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DISEASE SCREENING AND POST-ENTRY QUARANTINE PROGRAM FOR SAFE INTRODUCTION OF SUGARCANE VARIETIES IN THE PHILIPPINES

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ABSTRACT

A three-year post-entry quarantine pathogen testing protocol for foreign sources of sugarcane was developed for the Philippines. A total of 284 varieties acquired from Australia, Bangladesh, China, France, Indonesia, Malaysia, Mauritius, Thailand, USA, Japan, Vietnam and Pakistan were subjected to pathogen testing inside the post-entry quarantine glasshouse for 24 months at the Institute of Plant Breeding – College of Agriculture and Food Science, University of the Philippines Los Baños and another 10 months under the open-field conditions at Guimaras, Visayas for the period of 2001-2012. Leaf scald, ratoon stunting disease and sugarcane mosaic were among the most common diseases that were detected on foreign varieties imported from Thailand, Malaysia, Indonesia, Bangladesh, China, France, USA, Vietnam and Pakistan. The presence of other diseases such as smut, red rot, grassy shoot, sheath rot, Pokkah boeng and yellow leaf disease were also observed on some foreign varieties from Thailand, Indonesia, China, Australia, USA and Pakistan. A cold soak and hot water treatment was found effective in eradication of pathogens associated with seed pieces received from foreign countries. Furthermore, the use of an optimized and standardized scheme of quarantine pathogen testing through antibody and nucleic acid based assays proved to be a reliable practice in checking the introduction of new pathogens in new and promising sugarcane varieties received from other countries. These preliminary quarantine practices were found effective in protecting the Philippine industry from unwanted quarantine pathogens.

Key words: pathogen testing, antibody, nucleic acid, cold soak and hot water treatment

INTRODUCTION

Introduction of foreign germplasm increases the genetic diversity and desirable agronomic traits (Croft, 1996). As a result of sugarcane germplasm exchange and plant breeding, sugarcane varieties that are high yielding, adapted to changing environmental conditions, and resistant to pests and diseases can be developed. Sugarcane (*Saccharum* spp. hybrid) is the major crop cultivated in the Western Visayas (Moog, 2006) and is an important cash and exportable crop in the Philippines along with coconut, pineapple, banana, coffee and mango (Espino and Atienza, 2001). The Philippines is second in terms of sugarcane production in Southeast Asia (ASEAN Food Security Information System, 2014). The feasibility of sugarcane as a source of special sugars, bio-plastics, bio-fertilizers, among other products, is also being studied to further increase its input to the Philippine economy (www.sra.gov.ph).

In the Philippines, diseases have always been a major biotic constraint in sugarcane production. About 75-100% loss of sugarcane yield has been recorded in fields when cuttings infected by *Sporisorium scitamineum* syn. *Ustilago scitaminea* are planted (Reyes *et al.* 1980). In addition,

there are sugarcane diseases caused by fungi, bacteria and viruses which are also important for quarantine purposes and therefore, should be regulated.

Foreign sugarcane varieties are imported into the Philippines from: Thailand, Malaysia, Indonesia, China, Australia, France (CIRAD) and Mauritius. Therefore, sugarcane germplasm exchange in the Philippines poses a risk of introducing exotic diseases. Reports are available that pathogens can be disseminated and distributed through sugarcane seed pieces, without external symptoms or in the case of latent infections (Croft, 1996).

With the implementation of the Common Fund for Commodities (CFC) Varietal Exchange Agreement in 2000, the Institute of Plant Breeding of the University of the Philippines Los Baños (IPB-UPLB), Philippine Sugar Research Institute Foundation, Inc. (PHILSURIN), and the Plant Quarantine Services of the Bureau of Plant Industry (BPI-PQS) have collaborated in studying and indexing pathogens in introduced varieties through developing pathogen testing protocols and constitution of the sugarcane quarantine program. Quarantine and pathogen testing for sugarcane have been implemented in South Africa (Bailey and Bechet, 1988) and Australia (Thompson *et al.* 2011), and these measures must also be established in the Philippines. Hence, this study was initiated to develop and optimize pathogen testing procedures for the detection of pathogens such as *Xanthomonas albilineans*, the cause of leaf scald (LS), *sugarcane mosaic virus (SCMV)*, the cause of sugarcane mosaic (SCM) and *Leifsonia xyli* ssp. *xyli (Lxx.)*, the cause of ratoon stunting disease (RSD) in foreign varieties of sugarcane, to safeguard the Philippines against these pathogens. An open-field quarantine system under natural conditions, situated on a remote location, was also employed as part of the protocol to check for possible occurrence of any other new disease which did not manifest in the post-entry glasshouse condition.

MATERIALS AND METHODS

Acquisition of introduced sugarcane varieties

From 2001 to 2012, a total of 284 sugarcane setts or seed pieces were acquired from different sugarcane growing countries including Australia, Bangladesh, China, France, Indonesia, Malaysia, Mauritius, Thailand, USA, Japan, Vietnam and Pakistan (Table 1). Upon arrival of the planting materials at the Post-Entry Quarantine in IPB-CAFS, UPLB, visual inspection was done to detect readily observable symptoms and for proper documentation. The general appearance and condition, as well as number of buds per variety, presence of initial signs and symptoms were noted.

A hot water treatment for the newly imported materials was done prior to planting following the BSES protocol (Croft *et al.* 2011). Sugarcane seed pieces were exposed to an overnight cold soak treatment at an ambient temperature with occasional addition of fresh water. Then, these were given a short hot water treatment (SHWT) for 30 min at 50°C. Five minute (5.6 g/L) Mancozeb (fungicide) dip treatment was also done following the HWT. The seedpieces were then potted in plastic containers filled with sterile soil (5:1 soil:coconut coir dust) and allowed to germinate under glasshouse conditions. Upon germination, the varieties were transferred into individual 24-liter plastic utility pails with newly sterilized soil. These were labeled separately with variety names and date of planting. Two months after germination, fertilizer application using urea (46-0-0) was done. Monitoring for occurrence of diseases was conducted on a regular basis during a two-year quarantine cycle.

Closed quarantine

Post-entry quarantine inspection of the introduced sugarcane varieties were divided into two cycles: cycle 1 and cycle 2, corresponding to the number of years the varieties were maintained under

greenhouse quarantine conditions (Figure 1a). During quarantine testing, at 4 months after planting, the plants were visually observed for LS, SCM, yellow leaf syndrome, downy mildew, smut and other diseases. Also, plants were tested for the presence of *SCMV*, *sorghum mosaic virus (SrMV)* and *Xanthomonas albilineans* using either enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR). The tests were repeated. At 12 months, the plants were ratooned and stalks were observed for the presence of reddish or pinkish discoloration as an indication of RSD. After 10-12 months of the first pathogen testing cycle, sugarcane stem-cuttings or "setts" from the ratooned plants were processed and exposed to an overnight-soak in running cold water. Following the overnight soak, setts were subjected to a long hot water treatment (LHWT) for 2h at 50°C, followed by soaking in running water for an hour, and dipping in (5.6 g/L) Mancozeb solution before planting in a sterile soil-filled plastic container. The crop plants and the ratooned plants were allowed to grow for another 12 months for the second cycle of pathogen testing. Sugarcane varieties that were tested positive for the pathogens associated with diseases such as LS and RSD in diagnostic procedures underwent cold soak and long hot water treatment again to eradicate the said pathogens. Plants infected with *SCMV* were subjected to LHWT. Moreover, plants that still exhibited symptoms of diseases even after hot water and fungicide treatment were rogued out and burned.

Fig. 1. (a) Post-entry Quarantine Greenhouse at the Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños; (b) Open-field quarantine in the province of Guimaras in the Visayas.

Open-field quarantine

After 18-24 months of pathogen testing in the post-entry quarantine glasshouse, introduced sugarcane varieties that were free from diseases were ratooned. Cane setts were subjected to cold-soak for 12 hours and LHWT (50°C for 2 hours). All the cane setts were treated with (5.6 g/L) mancozeb solution. Ratooned cane setts were transferred and planted to an isolated, open-field natural condition in Guimaras province in the Visayas located at a very remote area (Figure 1b). The island is distant from commercial sugarcane plantations; hence the probability of introducing new diseases to existing sugarcane plantations is very low. Solarization and fallowing for 2-3 months are practiced to prevent the chance of varietal contamination. During the 3rd year of pathogen testing, materials were planted and allowed to grow in the field for another 12 months. The plants were regularly observed for the occurrence of pests and diseases. Sampling of leaves to test for the presence of *Xanthomonas*

albilineans and *SCMV* was done at 5-10 months after planting, while stalk sap sampling was used for *Lxx.* detection. The presence of other important diseases was also monitored. Plants that expressed visible symptoms and/or positive to indexing were rogued out and destroyed.

Pathogen testing

An optimized and standardized procedure for the detection of disease-causing organisms for all of the 284 introduced sugarcane varieties in the post-entry and open-field quarantine was adopted with modifications (BSES Quarantine Training Manual, 2001). For the antibody-based tests, leaf sampling was done using the third to the youngest fully expanded leaf at 4, 8 and 12 months for *Xanthomonas albilineans* and *SCMV* detection. An approximate of 1x1 inch leaf portion was collected per plant. At 12 months, stalk sap sampling was done by obtaining one node per stalk per plant for *Lxx.* detection. All the cutting tools were disinfected using 10% NaOCl and 70% EtOH prior to use on each plant.

Modified enzyme-linked immunosorbent assay (ELISA) techniques such as Dot-blot immunoassay using nitrocellulose membrane for the detection of bacterial pathogens of *Xanthomonas albilineans* and *Lxx.*, and Indirect-ELISA for viruses such as *SCMV* and *SrMV* were employed. Nucleic acid-based technique (Polymerase Chain Reaction) utilizing specific primers against pathogens caused by bacteria and viruses, was also performed to ascertain the presence or absence of disease-causing microorganisms. In ELISA, locally produced polyclonal antibodies against *Lxx.* and *Xanthomonas albilineans* (Dela Cueva *et al*. 2010) were used routinely in pathogen detection while commercially available antisera from Agdia® were used to detect *SCMV* and *SrMV*.

Dot-blot immunoassay (DBIA)

For *Xanthomonas albilineans* detection, a dot-blot immunoassay technique was used (dela Cueva *et al.* 2010; Viswanathan *et al*. 1998; Wang *et al*. 1999) with the following modifications. An appropriate size (~10 cm x 10 cm) of the nitrocellulose membrane (Trans-Blot® Transfer Medium, Catalog 162-0115, Bio-Rad) was obtained. The membrane was dipped in 100 mL 1 x TBS buffer (0.05M UltraPure™ Tris, 0.15M NaCl, 100 mL ddH_2O, pH 8.0). Antigen loading was then done by acquiring 3 µl of the samples. Following 12 hours of incubation, the membrane was placed in blocking buffer (50 mL 1 x TBS buffer in 0.0005% v/v Tween-20 [polyoxyethylene (20) sorbitan monolaurate], 3% wt/v non-fat milk and 0.27M glycine [NH_2CH_2COOH]). Washing was done with 50 mL 1 x TBS-T buffer (0.05M UltraPure™ Tris, 0.15M NaCl, 0.0005% v/v Tween-20, 50 mL ddH_2O, pH 8.0) for three times at 10 min intervals, and every time the buffer solution was changed. Then, antibody loading was done in a 1:200 v/v dilution. The membrane was then incubated with the conjugate antibody, alkaline phosphatase-goat anti-rabbit IgG (GARAP) (Invitrogen) in 1:1000 v/v dilutions. After the washing step, it was incubated in BCIP (bromo chloro indolyl phosphate) and NBT (nitroblue tetrazolium) tablet (Sigma) and 10 mL of deionized water substrate solution.

Dot-blot enzyme immunoassay was also used to detect *Lxx.* causing RSD (Hoy *et al.* 1999; Gao *et al*. 2008) with the following modifications. Stalk sap samples extracted by mechanical grinding were added to TBS-T buffer (200 mL TBS-$Tween_{80}$ buffer, 0.30M NaCl, 0.10M Na_2EDTA, pH 7.4) in a 50 µL sap:50 µL buffer ratio. A portion of the nitrocellulose membrane (Bio-Rad) sufficient for the samples was soaked in PBS buffer (0.137M NaCl, 0.001M KH_2PO_4, 0.01M Na_2HPO_4, 0.003M KCl, 1000 mL ddH_2O, pH 7.4). Then, 10-20 µl per sample was applied on the assigned grids. The membrane was incubated in RSD specific (locally produced, 2nd bleed rabbit polyclonal) antiserum (1:2000 dilution polyclonal anti-*Lxx* IgG). Following the washing step, the membrane was added with 20 µL alkaline phosphatase-goat anti-rabbit IgG (GARAP) (Invitrogen).

Indirect ELISA (indirect enzyme-linked immunosorbent assay)

SCMV detection through indirect ELISA was used (Gemechu *et al.* 2004) with the following modifications. A 96-well microtiter ELISA plate was loaded with 100 µL per well of leaf extract and buffer (PBS buffer) solution. After incubation, 100 µL per well of the capture antibody anti-SCMV polyclonal antiserum (Agdia®) at 1:200 dilution in buffer solution (PBS buffer, 1% milk) was added. The wells were loaded with 100 µL per well of conjugate GARAP (Invitrogen) and buffer solution (PBS buffer, 1% milk) at 1:1000 dilution, followed by loading of the 100 µL per well substrate p-nitrophenyl phosphate (pNPP) (Sigma Aldrich) and diethanolamine buffer (diethanolamine, ddH_2O, pH 9.8) solution in 1:200 dilutions. Values were then measured in an optical density (OD) of 405 nm using an ELISA plate reader (Bio-Rad).

PCR-based techniques

PCR-based method using the specific primers PGBL1 (5'-CTTGGGTCTGTAGCTCAGG-3') and PGBL2 (5'-GCCTCAAGGTCATATTCAGC-3') with a product size of 288 bp for *Xanthomonas albilineans* detection (Pan *et al*. 1999; dela Cueva *et al.* 2010) was done. Leaf diffusate per sample was prepared and was used since the primers can work directly even without DNA extraction. PCR cocktails were prepared with a final volume of 25 µL.

For *SCMV* and *SrMV* detection, reverse transcription RT-PCR with the specific primers SCMV F3/R3 (5'-TTTYCACCAAGCTGGAA-3') (5'-AGCTGTGTGTCTCTCTGTATTCTC-3') and SrMV F3/R3 (5'-AAGCAACAGCACAAGCAC-3') (5'-TGACTCTCACCGACATTCC-3') with an expected product size of ~900 bp (Yang and Mirkov, 1997) was also conducted for confirmatory testing. PCR cocktails were assembled in a sterile microcentrifuge tube with a final volume of 50 µL.

RESULTS AND DISCUSSION

Acquired sugarcane varieties from Australia, Bangladesh, China, France, Indonesia, Malaysia, Mauritius, Thailand, USA, Japan, Vietnam and Pakistan were maintained in the PEQ facility, IPB-CAFS, UPLB from 2001 to 2012 (Table 1). A pathogen testing scheme specifically adapted and developed for the importation of sugarcane into the Philippines (Figure 2) was employed as a standard quarantine procedure to screen the samples. Hot water treatment did not affect the propagation of the planting materials. CS-HWT was effective for managing the pathogens, however, results varied depending on the varietal reaction to the treatment.

Using the protocol, pathogens of major quarantine significance such as *Xanthomonas albilineans*, *SCMV* and *Lxx.* were confirmed through ELISA and PCR in some varieties. The most common diseases observed and diagnosed on 284 sugarcane varieties were SCM (16), LS (11), Red rot (6) RSD (5) and Smut (5). Infected plants were observed to exhibit typical symptoms of LS such as white pencil-line streak that started to appear six months after planting (Figure 3a). Some plants were asymptomatic under glasshouse conditions but were also tested positive through DBIA and PCR during the first cycle, but not during the second cycle. These were treated through cold soak and long hot water treatment (CS-LHWT). The CS-LHWT could have eliminated the bacterium as PCR assay of the tested leaf samples showed negative results during the second cycle of pathogen detection.

At the open-field quarantine, symptoms of LS were noted. Some sugarcane varieties from France, though negative to *Xanthomonas albilineans* during the post-entry quarantine cycle, were found to express symptoms typical to leaf scald infection when planted in open-field quarantine. DBIA results confirmed the presence of *Xanthomonas albilineans*, hence, infected plants were rogued and destroyed. This could indicate that the natural conditions provided by the open-field quarantine may have influenced the disease to manifest from its latency period. LS has been previously reported in Thailand (Dookun-Saumtally *et al.* 2011), Malaysia (Rott and Davis, 2000), Indonesia (Ricaud *et al*. 1989). These countries are known to experience drastic changes in moisture and temperature such

as in continental climates (Ricaud and Ryan, 1989) which are conditions deemed favorable for the development of LS. Moreover, it is among the frequently reported diseases in sugarcane-growing areas in CIRAD, Montpellier, France (Daugrois *et al*. 2014; Rott *et al*. 1996). The appearance of LS in some varieties which originated from these countries may also indicate an environmental influence in disease expression. It is very serious in other sugarcane producing countries but has not been observed to occur at an epidemic level in the Philippines.

Table I. Total number of sugarcane varieties acquired and grown at post-entry quarantine greenhouse from 2001 to 2012 and types of diseases detected.

ORIGIN	YEAR ACQUIRED	NO. OF VARIETIES	DISEASES INTERCEPTED AND ELIMINATED (No. of infected varieties)	CAUSAL ORGANISM (Type of pathogen)	DISEASE INDEXING SCHEME		
					1ST CYCLE	2ND CYCLE	OPEN-FIELD
Thailand	2001	7	Mosaic **(1)**	SCMV or SrMV (virus)	x		x
			RSD **(2)**	*Leifsonia xyli* sp. *xyli* (bacterium)	x		
			Leaf scald **(1)**	*Xanthomonas albilineans* (bacterium)	x		
			YLS **(1)**	ScYLV (virus)	x		
			Pokkah boeng **(3)**	*Fusarium* spp. (fungus)	x		
			Grassy shoot **(1)**	*Candidatus phytoplasma* (phytoplasma)	x		
	2003	5	-				
	2006	6	Mosaic **(1)**	SCMV, SrMV (virus)	x		x
	2007	5	-				
Malaysia	2001	7	RSD **(1)**	*Lxx.* (bacterium)	x		
			Leaf scald **(1)**	*X. albilineans* (bacterium)	x		
			Mosaic **(2)**	SCMV or SrMV (virus)	x		x
	2003	4	-				
Indonesia	2001	6	Leaf scald **(1)**	*X. albilineans* (bacterium)	x		
			RSD **(1)**	*Lxx.* (bacterium)	x		
			Mosaic **(1)**	SCMV or SrMV (virus)	x	x	x
			Sheath rot **(2)**	*Cytospora sacchari* (fungus)	x		
	2003	5	-				
Bangladesh	2001	5	Mosaic **(2)**	SCMV or SrMV (virus)	x		x
			RSD **(1)**	*Lxx.* (bacterium)	x		
	2003	5	-				
China	2005	10	Smut **(1)**	*Sporisorium scitamineum* (fungus)	x	x	x
	2006	10	-				
	2007	10	Mosaic **(1)**	SCMV or SrMV (virus)	x		x
	2008	10	Mosaic **(1)**	SCMV or SrMV (virus)	x		x
	2009	10	-				
	2010	30	Mosaic **(3)**	SCMV or SrMV (virus)	x	x	x
			Smut **(2)**	*S. scitamineum* (fungus)	x	x	x
			Red rot **(1)**	*Colletotrichium falcatum* (fungus)	x		
			Leaf scald **(4)**	*X. albilineans* (bacterium)	x		
	2011	20	Pokkah boeng **(2)**	*Fusarium* spp. (fungus)	x		
			Red rot **(1)**	*C. falcatum* (fungus)	x		
	2012	10	Red rot **(1)**	*C. falcatum* (fungus)	x		
Australia	2002	10	-				
	2003	10	-				
	2005	10	-				
	2007	10	Smut **(2)**	*S. scitamineum* (fungus)	x		
France (CIRAD)	2005	21	Leaf scald **(1)**	*X. albilineans* (bacterium)	x		x
	2007	10	Leaf scald **(1)**	*X. albilineans* (bacterium)	x		
	2012	10	-				
Mauritius	2007	8	-				
Japan	2009	1	-				

ORIGIN	YEAR ACQUIRED	NO. OF VARIETIES	DISEASES INTERCEPTED AND ELIMINATED (No. of infected varieties)	CAUSAL ORGANISM (Type of pathogen)	DISEASE INDEXING SCHEME		
					1ST CYCLE	2ND CYCLE	OPEN-FIELD
USA	2010	9	Mosaic **(2)**	SCMV or SrMV (virus)	x		x
			Red rot **(2)**	*C. falcatum* (fungus)	x		
Vietnam	2010	10	Leaf scald **(2)**	*X. albilineans* (bacterium)	x		
Pakistan	2012	10	Mosaic **(2)**	SCMV or SrMV (virus)	x		x
			Red rot **(1)**	*C. falcatum* (fungus)	x		
TOTAL		**284**					

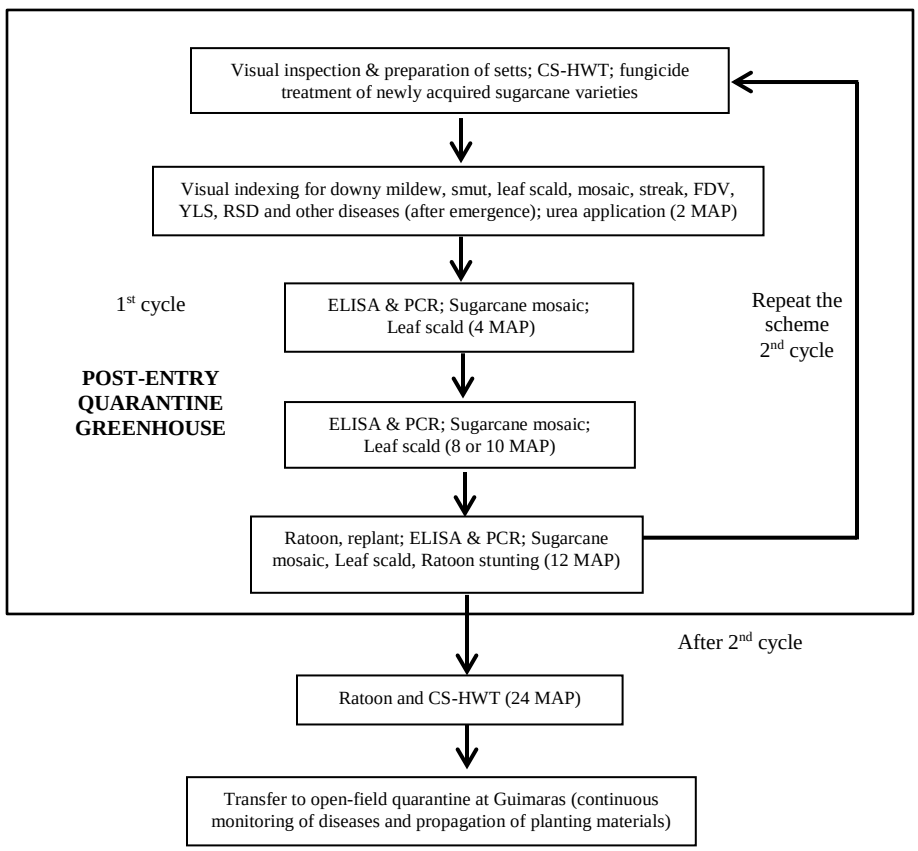

Fig. 2. Disease Indexing Scheme**.** Newly acquired sugarcane varieties were subjected to quarantine protocols for pathogen testing during the 1st cycle at the Post-Entry Quarantine Greenhouse. At first cycle, pathogen testing was done every 4, 8 and 12 MAP. Planting materials were ratooned and replanted, and the scheme was repeated for another year (2nd cycle). At 24 months, these undergone ratooning and CS-HWT, and brought to Guimaras for open-field quarantine and monitoring.

Some varieties from Bangladesh, Indonesia, and Thailand (Table 1) exhibited chlorotic and dark green discoloration on the leaf lamina (Figure 3b.1). SCMV-H strain of *SrMV* was detected among these varieties by RT-PCR. *SrMV* was detected in one variety from China which exhibited mosaic symptoms four months after emergence (Figure 3b.2). *SCMV* was also detected in varieties from Malaysia, USA and Pakistan. Presence of *SCMV* was confirmed through ELISA in some planting materials from Bangladesh, Indonesia, Thailand, Malaysia and China that showed chlorosis and dark green leaf discoloration under greenhouse conditions during the first cycle. Some varieties

from USA and Pakistan also manifested chlorotic streaks and leaf discoloration. Symptoms of SCM were not observed after exposure to CS-LHWT. This corroborates with the results of Smith (1996) where *SCMV* has been eradicated from sugarcane by serial hot water treatment of infected buds. However, the response to CS-HWT could be varietal reaction to the treatment as there were some varieties that still exhibited leaf chlorotic streaks and discoloration at the open-field quarantine. Moreover, it can be possible that the conditions provided during the two-year cycle of disease indexing inside the quarantine greenhouse are favorable for plant growth, resulting to the masking of symptoms of the viral disease. Symptoms of SCM were observed during the open-field quarantine in some varieties. All infected plants were rogued out and burned.

Mosaic disease is recorded in almost all sugarcane growing countries (Gonçalves *et al*. 2012), but only *SCMV*, *SrMV* and *SCSMV* can naturally infect sugarcane (Chatenet *et al*. 2005). The occurrence of SCM has been cited in Bangladesh (Ricaud *et al.* 1989), Indonesia (Putra and Damayanti, 2012), Thailand (Gemechu *et al*. 2004), Malaysia (Ricaud *et al.* 1989), China (Xu *et al*. 2008), USA (Ricaud *et al*. 1989) and Pakistan (Ricaud *et al*. 1989). The occurrence of SCMV strains in the Philippines has not been well-documented. Hence, *SCMV* is still considered as a disease of quarantine importance. The introduction of *SCMV* must be prevented to proscribe the spread of any strain.

The causal bacterium of RSD, *Lxx.*, was detected using DBIA in several sugarcane plants from Bangladesh, Indonesia, Malaysia, and Thailand during the first cycle. Conversely, after the second cycle and in the open-field quarantine, upon exposure of the ratoon setts to CS-LHWT, all the materials yielded negative results indicating the effectiveness of the CS-LHWT. External symptoms were not observed but reddish or pinkish discoloration on vascular bundles of the internodes were seen when the sampled stalks were sectioned (Figure 3c). Occurrence of RSD was noted in Thailand (Ricaud *et al*. 1989), Malaysia (Ricaud *et al*. 1989), Indonesia (Putra and Damayanti, 2012) and Bangladesh (Ricaud *et al*. 1989). Identification of RSD by visual inspection is often very difficult due to the fastidious nature of *Lxx.* and because the disease can be externally symptomless (Saumtally *et al*. 1996). Therefore, testing and heat treatment during quarantine are required to reduce the risk of importing infected planting materials. The results of this study also confirmed the method, as cited by Saumtally *et al*. (1996), of dual hot water treatment consisting of a SHWT followed by a three hour treatment and the CS-LHWT for eliminating the *Lxx* associated in RSD-cuttings.

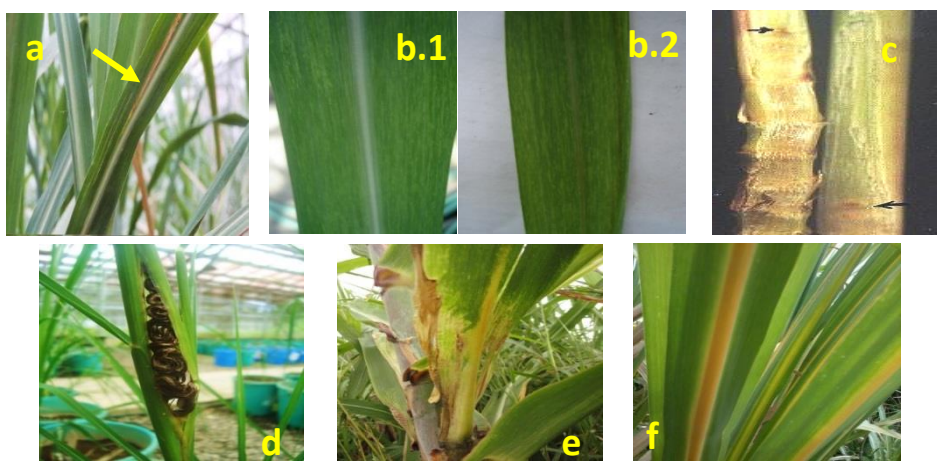

Fig. 3. Diseases of sugarcane observed in the Post-entry Quarantine, Institute of Plant Breeding - College of Agriculture and Food Science, University of the Philippines Los Baños.
(a) Leaf scald caused by *Xanthomonas albilineans* characterized by "white" pencil-line streak that developed into necrosis on leaf lamina; (b.1) *Sugarcane mosaic virus* (SCMV) with mosaic symptoms. (b.2) *Sorghum mosaic virus* (SrMv) with mosaic symptoms; (c) Ratoon stunting disease caused by *Leifsonia xyli sp xyli* characterized by pinkish discoloration as diagnostic internal symptom on infected plant; (d) Smut caused by *Sporisorium scitamineum* characterized by black, whip-like structure that developed on the spindle leaf; (e) Pokkah boeng caused by *Fusarium* spp. characterized by chlorotic discoloration at the base of young leaves and malformation/distortion of the apical shoot; (f) Yellow leaf virus (YLV) with yellowing of the midrib on the leaf underside

Visual symptoms of other diseases such as yellow leaf disease, pokkah boeng, grassy shoot, sheath rot, smut and red rot were also noted under greenhouse conditions in some varieties from Thailand, Indonesia, China, Australia, France, USA and Pakistan (Table 1). Smut was noted in some varieties from China and Australia (Figure 3d). Occurrence of Pokkah boeng disease (Figure 3e) was noted during the early stage of growth of some varieties from Thailand and China. The plants, however, recovered from infection during stalk elongation stage and the sp read of the disease inside the glasshouse was controlled by fungicide treatment. One variety from Thailand exhibited yellowing of midribs typical of *ScYLV* infection (Figure 3f). Grassy shoot disease was seen in one variety from Thailand. On the other hand, two varieties from Indonesia were observed to have sheath rot disease. All the infected plants were destroyed. The pathogens were eliminated by roguing out infected canes, and through CS-HWT of LS- and RSD-infected canes at the early stage of the disease. Smut was observed in the open-field quarantine. The infected plants were rogued out and burned.

The post-entry quarantine period inside the greenhouse provided enough time for the plants to grow and exhibit symptoms of diseases. Situation inside the greenhouse, however, sometimes did not simulate the actual field conditions. This inhibited the growth of undetected pathogens that are present in the planting materials, hence, growing of introduced materials in an open quarantine area which follows strict land management protocols, and is isolated from commercial sugarcane farms proved to be effective for disease expression. The natural environmental condition in the open-field quarantine may trigger the expression of symptoms not observed inside the post-entry greenhouse (Dodman, 1996).

CONCLUSION

Pathogen testing protocols for major diseases of quarantine importance such as LS, SCM and RSD that were employed in this study were beneficial especially for propagating clean planting materials. Likewise, using CS-HWT was a successful way to eradicate these pathogens. Since these methods were proven effective for pathogen detection and elimination, these will be used routinely for testing varieties that will be imported into the Philippines in the future, to ensure disease-free plants to increase sugarcane productivity and diversity.

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