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# Evaluation and quantification of digestive amylase enzyme and its relation with productive and economic traits of multivoltine silkworm genetic resources of India

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**Abstract**
Amylase is one of the key enzymes involved in the digestion and carbohydrate metabolism in insects. There is wide genetic variability in the activity of digestive amylase among the silkworm genetic resources of varying geographical origin and yield potential. The digestive amylase is generally very high in the non diapausing multivoltine silkworm races than that of the diapausing bivoltine stocks. Therefore in the present study an attempt has been made to evaluate and quantify the digestive amylase enzyme activity in the 73 multivoltine silkworm genetic resources conserved at Central Sericultural Germplasm Resources Centre, Hosur which is the only centre where all the multivoltine silkworm genetic resources were conserved following standard protocols. The study has been also carried out with the objective of revealing the quantum of variability of the amylase enzyme activity in the multivoltine silkworm genetic resources of the varying geographical origin and their association with that of the productive and economic traits. The study revealed that significant variability in the quantity of amylase 1600 µg to 2250 µg among the 73 MV silkworm genetic resources evaluated. Positive correlations in some of the traits like weight of 10 larvae, effective rate of rearing by number and single cocoon weight and cocoon yield per 100 dfls were observed. Some of the traits like total larval duration, effective rate of rearing by number, pupation and single shell weight showed negatively correlation. Significant negative correlations were noticed in fecundity, hatching, fifth age larval duration and shell ratio.

**Keywords:** amylase, silkworm genetic resources, correlation, economic traits

## 1. Introduction
Mulberry silkworm *Bombyx mori* L. feeds on the leaves of *Morus spp*. for its food and to grow and develop and completes its lifecycle. During the only active feeding stage it consumes large quantity of mulberry leaves for completion of its biological activities and processes. As mulberry a perennial tree plant grown although the year it is being influenced by the by the seasonal changes and also the inputs given to the plant. Probably most of the study of characteristics of an insect digestive enzyme has been made on amylase of silkworm (Mori, 1930; Ito *et al*., 1962) [19, 16]. Kanekatsu (1972) [17] purified amylase of the digestive fluid and studied properties of amylase in *Bombyx mori.* Banano *et al.* (1984) [4] reported the sex dimorphic and development changes in amylase of haemolymph of *Bombyx mori.* Amylases obtained from different origins have been characterized by Baker (1989) [3] and Bhawane and Bhanot (1989) [5]. In silkworm studies on the different amylase activity among silkworm strains and relationship between amylase activity of the larval digestive juice and economic characters has been done (Hirata, 1971; 1974) [13-14]. Amylase one of the key enzymes involved in the digestion and carbohydrate metabolism in insects (Horie and Watanabe, 1983) [15] and particularly carbohydrates available in the form of starch in mulberry leaves (Chatterjee *et al*., 1989) [7]. Abraham *et al.* (1992) [1] identified different series of amylases in both diapausing and non diapausing strains of *Bombyx mori*. The wide genetic variability in the activity of digestive amylase has been indicated when large number of silkworm stocks of wide geographical origin and yield status at different research centres and most of the high yielding, diapausing stocks and low amylase activity while most of the low yielding non diapausing stocks showed high amylase activity. In general the activities of amylase in multivoltine races were almost two fold higher than the bivoltine races of silkworms (Chatterjee *et al*., 1992) [9].

The information on the importance of digestive amylase activity in yield and biochemical parameters, of late molecular markers facilitated selection using isozyme / DNA markers is being widely explored and successfully used in the breeding of crop plants and livestock for the management of target traits (Young and Tanksley, 1989; Tankslet and Nelson, 1996; Visscher *et al*., 1996; Fulton *et al*., 1997) [27, 23, 26, 12]. The development profiles of amylase enzyme, its genetic variability and activity in the mulberry silkworm its significance and the correlation between yield and biochemical parameters were investigated by (Chatterjee *et al*., 1989; 1990; 1992; 1993) [7-10]. Patnaik and Datta (1995) [22] studied the type of amylase and their activity in the bivoltines and multivoltines strains. In view of the wide divergence in terms of activity and isozyme polymorphism and its role in better digestibility and close association with survival, digestive amylase isozyme was chosen as a marker in silkworm breeding for improving the potential of productive breeds (Ashwath *et al*., 2001) [2]. The function of the digestive fluid amylase is related to the digestion of starch contained in the food plant - mulberry (Kanekatsu, 1972) [17]. Muniv *et al.* (2011) [21] studied the characteristics of midgut amylase and made a comparative analysis in multivoltine races Nistari and Kolar Gold.

Therefore in the present study an attempt has been made to screen the amylase activity in the different multivoltine

silkworm germplasm resources conserved at CSGRC, Hosur which will indicate the extent of variability in the quantity of amylase among the 73 germplasm stocks of different geographical origins, productivity and economic important traits which can be used in future studies. Also the correlation between quantum of amylase and productivity, survival and also the deviations if any among the multivoltine genetic stocks of the contry.

## 2. Materials and Methods

The studies were undertaken at the Central Sericultural Germplasm Resources Centre, Hosur. Seventy three multivoltine silkworm accessions conserved at the centre were utilized for the investigations. Sixty three of these accessions are from different geographical regions of the country and 10 accessions were of exotic origin (Table-1). The accessions evaluated possess greater variability in terms of morphological and economic traits and also comprising many native breeds, land races, breeds under current use, breeding resources materials and also few obsolete breeds. The dfls of these accessions are conserved by cold preserving the eggs for 35 days under 5˚C and thereafter releasing them and incubating at 25˚C and 80 % RH. On attaining pin head stage they will be composited by taking 50 eggs from each of the 20 dfls preserved at two different cold storages and composite populations will be brushed after black boxing the composited eggs. (Thangavelu *et al*., 1997; 2000) [24, 25].

**Table 1:** Details of multivoltine accessions vis a vis their morphological traits.

| Accession No. | Race Name | Origin | Class | Egg colour | Yolk Colour | Larval pattern | Cocoon colour | Cocoon shape |
|---|---|---|---|---|---|---|---|---|
| BMI-0001 | PURE MYSORE | India/Karnataka | O(BCU) | Dull white | Light yellow | Plain | LGY | Spindle |
| BMI-0002 | SARUPAT | India/Assam | O(OB) | Light yellow | Creamish white | Plain | Creamy white | SA |
| BMI-0003 | MORIA | India/Assam | O(OB) | FYGT | Light yellow | Plain | Creamy white | SA |
| BMI-0004 | TAMILNADU WHITE | India/Tamil Nadu | E(OB) | White | Creamish white | Marked | White | SA |
| BME-0005 | C.NICHI | Japan | O(OB) | White | Creamish white | Marked | White | Constricted |
| BMI-0006 | HOSA MYSORE | India/Karnataka | E(BCU) | FYGT | Yellow | Plain | Greenish yellow | Elongated oval |
| BMI-0007 | MYSORE PRINCESS | India/Karnataka | E(OB) | White | Creamish white | Plain | White | Oval |
| BMI-0008 | KOLAR GOLD | India/Karnataka | E(OB) | White | Creamish white | Plain | White | Oval |
| BMI-0009 | KOLLEGAL JAWAN | India/Karnataka | E(OB) | White | Creamish white | Plain | White | Oval |
| BMI-0010 | MY-1 | India/Karnataka | E(BCU) | FYGT | Light yellow | Plain | LGY | Elongated |
| BMI-0011 | P2D1 | India/Karnataka | E(BCU) | FYGT | Yellow | Marked | Greenish yellow | Elongated oval |
| BME-0012 | RONG DAIZO | China | E(OB) | FYGT | Yellow | Marked | Greenish yellow | SA |
| BME-0013 | GUANGNONG PLAIN | China | E(OB) | White | Creamish white | Plain | Creamy white | Oval |
| BMI-0014 | OS-616 | India/West Bengal | E(OB) | FYGT | Yellow | Marked | Yellow | Oval |
| BME-0015 | RAJ | Bangladesh | O(OB) | FYGT | Light yellow | Marked | Creamy white | Spindle |
| BMI-0016 | G | India/West Bengal | E(BCU) | FYGT | Yellow | Marked | Golden yellow | Oval |
| BMI-0017 | NISTARI | India/West Bengal | O(BCU) | FYGT | Yellow | Marked | Golden yellow | Spindle |
| BMI-0018 | NISTARI(M) | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | SB |
| BMI-0019 | NISTARI(P) | India/West Bengal | E(BM) | FYGT | Yellow | Plain | Golden yellow | SB |
| BMI-0020 | ZPN(SL) | India/West Bengal | E(BM) | Faint yellow | Creamish white | Mixed | Creamy white | Spindle |
| BMI-0021 | CB5 | India/West Bengal | E(BCU) | FYGT | Yellow | Marked | Golden yellow | Oval |
| BMI-0022 | KW2 | India/West Bengal | E(BM) | Creamish white | Creamish white | Plain | Creamy white | Elongated oval |
| BMI-0023 | M2 | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | Oval |
| BMI-0024 | A23 | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | Oval |
| BMI-0025 | A25 | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | Elongated oval |
| BMI-0026 | OVAL | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | Oval |
| BMI-0027 | O | India/West Bengal | E(OB) | FYGT | Yellow | Marked | Golden yellow | Oval |
| BMI-0028 | M83(C) | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | Oval |
| BMI-0029 | B | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | Elongated |
| BME-0030 | GNM | China | E(OB) | White | Yellow | Marked | White | Oval |
| BMI-0031 | A14DY | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | Oval |
| BMI-0032 | A4e | India/West Bengal | E(OB) | FYGT | Yellow | Marked | Greenish yellow | Elongated oval |
| BMI-0033 | PA12 | India/Karnataka | E(BM) | FYGT | Yellow | Plain | Greenish yellow | Elongated oval |
| BMI-0034 | AP12 | India/Karnataka | E(BM) | FYGT | Yellow | Marked | Greenish yellow | Elongated oval |
| BMI-0035 | A13 | India/Karnataka | E(BM) | FYGT | Yellow | Plain | Greenish yellow | Elongated oval |
| BMI-0036 | PMX | India/Karnataka | E(BM) | FYGT | Yellow | Plain | LGY | Elongated oval |
| BMI-0037 | PMS2 | India/Karnataka | E(BM) | FYGT | Yellow | Plain | LGY | Elongated oval |
| BMI-0038 | MU-1 | India/Karnataka | E(BM) | FYGT | Yellow | Plain | LGY | Elongated oval |
| BMI-0039 | MU-11 | India/Karnataka | E(BM) | FYGT | Yellow | Plain | LGY | Elongated oval |
| BMI-0040 | WAI-1 | India/Maharashtra | E(BM) | FYGT | Yellow | Marked | LGY | Elongated oval |
| BMI-0041 | WAI-4 | India/Maharashtra | E(OB) | FYGT | Bright yellow | Marked | Yellow | Oval |
| BMI-0042 | MY23 | India/Karnataka | E(BM) | FYGT | Yellow | Plain | Greenish yellow | Elongated oval |
| BMI-0043 | MW13 | India/Karnataka | E(BM) | White | Creamish white | Plain | White | Oval |
| BMI-0044 | MHMP(W) | India/Karnataka | E(BM) | White | Creamish white | Plain | White | Elongated oval |
| BMI-0045 | MHMP(Y) | India/Karnataka | E(BM) | FYGT | Yellow | Plain | Greenish yellow | Elongated oval |
| BMI-0046 | P4D3 | India/Karnataka | E(OB) | FYGT | Yellow | Marked | Greenish yellow | Elongated oval |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| BME-0047 | NISTID(Y) | Bangladesh | E(OB) | FYGT | Yellow | Plain | Golden yellow | SA |
| BME-0048 | NISTID(W) | Bangladesh | E(OB) | White | Creamish white | Plain | White | SA |
| BME-0049 | NK4 | Japan | E(OB) | Light yellow | Light yellow | Marked | Yellow | SA |
| BME-0050 | CAMBODG | Japan | O(OB) | Creamish white | Light yellow | Marked | Yellow | SA |
| BME-0052 | DAIZO | China | O(OB) | FYGT | Yellow | Marked | Greenish yellow | Spindle |
| BMI-0053 | LMP | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | SA |
| BMI-0054 | DMR | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | Oval |
| BMI-0055 | LMO | India/West Bengal | E(BM) | FYGT | Yellow | Marked | Golden yellow | Oval |
| BMI-0056 | MY1(SL) | India/Karnataka | E(BM) | FYGT | Yellow | Mixed | LGY | Elongated |
| BMI-0057 | PM(SL) | India/Karnataka | E(BM) | FYGT | Yellow | Mixed | LGY | Elongated oval |
| BMI-0058 | BL23 | India/Karnataka | E(BCU) | Light yellow | FYGT | Plain | Greenish yellow | ENC |
| BMI-0059 | BL24 | India/Karnataka | E(BCU) | Light yellow | FYGT | Plain | Greenish yellow | ENC |
| BMI-0060 | MU303 | India/Karnataka | E(BM) | Light yellow | FYGT | Plain | Greenish yellow | ENC |
| BMI-0061 | MU520 | India/Karnataka | E(BM) | Light yellow | Colourless | Plain | White | Oval |
| BMI-0062 | MU10 | India/Karnataka | E(BM) | Light yellow | Colourless | Plain | White | Oval |
| BMI-0063 | TW x SK6 x SK1 | India/Tamil Nadu | E(BM) | Light yellow | Colourless | Plain | White | DB |
| BMI-0064 | SK6 x SK1 x TW | India/Tamil Nadu | E(BM) | Light yellow | Colourless | Plain | White | DB |
| BMI-0065 | BL43 | India/Karnataka | Evolved | Light yellow | Colourless | Plain | Greenish yellow | Elongated |
| BMI-0066 | APM-1 | India/Andhra Pradesh | Evolved | Yellow | Colourless | Plain | Greenish yellow | Oval |
| BMI-0067 | SLKSPM | India/Karnataka | Evolved | Light yellow | Colourless | Mixed | Greenish yellow | Elongated |
| BMI-0068 | M12(W) | India/West Bengal | Evolved | Light yellow | Colourless | Plain | White | Spatulate |
| BMI-0069 | M15 | India/West Bengal | Evolved | Light yellow | Colourless | Plain | White | Spatulate |
| BMI-0070 | M6DP(C) | India/West Bengal | Evolved | Light yellow | Colourless | Plain | Chrome yellow | Spatulate |
| BMI-0071 | M6DP(C)Green | India/West Bengal | Evolved | Light yellow | Light yellow | Plain | White | Spatulate |
| BMI-0072 | M6M81 | India/West Bengal | Evolved | Light yellow | Light yellow | Plain | Chrome yellow | Oval |
| BMI-0073 | BL-67 | India/Karnataka | Evolved | Light yellow | Colourless | Plain | Greenish yellow | Oval |
| BMI-0074 | MH-1 | India/Karnataka | Evolved | Light Yellow | Light yellow | Marked | Creamy White | Spindle |

The neonates were brushed upon hatching and were reared at standard rearing conditions (Krishnaswami, 1978) [18]. After third moult 250 larvae were counted and kept in 3 replications. The economic parameters like fecundity, hatching percentage, 10 larval weight, total larval duration, fifth age larval duration, effective rate of rearing by number, effective rate of rearing by weight, pupation rate, single cocoon weight, single shell weight, shell ratio and cocoon yield/ 100 dfls were recorded (Table-2) for analysis.

**Table 2:** The growth and economic traits of 74 multivoltine genetic resources alongwith Amylase quantity.

| Accession No. | Fecundity (No.) | Hatching (%) | Weight of 10 larvae g. | Total larval duration h. | Fifth age larval duration h. | ERR by No. | ERR By Wt. Kg. | Pupation (%) | Single Cocoon weight g. | Single Shell Weight g. | Shell Ratio (%) | Yield / 100 dfls kg. | Amylase quantity in µg /10µl /hour |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BMI-0001 | 432 | 94.0 | 29.89 | 576 | 144 | 9612 | 10.65 | 91.29 | 1.01 | 0.14 | 13.54 | 42.59 | 2050 |
| BMI-0002 | 376 | 90.8 | 31.50 | 504 | 120 | 8679 | 10.05 | 85.12 | 0.93 | 0.12 | 13.56 | 40.10 | 2103 |
| BMI-0003 | 428 | 93.2 | 29.00 | 500 | 116 | 9097 | 11.10 | 88.28 | 0.93 | 0.14 | 16.10 | 44.37 | 2000 |
| BMI-0004 | 342 | 93.6 | 27.00 | 492 | 108 | 9254 | 10.25 | 87.67 | 0.94 | 0.13 | 13.83 | 40.88 | 2100 |
| BME-0005 | 438 | 93.6 | 25.50 | 492 | 108 | 9379 | 10.10 | 92.37 | 0.86 | 0.12 | 14.14 | 40.32 | 1900 |
| BMI-0006 | 421 | 89.0 | 26.10 | 492 | 108 | 9780 | 10.25 | 96.33 | 0.90 | 0.13 | 14.38 | 40.88 | 1875 |
| BMI-0007 | 436 | 92.6 | 31.35 | 504 | 120 | 9473 | 12.45 | 92.66 | 1.06 | 0.18 | 16.56 | 49.80 | 1950 |
| BMI-0008 | 387 | 91.6 | 27.44 | 504 | 120 | 9791 | 10.00 | 94.94 | 0.90 | 0.13 | 14.35 | 40.04 | 1975 |
| BMI-0009 | 381 | 92.8 | 28.98 | 504 | 120 | 9547 | 11.10 | 93.38 | 0.92 | 0.13 | 13.79 | 22.32 | 2050 |
| BMI-0010 | 402 | 91.3 | 24.02 | 504 | 120 | 9733 | 10.15 | 96.39 | 0.91 | 0.11 | 12.22 | 40.66 | 2000 |
| BMI-0011 | 335 | 91.0 | 26.60 | 504 | 120 | 9720 | 10.55 | 94.34 | 0.92 | 0.13 | 14.75 | 42.25 | 2250 |
| BME-0012 | 436 | 94.5 | 26.56 | 576 | 144 | 9707 | 10.95 | 96.27 | 0.95 | 0.14 | 15.19 | 43.78 | 2150 |
| BME-0013 | 390 | 87.6 | 24.74 | 536 | 116 | 9712 | 10.45 | 94.42 | 0.76 | 0.10 | 12.89 | 41.93 | 1900 |
| BMI-0014 | 345 | 90.4 | 22.71 | 516 | 132 | 9804 | 11.30 | 97.13 | 0.92 | 0.14 | 15.29 | 45.04 | 1975 |
| BME-0015 | 441 | 93.2 | 26.41 | 516 | 132 | 9567 | 10.15 | 93.32 | 0.80 | 0.11 | 13.99 | 40.60 | 1650 |
| BMI-0016 | 295 | 92.2 | 26.73 | 500 | 116 | 9683 | 10.60 | 93.50 | 0.93 | 0.13 | 14.66 | 42.46 | 2053 |
| BMI-0017 | 342 | 95.3 | 25.86 | 500 | 116 | 9747 | 9.80 | 95.44 | 0.86 | 0.11 | 13.48 | 39.19 | 1950 |
| BMI-0018 | 409 | 95.0 | 24.53 | 500 | 116 | 9689 | 10.00 | 95.76 | 0.82 | 0.11 | 14.02 | 40.07 | 2000 |
| BMI-0019 | 449 | 93.5 | 25.69 | 500 | 116 | 9784 | 10.25 | 95.48 | 0.84 | 0.11 | 13.78 | 41.07 | 1950 |
| BMI-0020 | 353 | 90.0 | 23.93 | 528 | 124 | 9686 | 10.05 | 95.61 | 0.80 | 0.12 | 15.10 | 40.20 | 2000 |
| BMI-0021 | 397 | 91.6 | 24.31 | 500 | 96 | 9667 | 9.80 | 95.33 | 0.83 | 0.12 | 14.62 | 39.20 | 1953 |
| BMI-0022 | 306 | 89.9 | 24.83 | 504 | 100 | 9705 | 11.75 | 95.79 | 0.92 | 0.14 | 15.28 | 46.99 | 1975 |
| BMI-0023 | 469 | 87.0 | 25.36 | 528 | 124 | 9640 | 10.55 | 94.73 | 0.95 | 0.16 | 16.73 | 42.16 | 2050 |
| BMI-0024 | 520 | 95.9 | 27.93 | 528 | 124 | 9563 | 10.75 | 89.40 | 0.93 | 0.15 | 16.44 | 42.95 | 1900 |
| BMI-0025 | 424 | 92.9 | 29.57 | 516 | 112 | 9778 | 10.85 | 94.69 | 0.91 | 0.14 | 15.16 | 43.36 | 2103 |
| BMI-0026 | 345 | 92.5 | 29.85 | 516 | 112 | 9484 | 10.05 | 91.52 | 0.98 | 0.15 | 15.83 | 40.30 | 2050 |
| BMI-0027 | 365 | 92.3 | 25.64 | 528 | 144 | 9876 | 10.95 | 97.27 | 1.05 | 0.15 | 14.52 | 43.72 | 2250 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BMI-0028 | 306 | 89.8 | 26.46 | 500 | 116 | 9734 | 9.90 | 94.99 | 0.80 | 0.12 | 15.36 | 39.67 | 2050 |
| BMI-0029 | 361 | 92.2 | 25.11 | 500 | 116 | 9700 | 10.10 | 95.33 | 0.77 | 0.11 | 14.48 | 40.33 | 2050 |
| BME-0030 | 328 | 90.0 | 29.73 | 516 | 132 | 9390 | 11.80 | 92.96 | 0.89 | 0.13 | 14.93 | 47.13 | 2103 |
| BMI-0031 | 369 | 91.9 | 25.36 | 492 | 108 | 9752 | 10.05 | 96.19 | 0.76 | 0.11 | 15.05 | 40.19 | 1950 |
| BMI-0032 | 305 | 90.1 | 24.87 | 492 | 108 | 9650 | 9.75 | 94.17 | 0.98 | 0.15 | 15.25 | 39.13 | 1875 |
| BMI-0033 | 376 | 88.9 | 28.16 | 486 | 102 | 9800 | 10.00 | 96.87 | 0.97 | 0.13 | 14.07 | 39.92 | 2150 |
| BMI-0034 | 424 | 94.9 | 28.20 | 486 | 102 | 9718 | 10.05 | 93.89 | 0.99 | 0.16 | 15.99 | 40.17 | 2103 |
| BMI-0035 | 367 | 92.9 | 27.09 | 486 | 102 | 9768 | 9.95 | 94.71 | 0.91 | 0.12 | 13.26 | 39.80 | 2050 |
| BMI-0036 | 411 | 88.6 | 26.16 | 486 | 102 | 9669 | 10.25 | 94.97 | 0.94 | 0.13 | 13.86 | 41.14 | 2103 |
| BMI-0037 | 468 | 93.3 | 28.42 | 486 | 102 | 9746 | 10.05 | 96.06 | 1.01 | 0.10 | 11.04 | 40.18 | 1953 |
| BMI-0038 | 396 | 87.2 | 30.80 | 486 | 102 | 9666 | 9.95 | 94.87 | 0.92 | 0.13 | 14.52 | 39.85 | 2150 |
| BMI-0039 | 482 | 91.3 | 28.31 | 486 | 102 | 9677 | 10.15 | 95.20 | 0.95 | 0.14 | 15.02 | 40.62 | 2050 |
| BMI-0040 | 450 | 90.4 | 28.38 | 500 | 116 | 9699 | 10.20 | 94.99 | 0.98 | 0.13 | 13.83 | 40.84 | 2050 |
| BMI-0041 | 454 | 93.4 | 28.93 | 500 | 116 | 9679 | 10.65 | 96.25 | 1.02 | 0.15 | 14.64 | 42.64 | 2100 |
| BMI-0042 | 357 | 93.0 | 20.48 | 500 | 116 | 9619 | 10.10 | 93.00 | 0.89 | 0.13 | 14.94 | 40.29 | 2150 |
| BMI-0043 | 445 | 93.2 | 28.65 | 500 | 116 | 9544 | 11.10 | 94.14 | 1.07 | 0.17 | 15.72 | 44.49 | 2103 |
| BMI-0044 | 421 | 95.7 | 28.99 | 492 | 108 | 9747 | 10.50 | 95.70 | 1.03 | 0.15 | 14.57 | 42.07 | 1953 |
| BMI-0045 | 358 | 89.1 | 28.71 | 492 | 108 | 9776 | 10.35 | 95.66 | 0.99 | 0.14 | 14.38 | 41.31 | 2100 |
| BMI-0046 | 441 | 91.0 | 29.20 | 492 | 108 | 9691 | 10.55 | 95.07 | 1.09 | 0.15 | 13.32 | 42.19 | 2050 |
| BME-0047 | 315 | 88.7 | 23.81 | 492 | 108 | 9583 | 10.45 | 94.29 | 0.81 | 0.12 | 14.59 | 41.88 | 2100 |
| BME-0048 | 346 | 91.3 | 30.91 | 492 | 108 | 9721 | 10.35 | 93.31 | 0.89 | 0.14 | 16.13 | 41.45 | 1980 |
| BME-0049 | 393 | 90.3 | 29.02 | 492 | 108 | 9830 | 10.30 | 97.10 | 0.83 | 0.12 | 14.49 | 41.27 | 2100 |
| BME-0050 | 433 | 91.9 | 27.48 | 492 | 108 | 9657 | 10.80 | 95.12 | 0.92 | 0.13 | 13.78 | 43.21 | 2050 |
| BME-0052 | 460 | 93.3 | 27.41 | 528 | 144 | 9673 | 10.40 | 95.70 | 0.82 | 0.13 | 16.27 | 41.51 | 1650 |
| BMI-0053 | 322 | 86.9 | 27.78 | 500 | 116 | 9753 | 10.10 | 95.89 | 0.85 | 0.12 | 14.41 | 40.34 | 2050 |
| BMI-0054 | 366 | 90.3 | 25.54 | 500 | 116 | 9471 | 10.25 | 88.82 | 0.91 | 0.14 | 16.04 | 40.97 | 2100 |
| BMI-0055 | 474 | 92.2 | 29.20 | 500 | 116 | 9648 | 10.45 | 95.37 | 1.00 | 0.15 | 15.02 | 41.82 | 2100 |
| BMI-0056 | 400 | 93.2 | 25.55 | 500 | 116 | 9696 | 10.20 | 94.43 | 0.96 | 0.14 | 14.54 | 40.83 | 2000 |
| BMI-0057 | 359 | 87.5 | 25.65 | 500 | 116 | 9707 | 10.55 | 96.00 | 0.89 | 0.13 | 14.91 | 42.17 | 2100 |
| BMI-0058 | 460 | 90.7 | 27.39 | 486 | 102 | 9817 | 10.90 | 96.71 | 0.91 | 0.13 | 14.05 | 43.67 | 1950 |
| BMI-0059 | 471 | 90.8 | 28.57 | 486 | 102 | 9635 | 11.10 | 95.00 | 0.90 | 0.13 | 14.97 | 44.31 | 1950 |
| BMI-0060 | 360 | 90.6 | 27.33 | 486 | 102 | 9792 | 10.60 | 95.00 | 0.94 | 0.14 | 15.24 | 42.39 | 1950 |
| BMI-0061 | 384 | 91.9 | 29.88 | 516 | 132 | 9699 | 10.45 | 94.65 | 1.07 | 0.17 | 16.15 | 41.65 | 1950 |
| BMI-0062 | 381 | 89.0 | 28.84 | 500 | 116 | 9763 | 10.25 | 94.84 | 0.96 | 0.15 | 16.22 | 41.10 | 1953 |
| BMI-0063 | 363 | 92.2 | 30.18 | 486 | 102 | 9766 | 10.60 | 94.99 | 0.81 | 0.10 | 12.88 | 42.39 | 1950 |
| BMI-0064 | 391 | 93.3 | 25.98 | 486 | 102 | 9833 | 11.05 | 96.63 | 0.79 | 0.11 | 13.89 | 44.26 | 1875 |
| BMI-0065 | 450 | 94.5 | 31.23 | 516 | 132 | 9767 | 8.45 | 96.14 | 0.98 | 0.17 | 17.51 | 33.91 | 1900 |
| BMI-0066 | 378 | 92.5 | 31.77 | 516 | 132 | 9712 | 10.95 | 95.88 | 1.07 | 0.17 | 16.43 | 43.87 | 2100 |
| BMI-0067 | 444 | 93.6 | 29.68 | 596 | 164 | 9595 | 10.50 | 94.74 | 0.91 | 0.13 | 14.38 | 42.07 | 1950 |
| BMI-0068 | 432 | 94.9 | 23.18 | 528 | 144 | 9810 | 10.80 | 96.35 | 0.82 | 0.13 | 15.67 | 43.12 | 1650 |
| BMI-0069 | 449 | 92.6 | 24.25 | 528 | 144 | 9567 | 10.85 | 94.12 | 0.84 | 0.14 | 16.50 | 43.34 | 1875 |
| BMI-0070 | 345 | 90.0 | 23.66 | 528 | 144 | 9704 | 10.80 | 96.11 | 0.95 | 0.16 | 16.64 | 43.26 | 1600 |
| BMI-0071 | 455 | 96.0 | 21.77 | 528 | 144 | 9651 | 10.15 | 93.93 | 0.83 | 0.14 | 16.46 | 40.61 | 1650 |
| BMI-0072 | 385 | 91.4 | 25.92 | 500 | 116 | 9800 | 10.30 | 96.03 | 0.90 | 0.13 | 15.01 | 41.31 | 1898 |
| BMI-0073 | 342 | 93.3 | 30.49 | 500 | 116 | 9686 | 10.70 | 93.86 | 0.90 | 0.16 | 17.63 | 42.89 | 1950 |
| BMI-0074 | 488 | 95.9 | 34.10 | 528 | 144 | 9698 | 9.95 | 93.74 | 1.25 | 0.22 | 18.04 | 39.89 | 1650 |

The amylase estimation was carried by using the fourth day old fifth instar larvae starved for 2 hours and kept in a clean container with dried tobacco leaves on the plastic paper over the mulberry leaves. After 20 minutes, regurgitated digestive juice was collected in syringes and stored the sample at –20º C to avoid oxidation. Centrifuged at 5000 rpm in 4º C to get clear solution, this is used for further analysis. DNS was added to give colour (reddish orange) and to stop the reaction of enzyme over the substrate. 10 µl of digestive juice was taken in test tubes and 2ml of 0.2 per cent starch solution was added and incubated at 37 º C for 60 minutes. Then 2ml of DNSA solution was added to each tube and kept in hot water bath 5 minutes. The optical density (OD) values were measured at 525nm (Manickam and Sadasivam, 1992) [20].

Further to determine concentration of maltose, serial dilutions were prepared from the stock solution of maltose from 100µl upto 2ml. For each sample of 2ml, 2ml of DNSA was added and kept for 5 minutes in water bath. After cooling, recorded the OD values of the serially diluted maltose solutions at 525nm. A standard graph was prepared by plotting OD values against the serial dilutions. The amylase activity was calculated using the standard graph and expressed as µg/100ml of digestive juice in 60 minutes. The amylase activity values obtained were correlated with the growth, productive and economic traits (Table-3) using SPSS statistical packages. The amylase quantity for all the 73 MV accessions studied were represented graphically (Fig 1-3).

**Table 3:** Correlation of growth and economic traits with digestive amylase activity

| | Fecundity | Hatching | Weight of 10 larvae | Total Larval duration | Fifth age larval duration | ERR by No | ERR by Wt. | Pupation | Single cocoon weight | Single shell weight | Shell Ratio | YIELD/ 100 dfls | Amylase activity mg/ml/h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fecundity | 1 | | | | | | | | | | | | |
| Hatching | 0.408** | 1 | | | | | | | | | | | |
| Weight of 10 larvae | 0.129 | 0.012 | 1 | | | | | | | | | | |
| Total Larval duration | 0.210 | 0.248* | -0.383** | 1 | | | | | | | | | |
| Fifth age larval duration | 0.197 | 0.322** | -0.272* | 0.858** | 1 | | | | | | | | |
| ERR by No. | -0.020 | -0.068 | -0.150 | -0.057 | -0.065 | 1 | | | | | | | |
| ERR by Wt. | 0.005 | -0.037 | 0.030 | 0.147 | 0.129 | -0.114 | 1 | | | | | | |
| Pupation | 0.011 | -0.170 | -0.139 | -0.061 | -0.026 | 0.870** | -0.036 | 1 | | | | | |
| Single cocoon weight | 0.261* | 0.168 | 0.465** | 0.058 | 0.122 | -0.050 | 0.133 | -0.086 | 1 | | | | |
| Single shell weight | 0.217 | 0.198 | 0.442** | 0.185 | 0.312** | -0.043 | 0.147 | -0.113 | 0.808** | 1 | | | |
| Shell Ratio | 0.077 | 0.144 | 0.219 | 0.232* | 0.379** | -0.033 | 0.101 | -0.107 | 0.246* | 0.762** | 1 | | |
| Yield/100 dfls | 0.035 | -0.068 | -0.046 | 0.113 | 0.073 | -0.011 | 0.576** | 0.028 | 0.092 | 0.148 | 0.157 | 1 | |
| Amylase activity mg/ml/h | -0.280* | -0.308** | 0.135 | -0.204 | -0.355** | -0.099 | 0.070 | -0.087 | 0.165 | -0.067 | -0.282* | 0.004 | 1 |

** Significant at 0.01 level (2 tailed) P = 0.01 n = 73

## 3. Results and Discussion

The productive and economic traits of the 73 multivoltine accessions revealed good variability. The fecundity varied from 520 (BMI-024) to 295(BMI-016) numbers/ dfl with hatching of 96% (BMI-071) to 86.9 % (BMI-053). The weight of 10 grown larvae was high 34.1 g in accession BMI-074 and it was low in BMI-042 (20.5g). Accession BMI-001 had the longest total larval duration (596 h) and BMI-034 (486h.) had the least larval duration. The fifth age larval duration was longer (164 h) in accessions BMI-067 and it was shorter in accession BMI-021 (96 h.). The effective rate of rearing by number was high in accession BMI-027 (9876) and it was low in BMI-002 (8679). The effective rate of rearing by weight was higher 12.5 kg in accession BMI-007 whereas it was low in accession BMI-065 with 8.5 kg. Accession BMI-027 showed the highest pupation (97.3%) and it was low in accession BMI-002 (85.1 %). The cocoon weight was higher in accession BMI-074 (1.25g.) and it was low in accession BMI-031 (0.76g.). The shell weight (0.22g.) and shell ratio (18.04 %) were higher in accession BMI-074 and they were low in accession BMI-037 with 0.10g and 11.04 % respectively. The cocoon yield per 100 dfls was higher in accession BMI-007 with 49.8 kg and it was low in accession BMI-009 (22.3). The amylase quantity varied from 2250 in µg /10µl /hour in accession BMI-011 and BMI-027 to 1600 µg /10µl /hour in accession BMI-074. The variability of the amylase quantity among the 73 accessions studied was graphically represented in Fig: 1-3.

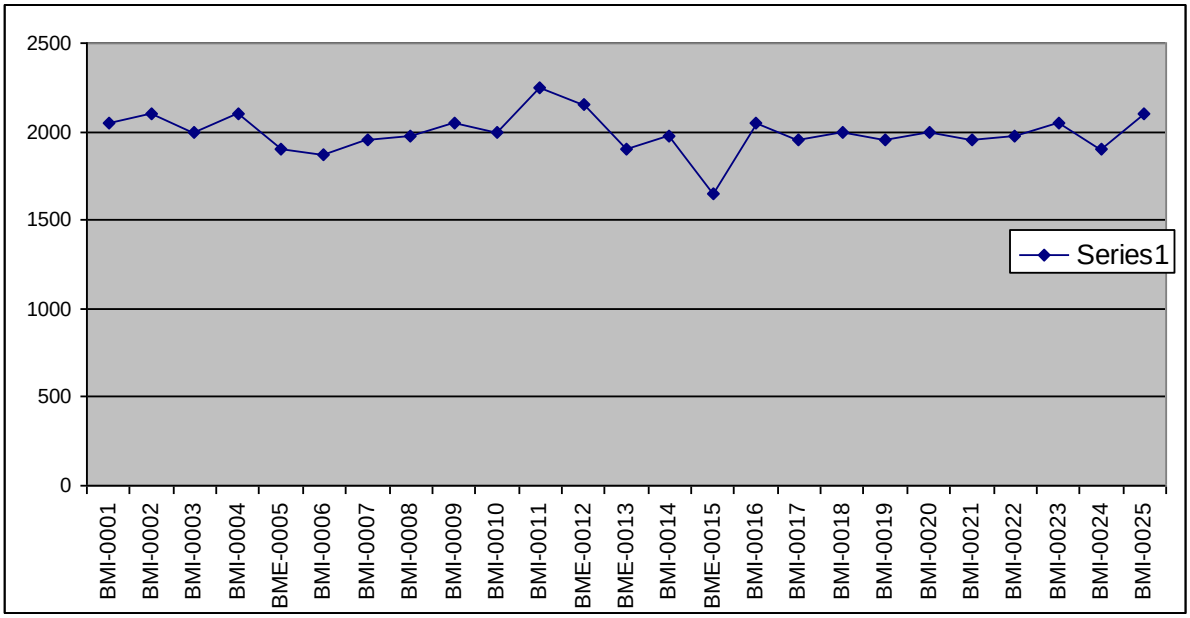


**Fig 1:** Amylase activity of MV accessions (1-25 Nos.).

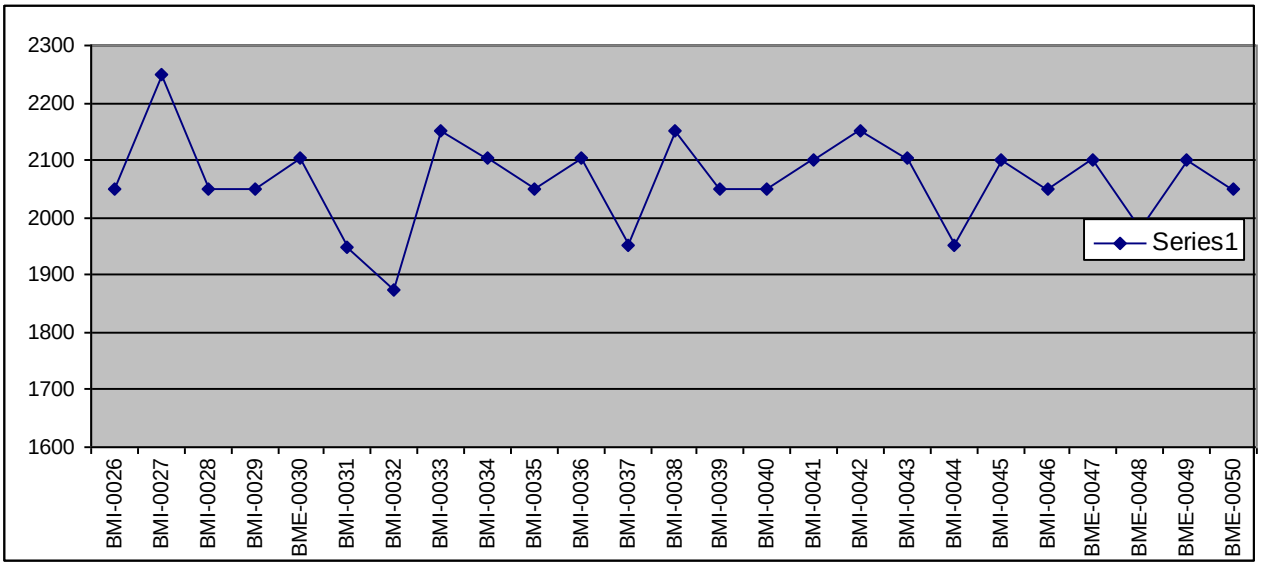


**Fig 2:** Amylase activity of MV accessions (26-50 Nos.)

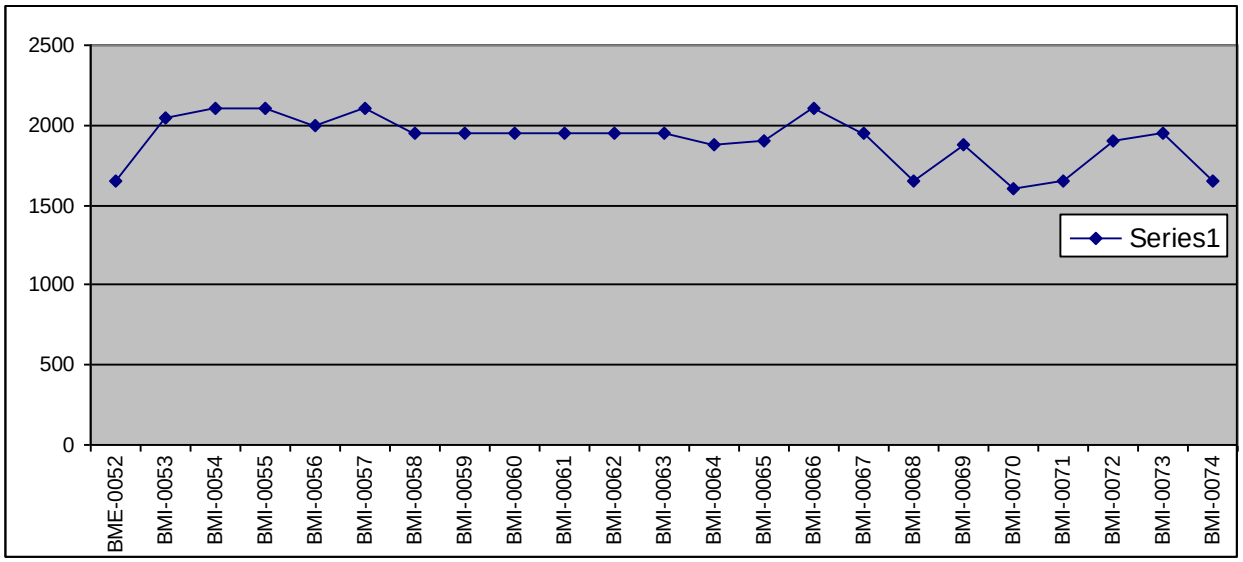


**Fig 3:** Amylase activity of MV accessions (52-74 Nos.).

The digestive amylase quantity was estimated among the 73 multivoltine accessions studied varied from a highest value of 2250 µg /10µl /hour to a lowest value of 1600 µg /10µl /hour by following the standard protocols. There is quite a high amount of variability among the accessions studied. The highest values of amylase activity was recorded in the accessions BMI-0011 and BMI-0027 (2250 µg /10µl /hour) followed by BME-0012, BMI-0033, BMI-0038, BMI-0042 (2150 µg /10µl /hour). Accessions BME-0030, BMI-002, BMI-0025, BMI-0034, BMI-0036 and BMI-0043 (2103 µg /10µl /hour). Nine accessions had 2100 µg /10µl /hour amylase activity viz., BME-0047, BME-0049, BMI-0004, BMI-0041, BMI-0045, BMI-0054, BMI-0055, BMI-0057 and BMI-0066. Accession BMI-0016 had 2053 µg /10µl /hour of amylase activity followed by twelve accessions BME-0050, BMI-001, BMI-0009, BMI-0023, BMI-0026, BMI-0028, BMI-0029, BMI-0035, BMI-0039, BMI-0040, BMI-0046 and BMI-0053. Five accessions BMI-0003, BMI-0010, BMI-0018, BMI-0020 and BMI-0056 showed 2000 µg /10µl /hour of amylase activity. The results observed are in concurrence with the results of differential digestive amylase activity among the silkworm strains recorded earlier by Hirata (1971) [12].

The multivoltine accession BMI-0070 showed the least amylase activity of 1600 µg /10µl /hour followed by five accessions BMI-0074, BMI-0071, BMI-0068, BME-0052 and BME-0015. Accessions BMI-0069, BMI-0064, BMI-0032, BMI-0006 revealed amylase activity of 1875 µg /10µl /hour and it was 1898 µg /10µl /hour in the case of accession BMI-0072. Similarly 23 accessions were in the range of 1980 to 1900 µg /10µl /hour of amylase activity. Accession BME-0048 showed 1980 µg /10µl /hour followed by three accessions BMI-008,BMI-0014, BMI-0022 with amylase activity values of 1975 µg /10µl /hour and then by four more accessions BMI-0021, BMI-0037, BMI-0044 and BMI-0062. The activity of amylase was 1950 µg /10µl /hour in eleven accessions BMI-0007, BMI-0017, BMI-0019, BMI-0031, BMI-0058, BMI-0059, BMI-0060, BMI-0061, BMI-0063, BMI-0067 and BMI-0073 followed by four accessions BME-0005, BME-0013,BMI-0024 and BMI-0065.

The relation between digestive amylase activity and economic traits among the silkworm races have been reported (Hirata, 1971) [13]. Some of the ruling hardy popular multivoltine breeds like Pure Mysore (BMI-0001), Sarupat (BMI-0002), Moria (BMI-0003) and Nistari (BMI-0017) were in the high digestive amylase activity category 2050, 2103, 2000, and 1950 µg /10µl /hour respectively. The high yielding accessions BMI-0070 and four accessions BMI-074 BMI-0081, BME-0052 and BME-0015 showed very least quantity of amylase activity 1600, 1650 µg /10µl /hour respectively where as some of the low yielding accessions like BMI-027 and BMI-011 had highest quantity of amylase of 2250 µg /10µl /hour which is in concurrence with the earlier studies conducted by Chatterjee *et al.,* (1992) [9] and Patnaik *et al.,* (1995) [22]. These accessions could be used as resource materials for inclusion in breeding programmes oriented towards amylase specific breeds and also in hard and robust breeds for the tropical regions to improve the silk productivity.

Correlation between amylase activity in 73 multivoltine silkworm genetic resources with that of the 12 productive traits (table-3) revealed positive and non-significant correlations between weight of larvae (g), ERR by weight (kg.) and single cocoon weight (g) and yield/100dfls (kg.) where as non-significant negative correlations were observed in the traits like total larval duration (h), ERR by number (no.) pupation rate (%) and single shell weight (g). There were negative and significant correlations at 5 % level in the case of fecundity (no.) and shell ratio (%) and significant negative correlations at 1 % level was observed in the traits like hatching (%), fifth age larval duration (h). The studies in line with that of the earlier studies by Chatterjee *et al.* (1988; 1989; 1990; 1993) [6-10].

The results obtained indicated there is much variability in the quantum of amylase recorded among the 73 multivoltine accessions studied as per the standard procedures which is in line with the earlier studies on the importance of amylase *viz*., Chatterjee *et* al., (1992) [9]; Abraham *et al.,* (1992) [1]; Patnaik and Datta, (1995) [22].

Datta and Ashwath (2000) [11] reported that the prospects of using digestive amylase as a marker for improving the viability in silkworm as it contributes for better digestibility and has close association with survival. Therefore the multivoltine accessions showing higher digestive amylase activity could be used as breeding resource materials for developing hardy breeds which could have higher abiotic stress tolerance and can digest even the mediocre/inferior quality of leaves which are bound to available in the not so conducive seasons also by withstanding stress conditions and higher survival.

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