characters of *Antheraea mylitta* Drury is presented in Table 2. Silkworms when fed with leaves of secondary mineral nutrients treated plants had a favourable effect on the full grown larval weight, effective rate of rearing (E.R.R.), cocoon weight, shell weight and shell percentage. Shankar *et al.* (1994) had conducted studies in the similar line and reported that secondary nutrients play an important role in silkworm larval growth, cocoon weight and silk quality. Among the ten treatments under the present study, combination SM₅ was the most effective in increasing the larval weight (10.29 %), effective rate of rearing (27.59 %), cocoon weight (20.17 %), shell weight (35.71 %) and shell percentage (13.01 %) followed by combination SM₆. Mineral

contents stimulate the metabolic activities in silkworm resulting in quantitative improvement of cocoon and silk (Bose *et al.*, 1995). Gunther (1981) revealed that about 300 enzyme reactions are influenced by Mg ions. When Magnesium is passed on to the silkworms, it accelerates the growth of silkworms through orientation of physiological activities. Moreover, Sulphur is known to have an important role in the synthesis of amino acids, proteins and vitamins. This may be the reason for improvement in commercial characters of cocoons due to the application of Magnesium sulphate. Viswanath and Krishnamurthy (1982) observed that silkworm larvae fed with Mg sprayed leaves resulted in better cocoon yield and cocoon weight. Loknath and Shivshankar (1986) could also reveal that when mulberry leaves fortified with Magnesium (1.25 kg/ha) were fed to silkworms, it favourably influenced the cocoon yield and shell quality. These findings are in agreement with that of Subburathinam and Chetty (1991) and supportive to the present study as well. Subburathinam *et al.* (1993) reported that enrichment of mulberry leaves with Calcium also results in improvement of the commercial characters of cocoons.

Table 3 presents the chemical composition of *T. arjuna* leaf under different combinations of secondary mineral nutrient treatments. Data indicate that application

Table 2: Influence of different combinations of secondary mineral nutrients on the commercial characters of *A. mylitta* reared on *T. arjuna*

Treatment	Weight of 5th instar larva (g)		E.R.R. (%)		Cocoon weight (g)		Shell weight (g)		Shell %	
	Crop I	Crop II	Crop I	Crop II	Crop I	Crop II	Crop I	Crop II	Crop I	Crop II
SM ₁	38.73	39.55	33.00	36.67	12.58	12.80	1.54	1.66	12.25	12.97
SM ₂	38.91	39.71	33.00	37.00	12.68	12.92	1.56	1.68	12.30	13.01
SM ₃	39.63	40.45	34.00	40.00	13.34	13.56	1.58	1.70	11.84	12.55
SM ₄	39.32	40.14	34.00	38.00	13.00	13.40	1.57	1.69	12.09	12.61
SM ₅	41.66	42.54	40.33	43.33	14.18	14.42	1.83	1.97	12.91	13.66
SM ₆	40.29	41.11	37.00	39.67	13.75	14.05	1.64	1.76	11.93	12.53
SM ₇	39.08	39.90	34.00	36.67	12.80	13.00	1.57	1.69	12.26	13.00
SM ₈	39.94	40.78	36.00	39.00	13.60	13.80	1.59	1.71	11.71	12.39
SM ₉	38.97	39.79	34.00	36.00	12.79	13.01	1.54	1.64	12.04	12.61
SM ₁₀ (control)	37.80	38.60	30.00	34.00	11.80	12.00	1.35	1.45	11.44	12.08
C. D. at 5 %	1.83	1.31	3.15	2.99	0.74	0.72	0.09	0.09	0.43	0.38

Table 3: Effect of different combinations of secondary mineral nutrients on the chemical constituents of *T. arjuna* leaf

Treatment	Moisture (%)		Total mineral (%)		Crude fibre (%)		Crude protein (%)		Total carbohydrate (%)	
	Crop I	Crop II	Crop I	Crop II	Crop I	Crop II	Crop I	Crop II	Crop I	Crop II
SM ₁	71.60	70.70	7.93	7.41	9.85	11.10	12.31	12.75	15.18	15.40
SM ₂	71.71	70.81	8.01	7.49	9.90	11.20	12.44	12.88	15.29	15.51
SM ₃	72.18	71.27	8.35	7.80	10.00	11.25	12.88	13.25	15.74	15.98
SM ₄	71.98	71.07	8.21	7.67	10.20	11.30	12.69	13.06	15.54	15.78
SM ₅	73.50	72.58	9.30	8.70	10.40	11.65	14.06	14.37	17.00	17.30
SM ₆	72.61	71.69	8.66	8.09	10.35	11.50	13.25	13.63	16.14	16.41
SM ₇	71.83	70.92	8.09	7.56	10.15	11.40	12.50	12.94	15.39	15.63
SM ₈	72.38	71.47	8.50	7.94	10.30	11.35	13.00	13.44	15.93	16.18
SM ₉	71.75	70.85	8.05	7.52	9.95	11.30	12.44	12.87	15.33	15.56
SM ₁₀ (control)	71.00	70.10	7.50	7.00	9.80	11.00	11.75	12.25	14.60	14.80
C. D. at 5 %	0.56	0.74	0.25	0.32	NS	NS	0.35	0.28	0.67	0.61

of secondary nutrient combinations has improved the quality of leaf. Except crude fibre, contents of moisture, crude protein, total carbohydrate and total mineral were significantly higher in treated plant leaves than control. Maximum increase in chemical constituents of leaves was observed for combination SM₅. The increase in chemical constituents may be due to the beneficial role of secondary nutrients in plant metabolism. Magnesium activates enzymes and Sulphur is involved in the formation of amino acids essential for protein synthesis (Pasricha and Sarkar, 2002). Calcium being an important mineral for plant nutrition, enhances the uptake of nitrogen in the form of nitrates and helps in protein synthesis (Das and Chaudhary, 2007).

From the present study it is, therefore, inferred that among all the treatments of secondary mineral nutrients under study, combination SM₅ is the best for boosting the production of tasar crops. This treatment brings in improvement in quality and quantity of leaves of *T. arjuna* as well as the commercial characters of cocoons of *A. mylitta*, thus functioning as a booster of tasar crops.

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UNE COMBINAISON DE NUTRIMENTS MINERAUX SECONDAIRES AMELIORE LE RENDEMENT ET LA QUALITE DES RECOLTES DE TASAR

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RESUME

Nous avons étudié le rôle des nutriments secondaires dans l'amélioration des récoltes tasar. Différentes formules de nutriments minéraux secondaires ont été combinées et leur effet sur la qualité et la quantité de feuilles de *Terminalia arjuna* Bedd, la plante hôte du tasar, a été évalué. Les résultats montrent que les combinaisons de nutriments secondaires ont un effet positif sur l'amélioration des récoltes en accroissant la qualité et le rendement en feuilles ainsi que les caractères commerciaux des cocons. Parmi les différentes combinaisons testée, SM₅ [Application foliaire de sulfate de Magnésium (2 %, poids/poids) et une application basale de carbonate de calcium (3 quintaux/ha)] s'est avérée la meilleure. Avec cette combinaison, le rendement foliaire est accru de 27.45 % par rapport au témoin. De plus, les essais chimiques révèlent une amélioration significative de la qualité des feuilles. La teneur en eau, la quantité totale de minéraux, les protéines brutes et la quantité d'hydrates de carbone sont augmentées respectivement de 3.53, 19.44, 18.50 et 16.66 %. L'amélioration de la qualité des feuilles a été confirmée par des tests biologiques puisque le poids du cocon, le poids de la coque et la richesse soyeuse sont supérieurs au témoin à raison de 20.17, 35.71 et 13.01 % respectivement.

Sericologia India Edition Released



After decades long publishing history from Lyon, France, SERICOLOGIA moved to Bangalore, India consequent upon the change in the Headquarters of International Sericultural Commission. This was an opportunity for the journal to overhaul its appearance and presentation style. The new-look SERICOLOGIA was released by the Honourable Chairperson, Central Silk Board on 30th July 2013, in the presence of Ms. Ishita Roy, IAS, Secretary General of ISC, Mr K. K. Shetty, Officer on Special Duty, ISC, Dr. Julia S. Nair, Chief Editor, SERICOLOGIA, Directors of various institutes of Central Silk Board and members of Administrative Council of SERICOLOGIA.

CHAIRMAN, BANGLADESH SERICULTURE BOARD VISITS ISC HEADQUARTERS



Mr. Manontosh Dhar, Chairman, Bangladesh Sericulture Board, Dhaka met Secretary General, ISC on 14.08.2013 at the ISC headquarters, Bangalore. He has been deputed by the Government of Bangladesh for initiating the process of Bangladesh enrolling as a member country of ISC. The discussions were mainly



focused on the procedural formalities to be followed for the country membership and also the various facilities that can be availed of once Bangladesh become a member country. Twenty one officials from the Bangladesh Sericulture Board accompanied Mr. Dhar to undertake training on sericulture at Central Sericultural Research and Training Institute, Mysore, the premier research institute under Central Silk Board, Govt. of India. The training programme scheduled for a period of 35 days commenced on 12th August 2013.

A HIGH LEVEL DPR KOREAN DELEGATION PAYS A VISIT TO THE HEADQUARTERS OF ISC



A high level 5 member DPR Korea delegation headed by Mr. Kim Jong Bin, Director General, Sericultural and Silk Industry Guidance Bureau, Pyongyang and comprising members, Mr. Ri Chong, Director and Mr. Ri Chol Ho, Director from Sericultural and Silk Industry Guidance Bureau, Mr. Rim Myong Su, Managing Director and Ms. Jang Hye Song, Manager from Korea Silk Trading Corporation, Pyongyang visited India from 11th to 14th June 2013. An interactive meeting between the delegation and Secretary General was held at ISC headquarters on 11th June 2013 on the possible collaboration with ISC for the development of sericulture and silk industry in DPR Korea. The Secretary General, while offering to extend all possible supports available from ISC for the development of silk industry, appealed them to enroll DPR Korea as a member of ISC. The delegation also visited sericulture areas of Karnataka, research institutes, cocoon market, seed production facilities and silk companies to study and understand the sericulture development in India.

STATISTICS

Global Silk Production (MT)

Country	2006	2009	2010	2011	2012
Brazil	1177	811	770	558	614
Bulgaria	7.5	6.3	9.4	6	8.5
China	98620	84000	115000	104000	126000
Colombia	0.6	0.6	0.6	0.6	0.6
Egypt	3	3	0.3	0.7	0.7
India	18370	19690	21005	23060	23679
Indonesia	37	19	20	20	20
Iran	180	82	75	120	123
Japan	96	72	54	42	30
Korea (North)				300	300
Korea (South)	3	3	3	3	1.5
Madagascar	15	16	16	16	18
Philippines	1	1	1	1	0.89
Syria	0.4	0.6	0.6	0.5	0.5
Thailand	1100	665	655	655	655
Tunisia	0.08	0.04	0.12	3	3.95
Turkey	15	20	18	22	22
Uzbekistan	770.5	780	940	940	940
Vietnam			550	500	450
Total	120398	106169	139118	129684	152898



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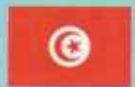
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