

## BAR Research and Development DDDDCCST Official guarterly publication of the Bureau of Agricultural Research

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**BAR R&D Digest** is the official quarterly publication of the Department of Agriculture-Bureau of Agricultural Research (DA-BAR). A staff bureau of DA, it was established to lead and coordinate the agriculture and fisheries research and development (R&D) in the country. Specifically, BAR is tasked to consolidate, strengthen, and develop the R&D system to improve its effectiveness and efficiency by ensuring customer satisfaction and continous improvement through work excellence, teamwork and networking, accountability and innovation.

This publication contains articles on the latest technologies, research results, updates, and breakthroughs in agriculture and fisheries R&D based from the studies and researches conducted by the member-institutions of National Research & Development System for Agriculture and Fisheries (NaRDSAF).

BAR R&D Digest welcomes comments and suggestions from readers.

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R&D NOTES

# Creating fresh opportunities for improved high value crop production

by Dr. Nicomedes P. Eleazar, CESO IV

n the year after being appointed as the head of the Department of Agriculture (DA), Secretary Emmanuel F. Piñol issued directives to DA units and agencies on issues and concerns that will help improve production and facilitate smooth operations, urging its officials and staff to respond and get things done the soonest possible.

One of the marching orders given was to explore possible research and development (R&D) interventions for specific crops that include onions, garlic, Spanish Red Pineapple, banana and water lily, and jute. Accordingly, efforts on the order of high value-crops were mapped out by BAR and other R&D implementing agencies of DA for the crop commodities identified for research interventions.

Closer attention is now being given to protect onions after a spate of armyworm attacks that devastated the main onion-growing areas in Central Luzon and in Pangasinan. Spanish Red Pineapple may be famous as the source of piña cloth for making *Barong Tagalog* but the profitability of the growers of this variety is dwindling and fruit size and quality must be improved for farmers to make additional income aside from the fiber. Banana stalks become waste once the fruits are harvested while water lily is considered a nuisance in freshwater bodies and waterways. They have untapped potentials as extenders for ruminant feeds and as sources of fiber to help lower the cost of feed and create new fiber resources while eliminating unwanted plants or plant parts at the same time.

Jute was present in the country in the last century but its cultivation is practically gone. With a resurgence in the use of natural fiber for sacks and other products, the revival of this industry can lead to job generation, improved farmers' income, increased by-product utilization and development, and independence from other countries for this material.

Average yield of garlic in the Philippines is very low due to the poor state of planting materials. The crop can only be propagated asexually and, in the absence of good seed management, it has accumulated diseases through the years. To produce disease-free planting materials, tissue culture, with strict quality protocols, has been developed.

The push for high-value crops is a key DA strategy. But what exactly are high-value crops? The DA Cordillera Administrative Region (DA-CAR), a high-value crop area, mentioned that these are "those crops that have competitive returns on investment when traded in fresh form vis-a-vis alternative investment opportunities which are characterized by defined regular or niche market or potential domestic and/or export markets, command high prices, with value added or are good foreign exchange earners." They are also called non-traditional crops.

A prominent agribusiness figure, Dr. Rolando Dy of the University of Asia and the Pacific, has a different take on what is a high-value crop seeing that "value is derived from productivity, expressed in quantity per hectare multiplied by farmgate price." In comparison with our Asian neighbors, it is the generally low level of agricultural productivity in the country that makes a third of Filipino farmers poor, even if farmgate prices are high, and is true even for major crops said Dr. Dy. Conversely, quoting Dr. Cesar Virata, a highly-placed economist from decades back, Dr. Dy said that "high-value crops become low value when prices go down because of oversupply." In short, a crop can become either low or high in value depending on developments in supply and demand.

Clearly, Secretary Piñol had these ups-and-downs in value in mind when he eyed the six crops for further development. With the exception of onions and garlic, the products from the other crops in development may not yet be high-value but can quickly transform once issues in productivity and in farmgate price/demand get addressed with the full backing of the DA and its instrumentalities. Much technology updating and sharing, modernization and mechanization, and market intelligence need to be in place to enhance productivity in the identified products and this is where R&D comes in.

Allied to the cause of highvalue crops is biotechnology. All around the globe, biotechnology has been hailed as providing developing countries with the tools to attain food security, increase farm productivity and profitability, and even minimize the environmental damage caused by conventional agricultural practices. Filipino farmers stand to benefit from its applications with better means to improve farm productivity.

Examples of biotechnology products are many. Bio-actives from onions can be extracted for the development and production of health products. The development of fruit and shoot borer-resistant Bt eggplant is led by the Institute of Plant Breeding of the University of the Philippines at Los Baños (IPB-UPLB). The institute has also been engaged in research on biotech papaya with delayed ripening and papaya ring spot virus (PRSV) resistance. Bt cotton for the country is being worked on by the Philippine Fiber Development Administration (PhilFIDA).

Golden Rice is a beta carotene-fortified rice being collaborated on by the Philippine Rice Research Institute (PhilRice) and the International Rice Research Institute (IRRI). Biotechnology has shortened breeding work in rice from 10-12 to as short as 5-7 years. It has also enabled PhilRice to produce a number of climate change-ready varieties.

The benefits of biotech corn to farmers' livelihood, income, the environment and health have been closely studied and documented. Those that have used it have reported, not only higher yields, but also ecological stability with higher populations of beneficial insects and spiders compared with the use of conventional hybrid corn.

Under the Philippine Biotechnology R&D program, microorganisms have been harnessed to maintain soil fertility, protect crop plants, add value to agricultural byproducts, and improve traditionally fermented foods. Tissue culture is making possible the rapid propagation of desired plant varieties.

This issue of the *BAR R&D Digest* highlights new technologies on particular crops that have been developed following the directives of Secretary Piñol. It also includes notable developments in biotechnology, with BAR's assistance, that improve productivity in highvalue and regular crops. ###

n the early 19th century, Western Visayas was a center for fashion for both colonizer and colonized. *Piña* fiber, which is the raw material used in making *barong Tagalog* was mainly sourced out from Aklan and brought down south to Iloilo where the fabric was designed and weaved into sophisticated pieces of clothing worn only by the elite. The climate in Aklan and its soil is a sweet spot for the mass production of Red Spanish pineapple whose leaves contain the fiber to make *Piña* cloth.

The Red Spanish pineapple is the first pineapple variety to flourish in the Philippines. It wasn't just known for its fiber but locals also grew to love eating its fruit. It eventually lost its appeal when the Hawaiian variety was introduced in the country.

Compared to the Red Spanish variety, Queen Formosa has a larger fruit size by average, and has fruit that's juicier and sweeter. With the introduction of these newer varieties to the market, Red Spanish production focused on leaf harvesting that supplies for the demand for piña fabric and at the same time overlooked the declining market potential of its fruits.

In March 2017, Agriculture Secretary Emmanuel Piñol visited Western Visayas for his *Biyaheng Bukid* television program. There, he saw that the pineapple fiber

# Improving fruit and fiber qualities of Red Spanish pineapple

by Ephraim Joseph J. Gestupa

industry is facing an economic slump. "In recent years...the industry has suffered from very low supply of the fiber and the dwindling number of weavers who only earn as much as P300 a day for the difficult work which strains the eyes," Sec. Piñol mentioned in his Facebook post.

With the Red Spanish pineapple being the only source for *Piña* fiber, Sec. Piñol saw an urgent need to support the industry by providing more quality planting materials and sound protocols that can maximize the growth of Red Spanish, both its leaves and its fruits.

"Yung tanginess ng bunga ng Red Spanish, medyo may kati at parang matabang sabi ng iba, varietal characteristic niya ito, ibigsabihin iyon talaga ang kanyang bunga," said Mr. Innocencio Obredo, pineapple expert and chair of the Bicol Pineapple Board for the Province of Camarines Norte. When asked about why Red Spanish pineapple no longer appeals to consumer preferences, Obredo mentioned that, "siguro mas lalo siya hindi sumasarap kasi yung practice sa Aklan ay inaalisan ang kanyang mga dahoon bago pa siya maging hinog. So lumalaki ang prutas na walang na siyang 'lutuan' ng itinanim."

Secretary Piñol further instructed the Bureau of Agricultural Research (BAR) and the Bureau of Plant Industry (BPI) to come up with research projects geared towards revitalizing the economic value of the crop.

In response, BAR, as the lead agency for research and development (R&D) in agriculture, immediately convened concerned stakeholders along with its pool of experts, particularly, representatives from the Aklan State University (ASU), DA-Regional Field Office (DA-RFO) 5, DA-RFO 6, and the Philippine Fiber Industry Development Authority (PhilFIDA) to discuss and finalize the R&D component studies to improve the size and quality of the Red Spanish pineapple.

According to BAR Director Nicomedes Eleazar, the group was able to come up with a concept and an action plan showing specific R&D activities to be implemented by concerned agencies specifically on how to improve the fruit size without compromising the quality of its fiber. He added that initial discussion was also facilitated on the issues and concerns of the textile fiber production from the Red Spanish pineapple as this is the main use of the plant.

The R&D component of the program titled, "Fruit Size and Quality Enhancement of Spanish Red Pineapple through Cultural Management Practices," includes the profiling and marketing research of Red Spanish pineapple production and looking into the cultural management studies for production of large and sweet variety, including cost-benefit analysis of processing products.

The projects that are being conducted by DA RFO 5 and ASU have now plotted Red Spanish pineapple in their own experimental fields. Each plot was treated differently, varying in planting density, leaf harvesting, and fertilizer management.

The initial data results that were collected one year after the project started showed the nuances on leaf sizes depending on the fertilizer management and planting distance. Fertilizer treatments were based on the recommendations of the Bureau of Soils and Water Management and PhilFIDA. ###

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# Comeback of the jute sack

n the context of sustainable agriculture, society should be able to meet both food and textile needs without compromising the future generations to do the same. While sustainable farming practices is relevant, it is also important to pay attention to the materials that we use to package agricultural products.

With the introduction of synthetic polypropylene as packaging material, sacks made from natural fibers including jute and kenaf are slowly being neglected by farmers. The preference for cheaper alternative became the new norm.

As the world starts to realize the negative effects of using synthetic materials to the environment, using eco-friendly materials becomes an by Rena S. Hermoso

efficient and sustainable alternative. Sacks made from jute, kenaf, and other natural fibers are slowly making a comeback.

Aside from being biodegradable and reusable, jute sacks can preserve the quality and germination capability of grain commodities intact as air can pass through the bags easily. It can protect grains from heat and sunlight as well. Unlike its synthetic counterpart, jute sack is easier to handle because it does not slip off when stacked. Another advantage is that its production does not require the use of harmful chemical.

"These natural fibers (i.e. jute, abaca, coir kenaf, and sisal) are of vital importance to the livelihood and food security of farmers in some of the poorest regions of the world. They provide employment for lowincome populations in rural areas while contributing to food security in times of drought," said Kaison Chang, senior economist at the Food and Agriculture Organization of the United Nations (FAO). It is a cash crop for millions of poor and marginal farm families of South Asian countries according to reports.

#### Jute production in the Philippines

Known as the "golden fiber," jute fiber is extracted from the bark of two closely related annual herbaceous species, *Corchorus capsularis* L. and *Corchorus olitorius* L. belonging to the Tiliceae family. It thrives in tropical lowland areas with humidity of 60-90 percent. Jute is a rainfed crop with little need for fertilizers or pesticides.Cultivating jute also enriches the fertility of soil for the next crop.

In the Philippines, the last recorded jute production was in 2008 according to the Philippine Statistics Authority. In their database, North Cotabato and Cagayan were the only provinces that have reported jute production. According to the Department of Agriculture-Bureau of Agricultural Statistics, the top producers of saluyot in 2006 were Ilocos region, particularly Pangasinan; and Western Visayas.

Under Agriculture Secretary Emmanuel F. Piñol's directives, the Philippine Fiber Industry Development Authority (PhilFIDA) and the Bureau of Agricultural Research (BAR) were tasked to study the jute industry particularly jute as a source of natural fiber for packaging material. The instruction was to explore the commodity and the mechanisms that will facilitate import substitution of jute sack. Likewise, the Bicol Integrated Agricultural Research Center (BIARC) of the Department of Agriculture-Regional Field Office (DA-RFO) 5, embarked on a study that aims to determine the current status of jute production in the Bicol region.

### PhilFIDA's research initiative on jute as materials for packaging

According to Dr. Remedios V. Abgona, chief of the Fiber Utilization and Technology Division of PhilFIDA and project leader, "in the Philippines, the coffee and cacao growers are one of the major users of sacks for packaging coffee and cacao beans. With the growing demand for these commodities, requirement for packaging materials will also follow." However, the only jute sack mill, Mackie Industries, in the Philippines closed down many years ago. According to Joel Lumagbas, one of the board members of Philippine Coffee Board Inc., there is a law by the International Coffee Organization specifies that the packaging material to be used for exportation of coffee is jute sack with 60 kilogram capacity. The project found that local coffee and cacao growers import jute sacks from other countries such as Pakistan, Sri Lanka, and India.

Part of PhilFIDA's study is to determine the availability and consumption of jute, jute sack, and other products (imported and local) in the Philippines. The research team found that in Nueva Ecija, jute is primarily cultivated for food consumption. Moreover, interviewed coffee growers and associations that use jute sack as packaging material for their commodity expressed their concerns. Jute sacks tend to grow molds faster. The fabric easily loosens even if they use brand new sacks. It also gets heavier when wet. Most of the interviewed participants shared that the lack of affordable and quality jute sack in their area is their main problem with jute sack as packaging material for their commodities.

According to Dr. Abgona, "one of the advantages of using jute sack is it prevents moisture accumulation in dried beans and the air in the sack can circulate freely. Molds tend to grow faster only in wet jute." She also shared that, "woven jute fabrics are more durable compared to synthetic materials. [However,] improper handling can lead to the early deterioration of the quality of jute sack." She also explained that, "jute sack is porous, so it will easily absorb and retain water; [thereby making it heavier]. But, this situation happens only if the sacks are not properly stored," shared Dr. Abgona.

Hopefully, through the study, PhilFIDA aims "to provide the government as well as prospective investors the needed information on jute fiber production for policy formulation, decision making and needed interventions for jute production and its consideration as packaging material," ended Dr. Abgona. ###

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# Exploring banana stalk and water lily as ruminant feeds

Whe availability and costs of animal feeds are two of the most pressing challenges faced by the livestock industry. The readiness of quality forage for feeding livestock is normally weather dependent. Forage is usually scarce during dry season and more often, farmers opt to rely on feeding the animals with farm byproducts with lower nutritive values resulting to deprived growth, poor reproduction, low milk production, and high mortality. Animal feeds account for 60-75 percent of the overall inputs in livestock production. Feed is typically the single largest cost item in

livestock farm operations.

The importance of these animals as one of the primary sources of protein food for humans is so vital that one cannot afford to overlook livestock and livestock-products shortages and in addressing the increasing costs of commercial feeds.

Driven by the increasing population and urbanization, the demand for livestock products also increases. Increased availability of meat, milk, cheese, and other dairy products offer huge opportunities as

by Patrick Raymund A. Lesaca

well as improvement in diets addressing malnutrition.

Livestock-related businesses could also boost the incomes of farmers and other stakeholders. For instance, tapping banana (*Musa sapientum*) stalk and water lily or water hyacinth (*Eichhornia crassipes*) (Mart.) as an alternative livestock feeds or feed ingredients is an option worth considering.

Banana stalks and water lilies are often considered as farm waste. Although they are already being used as feeds, extensive studies and promotion of their use have not yet been fully explored. Majority of the available forages are not fully utilized as fodder. Exploring alternative and underutilized feed resources can be promoted for smallholders and commercial livestock farmers.

Considering these scenarios, the Bureau of Agricultural Research (BAR) embarked in an on-going research and experiment studies to tap unconventional feed resources like the dehydrated banana stalks and water lilies as feed extenders for small and large ruminants.

On this research effort, BAR collaborated with the Philippine Carabao Center (PCC) to conduct a study on the "Nutritive Value, Digestibility and Performance of Buffaloes using Banana by-products and Water Lily as Alternative Feed Sources". Another initiative, this time with the Dairy Training and Research Institute (DTRI) of the University of the Philippine Los Baños (UPLB) was also conducted to study the "Feeding Value of Banana Stalk and Water Lily in Dairy Cattle". These studies aimed to explore the nutritional feeding values of banana stalk and water lily as alternative fodder for growing and lactating buffaloes, and dairy cattle. The BAR-funded projects also aimed to address feed scarcity and the nutritional gaps in the ruminant feedings.

Experiments on the nutritive and feeding value, digestibility and performance of animals, and the economics using simple cost analysis as well as the situational studies on the potential uses of such plants have been conducted through the projects.

If found effective on enhancing animal performance and farmers' profitability, this could be promoted and commercialized, thus contributing to the development of the livestock feed milling industry. To analyze the nutrient formulations of banana stalk and water lily, proximate analysis and fiber fractions were separately conducted by the proponents. The analysis will show the moisture, crude protein, crude fiber, crude ash, and nitrogenfree extract content levels of banana stalk and water lily.

The five banana varieties (*latundan, saba, lakatan, tampuhin, balayang*) and water lily samples collected by PCC came from Regions 1, 2, 3, and from the different swampy areas in Nueva Ecija and Pampanga, respectively. The analysis was done at the PCC following the conventional procedures. Meanwhile, the UPLB sourced their water lily samples from the different tributaries in Pakil, Paete, Lumban, and Los Baños in Laguna.

Results obtained from PCC showed that the five banana varieties had an average dry matter (DM) content of 10.68 percent indicating that this feed material contains very high moisture content. The average crude protein (CP) content was 11 percent.

The *tampuhin* variety has the highest CP of 15.59 percent, while *lakatan* has the highest organic matter (OM) content of 88.62 percent. As regards to the water lily, low DM content was observed (8.20 percent), but with higher CP content of 15.10 percent compared to CP content of the banana. Based on UPLB's proximate analysis, waterlily has 11 percent DM, while banana stalk had 8 percent DM. The CP of banana stalk was low at 2 percent compared to water lily at 11 percent.

The relevance and importance in determining higher CP could mean more nutrients are available for the animal's growth and muscle/lean development. Higher DM, on the other hand, is an indicator of the amount of nutrients that are present in the feed source.

Despite speculations that water lily may contain considerably high concentration of heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As); PCC and UPLB conducted separate tests. Laboratory results showed that no heavy contaminations were detected from the water lily samples obtained by PCC and UPLB.

According to Dr. Amado L. Angeles of DTRI-UPLB, project leader, based on the feeding experiments conducted, calves fed with Total Mixed Ration (TMR) with 10 percent banana stalks had the greatest bodyweight gain (BWG) during the 2-6 trial weeks. It was also observed that during 12-14 trial week, calves fed with TMR with 10 percent banana stalk, had the best Feed Conversion Ratio (FCR) among treatments. Milk production was also observed to be increasing during the early trial.

Given the data, Dr. Angeles said, banana stalk can be fed to calves up to 10 percent inclusion as a substitute of Napier grass. The team from UPLB also fed the test animals combined with salt and molasses. Results showed that the animals consumed water lily in whole form. Test animals consumed more banana stalks especially when added with molasses.

Dr. Daniel L. Aquino of PCC, project leader, said that at PCC, they were able to develop four fermented TMRs or what PCC calls as "Chow Fan for Buffaloes" using banana byproducts and water lily as the basal fodders. The TMRs consisted of 25 percent and 50 percent BS and 25 percent and 50 percent water lily. Fifteen growing buffaloes and 15 lactating buffaloes were fed with TMRs.

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# R&D initiatives on onion armyworm deliver results

deadly pest, also locally known as onion armyworm (*Spodoptera exigua* Hubner) has been causing a threat to onion farmers in Luzon that could as well affect the whole onion industry.

Reports, which were published in the national dailies, showed that some municipalities and towns in the provinces of Tarlac, Nueva Ecija, and Pangasinan have experienced onion armyworm attacks recently. And thus, quick and rapid measures must be put in place to avert its occurrence.

The Department of Agriculture (DA), through the High Value Crops

#### by Patrick Raymund A. Lesaca

Development Program (HVCDP) and the Bureau of Agricultural Research (BAR), has created an *ad hoc* group to address the situation. The group, composed of scientist-researchers from the National Crop Protection Center (NCPC) and Postharvest Training and Research Center of the University of the Philippines Los Baños (UPLB); and the Central Luzon State University (CLSU) was commissioned to conduct an indepth and science-based studies on the behavior and management of onion armyworm.

The partnership, which was institutionalized during the mid-part

of 2017, resulted in the creation of a BAR-funded and assisted program known as the "Comprehensive Research and Development on Integrated Pest Management for Onion Armyworm". The general objective of the program is to develop an integrated pest management options for onion armyworm and to come up with science-based approach and recommendations to stop.

The program has seven sub-studies. These include: 1) Early Detection and Warning: Surveillance and Monitoring of Different Crops/ Areas Affected by Onion Armyworm; 2) Detection, Spatial Tracking, Damage, and Yield Assessment and Mapping of Armyworm Infestation and Diseases of Onion Using Remote Sensing Technology; 3) Biological Studies of Onion Armyworm; 4) Efficacy Test of Bio-pesticides and Microbials against Onion Armyworm; 5) Insecticide Management and Resistance Studies for Onion Armyworm; 6) Quality and Safety Assessment and Postharvest Behavior of Onion Grown under Integrated Pest Management Program against Armyworm; and 7) Enhancing Cultural Management Practices in Reducing the Infestation and Damage of Onion Armyworm.

These studies aimed to provide onion farmers and the onion industry players with vital information on the behavior of the pest and how to eliminate it, as well as produce updated reference materials to be used in a region-wide farmer education campaign on armyworm integrated pest management (IPM).

#### **Results and undertakings**

Among the significant outputs of the program was the placement of sex pheromone traps in the affected areas of Nueva Ecija, particularly in Barangay Dolores in Sto. Domingo. The trap is primarily intended for monitoring when the adult starts to come or when the peak population occurs. This enables onion farmers to prepare when to apply management options against the pest. The trap catches the male moths trapped in the water with detergent inside the trap. Field trappings have shown that a single sex pheromone can be effective in lurring male moths up to three months.

Apart from setting of pheromone traps, onion farmer cooperatives in Sto. Domingo and in Bongabon, Nueva Ecija were trained on pheromone trapping workshops. Further, the local government units of Region 3 and the Regional Crop Protection Center (RCPC) were given synthetic pheromone traps as an initial step in their monitoring activities.

Pheromone traps can be used in two ways: as control method or as monitoring method. They can be an effective control method if they are able to attract sufficient numbers of the insect pests. They are also used to monitor the presence and level of pest infestations to improve the timing of pesticide applications. Sex pheromone traps, unlike pesticides, are environment-friendly.

#### The use of

Nucleopolyhedrovirus (NPV), being environmentally safe and effective, is an ideal part of the IPM strategy being employed through the program. According to Dr. Bonifacio Cayabyab of ULPB, and one of the lead-project proponents, a local strain of onion armyworm NPV was collected from diseased larvae of onion armyworm in Brgy. Abar, San Jose City in Nueva Ecija.

The NPV was isolated from the field collected infected larvae and laboratory reared larvae were re-infected with NPV. The NPV infected larvae showed apparent feeding cessation, soft and shiny bodily appearance, and are fragile. Upon death of the larvae, the body liquefy and disintegrate having a characteristic foul odor.

NPV infected larvae were placed in microcentrifuge tubes and stored in the refrigerator until ready for use. These microcentrifuge tubes with infected larvae were given to onion farmers, selected LGUs, Crop Pest Management Division of BPI, RCP Centers 1, 2, and 3, and farmer cooperatives to serve as a source for NPV production and utilization in onion fields and other alternate crops that the OAW might infest. An IEC material on NPV was also developed by Dr. Cayabyab.

Another significant result of the program was the production two Information, Education, and Communication (IEC) materials produced by NCPC-UPLB. The materials, which were written in the vernacular, are: "Gabay sa Paggamit ng Pestisidyo sa Pangangalaga ng Sibuyas Laban sa Harabas" and "Harabas ng Sibuyas". Harabas is the local term of onion armyworm. According to the program proponents, Scientist Mario Navasero and Scientist Marcela Navasero of NCPC, the IEC materials are aggressively being distributed to onion farmers and other stakeholders.

High resolution maps of onion affected and non-affected areas were generated and turned over to different municipalities covered. Under the program, as well, the conduct of quality profile and storage behavior of onion from Nueva Ecija and Pangasinan to serve as baseline data were established.

The lead-project proponents of the program were Dr. Ronaldo Alberto and Dr. Marilyn Patricio of CLSU; and Dr. Melvin Ebuenga, Ms. Marcela Navasero, Dr. Bonifacio Cayabyab, Mr. Mario Navasero, and Dr. Elda Esguerra of UPLB.

The knowledge and information generated and the progressive variables identified, so far, as the program progresses will immediately help onion farmers and onion industry players in understanding OAW prevention, reduction, and or possibly complete eradication. Furthermore, the other accomplishments of the projects on early detection and warning systems as well as the safety assessment and postharvest behavior of onion armyworm are being still being documented.

The implementation of the project is in line with the instruction *turn to page 31* 

## From waste to wealth: Developing new products from onion leaves

by Rita T. dela Cruz

Anaging agricultural waste continues to be one of the key challenges in the agriculture sector. It continues to accumulate as more crops are being grown to produce food for the growing population. Agricultural waste could be an untapped biomass resource that can ease the country's environmental burden or source of profit if converted into new and valuable products.

This was the challenge posted by Department of Agriculture (DA) Secretary Emmanuel Piñol to the onion industry, specifically the research and development (R&D) sector.

"What we want to do is to make our onion farmers more competitive so that they can produce more even at current operating costs so that they can earn more," Secretary Piñol said. To do this, the agriculture chief mentioned strategies such as adopting new farming technologies and techniques to lower production costs and increase farm yields of the local onion farmers.

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BAR R&D DIGEST

Upon posting this challenge, DA has embarked on various initiatives to protect the country's onion industry. Specifically for the Bureau of Agricultural Research (BAR), the national R&D arm of DA, it was tasked to spearhead research initiatives on how to make the most out of onion leaves that are usually considered waste materials after the harvest season. "[They will] study if it could be dehydrated and used in arroz caldo, mami and as spice in Oriental dishes," Secretary Piñol instructed.

#### **Onion leaves as waste**

Onion (*Allium cepa*) is an important crop which is highlyvalued for its flavor, nutrients, and medicinal properties. No dish will ever taste the same without that distinct flavor and aroma of onion.

In 2016, the Philippines ranked 70th among the 150 countries in the world production of producing 122,595 tons of onions. China, India and Egypt are the top three producing countries (FAO Statistical Database, 2016). In the Philippines, Nueva Ecija continues to be the top producer of onion accounting to at least more than half of the country's production. The onion varieties commonly planted are red onion, yellow or white onion, and shallot. The Philippines' onion exports, mainly consisting of the red shallot type, are mostly coming from Nueva Ecija.

Onions are harvested manually by pulling the matured bulbs. After harvesting the bulbs, the onion roots and leaves become waste. Due to lack of proper disposal of organic waste, the accumulation of large quantity of organic wastes has become a challenge.

The onion leaves are left behind in the field and are allowed to decay producing foul odor and a repository for pests and insects causing possible contamination and environmental hazard in the area.

Seeing the value from these agricultural wastes, particularly the onion leaves, Secretary Piñol instructed BAR



to look into the possibilities of finding new uses or converting new products from these waste onion leaves. Specifically, he instructed that the onion leaves, upon dehydration, be used as spice in Oriental dishes, including *arroz caldo* and *mami*. By doing so, farmers will be able to earn more and address the problem of managing farm wastes.

### Turning onion leaves into new products

In response to the directives of Secretary Piñol to explore research interventions that can optimize the uses of onion, BAR met with project implementers from various R&D implementing agencies, including the University of the Philippines Los Baños (UPLB), during the last quarter of 2017. The meeting was set to come up with an integrated program that will cover various researchable areas and the development of technologies on onion.

Implemented in 2017, UPLB's project titled, "Increasing Farmers' Income through the Utilization of Waste Onion Leaves for Various Applications" aimed to develop package of technologies (POTs) for the utilization of onion leaves into different products, and to promote the commercialization of POTs to provide additional income to farmers.

Dr. Myra G. Borines, project leader from UPLB, mentioned that one of the potential impacts of this research initiative is to produce high-value products from waste onion leaves, thereby increasing the profit of onion farmers. She added that with this study, there will be a reduction of postharvest losses, and increase competitiveness of onion industry.

According to Dr. Borines, similar to spring onion, onion leaves may have the same components that can be processed for food applications. With proper research and processing technique, onion leaves can be used as spice in local dishes. One of the challenges that the group of Dr. Borines addressed was the pesticide residues or traces present in the onion leaves. Onion's vulnerability to a wide range of pests has resulted to the use of chemical pesticide by onion growers.

Prior to the processing of onion leaves for food application, the samples were analyzed for residual pesticides and various methods to remove the residues were explored. Among the methods used to remove pesticide residues include: washing with tap water, soaking in 1% vinegar solution, boiling, washing with 0.02% liquid detergent solution, and soaking in baking soda and lemon juice. These methods were based on the study of Dr. Susan May F. Calumpang of the National Crop Protection Center of the College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños on the removal of pesticide residues in vegetables.

Storability studies were also conducted to determine as to what extent the leaves can be stored. "Different factors affecting the shelf-life of onion leaves will also be considered. Characterization and monitoring of the bioactive components of onion leaves has to be done to investigate the different potential products from its utilization," explained Dr. Borines.

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## **Developing sustainable seed system for garlic through R&D**

arlic, scientifically known as Allium sativum L., is considered one of the fundamental culinary ingredients because of its distinct pungent aroma and tangy taste. It is mainly used as a condiment for flavoring meat, fish and salads, in fresh and dehydrated forms. Aside from being an indispensable ingredient, garlic is also commonly used as medicine known to lower blood sugar and cholesterol levels.

Garlic, considered as a superfood, is not just a food seasoning, but a health-promoting ingredient needed by the human body. This makes garlic a highly valued crop contributing to the economy of the country.

In spite of having many uses and its increasing demand, the Philippines remains to be dependent on imports for its domestic garlic needs. Recent data from the Philippine Statistical Authority (PSA) showed that, in 2017, the country's self-sufficiency ratio (SSR) for garlic was about 10.34 percent, which meant there an import dependency ratio (IDR) of 89.66 percent for the crop.

In 2015, the country's garlic production was about 10.4 thousand metric tons (MT), and it even decreased to about 7.5 thousand MT in 2016. In 2017, garlic production was at about 7.8 thousand MT, as reported by PSA.

From 2013-2017, the average production level of 8.74 thousand MT was enough to meet only 8 percent of the local demand for garlic estimated at by Leoveliza C. Fontanil

43.7 thousand MT annually. The PSA stated that the gap increase is at 11 percent based on a higher demand of 130 thousand MT per year.

Today, among the challenges facing the country in terms of garlic production, is the limited supply of quality planting materials, low volume of production due to season ability of crops, decreasing production areas, and cheapest cost of imported garlic compared to local varieties results to discourage smallholder farmers in venturing into garlic production.

The immediate and major concern is at the production system which needs to be addressed through research and development (R&D). One area that is being looked at is the development of a sustainable seed system through varietal evaluation of garlic cultivars.

#### **R&D** interventions on garlic

To increase the country's sufficiency level on garlic, the Department of Agriculture-High Value Crops Development Program (DA-HVCDP) and the Bureau of Agricultural Research (BAR), supported four research and development (R&D) projects on garlic. These projects aimed to identify other possible growing areas in the country through adaptability trial and develop package of technology for the identified regions.

In 2017, the project, "Selection, Purification and Multiplication of Garlic Cultivars for Multi-Location Trials" (Phase 1) was implemented in various areas of the country. The project is being implemented by DA-Regional Field Office I (DA-RFO 1), Mariano Marcos State University (MMSU), University of the Philippines Los Baños (UPLB), and Bureau of Plant Industry- Los Baños National Crop Research, Development, and Production Support Center (BPI-LBNCRDPSC).

Each agency was assigned study areas: DA-RFO 1 for region 1, MMSU for Cordillera Administrative Region (CAR) and region 2; UPLB for region 4A and 4B; and BPI-LBNCRDPSC for regions 3, 5, and 6.

The specific objectives of the projects were to: 1) collect garlic cultivars/strains from the selected regions in the country; 2) evaluate the purity of selected garlic cultivars through agronomic and multiplex microsatellite analysis; and 3) multiply garlic planting materials for the multi-location trials in identified possible areas of expansion.

The potential impact of these studies was aimed to contribute to the decrease in garlic importation, create expansion of garlic production areas and more farmers will venture into garlic farming due to profitability. Garlic growers may have access to quality planting materials that has desirable characteristics such as good quality bulbs, long shelf-life that is adapted to local conditions.



Initial findings revealed that the conventional planting materials locally used were highlyinfected by viruses such as onion yellow dwarf virus, a garlic strain (OYDV-G), leek yellow stripe virus (LYSV), garlic latent virus (GLV), and garlic common latent virus (GCLV). These garlic viruses often occur in complex infections due to environmental and climatic factors. Through the projects, continuous indexing and micropropagation through tissue culture technology were done producing clean, good quality planting materials for garlic.

The UPLB is currently producing true-to-type and certified virus-free garlic from the 26 different garlic accessions that they've collected. There were 1,895 cultures being maintained at the IPB-UPLB laboratory, 361 of which are in the bulbing stage.

The DA-RFO 1 collected three garlic cultivars/ strains namely: Sarang, Native and Batanes White. The three collected garlic cultivars were already planted with its plan cropping season and will be subjected for characterization using the International Plant Genetic Resources Institute (IPGRI) descriptors for Alliums. From the collected cultivars, 424.78 kg of the six NSIC garlic varieties were produced for planting materials. These will be tried in different sites of region 1 such as in the provinces of La Union and Pangasinan to determine its adaptability.

Meanwhile, nine local and introduced garlic cultivars were collected by BPI, these are Tan Bolters, Mexican, Romblon, Ilocos Pink, VFTA 275 M76, Batanes Red, Batanes White, Native Ilocos White, Ilocos White that were subjected to further purification trials. While MMSU collected 17garlic varieties/ accession producing 422 kg of the NSIC garlic varieties.

The next activities is through multi-location trial of the quality garlic varieties that were collected, selected, and purified. A second phase of the garlic R&D project titled, "Multi-location Adaptability Trial of Registered Garlic Varieties and Other Cultivars from the Regions" will be conducted. It aimed to identify expansion areas for garlic production and develop a package of technology for each identified area using the traditional garlic varieties. ###

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The big bang in bawang

by Victoriano B. Guiam

o, it's not about New Year's Eve *bawang* firecracker that is the topic of this article but native garlic, the condiment that is so indispensable for many of our dishes as it adds just the right zest and tanginess to them. What is explosive is the quick multiplication of garlic planting materials in big quantities now possible with advances in Filipino knowhow.

Garlic, scientifically known as *Allium sativum* L., is a perennial herb that is grown throughout the world. It produces a bulb that is surrounded by sheaths that is actually composed of thin-shelled bulblets, cloves, or set, all of which are capable of forming a new plant. It is the bulblet in fresh or in processed form that is used as food, condiment, and for medicinal purposes.

In all the places that have garlic, the bulblet, either in fresh or in processed form, finds use as a condiment and as medicine (speak hypertension). It is also said to be an ingredient in the preparation of insecticides. In the Philippines it is an indispensable recado. It is simply unthinkable to have *sinangag* (fried rice), *adobo* or *longanisa* that does not have garlic. The crop is widely cultivated in the Ilocos region where the green tops are used for preparing the Ilocano *pinakbet*.

According to the Philippine Statistics Authority (PSA), garlic production in the Philippines in 2017 amounted to about 7.8 thousand mt. Production area was maintained at 2.6 thousand ha mainly in the Ilocos region. Other growing areas are Southern Tagalog, Mindoro Occidental, Central Luzon particularly Nueva Ecija, Cagayan Valley, Batanes, Bicol, and provinces in Western Visayas.



All varieties grown in the Philippines are native ones and include Batangas White, Ilocos White, and Batanes White. It is this locally-produced garlic, though smaller, that is stronger in flavor and aroma and a bit more expensive. To the discerning Filipino consumer, the cheap imported garlic that is dumped in the country lacks life and is nearly flavorless.

Garlic can only be produced vegetatively as it is sterile. As the planting materials are merely clones of one another, their use renders commercial garlic vulnerable to viral infections and pests and diseases that can cause as much as 70 percent yield loss.

Average yield of garlic in the Philippines is very low at 2.78 t/ha compared to about 10.6 t/ha in Thailand. This is due to the state of garlic planting materials which, through the years, have accumulated diseases through asexual propagation. Up until 1970, the only virus disease known was the tangle top disease. The Asian Vegetable Research and Development Center (AVRDC) has since identified onion yellow dwarf virus, garlic common latent virus, shallot latent virus, and other viruses as also present.

Local garlic production is reported to be in a decreasing trend. Simultaneously, there have been increases in the price of garlic in the local market to as much as P200 per kilo. Even as local production is exceeded by demand, the cost of production has remained high. The country, therefore, is heavily dependent on cheaper garlic imported from countries where production is more efficient. With high demand, smuggling has also thrived. Imports reached 74,000 mt in 2015, according to PSA, representing more than 90 percent of total supply, valued at \$25.43 million.

For Filipino garlic producers to compete with garlic imports and thrive, productivity needs to be raised and costs reduced. One thing working in their favor is the Filipino consumer's preference for the local garlic. Smaller in size, Philippine garlic is more potent in taste. Obviously, producing garlic planting materials free from viral and other infections and quickly multiplied in large numbers on a sustained and regular basis is desirable. In other crops, the proven way to do this is through plant tissue culture.

Tissue culture (TC) has several applications such as cell behavior studies (cytology, nutrition, metabolism, morphogenesis, embryogenesis, pathology, etc.), plant modification and improvement, and product formation. Of immediate interest to us is the production of disease-free plants and clonal propagation of the preferred varieties.

With TC, disease-free planting materials are mass produced in

capable laboratories for eventual field planting. Different parts may be taken from parent plants and "grown" under aseptic and controlled environments. A bonus is that it is not affected by the seasons as it can be done anytime.

In developing TC for garlic, researchers at the Institute of Plant Breeding of the University of the Philippines Los Baños, submitted to the Bureau of Agricultural Research (BAR) the project titled, "Utilization of the Technology of Producing True-to-Type and Certified Virusfree Garlic (*Allium sativum* L.) for Economic Production of Planting Materials for the Farmers". It sought to optimally develop TC technology with economy in garlic production in mind.

The project used the tissue culture technique to micropropagate (rapid multiplication of a small amount of plant material to produce more progeny) garlic; conduct serology, molecular markers development for genetic fidelity tests, and cytology to determine if the plant materials are true-to-type; and carry out a feasibility study to determine if the technology is indeed commercially feasible. It also sought to determine the production rate of different tissue-cultured garlic varieties/ cultivars in terms of shoot and bulblet production and in terms of bulb production under greenhouse and field conditions. Several concerns to be addressed were: evaluation and utilization of local genetic diversity of garlic, the establishment of an effective seed system of garlic, and development of a standard indexing protocol for virus-free certification of garlic for effective management of the major garlic virus-diseases.

Eight studies have been carried out. Study 1 involved the collection of representative materials of the different garlic cultivars for

TC. Study 2 was on in vitro culture of different cultivars. Study 3 was on virus-free certification of the different cultivars. Study 4 focused on genetic fidelity testing of different cultivars with the use of molecular markers. Study 5 was also about genetic fidelity testing but using cytological techniques. For Study 6, different varieties/cultivars were acclimatized and transferred to greenhouse and field production conditions. In Study 7, different tissue-cultured cultivars in the form of certified clean bulblets were distributed to farmers for evaluation under actual farming conditions.

Finally, Study 8 was on the economic feasibility of producing good quality planting materials of garlic, i.e., the production of trueto-type and certified virus-free bulbs of the different garlic varieties/ cultivars. This was of two parts: a) feasibility study of producing in vitro bulblets from multiplied shoots, and b) feasibility study of producing bulbs under greenhouse and field conditions using tissue-cultured materials.

Micropropagation of 18 accessions of garlic has been done on a continuous basis and conserved in vitro and subjected to virus-indexing and karyotyping (a test to examine chromosomes in a sample of cells). These tissue-cultured accessions also became the basis for the production of true-to-type and certified virusfree garlic bulblets.

With the initial data collected on the field performance of TC and non TC garlic under greenhouse and field conditions, yield performance of TC garlic under field conditions (Ilocos) were already noted to increase by 65 percent. The media used for garlic shoot and bulblet production are undergoing optimization.

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## Molecular markers seal cacao authenticity

by Daryl Lou A. Battad

ith cacao being a multibillion dollar industry, one cannot simply afford mislabeling and misidentification of cacao varieties which can have significant implications against the industry. Lack of knowledge on the basic morphological characters of cacao clones among commercial nursery operators in the country was identified as among the hindrances in furthering varietal development efforts of this high-value commodity.

To address this growing concern, a team from the Philippine Agriculture and Resources Research Foundation Inc. (PARRFI) initiated a project titled "Collection, Characterization and Identification of Cacao Clones in Major Growing Areas using Morphological and Genetic Markers" that aimed to confirm the identity of cacao clones grown in the Philippines, and conserve genetic diversity of accessions at the farmers' fields and laboratory levels in support to cacao's varietal development efforts.

Funded by the Bureau of Agricultural Research (BAR), the project used the single sequence repeats (SSR) technology, a powerful tool in identifying, authenticating, and differentiating cacao clones. It can determine genetic diversity and identify important genetic traits, which are crucial in efficiently utilizing cacao germplasm for breeding and transplanting.

#### **Collection of plant materials**

The material samples consisting of leaf and scions of cacao clones were collected from various germplasm sources in Davao, North Cotabato, Quezon province, Batangas, Palawan, Zamboanga del Norte, and Camarines Sur.

The collected clone samples were propagated asexually through grafting and budding and reared

### DNA extraction and PCR amplification

For improved plant breeding, the project optimized the conditions in carrying out DNA extraction and purification, as well as the polymerase chain reaction (PCR) amplification – an extremely versatile technique for copying DNA.

The project used the cetyltrimethylammonium bromide



at a screen house. The accessions' passport data, relevant environmental conditions, and chorography were carefully noted to expound on the genetic possibilities that caused genetic variation within the collection. The samples were analyzed to determine the authenticity of each clone being used in an area as compared to the typical representative of the clone planted at the cacao gene bank in the University of Southern Mindanao, Kabacan, North Cotabato. (CTAB) protocol for purifying DNA from the sample plant tissues. The quantity and purity of the extracted DNAs were then checked using DeNovix DS-11 spectrophotometer, by which the absorbance ratio of 260/280 values within the 1.8 - 2.0 range were considered pure.

PCR amplification – using the 15 standard SSR markers – resulted to variations within and between clones.







This means that the primers used were highly informative, detecting genetic differences between putative clones. It was observed that the diversity within samples may be attributed to mislabeling during transport or through mutations that occurred within the genome.

As a result, the similarities and differences of various cacao clones and germplasm collections were carefully and sophisticatedly determined, for higher reproductive success and improved conservation measures of each variety.

At 75 percent similarity index coefficient, samples collected from Kabacan clustered with those from the sample sites served as mother plants to produce planting materials that closely resemble the typical representative of the clone. Moreover, a considerable amount of genetic variability is present in the population of each clone bearing a buffering effect against pests and climate deviations and abnormalities.

#### DNA fingerprint database

The DNA fingerprint database is the major output of this project. It aims to establish proper identification of cacao for future varietal development efforts. 84 alleles were observed for 15 polymorphic primers designed for cacao fingerprinting. The map provided information on the number of alleles per locus, size of alleles, and their banding patterns.

A haplotype map – a catalogue where clones are presented accordingly with the loci that were observed – can serve as a guide in varietal authentication and registration using the same set of markers. The project team, led by Josephine Ramos, recommends the use of this technology especially to the National Seed Industry Council (NSIC) and Bureau of Plant Industry for the registration of new clones and certification of cacao planting materials.

The study also noted a good amount of genetic variation of several accessions of *Criollo*, a type of cacao that is considered the 'new wave' in the chocolate industry with its fine flavor attributed to its genetic makeup. However, there is still a need to conduct a more extensive study on Criollo to identify superior clones and authenticate its identity for commercial use of cacao farmers and growers. ###

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by Rita T. dela Cruz

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The use of biological control agents and botanical pesticides as organic crop protection strategies are among the most promising technologies for sustainable agriculture. They reduce the dependence on synthetic pesticides, minimize the negative impact on the environment, and promote safety while at the same time maintaining the economic viability of crop production. But even with the availability of these technologies, these organic pest control methods remain marginal and are not widely-used.

To strengthen the production and utilization of biological control agents and botanicals, the Bureau of Plant Industry- La Granja National Crop Research, Development and Production Support Center (LGNCRDPSC) implemented the project, "Enhancing the Utilization of Biological Control Agents, and Botanicals for Organic Agriculture in Support to Organic Stakeholders in the Visayas".

Funded by the Bureau of Agricultural Research (BAR) under its Organic Agriculture R&D Program, the project has four components: 1) inventory of beneficial predators, parasites, microbials, botanicals and organic agricultural practices of selected organic farms in the Visayas; 2) efficacy test of promising microbials and botanicals; 3) mass production and propagation of selected biological control agents and botanicals for organic stakeholders and farmers; and 4) pilot testing of selected promising microbials and botanicals at farmer's field.

With the information and technologies generated from the project, it is hoped that the use of biocon and botanicals will be greatly promoted to the stakeholders providing them with more options in producing chemical-free healthy food. This will likewise broaden the science-based knowledge on organic crop protection management technologies and capacitate the stakeholders on organic farming.

#### Biocon, natural enemies of crop pests

Biological control agents are natural enemies or competitors of crop pests. They prey on or compete with crop pests without harming the crops and they occur naturally in the farm. They can be bred in commercial insectaries or can be purchased and released into crops to control specific pests. These biological control agents include predators, parasitoids, and pathogens. Predators eat the eggs and/or larvae or grubs of the pest; parasitoids lay eggs in the eggs or larvae of the pest; and pathogens are diseases which affect pests.

Being able to determine the presence of these beneficial organisms is important as they occur naturally in the farm and is a free method of controlling crop pests. Using biocon requires good production and management skills. As part of an integrated pest management program, it can significantly reduce the need for chemical pesticides which are harmful both to humans and environment.

The BPI-LGNCRDPSC study mentioned some biological control agents that are often found in the farm and can be implemented as effective crop protection strategies. These include:

*Trichogramma ostriniae* is a parasitoid that can be used against corn borer and Braconid wasp, a parasite to other arthropods.

Beuveria bassiana or white muscardine fungus is an insect pathogen that can serve as microbial agent controlling or suppressing the growth and multiplication of insect pests including aphids, bugs, leafhoppers, moth caterpillars and migratory locusts.

*Metarhizium anisopliae* is an entomopathogenic fungus that infects whatever it comes in contact with it. Once it attaches to the surface of the insect, it germinates and begins to grow penetrating the exoskeleton of the insect causing it to die.

Trichoderma is one of the best known mycoparasites that can be used as biocontrol agent against soil-borne plant

#### pathogens.

*Chrysoperia carnea* or green lacewing is a predatory insect kknown to feed on a wide variety of soft-bodied arthropods including many aphid species, caterpillars, insect eggs, spiders and mites. The use of lacewings to control arthropod pests has been reported for several crops, worldwide.

#### Botanicals, natural death for pests

Botanical pesticides or simply "botanicals" are naturally-occurring chemicals extracted from indigenous plants that can kill or inhibit the growth of pests. Botanicals degrade more rapidly than most chemical pesticides, and are, therefore, considered relatively environment-friendly and less likely to kill beneficial pests than synthetic pesticides with longer environmental retention.

Among the commonly-found botanicals as effective crop protection strategies are:

Citrus oil from citrus peels can be used as flea dips while tobacco which has nicotine can control soft bodied insects.

Pyrethrin from Pyrethrum daisy is effective in most insect but does not control mites and Sabadilla from the seeds of South American lilies is most effective against true bugs such as harlequin bugs and squash bugs.

Neem extracts from Neem tree is reported to control over 200 types of insects, mites, and nematodes while the Rotenone from the roots of over plant species has a broad spectrum poison that can control leaf-eating caterpillars and beetles. However, direct contact may cause skin and mucous membrane irritation and more toxic when inhaled. ###

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## Fertilizing embryos through *In vitro*

Photos: Dr. Eufrocina Atabay/PCC

by Ephraim John J. Gestupa

itrification is an indispensable tool in the preservation of livestock diversity in the agricultural sector. Achieving a healthy, sufficient, and diverse livestock population is made possible through advancements in biotechnology such as the ability to harvest and preserve the reproductive cells of species so that fertilization can happen inside the laboratory.

Why is there a need to preserve the egg cells and sperm cells of livestock?

Being able to cryopreserve egg cells (oocytes), sperm, and embryos, will provide reproductive materials generally in support to genetic conservation, propagation and expanded utilization in livestock. Due to changing market demands and increasing dependence on agriculture-based products, genetic diversity among farm animals has declined. If there is little diversity in a species, it makes them more vulnerable to disease epidemics and, in worse cases, extinction.

The conservation effort in the Philippines has now involved the process of vitrification and cryopreservation. Gabor Vajta of the Centre for Early Human Development at Monash University in Australia defined vitrification as the "solidification of a solution at low temperatures without ice crystal formation." Before vitrification, livestock oocytes were once preserved through a delicate and inefficient slow freezing process wherein ice crystals that developed within the cells render it unfit for fertilization or further use for research. With vitrification, there is a higher success rate in laboratory for buffalo and cattle embryo production as compared to slow freezing, as it uses a more rapid way of preserving egg cells.

Since 2013, the Philippine Carabao Center (PCC), in partnership with the Bureau of Agricultural Research (BAR), has been improving the production of cryopreserved buffalo and cattle oocytes and embryos through the optimization of vitrification protocols. Egg cells that were cryopreserved are said to have a hardened outer covering compared to fresh oocytes. This phenomenon limits the permeation of sperm during natural fertilization.

The major component of the project titled, "Enhancing *In-Vitro* Production of Embryos from Vitrified Buffalo and Bovine Oocytes by Intracytoplasmic Sperm Injection (ICSI) Technique," is testing to see if ICSI provides a more efficient way of fertilizing vitrified cattle and buffalo oocytes compared to In vitro fertilization (IVF). IVF happens when in a laboratory dish, an egg cell is exposed to a sperm sample until one sperm cell fertilizes the egg. ICSI differs in that an individual sperm cell is injected to an egg cell through the use of a piezo-driven micromanipulator.

By improving the protocols that happen before and after fertilization such as activation of oocytes by chemical stimuli, sperm pretreatment by tail immobilization, and in vitro culture systems for the production of embryos, PCC was able to increase the rate of success of ICSI for cattle and buffalo.

Part of the research project was also the characterization of the biochemical and physiological events between cattle and buffalo oocytes during cryopreservation. It was revealed through the tests that buffalo oocytes showed better cryobiological resilience than cattle sex cells.

ICSI presents its own set of challenges, "It is technically-

demanding work and ICSI demands a large number of available oocytes either in fresh or frozen form, which is considered a concern if mass production of in-vitro derived embryos is desired," says Dr. Eufrocina Atabay, the main proponent of the PCC project.

So far, ICSI has been proven to produce embryos in-vitro. These will be mainly used for research and embryo conservation purposes. Embryo transfer to live recipient or surrogate animals is considered given that PCC further establishes an effective reproductive management program for surrogate animals.

One offshoot project that arose from Dr. Atabay's study on ICSI is the use of sex-sorted sperm in fertilization which can lead to the production of offspring with pre-determined sex. "For example, X chromosome-bearing sperm can be used to produce female calves for dairy, while Y chromosome-bearing sperm can be used for ICSI to produce male calves for meat production purposes.

The prospect of Sperm Sexing Technology together with ICSI/IVF and Artificial Insemination technologies is herein emphasized which can enhance dairy production in livestock species," says Dr. Atabay. ###

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#### Exploring banana stalk...from page 11



The TMR is a method of feeding ruminants that combines feeds formulated to a specific nutrient content into a single feed mix. The mix may contain foraged, grains, proteins, minerals, and vitamins. TMR containing 50 percent banana by-products has 61.94 percent DM digestibility (DMD) and 45.98 percent OM digestibility (OMD), while the TMR containing 25 percent banana-byproducts has lower percentage DMD of 51.4 percent and 47.3 percent OMD. With the water lily, the TMR with 25 percent water lily has 55.9 percent DMD and 42.8 percent OMD, while the 50 percent water lily in the TMR gave lower percentage DMD but with higher

coefficient of OMD equivalent to 45.2 percent.

The feeding results also suggested that 50 percent bananaby-products can support the nutrient needed for almost one-kilogram average daily gain (ADG) of the growing buffaloes. The growth rates of growing buffaloes fed with TMR composed of 25 percent water lily gave the highest ADG of 0.67 kg.

For the lactating buffaloes, feeding trials revealed that the animals fed with 25 percent water lily gave the highest milk production of 609kg or equivalent to a daily milk yield of 6.77 kg. The highest milk fat was 7.52 percent observed from the milk samples of buffaloes given 50 percent water lily and 25 percent banana-by products. Likewise, the highest milk protein was recorded in milk animals given 25 percent banana-by products.

In the studies conducted, banana stalks and water lily can potentially supply dry matter (nutrients) for ruminants and can be utilized as feeds. ###

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#### Dr. Amado A. Angeles

Dairy Training and Research Institute University of the Philippine Los Baños Los Baños, Laguna Tel. No.: (+049) 536- 2205 Email: *aaangeles8@up.edu.ph*  assava is the most important tropical root crop and the third most important source of calories in the tropics (after rice and corn) and generally grown by poor farmers often on marginal land, according to Food and Agriculture Organization of the United Nations (FAO).

In the Philippines, cassava is regarded not only as food but also as ingredients for feeds, and for numerous industrial uses including starch, flour and bioethanol. Due to the many important uses and demand for this crop, the areas for cassava plantation is ever-increasing.

One of the most drought-tolerant crops, cassava is capable of growing on marginal soils. Despite its adaptable nature, cassava "extracts more nutrients from the soil than most other crops leading to nutrient depletion and a decline in soil fertility and unsustainability" said Dr. Marilyn B. Brown, director of National Institute of Molecular Biology and Biotechnology (BIOTECH), University of Philippines Los Baños (UPLB).

Recently, cassava plantation covers about 25 millions of hectares worldwide according to FAO. This is a threat to aboveground and underground biodiversity especially when the cassava is planted as monoculture, (the impacts of agriculture on the environment become very large). One way to reduce the negative impact of cassava monoculture to biodiversity is to practice intercropping (plant diversity) and the utilization of biofertilizers and biopesticides (microbial diversity).

#### UPLB's initiatives on biofertilizers

The BIOTECH-UPLB has been promoting the use of microbial inoculants and inoculated organic fertilizers of biofertilizers for crop production in the country. Through the financial aid of various international and local funding agencies, the institution was able to develop various bioorganic fertilizers such as VAMRI, NitroPlus, and BIO-GREEN.



# Increasing cassava pro with **biofertilizers**

VAMRI is composed of chopped dried corn roots infected with arbuscular mycorrhizal fungi. These fungi assist the plant roots in absorbing water, phosphorus, and other nutrients. Further, NitroPlus is a biofertilizer for legumes which consists of pure effective rhizobia grown in a suitable carrier. The symbiosis between rhizobia and the legume is a cheap way of supplying crops with nitrogen. Lastly, BIO-GREEN is a bioorganic fertilizer that can increase the population density of beneficial nitrogen-fixing bacteria, can eliminate phytotoxic effects, can optimize the level of nutrient enrichment, among others.

### Enhanced biofertilizers for cassava and its intercrops

The improved biofertilizers were subjected to efficacy trials on cassava and selected intercrops in different areas in Luzon through the study, "On-Farm Application of New and Improved Biofertilizers on Cassava and Appropriate Intercrops in Different Areas," conducted



## oduction profitability and intercrops

by the Institute. Funded by the Bureau of Agricultural Research, the project aimed to "harness improved biofertilizers for sustainability, increase cassava and intercrop production, and increase farmers' income by reducing chemical fertilizer input by as much as 50-80 percent," said Dr. Brown. The study found that the most suitable intercrops for cassava were mungbean, peanut, bush *sitao*, corn, and okra. All these crops except the last one supported the multiplication of VAMRI and affect higher microbial activity. Meanwhile, okra seemed to affect cassava positively during its first three months regardless of biofertilizer treatments during dry season. It also found that cassava without intercrop was more prone to drought and weeds compared to cassava with intercrop. According to Dr. Brown, "cassava plants with intercrops treated with biofertilizers were healthier and greener compared to cassava without intercrop and consequently, the yield was significantly higher compared to those without intercrop." Part of the study is the capacity building of the farmers on the use of appropriate intercrops handling mycorrhizal inoculants and other biofertilizers and processed inoculated compost. Technologies developed through the project were packaged into information and education materials which were distributed to participants during seminars and trainings and visitors to at BIOTECH, UPLB.

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Through this research, the **BIOTECH-UPLB** research team hopes that cassava farmers' can earn more through savings from reduced use of chemical fertilizer and an additional income from the use of appropriate intercrops. They also hope that through the technologies introduced to the farmers in the project, soil nutrient can be conserved/sustained, land productivity can be maximized, soil biodiversity can be enhanced, pest can be suppressed, degraded soil can be rehabilitated for sustainable crop production and improve soil quality thus contributing to the maintenance of the ecologically safe environment. ###

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## FAST DETECTION OF DISEASE AND PEST-RESISTANT NATIVE CORN THROUGH MOLECULAR CHARACTERIZATION

by Victoriano B. Guiam

The Department of Agriculture (DA), together with its bureaus and attached agencies are, therefore, hard pressed to meet the demands of this population. Even marginal lands are being marshalled to be more productive and be a source of employment. Under these challenging conditions, the usefulness of biotechnology comes to the fore.

Corn is one of the most phenotypically diverse of cultivated crops as it can grow over a wide range of environments. Breeders emphasize selection for a desired mix of traits that are controlled by multigenes. Known as quantitative traits, these include important agronomic characteristics such as yield and yield components, and resistance to pests and diseases. In looking for these, the application of biotechnology is vital.

The effectiveness of selection for different quantitative traits lies in the effectiveness of screens used in determining the traits. Variations among corn lines can be determined using phenotypic markers but their performance is strongly affected by environmental factors. Molecular markers for detecting total genetic variation of corn lines are preferred when environmental influences are not desired.

The use of molecular markers, or microsatellites, can facilitate the breeding process. The time needed to reach breeding objectives is much reduced as it entails less field assays. For many crops, molecular markers have been determined. With the use of molecular markers, genetic variation and the genome dynamics of many plants including corn are now better understood, leading to improved breeding efficiency.

With the biotechnology tools available at their disposal, a team of researchers at the Institute of Plant Breeding of the University of the Philippines Los Baños led by Ms. Alma Canama, set out to assess the genetic diversity among the country's native corn populations using SSR DNA markers. Guided by the institute's aim for its corn breeding program which is to develop corn varieties for biotic and abiotic stress resistance and nutritional properties, a proposal titled, "Molecular Characterization of Philippine Native Maize Populations (Year 2)", was submitted to the Bureau of Agricultural Research and was approved for funding in 2016.

No study on molecular genetic diversity analysis of native corn populations had previously been done. Knowledge about diversity and relationships among the Philippine native corn populations is important for the corn breeding program of UPLB-IPB and will benefit the corn program of DA. The new study built up on the assessment done under the project's Year 1 for genetic diversity among native corn populations on resistance to corn borer infestation and downy mildew infection with the use of SSR DNA markers and dendrogram (a tree diagram used to represent data where each group or "node" links to two or more successor groups based on similarity of traits).

Among the molecular markers, simple sequence repeats (SSR) microsatellites are commonly used for genetic diversity analyses due to their high level of polymorphism, repeatability and low cost. SSRs are abundant and their chromosomal assignments have been established, thus, the corn genome can be uniformly sampled and analyzed.

Polymorphic SSR markers can distinguish the allelic profiles of resistant lines over susceptible lines to particular pests and diseases. With knowledge about the allelic profiles of resistant/ tolerant corn, molecular screening criteria can be used to sort out various crop lines as to resistance. It can be expected that high heterosis in yield and its components could be obtained from crosses among those lines belonging to different heterotic groups.

In the Project Year 2's Activity 1, a total of 20 SSR markers were used to screen the inter-population diversity among 26 native corn populations. These populations were chosen based on a Project Year 1 constructed dendrogram (a tree diagram used to represent data where each group or "node" links to two or more successor groups based on similarity of traits eventually creating a viewable clustering) that assessed the genetic diversity among native corn populations using SSR DNA markers.

A new dendrogram was created using 20 representative populations with five samples each utilizing 12 SSR markers. The dendrogram showed high diversity within a population. From this, the researchers infer that the samples within a population are very diverse owing to corn's open pollinated nature.

For Activity 2, allelic diversity between susceptible and resistant populations on downy mildew infection and corn borer infestation were studied. Unique alleles were found to be associated with either downy mildewresistant or susceptible populations with the use of an SSR marker.

As for corn borer resistance and susceptibility, populations that exhibited high susceptibility showed a more complex banding pattern and a monomorphic pattern. Also, more alleles were observed compared to the populations that are highly resistant to the pest. The populations that exhibit high resistance to corn borer infestation tended to exhibit a more polymorphic pattern.

The researchers conclude that the results indicate the reliability of the information provided by the dendrogram from Project Year 1 and can be the basis for breeders to devise better breeding programs and choose populations which are distant from one another to create better breeds or varieties. The SSRs used were also found to be informative markers that revealed genetic variation among the inbred lines studied and that SSR markers tightly linked and associated with pest and disease resistance can be utilized to screen populations at the DNA level.

The knowledge generated about diversity and relationships among Philippine native corn populations, through the use of SSRs in the search for resistance to corn borer infestation and downy mildew infection, will lessen the time and cost it will take to conduct breeding efforts for native corn. ###

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### The big bang in bawang...from page 19



In developing a standard protocol for virus-free certification, several viruses (onion yellow dwarf virus, garlic common latent virus, shallot latent virus, and leek yellow stripe virus) were detected in the samples collected. Only six of the accessions were found to be virus-free and the rest have to be "cleaned".

A number of molecular markers (22 SSR primer pairs) were tested and used for genetic fidelity tests of the different garlic cultivars. Cytological tests for genetic fidelity were also conducted.

Current results show that at 13 SSR loci, the tissue-cultured garlic are genetically the same as the non-tissue garlic. The researchers are proposing to try additional ones. With more markers tested, DNA markers can be established for variety identification of our local cultivars and for validating the genetic fidelity of tissue-cultured garlic. A diagnostic kit for genetic fidelity and cultivar identification can then be developed. Farmer-cooperators in Iloilo and Ilocos were certified virus-free TC planting materials (bulblets) for initial field testing along with a series of trainings on the production of TC garlic, and on its planting and maintenance in the field.

In Ilocos, the results were encouraging with the excellent farmercooperators' performance and their readiness to adapt the tissue culture technology for commercial garlic production. Technology transfer was partially successful and needs to be intensified according to the researchers. Plans are being made to spread the technology to other farmercooperators in Batanes, Mindoro, Cagayan, and some areas in Mindanao.

Already, in the Department of Agriculture, TC is already being done by various agencies and Regional Field Offices (RFOs) along with partner state universities and colleges (SUCs) various crops.

The contribution of BAR has been

in equipping a number of these RFOs and SUCs with tissue culture facilities and laboratories. With TC technology for garlic a reality, these facilities can turn out the production of virus-free planting materials in rapid fashion. Once the tissuecultured planting materials reach the production areas, native garlic production can increase drastically as the cost of production shall be reduced. This will also make possible the development of a seed system that shall lead to a revival of the native garlic industry. We will thus be getting a bigger bang for our R&D buck. ###

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#### **R&D initiatives on onion...** from page 13

of Agriculture Secretary Emmanuel Piñol to BAR to look into the armyworm infestation of onions and to come up with research agenda program and deliverables in addressing the infestation in the affected provinces.

The onion armyworm is one of the most important species

of Noctuid moths whose larvae are called Spodoptera exigua (Hübner). It has a wide host range throughout tropical and subtropical regions of the world and occurring as a serious pest of vegetable, field, and flower crops. Among susceptible vegetable crops are asparagus, broccoli, cabbage, eggplant, lettuce, onion, potato, radish, spinach, tomato, among others. ###

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#### From waste to wealth...from page 15

In the study, various dehydration (drying) techniques were explored to enhance the shelf-life of onion leaves. Dehydration is the process of removing water or moisture from a food product. Dr. Borines explored four dehydration techniques for this study. These include: 1) sun drying (cheapest and simplest method), 2) conventional drying (air is heated with steam, gas or hot water and then circulated over the wet product), 3) freeze drying (involves the sublimation process, where solid turns into gas without becoming a liquid), and 4) vacuum drying (involves indirect heating generally used for heat-sensitive materials).

Initial results of the study showed that conventional drying and vacuum drying were the preferred methods in preserving total phenolic content (TPC) and total flavonoid content (TFC) of onion leaves. TPC and TFC are the phytochemicals present in onion leaves. In terms of color and appearance, onion leaves were best preserved using freeze drying. Likewise, desired significant reduction of moisture in onion leaves was achieved using this drying technique.

As for the products developed from onion leaves, the group of Dr.



Borines was able to produce at least five POTs. These were: 1) dried onion leaves (can be further processed as tea); 2) powdered onion leaves (can be further processed as seasoning/salt, kropek, noodles, pandesal); 3) pickled onion leaves; 4) onion leaves extract (can be further processed as puree and juice); and 5) vacuumfried onion leaves (can be further processed as garnish).

With the new products and technologies developed from waste

onion leaves, Dr. Borines cited that not only the problem on postharvest losses is being addressed but it may help improve the productivity and income of onion farmers thereby providing significant impact to the onion industry. ###

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#### **RED SPANISH PINEAPPLE FIBER**

The Red Spanish pineapple is the first pineapple variety to flourish in the Philippines. In the early 19th century, Western Visayas was a center for fashion and *Piña* fiber, which is the raw material used in making *Barong Tagalog* was mainly sourced out from Aklan and brought down south to Iloilo where the fabric was designed and weaved into sophisticated pieces of clothing worn only by the elite. With the Red Spanish Pineapple being an excellent source for *Piña* fiber, Secretary Emmanuel Piñol saw an urgent need to support this fading industry by providing more quality planting materials and sound protocols that can maximize the growth of Red Spanish, both its leaves and its fruits. (*Photos: EJGestupa*)



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