JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

REVIEW ON BIO-CHEMICAL PARAMETERS IN EFFECTIVE GROWTH ON SILKWORM

[1]Arumugavel.S, [2] Babila Jasmine .J

[1]Research Scholar, Department of Zoology, St. Xavier's college, Palayamkottai. [2]Assistant Professor, Department of zoology, St. Xavier's College, Palayamkottai.

Abstract: Improving the quantity and quality of silk produced by the silk worm, using exogenous nutrients and minerals has been practiced as a traditional scientific method in the sericulture industry. The present study is to search base line data of significance of some important biochemical parameters in silk yield. Bio-chemical parameters are very essential to understand the inbuilt mechanism of any organism and silkworm is no exceptional. The review finding prominence the roles of biochemical parameters for better yield of silk.

Key words: Biochemical parameters, Silkworm, silk production.

I. INTRODUCTION

Sericulture industry deals with the production of silk which plays an important role in the rural economics of the developing countries like India and China. Six million people in India alone are engaged in sericulture and the main source of improving rural economy. However, the sericulture industry mainly faces the constraints like diseases to mulberry and silkworm and abiotic stresses which leads to poor yields both quality and quantity wise. Nutrition is the most important and very much essential component for all the living being. Without nutrition or nutritious food, it is impossible for any living organism to survive and attain a healthy disease-free growth. Like other organisms' silkworms are also very much sensitive towards nutritional factor. It is well known that the amount and quality of leaves affects growth rate, developmental period, body weight and survival rate of larvae, as well as influencing the subsequent fecundity, longevity, movement and competitive ability of the adults. If the optimum required nutrients are not present in the leaves then silkworm acquires diseases and the percentage of effective rate of rearing will be decreased as the mortality rate increases in silkworm which is reared in poor foodstuff.

Nutritional requirements are the chemical factors of ingested food is essential for normal metabolism and development of the insect. In general, there is an evidence that nutritional requirements of young insects may vary with sex, changes with developmental stage. Insects generally have the same nutritional requirements as large animals. The balance of nutrients is very important in most studied insects. Balance may be important due to small body size.

Insects may respond to imbalance diet in one of the three ways- They can alter the total amount of ingested food; they can move from one food to another with a different nutrient balance; or they can regulate the effectiveness of the nutrients. The required essential nutrients such as carbohydrates, proteins, lipids, vitamins and amino acids, water etc., are generally present in the natural food plants of the species.

The main objective of the present review is to analyse the significance of biochemical parameters on the quality and quantity of the silkworm.

Need for adequate nutrition

There is great need to develop resistance to biotic and abiotic stresses in mulberry and silkworm to augment productivity and quality which can be achieved by the application of sophisticated tool like biotechnology. Silkworm *Bombyx mori* is completely domesticated tiny insect with economic significance. It mainly feeds on mulberry leaves, although *Tricuspid cudrania* leaves can also act as the food of the larvae (Li, 1987). Due to centuries of this domestication, it has lost much of its natural resistance and shows neither morphological nor behavioural adaptations to escape predators, parasites, pests and pathogens. Diseases in silkworm *Bombyx mori* are fairly common in occurrence and are serious in inflicting losses. Silkworm breeds within a voltine group also vary widely in many of the quantitative traits. The multivoltine breeds are known for their poor productivity and higher survival. However, advancement in breeding techniques resulted in the improvement of quantitative traits with better silk yield in multivoltine breeds and resulted in the development of productive multivoltine breeds (Marvyn *et al.,* 2016; Larsson *et al.,* 2016).

The production of digestive enzymes is often dependent to a large extent upon the type of food consumed by the insets. In the silkworm, *Bombyx mori*, the presence of intestinal proteinases has been reported by Shinoda (1930) and several investigations have been done in this direction mainly in Japan (Horie *et al*., 1963; Eguchi and Yoshitake, 1967; Hamano and Mukaiyama, 1970). Enzymes provide the

energy needed for metabolic reactions essential to immune health. Enzymes have been shown to stimulate the body's natural defences while breaking down offending pathogenic immune complexes. This helps in relieving stress on the body and strengthen the immune system, which can speed the healing process.

Biochemical parameters

Bio-chemical parameters are very essential to understand the inbuilt mechanism of any organism and silkworm is no exceptional (Senthil Nathan *et al*., 2006; Chandrasekaran *et al.,* 2012; Chandrasekaran *et al.,* 2014; Djebbi *et al.,* 2016; Hara *et al.,* 2016) Proteins are essential molecules used in all types of cellular activity and life. Enzymes, antibodies, regulatory molecules, contractile molecules responsible for movement, hormones, cellular structural components are some of a few of the proteins needed to sustain life. Almost all proteins in the body are in continuous form of dynamic: breakdown (catabolism) and formation (anabolism).Thus, in order to maintain vitality, viability and health the silkworm pupae must have a supply of building blocks to help replenish the proteins that are broken down. Considering that even the proteins involved in regulating and making other proteins need also to be made and replenished, protein in the diet (i.e., amino acids) is an obligatory demand by the silkworm. Proteins contain different amino acids and in various proportions.

Some amino acids may be absent or only in a trace amounts in a particular protein. In the synthesis of proteins, if one essential amino acid is missing, then the cell will stop the process of synthesizing the protein and the result is a truncated polypeptide. Vijayaragavan *et al*. (2010) this is because the amino acid is not provided in the diet, and the cell cannot synthesise it. Thus, the lack of a single essential amino acid, a potentially vital protein will not be synthesized.

Additionally, proteins are needed to help provide the nitrogen necessary for the synthesis of other nitrogenous molecules, including the nonessential amino acids. This is accomplished by the proteins in the diet that the body breaks down into amino acids that can be incorporated into the building process of new molecules.

Significance of Enzymes

Enzymes are large biological molecules responsible for the thousands of biochemical inter-conversions that sustain life. Enzymes serve a wide variety of functions, act as catalysts and help in complex reactions occur everywhere in life. Without enzymes, metabolism would neither progress through the same

steps, nor be fast enough to serve the needs of the cell. In the absence of enzymes, this occurs so slowly or insignificant. Metabolic pathways within each cell depend on the set of functional enzymes that are present. Enzymes can also reduce free radical damage, inhibit pathogens and potentially provide support for the most challenging health disorders. The biochemical studies are highly valuable to understand the genetic variation in natural population and have been used as useful indicator in plant and animal breeding programmes (Tanksley *et al*., 1982). Among many digestive enzymes, protease enzyme in silkworm plays a key role in converting the mulberry protein to silk protein. It has been reported that the protease enzyme activity was observed to be higher.

Significance of Proteases

Proteases and serine proteases homologs (SPHs) are a large group of proteolytic enzymes, with important roles in a variety of physiological processes, such as cell signalling, defence and development. Serine proteases constitute nearly one-third of all the known proteolytic enzymes and modulate the bioactivity of target proteins in a timely manner through proteolytic cleavage (Tripathi *et al*., 2008). When these serine proteases are no longer in need, they will be inactivated by serine protease inhibitors (SPIs). Many SPIs from insects have been purified from the integument, genital tract, salivary gland, and heamolymph. SPIs not only play key roles in insect digestion, metamorphosis and development, but also are important components of immune system (Abraham *et al*., 2005; Anderson *et al.*, 2010; Coleman *et al*., 1995). SPIs are named as trypsin inhibitors (TIs), chymotrypsin inhibitors (CIs), elastase inhibitors (EIs) and subtilisin inhibitors (SIs) according to their different inhibitory target proteases (Friedrich *et al*., 1993; Saha and Joy, 2016; Ligoxygakis *et al*., 2003). SPIs also can be classified into three types, namely, canonical inhibitors, non-canonical inhibitors, and serpins based on the mechanism of action (Reichhart *et al*., 2005; Otlewski *et al*., 1999). Canonical inhibitors are usually small proteins (14,200 amino acid residues) and can bind to protease through an exposed convex binding loop.

Significance of Amylase

Amylase is one of the key enzymes involved in digestion and carbohydrate metabolism in insects (Ishaaya and Swirski, 1970; Buonocore *et al*., 1976; Horie and Watanabe, 1980). Amylase refers to a group of enzymes whose catalytic function is to hydrolyze sugar and starch. Chatterjee *et al*., (1989) reported that

the presence of two different forms of amylase activity in digestive fluid and haemolymph. Abraham *et al*., (1992) noticed that the amylase activity of digestive fluid was 40 fold higher than that of haemolymph.

Christopher *et al.*, (1985) suggested that the rational food consumped by a lepidopteran larvae correlated directly with the activity of amylase. The larva which received 100% food found to have the highest amylase activity, which declined as the percentage of food offered was reduced. He also suggested that the balanced food consumption by a lepidopteran larvae correlated directly with the activity of amylase.

Significance of Lipases

Lipases are among the group of proteins that regulate several invertebrate immune defences, conceivably acting directly against invading microorganisms. Lipase activities were also described in the fat body of some insects, where they are important for the mobilization of stored lipids. Information on lipid digestion and absorption in insects is scarce compared to the amount of data published on lipid requirement or composition. Some of the analogies found between insect and mammalian lipid metabolism have suggested that the processes of digestion and absorption in insects might be similar to those in mammals. Triacylglycerol (TAG) constitutes a major lipid component of the diet and the main form for fatty acid storage (Arrese *et al*., 2001). After a meal, TAG is hydrolysed in the mid-gut lumen, products of digestion are absorbed and, at mid-gut epithelium, used for the synthesis of complex lipids, such as TAGs, diacylglycerols (DAG) and phospholipids (PL) (Tsuchida and Wells, 1988; Turunen, 1993; Turunen and Crailsheim, 1996; Canavoso *et al*., 1995).

Products of the digestive process are mainly used inside the mid-gut epithelium for de novo synthesis, via the phosphatidic acid or the monoacylglycerol (MAG) pathways (Arrese *et al*., 2001; Canavoso et al., 2003; Canavoso *et al*., 2004). TAG-lipases are enzymes that preferentially hydrolyze the outer ester links of TAG and act on the water–lipid interface. Available data suggest that in insects these enzymes preferentially release fatty acids from the a-positions (Hoffman and Downer, 1979), prefer unsaturated fatty acids (Weintraub and Tietz, 1973) and are activated by calcium ions (Gilbert *et al*., 1965), thus resembling the action of mammalian pancreatic lipase(Cherry and Crandall, 1932). Information on TAG-lipases in insects has been obtained from several species. Smith *et al*., (1994) showed the presence of lipase activity in the gut of male and female flies, where it presumably acts in the utilization of dietary TAG.

Significance of Zinc

According to Naik and Asana (1961), Zinc deficiency resulted in reduced rate of protein synthesis and increased accumulation of non-protein intermediates in plants. Under zinc deficiency, the uptake of nitrogen, total ash, copper and zinc was relatively less, that of phosphorus almost completely inhibited and manganese slightly increased as compared with control. Zinc deficiency increased the activity of RNA only in leaves. (Ishaaya and Swirski, 1976), conducted an experiment on C7al dose 76 mulberry variety using two doses of copper, zinc and boron along with a basal dose of 300 N: 100, P 205:100, K 20 KG/hac. The yield data showed that zinc treated plots at lower dose provided maximum amount of leaf and the increase over fertilization without micronutrients and NPK alone was 73.9% and 95% respectively. Higher dose treated plot with zinc also yielded maximum amount of leaf and the increase over control and NPK was 73.9% and 12.9% respectively.(Zibaee and Bandani, 2010) With regard to chemical analysis, the percentage of protein was minimum and minerals, starch and total carbohydrates was maximum in control plots.

Significance of Boron

Patnaik (1950) studied the effect of Boron on catalase activity in plants. Carbohydrates and their utilization in the normal physiological metabolic processes in the plants were affected by boron. All the carbohydrate fractions decreased with the increasing concentrations of the element. The results indicate that 0.5ppm boron application in V1 mulberry variety showed better performance in context with fecundity and egg recovery. This information from the results indicates the metabolic enzymes influencing silkworm growth and reproductive characters.

However, the foliar application in S36 mulberry variety showed less fecundity and egg recovery compared to V1 variety. Both the races namely CSR2 and CSR4 have performed differently with the treatment of boron given to them, in respect to their enzyme activity. The reproductive parameters of any living organism are dependent on the quality of food intake with appropriate nutrients.

Conclusion

Sericulture is an ancient agro based ample employment providing area irrespective of gender and age. Research regarding silkworm nutrition experienced a real progress in recent years because of the development and introduction of artificial diets, which lead to the achievement of important benefits in the textile industry. Silkworm requires certain essential sugars, proteins, amino acids, fatty acid, vitamins and

micronutrients for its growth and higher production of good quality of silk. Low productivity is mainly due to inadequacy of nutrients. This review emphasis on the importance of biochemical parameters in silkworm cultivation. Henceforth, the application of tools and tricks of biotechnology in sericulture will bring an explosive growth in mulberry and silkworm and opens new prospects in silk industry.

REFERENCES

(1) Abraham, C., Sheeran, P. and Johnston, M. 1992. From health beliefs to self-regulation: theoretical advances in the psychology of action control. Psychology and Health, 13, 569–591.

(2) Abraham, E**.**, Laterre, P.F. and Garg, R. 2005. *Drotrecogin alfa* (activated) for adults with severe sepsis and a low risk of death. *N Engl J Med.* 353 (13):1332-41.

(3) Anderson, C. A., Shibuya, A., Ihori, N., Swing, E. L., Bushman, B. J., Sakamoto, A. and Salaam, M. 2010. Violent video game effects on aggression, empathy, and prosaically behavior in Eastern and Western countries. Psychological Bulletin, 136-139.

(4) Arrese, E. L., Canavoso, Z. E., Jouni, J. E., Pennington, K., Tsuchida, L. E. and Wells, M. A. 2001. Lipid storage and mobilization in insects: current status and future directions. Insect *Biochem. Mol. Biol*. 31: 7-17.

(5) Arrese, E.L., Flowers, M.T., Gazard, J.L., Wells, M.A., 2001. Calcium and cAMP are second messenger in the adipokinetic hormoneinduced lipolysis of triacylglycerols in *Manduca sexta* fat body. *J. Lipid Res*. 40, 556–564.

(6) Buonocore, V., Caporale, C., De Rosa, M., Gambacorta, A. 1976. Stable, inducible thermoacidophilic α amylase from Bacillus acidocaldarius. *J. Bacterial*. 128: 515-521.

(7) Canavoso, L. E. and Rubiolo, E. R. 1995. Interconversions of lipophorin particles by adipokinetic hormone in hemolymph of *Panstrongylus megistus*, *Dipetalogaster maximus* and *Triatoma infestans* (Hemiptera: Reduviidae). Comp. *Biochem. Physiol*. 112A: 143–150.

(8) Canavoso, L. E., Frede, S. and Rubiolo, E. R. 2004. Metabolic pathways for dietary lipids in the midgut of hematophagous *Panstrongylus megistus* (Hemiptera: Reduviidae). *Insect Biochem. Mol. Biol*. 34: 845–854.

(9) Canavoso, L. E., Stariolo, R. and Rubiolo, E. R. 2003. Flight metabolism in *Panstrongylus megistus* (Hemiptera: Reduviidae): The role of carbohydrates and lipids. Mem. Inst. *Oswaldo Cruz*. 98: 909–914.

(10) Chandrasekaran, R., Revathi, K., Nisha, S., Kirubakaran, S.A., Sathish-Narayanan, S. and SenthilNathan, S. 2012. Physiological effect of chitinase purified from *Bacillus subtilis* against the tobacco cutworm *Spodoptera litura* Fab. *Pesticides Biochemical and Physiology*, 104: 65–71.

(11) Chandrasekaran, R., Revathi, K., Thanigaivel, A., Kirubakaran, S.A. and Senthil-Nathan, S. 2014. *Bacillus subtilis* chitinase identified by matrix-assisted laser desorption/ionization time-of flight/time of flight mass spectrometry has insecticidal activity against *Spodoptera litura* (Fab.). *Pesticide Biochemistry and Physiology*, http://dx.doi.org/doi: 10.1016/j.pestbp.2014.09.013 February 2016.

(12) Chatterjee, I., Kriegeskorte, A., Fischer, A., Deiwick, S., Theimann, N., Proctor, R. A.1989. In vivo mutations of thymidylate synthase (encoded by thyA) are responsible for thymidine dependency in clinical small-colony variants of *Staphylococcus aureus*. *J. Bacteriol.* 190, 834–842.

(13) Cherry, I. S. and Crandall, L. A. 1932. The specificity of pancreatic lipase: its appearance in the blood after pancreatic injury. *American Journal of Physiology-Legacy Content*, 100(2): 266-273.

(14) Christopher, S. A., Li, R. M., Welch, J. S., Reid, P. V., Hobbs, T. F., and Holben, B. 1985. Estimation of surface and top-of-atmosphere (TOA) shortwave irradiance in biomass-burning regions during SCAR-B, *J. Appl. Meteorol*., 39, 1742 – 1753.

(15) Coleman, M.E.,DeMayo, F., Yin, K.C., Lee, H.M., Geske, R..C., Montgomery, R.J. Schwartz, S.1995. Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice Biol. hem. 270 (10), pp. 12109-12116.

(16) Djebbi, M.A., Braiek, M., Hidouri, S., Namour, P., Jaffrezic-Renault, N. and Amara, A.B.H. 2016. Novel biohybrids of layered double hydroxide and lactate dehydrogenase enzyme: synthesis, characterization and catalytic activity studies. *Journal of Molecular Structure*, 1105: 381–388.

(17) Eguchi, M., Yoshitake, N., and Kai, H. 1967. Genetic Studies on Isozymes of the Integument Esterase in the Silkworm, *Bombyx Mori* L *Japan. J. Genet.* 40, 15.

(18) Friedrich B, Gronberg H, Landstrom M, Gullberg M, Bergh A .1993. Differentiation stage specific expression of oncoprotein 18 in human and rat prostatic adenocarcinoma. *Prostate* 27: 102–109.

(19) Gilbert, D., Sundby, B., Gobeil, C., Mucci, A.and Tremblay, G.-H. 1965. A seventy-two-year record of diminishing deep-water oxygen in the St. Lawrence estuary: The northwest Atlantic connection, Limnol. Oceanogr, 50, 1654–1666.

(20) Hamano, K. and Mukaiyama, F. 1970. Some properties of digestive fluid proteinase in the silkworm *Bombyx mori* with references to the relation between dissociation degree and nutritive value of some proteins. *J. Sericult. Sci. Jpn* 39 371-376.

(21) Hara, M., Monna, S., Murata, T., Nakano, T., Amano, S., Nachbar, M. and Wätzig, H. 2016. The *Arabidopsis* KS-type dehydrin recovers lactate dehydrogenase activity inhibited by copper with the contribution of His residues. *Plant Science*, 245: 135–142.

(22) HOFFMAN, A. G. D. AND DOWNER, R. G. H. 1979. SYNTHESIS OF DIACYLGLYCEROLS BY MONOACYLGLYCEROL ACYLTRANSFERASE FROM CROP, MIDGUT AND FAT BODY TISSUES OF THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA* L. *INSECT BIOCHEM.* 9, 129-134.

(23) Horie, Y. and Watanabe, H., 1980. Recent advances in sericulture. Ann. Rev. Entomol., 25: 49-71.

(24) HORIE, Y., TANAKA, M., AND ITO, T. 1963. ELECTROPHORETIC VARIATION OF PROTEINASE IN THE DIGESTIVE JUICE OF THE SILKWORM, *BOMBYX MORI* L *J. SERICULT. SCI. JAPAN*. 32, 8.

(25) Ishaaya, T. and Swirski, E. 1970. Invertase and amylase activity in the armoured scales Chrysomphalus aonidum and Aonidiella auantii. *Journal of Insect Physiology*. 16:1599-1606.

(26) Larsson, S., Jones, H.A., Goransson, O., Degerman, E. and Holm, C. 2016. Parathyroid hormone induces adipocyte lipolysis via PKA-mediated phosphorylation of hormone-sensitive lipase. *Cellular Signalling*, 28(3): 204–213.

(27) Li, J. 1987. Multi-Scale Modeling and Method of Energy Minimization for Particle-Fluid Two-Phase Flow. Doctor thesis, Institute of Chemical Metallurgy, Chinese Academy of Sciences, Beijing. 12, 20.

(28) Ligoxygakis, P., Roth, S., Reichhart, J. M .2003. A serpin regulates dorsal–ventral axis formation in the *Drosophila* embryo. *Curr Biol.* 13: 2097–2102.

(29) Marvyn, P.M., Mardian, E.B., Bradley, R.M., Marks, K.A. and Duncan, R.E. 2016. Data on hepatic lipolysis, adipose triglyceride lipase, and hormone-sensitive lipase in fasted and non-fasted C57BL/6J female mice. *Data in Brief*, 7: 721–725.

(30) Naik, M. S. and Asana, R. D. 1961. Effect of zinc deficiency on the synthesis of protein. mineral outpace and ribo nuclease activity in cotton plant. llUlian *J. Plant Physiogy* 4: 103-111.

(31) Otlewski, J., Whatley, H., Polanowski, A., and Wilusz, T. 1999. Amino acid sequences of trypsin inhibitors from watermelon (Citrullus vulgaris) and red bryony (*Bryonia dioica*). *Biol. Chem. Hoppe-Seyler*. 368, 1505–1507.

(32) Patnaik. U. 1986. INeo-Marxian theories of capitalism and under development ón Towards a critique in P. Patnaik (ed.), Lenin and Imperialismó An Appraisal of Theories and Contemporary Reality, Orient Longman, Delhi,205-209.

(33) Reichhart, J.M., Ferrandon, D., Rämet, M., Ezekowitz, R.A. 2005. Eater, a ransmembrane protein mediating phagocytosis of bacterial pathogens in Drosophila. Cell 123(2): 335-346.

(34) Saha, I. and Joy, V. C. 2016. Short-term biochemical ill effects of insect growth regulator (IGR) pesticides in *Cyphoderus javanus* Borner (Collembola: Insecta) as potential biomarkers of soil pollution. *Environmental Monitoring and Assessment*, 188: 98-102.

(35) Senthil Nathan S., Chunga, P.G. and Murugan, K. 2006. Combined effect of biopesticides on the digestive enzymatic profiles of *Cnaphalocrocis medinalis* (Guenee) (the rice leaffolder) (Lepidoptera: Pyralidae). *Ecotoxicology and Environment Safety*, 64: 382–389.

(36) Smith, M.V, Hazucha, M. J, Benignus, V. A 1994. Effect of regional circulation patterns on observed HbCO levels. J Appl Physiol. 77(4):1659–1665.

(37) Tanksley, S. D., Medina-Filho, M. and Rick, C. M. 1982. Use of naturally-occurring enzyme variations to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity 49 (1):11-25.

(38) Tripathi, L., Odipio, J. and Tripathi, J.N. 2008. A rapid technique for screening banana accessions for resistance to Xanthomonas wilt. European Journal of Plant Pathology 121, 9–19.

(39) Tsuchida, K., Arai, M., Tanaka, Y., Ishihara, R., Ryan, R.O., Maekawa, H., 1998. Lipid transfer particle catalyzes transfer of carotenoids between lipophorins of Bombyx mori. Insect Biochem. Mol. Biol. 28, 927–934.

(40) Turunen, S., 1993. Metabolic pathways in the midgut epithelium of Pieris brassicae during carbohydrate and lipid assimilation. Insect Biochem. Mol. Biol. 23, 681–689.

Turunen, S., Crailsheim, K., 1996. Lipid and sugar absorption. In: Lehane, M.J., Billingsley, P.F. (Eds.), Biology of Insect Midgut. Chapman and Hall, London, pp. 293–320.

Vijayaragavan, C., Sivakumar, C., Kavitha, Z. and Sivasubramanian, P. 2010. Effect of plant extracts on biochemical components of cabbage leaf webber, *Crocidolomia binotalis* Zeller. *Journal of Biopesticides*, 3(1): 275-277.

Weintraub H, Tietz A 1973. Triglyceride digestion and absorption in the locust. *Locusta migratoria. Biochim Biophys Acta* 306: 31-41.

Zibaee, A. and Bandani, A.R. 2010. Effects of *Artemisia annua* (Lin.) (Asteracea) on digestive enzymes profiles and cellular immune reactions of sunn pest, *Eurygaster integriceps* (Heteroptera: Scutellaridae), against *Beauvaria bassiana*. *Bulletin of Entomological Research*, 100: 185-196.