Current Cacao OMICS and Future Prospects

Ian Marc G. Cabugsa
Mindanao State University – Iligan Institute of Technology, Chemistry Department, Philippines
Ateneo de Davao University, Chemistry Department, Philippines
Email: imgcabugsa@addu.edu.ph

Joval C. Afalla[1], Marvin Jose F. Fernandez[2], and Zarine H. Cabugsa[3]
[1]Ateneo de Davao University, Chemistry Department, Philippines
[2]Mindanao State University, Chemistry Department, Philippines
[3]San Pedro College of Davao, Science Department, Philippines
Email: Jcafalla@addu.edu.ph, marvinjose.fernandez@g.msuiit.edu.ph, marymaxmara88@gmail.com

***Abstract*—*Theobroma cacao* is one of the most highly valued and consumed agriculture products in the world with Europe as the top consumer and Africa as the top producer. In recent years, there has been an observed constant increase in cacao consumption and demand in the world and this trend will continue to 2020 as the International Cacao Organization (ICCO) has forecasted. This in turn, caused massive campaign by both the current chocolate producing countries and other tropical countries where cacao can thrive to have it cultivated along with their existing crops on the field. With the high demand for cacao, the technology in cacao farming must keep with the production pace. The traditional farming will not suffice anymore as persistent problems like poor yield, pest and diseases, flood, drought and the heavy metal acquisition of the plant continues to hamper production. To address these issues in cacao production, crop scientists are looking for alternative and modern ways to provide answers and one of these prospects is the application of omics in cacao science. After the complete cacao genome sequence was published, numerous researches in cacao omics have been conducted and this has slowly dissected the molecular mechanisms of cacao in terms of disease resistance, growth and development and its molecular composition. The future of cacao omics is bright, with the chocolate industry will ultimately benefit from this advancement along with the other developing countries where cacao farming is present.**

Index Terms*—Cacao Metabolomics, Cacao Proteomics, Cacao Secretomics, Omics and *Theobroma cacao

I. Introduction

Food is an essential component for any man to continue living on Earth. To have something to eat might be common to the "*haves*" but a luxury to the "*have nots*". To the "*haves,*" they can eat at least thrice a day while others or the "*have nots*" can barely have anything to eat. For those who have the luxury to eat whatever they want and whenever they can, they have this common preference to something special. One of the most special type of food one can have are chocolates. Chocolates are derived from carefully fermented cacao beans [1]. This fermentation process is the key to produce that aromatic and distinct flavor of chocolates that majority of the world, if not all, loves. This chocolate craving of humans is the one of the key Gross Domestic Products (GDP) in some countries in Europe which keep their economy robust. One these countries known for their high-quality chocolates is Switzerland where chocolates are used almost everywhere and are also sold as souvenir items to tourists. Interestingly, these chocolates made and sold by European Union (EU) countries came from *T. cacao* plants that can never be cultivated in any EU member country as their climate and geographical position with respect to its latitude, cannot support cacao agribusiness. The cacaos that these countries process came from developing countries from neighboring continents of Africa, Latin America and Asia particularly Southeast Asia [2]. Countries such as the Ivory Coast, Ghana, Indonesia, Peru, Colombia, Philippines and the other tropical countries along the equator have the needed geographical position where enough sunlight is present integral to cacao production.

II. *Theobroma Cacao* or *"Kakaw"*

Figure 1. Clockwise from the left. a) Photo of mature T. cacao tree, b) photo of unripe Trinitario pod, c) photo of unripe criollo pod.

Theobroma cacao or cacao belongs to the subfamily *Sterculioidea* [3] and of the family *Malvaceae* [4]. It is a small tree that grows at around 7 meters and is endemic in the Amazonian basin [5]. The *Theobroma* genus has 22

Manuscript received April 15, 2019; revised August 20, 2019.

doi: 10.18178/joaat.6.3.194-199

known species and only *T. cacao* is cultivated extensively [3], [4]. The word *Theobroma* is from the Greek words "*theos*" meaning gods and "*broma*" meaning food, thus indicating that it is "food for the gods". The name cacao was derived from the native name of the plant which is "kakaw" used in indigenous Mesoamerican languages. There are currently several cultivars of cacao to which some of them are shown in Fig. 1 which are found in the Hawaiian Peninsula. The green pod is from a typical trinitario variety while the red one is from the flavorful variety of criollo.

The species of cacao has 3 main genetic groups based on the morphological and anatomical characteristics which are criollo, forastero and trinitario [4], [5]. However, this classification was recently reclassified based from the work of Montemayor [6]. The reclassification was attributed to several factors such as, location, phenotype and its geneotype. From 3, there are now 10 classes and they are; Amelonado, Contamana, Criollo, Curaray, Guina, Iquitos, Marañon, Nacional, Nanay and Purús [6]. Despite the new classifications, majority of cacao farmers still use the three original names to classify their trees. This must be due to the degree of knowledge transfer is not adequate to reach the farmers or perhaps an advance technology needed for genetic research in cacao is not available particularly in some developing countries where cacao is cultivated.

Cacao is mainly cultivated in tropical regions as this tree can only produce fruits at certain degrees in latitude along the equator in the globe. These places are Africa, Southeast Asia, Central and South America and even to some countries in Oceania. The current production of cacao is currently progressing linearly from 1961 to 2013 [7]. However, the tensions in Africa created an unsustainable cacao production and is disturbing the total world cacao production [8]. This led to the massive advertisements and campaigning for cacao farming to other feasible areas in the tropics. This in turn created massive conversion of lands to cacao orchards [9] or combining cacao with other existing crops on the field. This resulted to a significant increase in revenue from cacao farming in the last few years. The cacao farming industry has provided sustainable economic and environmental benefits to some of the poorest areas in the world [10]. Furthermore, the popularity of cacao made farmers to intercrop the tree with other commodities not usually seen in orthodox intercrop farming. There are now farmers that plant cacao along with fast growing timbers such as falcate and currently existing trees like coconut and rubber so they can have additional revenue and when prices of cacao are unfavorable [11]. This agroforestry technique dominated the current cacao farming with 70% of cacao farms are now associated with trees or with annual or perennial crops [12].

The cacao farming industry has been plagued by several problems. Some of these problems are the accumulation of heavy metals particularly cadmium on farm lands causing massive price drop in some key markets [13]. Another factor that affected cacao farming is the severe weather and climate conditions like prolonged droughts. The most notable factor that lead to the demise of cacao farms is the presence of pest and diseases. Pests and diseases in cacao had reduced the harvest of some farms by up to 90%. In the Philippines, the most prevalent cacao disease is the black pod [14], or pod rot caused by the fungus *Phytophthora spp*. This disease single handedly devastated at least 50% of the total cacao worldwide.

III. CURRENT CACAO OMICS

One of the new techniques explored to answer problems associated with agriculture and particularly cacao farming is omics. Omics is a generalized term used to describe complete analysis of components such as the genome (genomics), transcriptome (transcriptmics), proteome (proteomics, metabolome (metabolomics), lipidome (lipidomics), and secretome (secretomics) to name a few. The aim of omics is to provide vital information on the target substance such as proteins and its environment at a molecular level. This would mean, one may be able to understand the important mechanisms that support the life of plants. How they interact, how they respond to abiotic and biotic influence are some of its aims. Using this knowledge, one may be able to generate better plant species that will have resistance to *Phytopthora spp*, the fungi that devastate cacao and other plants. Omics studies products may also produce antifungal agents that may interact with the disease. Because of the seemingly limitless application of omics in agriculture, this technique is now being used to study cacao and its main product –chocolates.

The scientific publication of the complete genome of cacao by Argout *et al.* [15] enable cacao scientists to study its transcriptomics, proteomics and metabolomics. The cacao omics researches can be divided into two types, the first one is related to cacao and health relations [16] to which the primary subject of the analyses are chocolates. The value placed on chocolates is one of the main reasons for the numerous studies associated with it. The other focus is on the agriculture of cacao.

Currently, several researches are published related to cacao omics. Some of these studies are listed in Table I.

TABLE I. RETRIEVED CACAO OMICS RESEARCHES

Omics	Research Overview	Reference
Genomics	Mapping of resistance factors to *P. palmivora* in cocoa	[17]
	Cacao pathogenesis and gene expression dynamics	[18]
Metabolomics	Metabolome changes in cacao-pathogen interaction	[19]
	Metabolome changes in cacao fermentation	[20]
Proteomics	Cacao protein analysis: pods	[21]
	Cacao protein analysis: pods II	[22]
	Cacao protein analysis: leaves	[23]

	Determination of proteome changes for developing cacao pod	[24]
	Cacao protein analysis: beans	[25]
	Cacao protein-pathogen analysis: *Moniliophthora perniciosa*	[26]
	Cacao protein analysis: pod husk	[27]
Combined Metabolomics and Proteomics	Analysis of metabolomes and proteomes in the ripening of cacao pods	[28]

Majority of the cacao researches conducted so far are in the proteomics area. The sample material used in the analyses varies from the entire cacao pod to leaves. There are cacao-pathogen interaction studies and even combined metabolomics and proteomics studies. Based from the researches published, there are still several areas that need extensive research. These areas of research can help improve farming techniques for cacao. Furthermore, combinatorial omics is not yet thoroughly explored as most combinatorial omics analyses usually involve only proteomics and metabolomics. The numerous type of omics can help in the comprehensive analysis of cacao. Cacao studies that dominate the world are usually from countries from Africa, Latin America and some from Malaysia, Indonesia and Papua New Guinea. Unfortunately, cacao studies in the Philippines is still lacking much more in the omics side. The potential use of omics in cacao needs to be explore in order to get the most revenue from its cultivation.

IV. Future Prospects of Cacao Omics

More researches in cacao is encouraged in the coming years. The diversity of cacao species from the different cacao-producing countries combined with the unique biotic and abiotic exposures of these plants, the application of omics in cacao research may provide insights to never before explored or explained phenomena. The use of omics to these field has a wide range of application and will prove very promising. The comprehensive study of cacao omics might lead to the creation of better species of cacao for the different countries in the world. The potential application of omics studies/research are described in the succeeding paragraphs.

V. Comparative Omics

Comparative omics such as, but not limited to genomics, transcriptomics, lipidomics, proteomics, metabolomics, ionomics, and secretomics are useful tools to determine the characteristics of healthy-normal cacao compared to the condition studied at a molecular level. The vast potential of comparative omics makes comprehensive investigations on cacao possible. The use of different omics approach to study a phenomenon or condition in cacao may unravel never explored properties and reactions of cacao. One of the possible uses of this comparative study is in the holistic investigation of the attraction and reaction of pests and diseases to cacao. The determination of the specific protein, gene or metabolite needed for a pathogen to attack cacao may be determined using comparative omics research. Furthermore, the use of the technology will allow one to determine what is/are the conditions to which cacao will be more susceptible to certain pests or diseases. Pests and diseases have preferred target, regardless of it, the result is the disruption of normal metabolic function of the plant leading to its underdevelopment, low productivity or even necrosis is expected. These targets or sites interact with the pathogen and thereby promote its pathogenicity. Comparative omics research of the healthy and infected cacao will provide insights on the extent and precursors of infection. Example would be the analysis of pod borer-infected cacao vs normal or a pod borer resistant cacao species. These analyses at the molecular level may provide clues that will help cacao scientist or agriculturist formulate better strategies on produce pathogen resistant farms.

VI. Potential Use of Secretomics

Secretomics is an offshoot from proteomics on the same level as metabolomics, wherein it studies the secreted compounds or substances from a biological source [29]. Some of the secreted products are volatile or Volatile Organic Compounds (VOC) to which these substances may produce odor that may attract insects and/or other organisms in the environment that may lead to the infection of cacao. One of the most notable insects infecting cacao are the pod borers [30]. These insects bore the sturdy pods of cacao until it reaches the beans where it will stay and causes the blackening of beans. These insects might have been attracted to the odor produced by the pods or the cacao beans itself. Researches on the pod/bean odor to the action/interaction with the pod borers are items that can be investigated using secretomics techniques. Typically, the VOCs released by cacao is trapped and collected then will be subjected to spectroscopic analysis. Different techniques can be used for this analysis depending on the type of sample analyzed or the target compound investigated. Because of the variety of methods applicable and the different properties of the target, a number of applications for secretomics is possible. What makes this exciting is that the advances in VOC trapping and extraction has evolved to which on site extraction with the use of SPME or the Solid Phase Microextraction is possible with very promising results.

VII. Chocolate Flavor

The flavor of chocolates makes cocoa and its source-the cacao, a prized commodity. Several studies have been conducted in cacao and chocolate in order to determine the compound responsible for its flavor and aroma [31]-[35]. However, after several extensive studies, the compounds associated to the chocolate flavor is still not conclusively determined. Several inputs suggest that there is actually no single compound necessary to the chocolate flavor but a myriad of compounds. Whatever the case may be, it is monumental if the actual flavor profile is determined. This determination quest may be solved using omics techniques. This is possible due to the holistic level analysis approach of omics which can be combined with other analytical techniques. The holistic and combinatorial use of omics may eventually isolate the flavor compound or compounds from cacao.

VIII. Omics for Improved Cacao Production

Farming in general has numerous trials as there are several effectors that contribute to the general productivity and sustainability of farms [36]. Moreover, the same or at least similar factors are present in the cacao farming. The productivity of cacao farming can be attributed to human and environmental factor [37]-[40]. Human factor in cacao farming mainly involves the farming practice. A well tutored and trained farmer will have better productivity than other farmers that are ill-tutored nor trained. Generally, cacao farming involves good practices such as constant pruning and the application of the necessary amount of fertilizers among others.

The environmental factors can be biotic and abiotic. The effect of other plants and animals in a cacao farm may improve or suppress its productivity. Such relationships can be explored using omics approach. The various plant-plant interaction via protein-protein pathway of the cacao with the other organisms, specifically, microorganisms in the field may provide changes in the productivity of the cacao. These organisms may improve the resistance of cacao to pest and diseases or promote the production of healthy cacao pods. Likewise, these plants and animals/microorganisms may also be detrimental to cacao as it may feast on any part of the tree or compete with the nutrients from the environment. Extensive molecular level analyses on these interactions have not been carried out from the authors' memory and thus such interactions analyses are potential subjects for omics research.

Abiotic factors such as temperature, water, light intensity, soil constitution and air quality can also affect the productivity of cacao [41]-[43]. Nowadays, production in cacao farms are susceptible to drought [44], flooding [45], presence of toxins [46] and even high salt concentration [47] among others. These phenomena may cause cacao growth retardation with the overall development being compromised. As these conditions are totally dependent on the environment, studies on the genetic level is pushed forward to breed cacao variants that can withstand these environmental conditions. In order to do such, a comprehensive analysis on the genes-proteins-metabolites interaction, relationship and property is vital. Resistant crops may be better engineered if the right proteins produced from the genes are generated. As such, the need to conduct combinatorial omics studies are key to such endeavor. Nevertheless, the omics is the first phase of this research with genetic engineering being second. The omics then works well with genetic engineering for future agricultural crops enhancement researches.

IX. Optimized Post-Harvest Cacao Production

The cacao industry is not limited only to farming as cacao beans are subjected to further processes after they are harvested. In a typical chocolate industry, harvested cacao beans are first subjected to fermentation to bring out the chocolate properties essential to its marketing. The process is then followed by drying, then the roasting to desired temperature the chocolate manufacturers prefer. After the beans are roasted, the flavor and texture development commence until the final chocolate product is produced. The crucial steps in the chocolate processes are the fermentation and the roasting as these processes will ultimately dictate the flavor [48].

The cacao fermentation is extensively studied resulting to a myriad of fermentation techniques developed for and by chocolate manufacturers. However, this fermentation process is difficult to standardize due to the multitude of environmental effectors. As such, the production of well fermented beans is necessary to cacao growers. It will be interesting to investigate the relationship of the fermentation products with the proteins and metabolites present in the beans as they are fermenting to it desired configuration. The potential of using omics in the fermentation procedure may provide the needed golden standard for cacao fermentation and may result to the ultimate automation of the procedure which is currently processed manually.

An equally important process in chocolate making, aside from fermentation of beans, is the roasting [49]. The development of the desired chocolate flavor is also attributed to the quality of the roasted beans. Cacao beans produce different flavors at different temperature, a phenomenon similar to coffee and all other products which undergo heating. Investigations on an omics level of the roasting of cacao beans may generate insights on the various reactions that happen on the beans and the eventual production of the desired product that produces the distinct chocolate flavor. This in turn will optimize the roasting process especially in areas where technology is still developing. Several studies have been conducted to the roasting of cacao such as [49] but researches on the molecular level or omics has never been attempted. This, however, is relative only from the best knowledge of the authors.

X. Exploration of the Other Uses of Cacao

The cacao farming industry is heavily reliant to the quality and quantity of the cacao beans. The pods are normally thrown away after pod breaking and extraction of the beans. Other farmers then, to make use of the pods, recycle it though this practice is not observed from other farmers. Aside from the pods, tree cuttings from to the constant pruning of the cacao tree is a common waste in the farm. With several waste materials generated from cacao farms and the limited cacao beans produced, studies on the alternative use of the waste products using omics approach are good research area to explore.

The dynamicity of omics combined with the number of unexplored omics of cacao entails numerous possibilities of cacao omics research. All of these omics researches will congeal and provide products or methods that may increase productivity of farms and provide additional use of cacao farm wastes ultimately may provide the needed improvement on the livelihood of farmers.

XI. Conclusion

Cacao omics is a promising study to explore, not only because the technique is new and that the commodity is important worldwide, but also because the application of this technique to cacao can be made as a template to analyze other important plants cultivated in the cacao growing countries. The aim of agricultural science is to increase yield, after investing a lot in farming technology, farm yields are still not improving tremendously. Probably, it is time to explore the molecular mechanism of plant system to answer this. The omics in cacao may pave the way for this establishment.

References

[1] R. Schwan and A. Wheals, "The microbiology of cacao fermentation and its role in chocolate quality," *Critical Reviews in Food Science and Nutrition*, vol. 44, 2004.

[2] G. Schroth and C. Harvey, "Biodiversity conservation in cacao production landscapes: An overview," *Bioversity Conservation*, vol. 16, pp. 2237–2244, 2007.

[3] J. E. Otoide, "Evaluations of vessel elements and rays in the trunk of Theobroma Cacao," *Bulletin of Pure and Applied Sciences*, vol. 36 B, no. 1, 2017.

[4] A. Wickramasuriya and J. Dunwell, "Cacao biotechnology: Current status and future prospects," *Plant Biotechnology Journal*, vol. 16, pp. 4-17, 2018.

[5] E. Tovar-Pérez, L. Guerrero-Becerra, and E. Lugo-Cervantes, "Antioxidant activity of hydrolysates and peptide fractions of glutelin from cocoa (Theobroma cacao L.) seed," *Journal of Food*, vol. 15, no. 4, pp. 489-496, 2017.

[6] X. Argout, *et al.*, "The Cacao Criollo Genome v2.0: An improved version of the genome for genetic and functional genomic studies," *BMC Genomics*, vol. 18, p. 730, 2017.

[7] W. Vanhove, N. Vanhoudt, and P. V. Damme, "Effect of shade tree planting and soil management on rehabilitation success of a 22-year-old degraded cocoa (Theobroma cacao L.) plantation," *Agriculture, Ecosystems and Environment*, vol. 219, pp. 14-25, 2016.

[8] S. Saj, *et al.*, "Lessons learned from the long-term analysis of cacao yield and stand structure in central Cameroonian agroforestry systems," *Agricultural Systems*, vol. 156, pp. 95-104, 2017.

[9] P. Oliveira, A. Gama-Rodrigues, A. Gama-Rodrigues, and M. Sales, "Litter and soil-related variation in functional group abundances in cacao agroforests using structural equation modeling," *Ecological Indicators*, vol. 84, pp. 254-262, 2018.

[10] A. M. Ramírez, H. J. Duque, and A. I. Trujillo, "Quality of cocoa (Theobroma cacao L.) DNA from foliar tissue at different stages of development," *Acta Agronómica*, vol. 67, no. 2, pp. 311-318, 2018.

[11] E. Somarriba and J. Beer, "Productivity of Theobroma cacao agroforestry systems with timber or legume service shade trees," *Agroforest Syst.*, vol. 81, pp. 109-121, 2011.

[12] J. C. Salazar, M. A. Bieng, L. Melgarejo, J. D. Rienzo, and F. Casanoves, "First typology of cacao (Theobroma cacao L.) systems in Colombian Amazonia, based on tree species richness, canopy structure and light availability," *PLOS ONE*, vol. 13, no. 2, 2018.

[13] M. R. Appiah and Y. Ahenkorah, "Arylsulphatase activity of different latosol soils of Ghana cropped to cocoa (Theobroma cacao) and coffee (Coffea canephora var. robusta)," *Biology and Fertility of Soils*, vol. 7, pp. 186-190, 1989.

[14] D. S. Tan, *et al.*, "AuToDiDAC: Automated tool for disease detection and assessment for cacao black pod rot," *Crop Protection*, vol. 103, pp. 98-102, 2018.

[15] X. Argout, *et al.*, "The genome of Theobroma cacao," *Nature Genetics*, vol. 43, no. 2, pp. 101-109, Feb. 2011.

[16] F. P. Martin, *et al.*, "Metabolic effects of dark chocolate consumption on energy, gut microbiota, and stress-related metabolism in free-living subjects," *Journal of Proteome Research*, vol. 8, pp. 5568-5579, 2009.

[17] M. H. Flament, *et al.*, "Genetic mapping of resistance factors to Phytophthora palmivora in cocoa," *Genome*, vol. 44, pp. 79-85, 2001.

[18] A. Fister, *et al.*, "Theobroma cacao L. pathogenesis-related gene tandem array members show diverse expression dynamics in response to pathogen colonization," *BMC Genomics*, vol. 17, p. 363, 2016.

[19] B. Bailey, "Dynamic changes in pod and fungal physiology associated with the shift from biotrophy to necrotrophy during the infection of Theobroma cacao by Moniliophthora roreri," *Physiological and Molecular Plant Pathology*, 2012.

[20] A. L. Mayorga-Gross, *et al.*, "An untargeted metabolomic assessment of cocoa beans during fermentation," *Food Research Internation*, 2016.

[21] A. Martínez-Márquez, *et al.*, "A comparison of tissue preparation methods for protein extraction of cocoa (Theobroma cacao L.) pod," *Acta Agronómica*, vol. 66, no. 2, pp. 248-253, 2017.

[22] F. Z. Bertolde, *et al.*, "Efficient method of protein extraction from Theobroma cacao L. roots for two-dimensional gel electrophoresis and mass spectrometry analyses," *Genetics and Molecular Research*, vol. 13, no. 3, pp. 5036-5047, 2014.

[23] C. Pirovani, *et al.*, "Protein extraction for proteome analysis from cacao leaves and meristems, organs infected by Moniliophthora perniciosa, the causal agent of the witches' broom disease," *Electrophoresis*, vol. 29, pp. 2391-2401, 2008.

[24] N. Niemenak, *et al.*, "Proteome analysis during pod, zygotic and somatic embryo maturation of Theobroma cacao," *Journal of Plant Physiology*, vol. 180, pp. 49-60, 2015.

[25] E. Scollo, *et al.*, "Characterization of the proteome of Theobroma cacao beans by nanoUHPLC-ESI MS/MS," *Proteomics*, 2017.

[26] J. Mares, *et al.*, "Proteomic analysis during of spore germination of Moniliophthora perniciosa, the causal agent of witches' broom disease in cacao," *BMC Microbiology*, vol. 17, p. 176, 2017.

[27] A. Awang, *et al.*, "Proteomic analysis of Theobroma cacao pod husk," *Journal of Applied Glycoscience*, vol. 57, pp. 245-264, 2010.

[28] L. Wang, *et al.*, "System level analysis of cacao seed ripening reveals a sequential interplay of primary and secondary metabolism leading to polyphenol accumulation and preparation of stress resistance," *The Plant Journal*, vol. 87, pp. 318-332, 2016.

[29] Y. Hathout, "Approaches to the study of the cell secretome," *Expert Review of Proteomics*, vol. 4, no. 2, pp. 239-248, 2007.

[30] [Online]. Available: http://cocoasafe.org/Resources/Datasheets/CocoaPod Borer.pdf

[31] P. Schnermann, *et al.*, "Evaluation of key odorants in milk chocolate and cocoa mass by aroma extract dilution analyses," *J. Agric. Food Chem.*, vol., 45, no. 3, pp. 867-872, 1997.

[32] A. C. Aprotosoaie, *et al.*, "Flavor chemistry of cocoa and cocoa products – An overview," *Comprehensive Reviews in Food Science and Food Safety*, vol. 15, pp. 73-91, 2016.

[33] S. Reed. Sensory analysis of chocolate liquor. [Online]. Available: https://studylib.net/doc/8851433/sensory-analysis-of-chocolate-liquor

[34] P. B. Liete, *et al.* Sensory profiles of chocolates produced from cocoa cultivars resistant to *M. Perniciosa*. [Online]. Available: http://www.worldcocoafoundation.org/wp-content/uploads/files_mf/1383661489Leite2013PostHarvestSensoryProfileResistantCultivars.pdf

[35] D. A. Sukha. Cocoa quality and flavour evaluation – A review and steps towards a harmonized international standards. [Online]. Available: https://www.icco.org/about-us/international-cocoa-agreements/cat_view/290-world-cocoa-conference-2016-

bavaro/291-presentations-world-cocoa-conference-bavaro-2016/293-fine-and-flavour-cocoa-forum-at-wcc3.html

[36] L. Musemwa, *et al*. Factors affecting efficiency of field crop production among resettled farmers in Zimbabwe. [Online]. Available: https://www.icco.org/about-us/international-cocoa-agreements/cat_view/290-world-cocoa-conference-2016-bavaro/291-presentations-world-cocoa-conference-bavaro-2016/293-fine-and-flavour-cocoa-forum-at-wcc3.html

[37] I. Nunoo, *et al.*, "Factors affecting adoption of organic cocoa farming in Ghana," in *Proc. Regional Conference on Marketing and Innovation in Organic Farming*.

[38] W. R. Fadzim, *et al.*, "Factors affecting efficiency of smallholder cacao farmers: A Tobit model application in Malaysia," *International Journal of Economics and Financial Issues*, vol. 6, no. S7, pp. 115-119, 2016.

[39] T. T. Amos, "An analysis of productivity and technical efficiency of smallholder cocoa farmers in Nigeria," *Journal of Social Sciences*, 2007.

[40] B. Duguma, J. Gockowski, and J. Bakala, "Smallholder Cacao (Theobroma cacao Linn.) cultivation in agroforestry systems of West and Central Africa: Challenges and opportunities," *Agroforestry Systems*, vol. 51, p. 177, 2001.

[41] F. Tsiboe, *et al.*, "Effects of biotic and abiotic stress on household cocoa yields in Ghana," *African Journal of Agriculture and Resource Economics*, vol. 13, no. 3, pp. 239-248, 2016.

[42] J. Diaz-Jose, *et al.*, "Cacao in Mexico: Restrictive factors and productivity levels," *Chilean Journal of Agricultural Research*, vol. 74, no. 4, pp. 397-403, 2014.

[43] A. A. Almeida and R. Valle, Ecophysiology of Growth and Production, Cacao, 2010.

[44] H. Bae, *et al.*, "The drought response of T. cacao and the regulation of genes involved in polyamine biosynthesis by drought and other stresses," *Plant Physiol. Biochem.*, vol. 46, no. 2, pp. 174-188, Oct. 2008.

[45] C. Delgado, *et al.*, "Status of cacao trees following seasonal floods in major watersheds of the Peruvian Amazon," *Agricultural Science*, vol. 4, no. 2, pp. 15-24, 2016.

[46] E. Arevalo, *et al.*, "Heavy metal accumulation in leaves and beans of cacao in major cacao growing regions in Peru," *Sci. Total Environ*, 2017.

[47] A. E. Akinkunmi, *et al.*, "Performance of T. cacao seedlings irrigated with water from different sources," *Biotechnology*, vol. 5, pp. 330-336, 2006.

[48] R. F. Schwan, *et al.*, "Microbiology of cacao fermentation and its role in chocolate quality," *Critrical Reviews Food Science and Nutrition*, vol. 44, no. 4, pp. 205-221, 2004.

[49] I. S. Rocha, *et al.*, "Effects of roasting temperature and time of cocoa beans on the sensory characteristic and acceptability of chocolate," *Food Science and Technology*, 2017.

Ian Marc G. Cabugsa has a Masters in Chemistry from Ateneo de Davao University and currently pursuing PhD in Chemistry majoring in Biochemistry with special application to agriculture at the Mindanao State University – Iligan Institute of Technology, Iligan City Philippines. Mr. Cabugsa was a recipient of the Norman Borloug cacao fellowship which made him passionate in cacao studies.