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Annual report

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1 Progress summary

Following the first report of Sri Lankan cassava mosaic disease (SLCMD) from a single, isolated plantation in Eastern Cambodia in December 2015 (Wang *et al.*, 2016[1]), the Short Research Activity (SRA) "*Developing and emergency response and long term management strategy for Cassava Mosaic Virus in Cambodia and Vietnam*" facilitated the completion of large-scale virus surveys covering the major cassava production districts of both Vietnam and Cambodia. The first phase of this SRA (2016-2017) survey detected SLCMD in two provinces of Eastern Cambodia in the 2016 cropping season. In 2017, Vietnamese authorities reported the virus in a province of Southern Vietnam, which was identified by our seed systems analysis as a major stake provider to other regions of both Vietnam and Cambodia. To date, SLCMD has been officially reported by national authorities for six provinces of Eastern and Central Cambodia, and two provinces of Southern Vietnam. A region-wide effort needs to be facilitated with diverse stakeholder to prevent further spread through the movement of infected stakes and insect vectors. While the challenge is huge, actual action needs to be led by national partners and few viable alternatives are currently available to deal with the situation.

During the second phase of the SRA (2017-2018), capacity building for SLCMD diagnosis was carried out in Cambodia, followed by a technical expert meeting in Vientiane, Lao PDR with participants from organizations in Thailand, Tanzania, and the United States. In addition, in CIAT Headquarters, a database for documenting the geographical occurrence and severity of pests and diseases was established. Diagnosis using Next Generation Sequencing was carried out on SLCMD symptomatic plants in CIAT-Headquarters. In parallel, communications activities were undertaken to develop basic extension materials for raising farmer awareness about this rapidly spreading disease, and to develop a blog post to voice key messages from our findings in the SRA and farmers' stories. In addition, CIAT has begun preparations for the undertaking of a business case study, which will evaluate existing business models for clean seed multiplication in Southeast Asia. Based on the SRA findings, a regional expert meeting for control of the disease with national institutions in Cambodia, Vietnam, Thailand, and Lao PDR will be held from 17th to 22nd September 2018 with support from ACIAR and GCP21.

During the second phase, key findings from the first phase were communicated both to scientific, governments and private sector stakeholders, and activities were undertaken to contribute to increase the capacity of young Cambodian scientists for responding to the spread of SLCMD. Highlighted progress and results include:

[1] Wang, H. L., Cui, X. Y., Wang, X. W., Liu, S. S., Zhang, Z. H., and Zhou, X. P. (2016). First report of *Sri Lankan cassava mosaic virus* infecting cassava in Cambodia. Plant Dis. 100, 1029-1029.

- Activity #1.4: Upon request from GDA, an SLCMD survey was conducted in Western Cambodia.
- Activity #1.7: Based on the results acquired from the first phase of the SRA, two journal articles on bi-national SLCMV surveillance and existing cassava seed systems were prepared for submission to Frontiers in Plants Sciences.
- Activity #1.8: The geographical distribution of Sri Lankan cassava mosaic virus (SLCMV) from the first phase was registered in the newly built database PestDisPlace (http://pestdisplace.org).
- Activity #2.1: An extension poster in local languages was designed for farmer extension, and series of video and blog post including CMD stories from Cambodian farmers were prepared for distribution to multiple stakeholders in the region.
- Activity #2.2: A capacity building event which consisted of field sampling and laboratory diagnosis training was carried out in Cambodia in June 2018. This activity trained four key trainees in the country to conduct sustainable SLCMV monitoring with molecular-based confirmation.
- Activity #2.4: A technical expert meeting was held in Laos. The results from the SRA were shared, and future activities for efficient disease control were discussed. A regional expert meeting is planned in November 2018, during the no-cost extension phase of this SRA.
- Activity #3.2: A total of 2,609,847 small RNA sequences were obtained four symptomatic plants. No other DNA/RNA viruses were detected besides SLCMV.
- Activity #3.3: Business feasibility case studies will be conducted during the no-cost extension phase of the project to cover 3 existing clean stake multiplication initiatives in Cambodia, Vietnam and Thailand. This study will seek to gather comparative data and analysis on the economic, organizational, technical, and social components of existing multiplication initiatives / stake production pipelines.
- Key results of the SRA phase I were presented in the special session of CMD outbreak in Southeast Asia, in IVth GCP21 conference held on 11-15th June in Benin.

Results of our 2-country survey indicated that by the 2016 cropping season, SLCMD had only spread within a limited geographical range from the site of the initial report. However, since then many reports of CMD-like symptoms have been made across Cambodia and Southern Vietnam, including the most intensive cassava-producing provinces of both countries. During the field sampling training in Cambodia, heavily infested fields were noted in which planting materials were indicated to have originated across the border via trader, suggesting that CMD continues to be spread throughout the region by stake trading, as well as by whitefly transmission. Socioeconomic feasibility analysis of clean seed production pipelines for feeding into new and existing seed supply networks will form a critical part of any mitigation strategy for SLCMV in the region, and the first stage of this work was planned within the second phase of the SRA based on the selected of three case studies ranging in complexity. Given that asymptomatic SLCMV-infected plants were detected in the SRA phase I survey, it is important for national research institutions to have a way to confirm viral infection by molecular biological methods in their own laboratories. The lab-based

capacity building exercises were conducted without using high-cost commercialized kits for DNA extraction process, allowing for ongoing sustainable diagnosis. As a first step in the establishment of national coordination platforms, a technical meeting with CMD experts from Africa and breeding experts from Thailand was held; an activity which lays the groundwork for the regional meeting to will be held in September.

2 Achievements against project activities and outputs/milestones

Objective 1: To generate an accurate, baseline diagnosis (including map) of the current geographical distribution of SLCMD in Cambodia and Vietnam (including measures of field-level incidence and severity) and baseline information on the insect / anthropogenic vectors involved in SLCMD spread

No	Activity	Outputs/ milestones	Completion date	Comments
1.1	Organize a multi-stakeholder workshop on emerging cassava plant health threats, with involvement of national actors (Cambodia: GDA, CARDI; Vietnam: PPD, PPRI) and representatives of ongoing, international cooperation programs on crop health (e.g., CRC Biosecurity project, CAVAC II, UWA) to share current knowledge and plan collaboration during the implementation of the SRA.	(a.) Plant and insect sampling protocols and farmer survey strategies were discussed and validated; (b) Target districts for large-scale plant and whitefly sampling were presented and adjusted according to expert knowledge; (c) Seed system survey methods were discussed and the tools validated; (d) Lead responsibilities and involvement in sub-components were divided among the project stakeholders in both Vietnam and Cambodia; (e) Initial work plan was developed and priorities were agreed upon.	Sept, 6, 2016	The actual involvement of partners changed once activities started to be implemented, based on real capacity to deliver in a short timeframe or within the possibilities of the budget
1.2	Develop a survey and sampling protocol following a customized sampling design that fully takes into account planting area per province and growth stage of the crop.	(a) Protocol developed and validated, (b) survey team collected: young leaf tissues, pictures of whole-plant and apex, whitefly population counts, whitefly bodies of nymphs and adults, and seed trading information at the household-level	Nov. 01, 2016	Based on 2014 cassava production data, 15 districts per country were selected to survey SLCMV occurrence and incidence in both countries. In Cambodia, Koun Mom district in Ratanakiri province was added as it was where the disease was first reported
1.3	Train a survey team in Vietnam and Cambodia (including government plant health officers) in the implementation of the standardized baseline diagnostics surveys & plant / insect sampling / coding, including field testing	(a) field survey training was conducted in (i) Hung Loc station in Dong Nai province, Vietnam and (ii) Phnom Penh & Dambae district, Tboung Khmum province, Cambodia.	Nov. 2016	3 survey teams in Vietnam (for Northern/Central/Southern region) and 2 in Cambodia (for Western / Eastern region) were organized. In total 6 governmental partners (3 / country)

1.4	Implement the baseline diagnostics surveys and conduct extensive plant / insect sampling in both countries	[2017] (a) Baseline survey for both seed systems and SLCMD covered 446 households (240 Cambodia / 206 Vietnam); (b) Samples collected for a total of 419 fields and 6,480 plants (15 fields per district, 16 plant samples per field) [2018] (a) CMD survey in five provinces of Western Cambodia with the coordination of GDA in July 2018 (b) Diagnosis of SLCMD on samples collected from the survey (a) in July-August 2018 (ongoing)	Nov. and Dec. 2016 (survey) June 2017 (PCR) July 2018 (Survey and Diagnosis training)	[2017] The surveys were conducted by the national partner teams, including PPD, PPRI and IAS in Vietnam and RUA, GDA and PDA in Cambodia. [2018] Upon request from GDA, a survey in Western Cambodia was immediately conducted after the first notice from PDAFF Oddar Meanchey. Two young trainees from GDA that participated in the training in June 2018 (see Activity#2.2) joined the survey.
1.5	Conduct centralized data entry and data cleaning of the completed diagnostics surveys.	(a) Data Entry and quality control for regional seed systems and diagnostics survey completed, (b) Translations, data entry and quality control for the zoom-in seed system surveys completed	May - June 2017	A MSc. intern from Wageningen University helped with data entry, organization and cleaning
1.6	Conduct centralized disease diagnosis on cassava leaf and insect samples, using existing protocols (serological and nucleic-acid based)	(a) SLCMD PCR-based diagnosis conducted at CATAS, China for a total of 6,480 plant samples, (b) Biotype identification of whitefly samples were completed with expert taxonomist in Thailand covering all districts and 35.7% of the fields	June 2017 (PCR)	We extracted total DNA from all the samples using a modified CTAB method and ran PCR-based diagnostics detecting the *AC1* gene of SLCMV. Given the large amount of samples and the limited capacity in Cambodian and Vietnam for processing an agreement was signed with CATAS, China.
1.7	Conduct statistical analysis, generate maps and draft a working paper on the baseline situation of SLCMD geographical incidence, severity / incidence, and direction of spread, as well as disease vectoring	[2017] (a) Write-shop for drafting manuscripts of two journal articles (one on virus geographical distribution, and another on seed systems analysis) held 18-23th June, (b) submission of manuscripts foreseen for October 2017 [2018] (a) Submission of two manuscripts on virus survey and seed systems in August 2018, (b) Uploading the seed systems survey results in CIAT Dataverse in June 2018.	N.A. (On track)	*Continued in 2017-2018.* A collaboration with experts on network analysis from the University of Florida was established. This will likely result in another publication elaborating scenario analysis. Submission of two articles with co-citation in the same journal, providing a cohesive picture of project results. Data uploading from conducted SLCMV surveys is in Activity 1.8 (see below).

1.8	Develop georeferenced global database (utilised by multiple stakeholders) that keeps accurate spatial and temporal dynamics of invading pathogen populations with the dynamics and intensity of a surveillance system	(a) Build-up the database on pests and diseases occurrence "PestDisPlace" in a CIAT Crop Protection team strategy, (b) uploading geographical distribution of SLCMD which was obtained from survey in the first phase of the SRA (Y1)	August 2017	PestDisPlace is available at: http://pestdisplace.org/ Depending on contributors' requests, data sensitivity, or manuscript submission, the data is open either to the public, or with restrictions defined by the data contributor.

PC = partner country, A = Australia

Objective 2: To generate broad-level awareness of the risks posed by SLCMD and to build critical capacity among multiple stakeholders, including researchers, plant protection officers and extension agents, to deal with disease

No	Activity	Outputs/ milestones	Completion date	Comments
2.1	Develop a highly didactical information-extension package on SLCMD, its symptoms and management for public or private sector actors.	(a) Design of the poster for farmers' awareness raising of cassava mosaic disease in June 2018, (b) distribution of the poster to the extension points (ongoing), (c) Development of the blog about the statement from experts on CMD outbreak in Southeast Asia in July 2018	N.A. (On track)	*Continued in 2017-2018.* Progress was made on 1 poster and 1 basic recommendation sheet for SLCMD recognition and on-farm management options. Posters in local language will be distributed the non-cost extension phase of the SRA (Y3).
2.2	Organize a technical training for plant health researchers and authorities on sampling protocols, laboratory-based diagnostics and recommended post-baseline-diagnostics surveillance.	[2017] (a) Technical training on sampling protocols provided in Vietnam for PPD, PPRI and IAS (2016), (b) Technical training on sampling protocols provided in Cambodia for RUA, GDA and PDA (2016), (c) Post-baseline diagnostics recommendations provided in Cambodia and Vietnam to RUA, GDA, PDA, PPD, PPRI and IAS (2017) [2018] (a) Technical training on laboratory-based SLCMV diagnosis conducted at PPSP of GDA, Cambodia from the 11th to 13th of June, 2018.	June 2018	*Continued in 2017-2018.* *Related to Activity No. 3.1.* [2017] Formal technical training was provided during dedicated events and backstopping a numerous meetings dealing with the disease outbreak situation. [2018] Training materials and DNA extraction protocols were translated and presented in Khmer language. In addition to chemicals, reagents and consumables, tablets used in the first phase of the national survey were provided for further GDA-led field monitoring of CMD.
2.3	Elaborate a focused strategy document for sector-wide sensitizing with actions, research needs, or targeted biosecurity measures, based on the baseline diagnostics data to devise SLCMD management / mitigation plans for the short, mid- and long-term.	(a) Short-/Mid-/Long-term strategies were discussed and proposed in the group work with national partners in a result-sharing workshop in Cambodia, on 19th July, (b) most of these strategies are incorporated into the national cassava strategy under development by UNDP and GDA in Cambodia (see section 8 on problems / opportunities for an overview of strategies).	19th July	In Cambodia the proposed strategies are incorporated into the national cassava strategy. In Vietnam progress is made at the level of MARD, yet buy-in from the starch factories via VICAAS is yet to be assured.

2.4	Organize a multi-stakeholder closing workshop, with involvement of government officials, plant health authorities and private sector actors to share the project's finding and present / discuss the strategy document.	[2017] (a) A results-sharing workshop for to share key findings, implications and discuss short- / mid- / long-term strategies for mitigation SLCMD held in Phnom Penh in July 2017. [2018] (a) Communication with Cambodian national authorities has been ongoing since the results-sharing workshop in July 2017 (Y1), (b) Technical meeting with experts on CMD in Africa and cassava breeding for discussing strategies and reviewing activities, Vientiane, 19-20th June, 2018.	June 2018	*Continued in 2017-2018.* [2017] High-level participation was achieved and recommendations taken forward by national institution. [2018] Dr. Graham Thiele, Director of the CGIAR- RTB research program, and Dr. Luis Augusto Becerra Lopez-Lavalle CIAT Cassava program leader, also joined the technical expert meeting.

PC = partner country, A = Australia

Objective 3: Enhance diagnostic capacity of national partners utilising existing technology, evaluate improved diagnostic technologies utilising NGS technology and the feasibility of different short-term control measures

No	Activity	Outputs/ milestones	Completion date	Comments
3.1	Provide hands-on capacity building on PCR-based, virus sequence analysis and field diagnostics that can be applied by national partners, and different primer options such that national programs can continue to contribute autonomously to surveillance and study of SLCMV	(a) Meeting with Deputy Director of GDA for discussion about training event in January 2018, (b) Leaf sampling of CMD symptomatic plants from Tboung Khmum and Kampong Cham province for lab training in January 2018, (c) Meeting with Director of Department of PPSP from GDA to discuss training aims and a plan for field survey of Western Cambodia in June 2018, (d) Combined diagnostics at the lab and field visit from 11th to 15th June 2018	June 2018	A close collaboration with GDA staffs both from department of Plant Protection Sanitary and Phytosanitary (PPSP) and department of Industrial Crop (DoIC) was established. This will likely result in another survey in Western Cambodia in August 2018, responding to farmer's symptom recognition in Oddar Meanchey in June 2018. For the training event, see details in *4. Training activities* of this report.
3.2	Evaluate Next Generation Sequencing (NGS) applied to diagnostics to track the occurrence of other cassava-infecting viruses in symptomatic and asymptomatic plants	(a) High-throughput sequencing of small interfering RNA (siRNA) by miSeq	March 2018	Both symptomatic and asymptomatic plants were used for siRNA sequencing.

3.3	Conduct a feasibility study of different practical and more advanced options (isolation, QDS, etc.) to support ongoing business models implemented by through private-public partnerships. This activity will be conducted in conjunction with AGB/2012/078 and ASEM/2014/053.	(a) confirmation and data gathering from three existing seed multiplication initiatives in Vietnam, Cambodia, and potentially Thailand, (b) compilation and analysis of comparative technological, organizational, social, and economic components of multiplications schemes, (c) completion of a report with descriptive and comparative analysis of (clean) seed multiplication pipelines in the project region	May N.A.	Three model cases will be evaluated. This activity will be carried out during the non-cost extension phase of the SRA (Y3), as a logical next step following our increased understanding of local seed systems

PC = partner country, A = Australia

See the **Annex 2** as a reference of activities done in the first phase of the SRA (2016-2017).

3 Impacts

3.1 Scientific impacts

3.1.1 CMD symptom observation and sample collection in Western Cambodia

Following the request made from GDA on June 11th, 2018 (see also section 3.3), Dr. Luis Augusto Becerra Lopez-Lavalle, Cassava program leader of CIAT had a discussion with GDA about the implementation of a survey in Western Cambodia in the last week of June. The survey team included two young GDA trainees who participated in the hands-on training of the SRA, Dr. Wilmer J. Cuellar (Virologist, CIAT-HQ), Dr. Nami Minato (Plant Pathologist, CIAT-Asia), and Mr. Sok Sophearith (Cassava Research Associate, CIAT-Asia). The survey was conducted in five provinces (Pursat, Battambang, Pailin, Banteay Meanchey, and Oddar Meanchey) from July 9th to 14th, 2018.

Cassava plants with CMD-like symptoms were found in four provinces: Pursat, Banteay Meanchey, Oddar Meanchey, and Kampong Thom (**Figure 1 and Photo A-E**). Fields with almost 100% in-field incidence were observed in Prusat province, Svay Chek district of Banteay Meanchey province, Trapeang Prasat district of Oddar Meanchey province, and Sam Tuk district of Kampong Thom province. Molecular confirmation will be completed from the end of July to early August, following the protocol shared in the training event (see details in section 3.2).

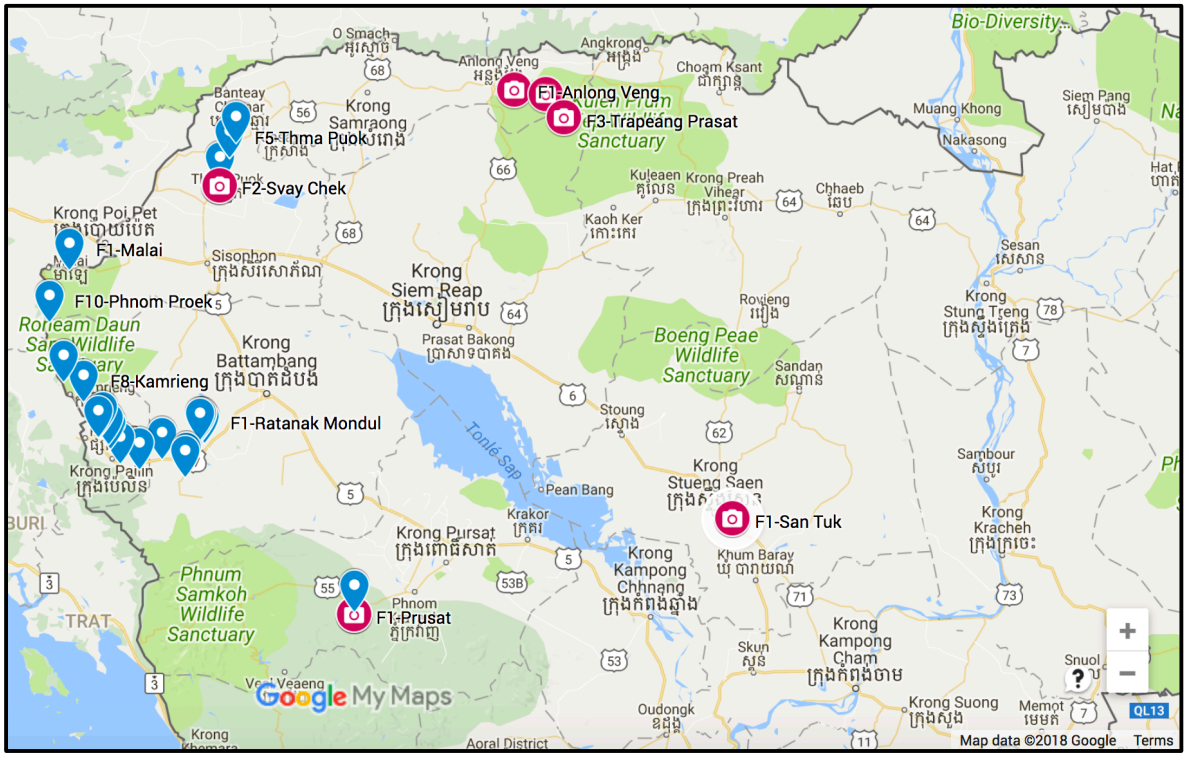

Figure 1. Location of surveyed fields in Western Cambodia. Red mark indicates the field site with CMD symptomatic plants, and blue one represents the field without symptomatic plants.

Photo A -E. Survey for quick response to CMD outbreak in Western Cambodia (July , 2018). (A) Survey team with two farmers that own diseased fields. (B) Two participating GDA researchers whom were trained at the capacity building event in June. (C) Plants displaying CMD-like symptoms. (D) Plants with typical foliar mosaic symptoms. (E) Severely symptomatic plant likely infected through planting material.

3.1.2 Key findings in submitted journal article #1: SLCMV occurrence in Cambodia

For SLCMV diagnostics leaf sample collection was conducted covering on total of 419 fields (**Figure 2;** Herrera Campo *et al*., 2011[2] and Herrera Campo *et al*., 2014[3]) and 6,480 plants (15 fields per district, 16 plant samples per field). We extracted total DNA from all of the samples using a modified CTAB method, and ran PCR-based diagnostics detecting the *AC1* gene of SLCMV.

Our study provides the first systematic baseline assessment of SLCMV presence and incidence for Cambodia and Vietnam, following the first report in Eastern Cambodia (Wang *et al.*, 2016[1]). Stung Treng, a neighboring province to the site of the original report, was found to have a higher incidence of the disease in our sampling, however positive detections remained restricted to Eastern Cambodia. The range of distribution of the virus was up to 70 km away from the initial 2015 detection site. We found asymptomatic infection of SLCMV on 16% of diseased plants. Some of the fields in Ratanakiri and Stung Treng consisted both of plants with systemic and non-systemic symptoms. Sseruwagi *et al.* (2014)[4] distinguished cutting- and whitefly-derived infections based on the fact that infection through planting material causes systemic mosaic symptoms, including on the lowest, oldest leaves, while insect vector transmission induced mosaic symptoms only on younger, upper leaves.

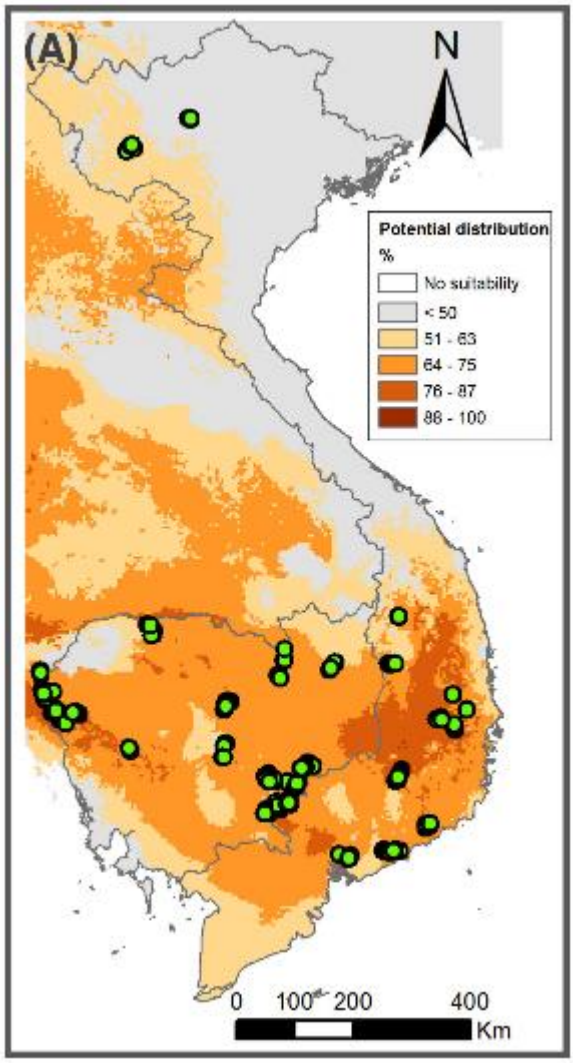

Figure 2. Survey sample locations from th current study (green dots) overlaying the potentia distribution of the whitefly vector of SLCM (Herrera Campo *et al*., 2011 and Herrera Camp *et al*.. 2014).

This suggests that SLCMV infection observed in Cambodia during the 2016 cropping season was derived from both infected cuttings, and whitefly transmission. Vectoring by whitefly may have already contributed to the dispersal

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[2] *Herrera Campo, B. V., Hyman, G., and Bellotti, A. (2011). Threats to cassava production: known and potential geographic distribution of four key biotic constraints. Food Secur. 3, 329-345.*

[3] *Herrera Campo, B. V., Hyman, G., and Bellotti, A. Data from: Replication data for: Threats to cassava production: known and potential geographic distribution of four key biotic constraints. CIAT - International Center for Tropical Agriculture Dataverse. (2014) https://dx.doi.org/10.7910/DVN/27490*

[4] *Sseruwagi, P., Sserubombwe, W. S., Legg, J. P., Ndunguru, J., and Thresh, J. M. (2004). Methods of surveying the incidence and severity of cassava mosaic disease and whitefly vector populations on cassava in Africa: a review. Virus Res. 100, 129-142.*

of SLCMV. In addition, planting materials from infected fields were mostly acquired from the farmer's own seed supply from the previous year, or from others within the community.

3.1.2 Key findings in submitted journal article #2: Seed systems

We conducted a first baseline characterization of existing cassava seed systems in 2016-17 through a farmer survey approach from national to community scales, focusing on identifying seed system actors, planting material management & exchange mechanisms, geographies, and variety use. Despite their status as farmer-organized 'informal' networks, the cassava seed systems in Vietnam and Cambodia are complex, connected over multiple scales, and include links between geographically distant sites (> 250 km). Cassava planting material was exchanged through largely informal farmer seed systems**,** in which re-use of farm-saved supply and community-level exchanges dominated, and participation by NGO, government, and private sector actors was relatively rare. At the national level, use of own saved seed accounted for 47 and 64% of seed use cases in Cambodia and Vietnam, respectively. Movement within communes was prevalent, with 82 and 78% of seed provided to others being exchanged between family and acquaintances within the commune in Cambodia and Vietnam, respectively. Yet, a proportion of seed flows, mediated mostly by traders, formed a robust inter-provincial and international exchange network, with 20% of Cambodia's seed acquisitions imported from abroad, especially neighboring Vietnam and Thailand. Province-province exchanges linked the high-intensity production areas of both countries, which in turn act as local redistributors in subsequent seasons.

Traders and local cassava collection points played important roles in the planting material distribution network at particular sites, with trader activity most prevalent in the highest production intensity site of Tay Ninh, Vietnam, where 27% of seed use and 69% of seed provided to others involving traders. Sales of planting material were important means of both acquiring and providing seed in both countries, and were more prevalent in high-intensity than in low-intensity production sites. Considerable variability existed in local seed exchange networks, depending on the intensity of production and integration with trader networks. At all sites varietal diversity was low, with an average of 1.38 and 1.09 varieties mentioned per household in Cambodia and Vietnam, respectively.
Adapted innovations are needed to upgrade cassava seed systems in light of emerging pests and diseases, taking into account and building on the strengths of existing systems. Key entry points for seed systems interventions in the Cambodia/Vietnam context include:

- **Improvement of farmer seed production and selection practices**, both in-field and in exchange transactions, including positive and negative selection, the use of a 'corner of prosperity' approach in which ~10% of the field is managed as a seed lot, and farmer and trader education about plant disease causes, symptoms, and eradication.
- **Combining seed network analysis with surveillance and biophysical models**, in order to identify key areas for surveillance and interventions, increasing their efficiency.
- **Technological innovations in clean seed multiplication and traceability** to increase the efficiency and cost of certified or quality declared cassava seed at centralized and/or decentralized scales, notably rapid virus indexing (including of stake tissues),

increasing cassava's low multiplication rate through technologies such as the use of mini-setts, and developing traceability technologies.

- **Integration of clean seed production schemes with informal seed networks** to ensure that the strengths of the existing informal seed systems (including cost-efficient dissemination networks and integration with trust-based social networks) are not lost.

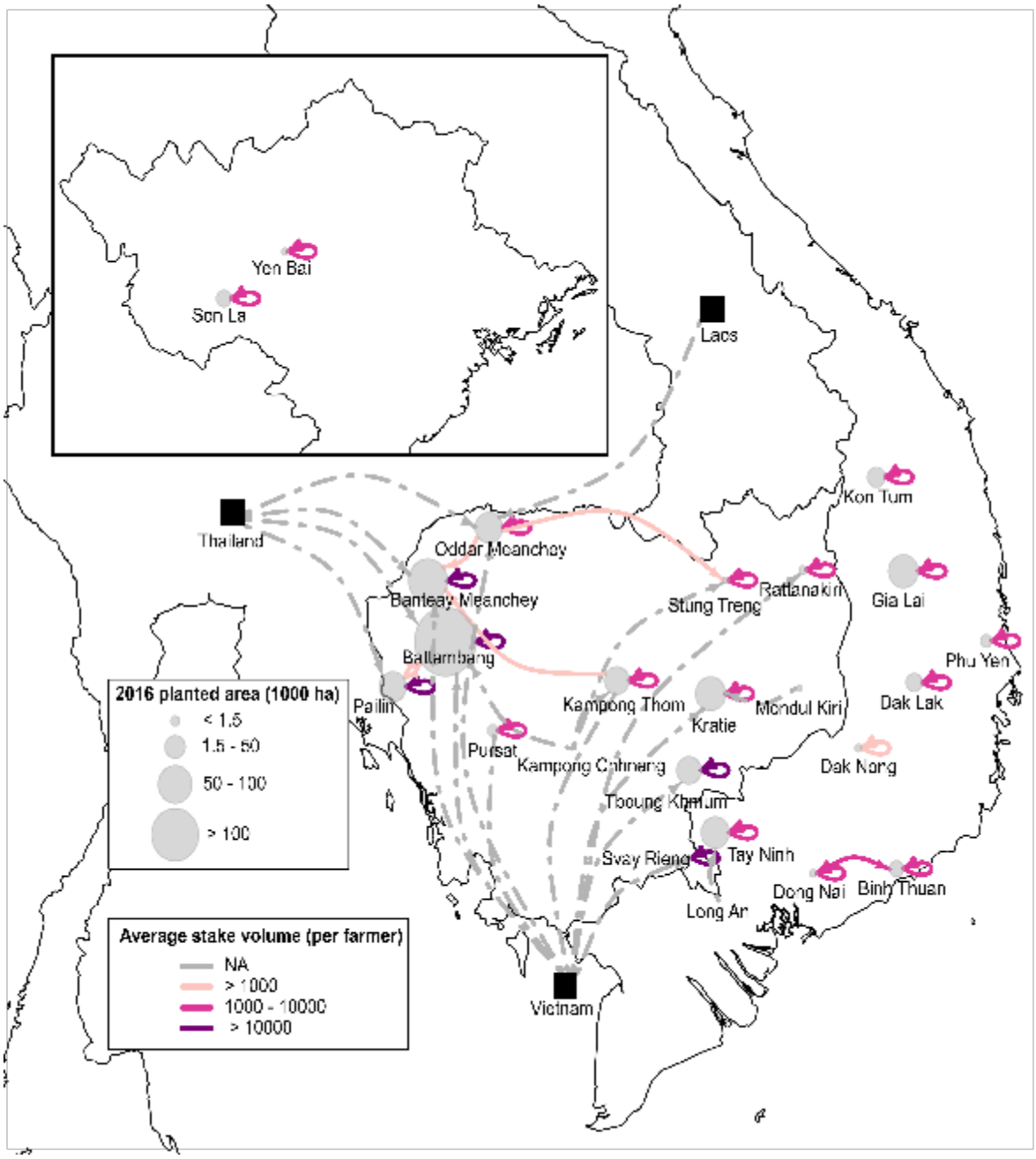


Figure 3. Inter-province stake exchange recorded in the 2016 farmer survey (average per farmer). Grey, hashed links do not reflect volume, but instances of stake transactions from unsurveyed regions, where there was no formal sampling effort. Country-level links indicate transactions in which farmers did not know where in the country the seed originated. Self-loops indicate within province exchange.

3.1.3 Establishment of the database on pest and disease distribution

PestDisPlace (http://pestdisplace.org/) is a database on crop disease and insect pest developed by CIAT researchers for continuous monitoring of their temporal and spatial distributions. This is a collaborative initiative to integrate the occurrence of symptoms and associated pests and pathogens, including confirmation by molecular diagnostics. To date PestDisPlace shows geographical distribution of pests and diseases on cassava, cassava mosaic disease and mealybug, and the data keeps being updated right after given the confirmation from national authorities and research publications.

3.1.4 Next Generation Sequencing (NGS)

Small interfering RNA (siRNA) sequencing

To discard the occurrence of other viruses in the samples we aim to sequence siRNA. Total RNA from a set of symptomatic and asymptomatic samples were extracted using CTAB and low molecular weight RNA was separated after electrophoresis in 4% agarose gels. Libraries were synthesized in-house from siRNAs following a standard protocol. After verifying concentrations and quality of the libraries using a Bioanalyzer (Agilent Genomics), sequencing was carried out using a miSeq (Illumina) at CIAT HQ.

A total of 2,609,847 small RNA sequences were obtained from 4 symptomatic plants and 226,641 sequences from 4 asymptomatic plants. Mapping and Assembly with Qualities (MAQ) analysis (maq-0.7.1) was performed to map reads to a list of all reported cassava infecting viruses (**Table 1**). This way we were able to simultaneously test for 22 viruses in the samples. The sequence assembly visualization of mapped reads to the virus reference sequences was done with TABLET software. A total of 7.4% of reads were mapped to SLCMV DNA-A (75%) and DNA-B (25%) segments in the pool of CMD symptomatic plants.

Confirmation by (RT-)PCR and Sanger sequencing

To verify the absence of other geminiviruses apart from SLCMV, we used generic PCR primers. Phylogenetic analysis of individual PCR products obtained corresponding to the capsid gene, showed that all sequences grouped in within the *Sri Lankan cassava mosaic virus* (*SLCMV*) group. To verify the absence of RNA viruses we used specific PCR primers for each virus as listed in **Table 1.**

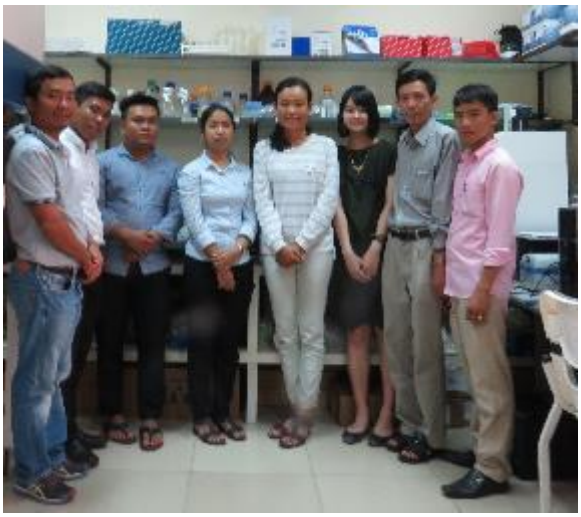
Table 1. Reference sequences used to test for the presence of different viruses in samples from Ratanankiri, Cambodia. Only SLCMV was detected in Ratanankiri province by using NGS of small interfering RNAs.

Virus species	Type	Genbank acc. nr.	Origin
Cassava brown streak virus (CBSV)	RNA	FN434436.1	Africa
Uganda Cassava brown streak virus (UCBSV)	RNA	FJ039520.1	Africa
Africa cassava mosaic virus (ACMV_Nigeria)	DNA_A DNA_B	X17095.1 X17096.1	Africa
Africa cassava mosaic Burkina Faso Virus	DNA_A DNA_B	HE616777.1 HE616778.1	Africa
Cassava mosaic Madagascar virus (CMMV)	DNA_A DNA_B	HE617299.1 HE617300.1	Africa
East African cassava mosaic Cameroon virus (EACMCV)	DNA_A DNA_B	AF112354 AF112355	Africa
East African cassava mosaic Kenya virus (EACMKV)	DNA_A DNA_B	AJ717580 AJ704965	Africa
East African cassava mosaic Malawi virus (EACMKV)	DNA_A DNA_B	AJ006460 AJ704965	Africa
East African cassava mosaic Zanzibar virus (EACMZV)	DNA_A DNA_B	AJ717562 AJ704942	Africa
South African cassava mosaic virus (EACMZV)	DNA_A DNA_B	AF155806 AF155807	Africa
Cassava mosaic Madagascar alphasatellite	DNAsat	HE984148	Africa
Cassava virus C	RNA	FJ157981	Africa
Cassava green mottle virus	RNA1 RNA2	MG581962 MG581963	Asia
Sri Lankan cassava mosaic virus-[Colombo] (SLCMV)	DNA_A DNA_B	AJ314737 AJ314738	Asia
Indian cassava mosaic virus	DNA_A DNA_B	NC001932 NC001933	Asia
Cassava common mosaic virus (CsCMV)	RNA	NC001658	America
Cassava vein mosaic virus (CsVMV)	DNA	NC001648	America
Cassava new alphaflexivirus (CsNAV)	RNA	KC505252	America
Cassava Polero-like virus (CsPLV)	RNA	KC505249	America
Cassava Torrado-like virus (CsTLV)	RNA1 RNA2	KC505250 KC505251	America
Cassava virus X (CsVX)	RNA	NC034375	America

3.2 Capacity impacts

A hands-on training on field leaf sampling and laboratory-based SLCMV diagnostics was organized in Cambodia from 11-15th of June, 2018 (**Photos F and G**; see also **section 4 on Training Activities**). On completion of the training, all the materials necessary to conduct regular surveillance of disease spread were transferred to GDA (**Table 1**), enabling them to follow the same survey and diagnosis protocols that were used in the SRA Phase I, such as collecting GPS coordinates and photographing of sampled plants with tablets, DNA extraction using laboratory-made buffer solution, and SLCMV diagnosis by PCR. Accompanying protocols were translated into Khmer language and provided to GDA.

Photo F & G. Hands-on training on cassava mosaic disease (CMD) diagnosis for the project "Developing and emergency response and long term management strategy for cassava mosaic virus in Cambodia and Vietnam" (June, 2018)



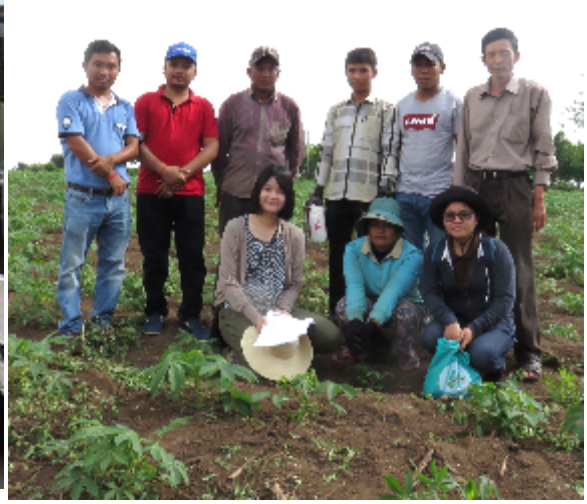


Table 1. List of chemicals, consumables, and reagents established in the laboratory of Department of PPSP of GDA in Cambodia.

#	Reagent/Chemical/Consumables	Unit
1	EDTA	-
2	Tris	-
3	HCl	2.5L
4	NaCl	1 kg
5	PVP40	-
6	CTAB	-
7	Chroloform	1.0L
8	Isopropanol	1.0L
9	Ethanol (100%)	2.5L
10	DreamTaq Green Master Mix (2x)	400 reactions
11	1kb DNA Marker	1mL

12	6x Loading Dye	1mL
13	Redsafe (20,000x)	1mL
14	agarose	-
15	tip - 1000µL	1000/bag
16	tip - 200µL	1000/bag
17	tip - 10µL	1000/bag
18	1.5mL tube	500/bag
19	Groves	1box
20	1.5mL tube rack	2 rack
21	0.2mL tube	1000/bag
22	2mL tube	500/bag
23	Primers (4 primers: 84mer)	

3.3 Community impacts

In the 12 months of implementation, SRA phase II has had an impact on the capacity of Cambodian national scientists to monitor SLCMD. The importance of capacity building for tackling the emergent CMD problem was discussed with the Deputy Director of GDA in January 2018, and diagnostic training followed in June 2018. The additional survey activity in Western Cambodia in July 2018 followed consultation with the Director of PPSP of GDA during the training, and was responsive to farmers' reports of suspicious CMD-like symptoms. The importance of building these open dialogues and relationships of trust among farmers, government, and international research centers is critical to a continued effective monitoring and response emerging pests and diseases.

3.3.1 Economic impacts

Like other, closely-related cassava mosaic viruses, it is anticipated that SLCMD will have an impact on yield, and therefore also on the economic performance of the cassava crop in Southeast Asia. Furthermore, the development of clean seed systems will require an economic rationale based on willingness to pay, and real demand. We also hope that our SRA activities will inform preventative measures by the respective governments involved, and thereby help to mitigate economic losses. Our current findings do not yet have the benefit of data on yield or economic impacts due to this disease.

Agronomic trials that conducted in ASEM/2014/053 and a small research activity funded by CAVAC, will help to assess yield penalties associated with the disease and changes in the response to fertilizer application. These studies will also help to understand the rate of infection when farmers plant clean material in locations where the disease is present and whether positive and negative selection of more resilient varieties can maintain yields at a viable level while resistant varieties are developed.

Farmer anecdotes have suggested yield losses of around 50%. A reduction in feedstock in southern Vietnam and eastern Cambodia will have serious implications for the starch sector. Root prices in Tay Ninh are around $150USD/t, which will make native cassava starch (that peaked at $550 USD/t) relatively expensive compared to substitutes.

While the SRA has not delivered any economic impacts to date, the outcomes are contributing to the planning and actions that will be required to minimize losses in the future. Failure to act will jeopardize the cassava industry in SE Asia.

3.3.2 Social impacts

There have been no significant social impacts of the SRA to date. The uncontrolled spread of CMD is expected to have significant impact on the livelihoods of cassava producers in the region. Impacts are expected to include indebtedness and forced asset sales. Once again, the SRA is contributing to plans and activities to minimise these losses.

3.4 Communication and dissemination activities

The SRA team has been actively communicating with national authorities to ensure the realities of this phytosanitary emergency are understood. The results of surveys under the SRA have been broadly shared and discussed with experts from various organizations during the technical meeting (in June 2018; **Photo H**), and as part of the International conference of Global Cassava Partnership for the 21st Century (GCP21) (in June 2018; **Photo I**). In the conference, Prof. Le Huy Ham, the Chairman of Science Council from Agricultural Genetic Institution (AGI) under the Vietnamese Academy of Agricultural Sciences (VAAS), presented results from the SRA, together with the results of their own research and appealed for further research to address this serious and growing problem. Such collaboration between the SRA project and national research institutions is a key strength of this initiative, and one built on years of co-operation, mutual respect, and trust. It is difficult to overestimate the sensitive nature of a phytosanitary issue affecting one of the top 3 crops for nearly every country in the region. Navigating the governmental and socio-political frameworks of Vietnam and Cambodia in the face of SLCMV has been a challenge, but one that ultimately strengthens co-operation among the multilateral actors involved in the project, and sets the stage for the future steps that will be necessary to implement corrective actions.

Photo H. Technical experts' meeting on cassava mosaic disease (CMD) in Vientiane, Lao PDR (June, 2018). Kasetsart University (Thailand), IITA (Tanzania), Cornell University (US), and the global director of RTB program participated in the event, hosted by CIAT-Laos staff.

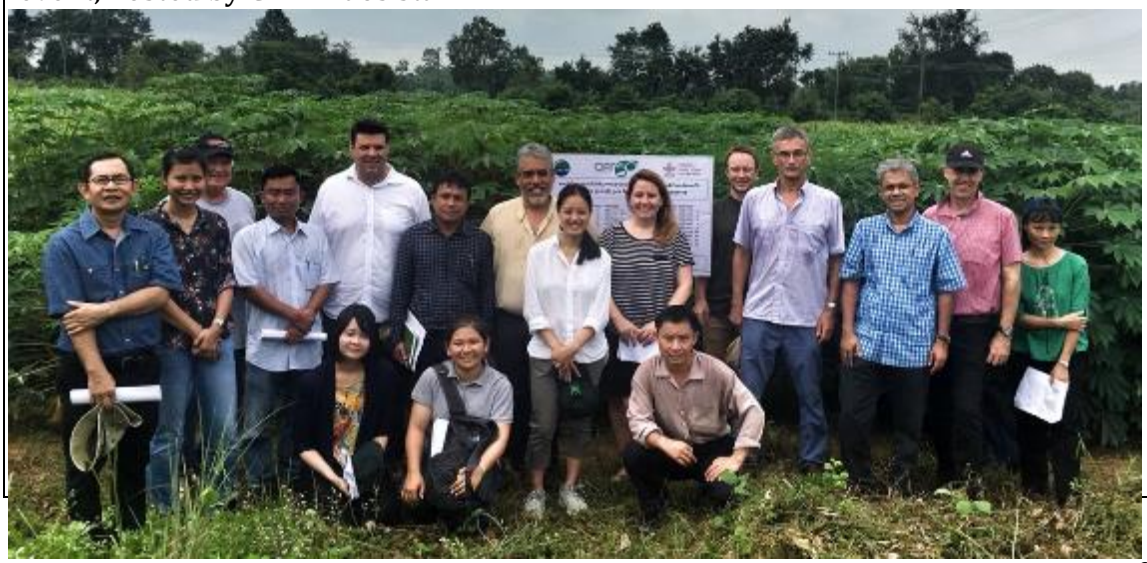


Photo I. Prof. Le Huy Ham presenting SRA results at the IV International Cassava Conference in Cotonou, Benin (June, 2018).

Communication materials were prepared and published in September 2018. Videos were developed based on farmer's story on the arrival of CMD in Cambodia and its threat. Both long version: https://youtu.be/P3quys_QDno and short version: https://youtu.be/jpCenlBYawg are available in YouTube. Blogs with five stories including Screen Shot 2018-09-11 at 15.11.40virus diagnostics and cassava seed systems as a topic are under development and going to be posted one by one within 2018. Extension material will be distributed in 2018 during non-cost extension period.

Photo J. One scene from the video on CMD in Southeast Asia. (September, 2018)

4 Training activities

During the second phase of the SRA (2017-2018), CMD training activities were organized to enhance the capacity of Cambodian partners in leaf sampling and molecular biological methods. To complement the training on monitoring & surveillance during the first phase of the SRA (2016-2017), a 5-day hands-on training event was organized at General Directorate of Agriculture (GDA) in Cambodia from June 11th to 15th 2018 (**Photos K-N**). Eight young researchers, including four key trainees from two departments (department of Plant Protection Sanitary and Phytosanitary (PPSP) and department of Industrial Crop (DoIC)) of GDA, were trained.

The first three days of the training focused on SLCMV diagnostics in the laboratory of PPSP Dept. in GDA. Total DNA extraction from samples were conducted using chemical buffers made in the lab, forgoing the use of prohibitively expensive commercial kits. This allows local scientists more sustainable diagnostics in a region lacking the resources and suppliers to acquire pre-mixed reagents. Using these DNA as a template, we ran polymerase chain reaction (PCR) for amplification of (i) viral genes to detect virus infection, and (ii) plant house-keeping genes to confirm DNA quality. Results were checked by electrophoresis, and on the final day data analytics were be run. On the first and second days of laboratory work, short presentations were given with additional critical information for successful CMD surveillance and diagnostics. Two days were spent visiting fields in Kampong Cham and Tboung Khmum to practice monitoring and sample collection methodologies. Leaves were dried with silica gel after collection.

Participants gained a common understanding of (i) sampling methodology in fields and (ii) molecular diagnostic techniques for confirming pathogen infection. Participants acquired diagnostic skills for working with plant samples collected systematically from fields, contributing to building a team working with common knowledge to generate epidemiological insights on emerging pests and diseases across the region. A standardized protocol was provided by CIAT, and translated into the local language (Khmer; see **Appendix. 1**). These methods and the skills they entail, although modified for specific application under the current project, consist of a robust methodology which can be applied in future monitoring & diagnostics scenarios.

Photo K. Electrophoresis for checking PCR products.

Photo L. Field photography and GPS collection using tablets.

Photo M. Plant sample collection.

Photo N. Farmer's field with CMD

5 Intellectual property

No intellectual property was derived on any of the research conducted in the SRA. All the data generated is open access and available as per the CGIAR Open Access and Data Management Policy (https://cgspace.cgiar.org/handle/10947/4488). The datasets pertaining to the two manuscripts that are about to be submitted will be also be published as data papers.

6 Variations to future activities

The new activities are planned as foreseen in the non-cost extension of the SRA, specifically within objective 1, related to Activity 1.4 of the first year. The following objectives and activities were added:

Activity 1.9 Implement a second round of surveys and diagnostics on cassava leaf samples in Cambodia and Vietnam, enabling time-line comparison of the spread of SLCMV in the region

Activity 1.10 Implement SLCMV survey and diagnostics in major cassava production areas of Lao PDR

Activity 1.11 Conduct statistical analysis, generate maps on the current situation and spread of SLCMD to inform subsequent actions of stakeholders

Activity 3.3 Conduct a feasibility study of different practical and advanced options (isolation, QDS, etc.) to support ongoing surveillance, diagnostics, and multiplication of clean materials through private-public partnerships. This activity will be conducted in conjunction with AGB/2012/078 and ASEM/2014/053.

7 Variations to personnel

No variations to personnel were required. Major roles in design, implementation, and completion of research were played by young CIAT staff, particularly Nami Minato and Erik Delaquis, who very effectively implemented activities on the ground with national partners. During the second phase the PI (Stef de Haan) changed responsibilities within CIAT, taking on the role of coordinator of CIAT new Sustainable Food System's research in Asia. Therefore, going forward after July 2018, he will no longer be involved in the SRA while Nami Minato will take over as the new PI.

8 Problems and opportunities

Challenges have arisen during the course of project implementation, including at the technical, organizational and institutional levels. Yet, these were to be expected when dealing with a new and potentially devastating disease for a regionally important economic cash crop. Based on the technical challenges specifically related to the in-country capacity to deal with PCR analysis for diseased samples in the first phase of the SRA, capacity development for CMD diagnosis was carried out in response, assisting country partners to sustain their efforts of disease monitoring with molecular-based confirmation. There have been several investments and initiatives in Cambodia from various organizations for diagnostics; however some of them demand high running costs which make them unrealistic for national partners beyond the scope of a given funded project. To achieve sustainable monitoring and disease management, uniform, cost-effective protocols for day-to-day virus detection beyond the scope of funded activities should form an integral part of each project.

Seed systems research is lacking in Southeast Asia in general; in Vietnam and Cambodia our study marked the first systematic attempt to characterize these systems for cassava. The farmer-led, informal nature of the seed system means that working with farmers and farmers' associations to better understand seed movement and behavior will continue to be imperative. However our study also underscores the role of traders in facilitating long-distance trade networks. In the present study interviewing these traders proved challenging. Especially in Cambodia, the highly mobile, seasonal nature of trading activities will require dedicated research to properly assess and understand. This will prove critical for future work aiming to introduce formal seed system elements, or to make use of existing trader networks for the dissemination of planting materials or information. Going forward, a significant challenge will be to ensure that perceptions of the role of the informal seed system do not fall prey to stereotypical blame for phytosanitary issues. All the strengths of the informal system will be required for effective intervention in local seed systems to address SLCMV.

9 Budget

See financial report provided by CIAT for details.

Annex 1: Training material of diagnostics

A CGIAR Research Center

CIAT
International Center for Tropical Agriculture
Since 1967 Science to cultivate change

ACIAR-SRA (AGB/2016/032)

Hands-on training on cassava mosaic disease (CMD) diagnosis

11th – 15th June 2018
Phnom Penh, Cambodia

Australian Government
Australian Centre for International Agricultural Research

RESEARCH PROGRAM ON
Roots, Tubers and Bananas
CGIAR

Regional Office for Asia
HANOI: : Agricultural Genetics Institute, Pham Van Dong, Tu Liem, Hanoi, Vietnam
HÀ NỘI: Viện Di truyền Nông nghiệp, Phạm Văn Đồng, Từ Liêm, Hà Nội, Việt Nam

Telephone: +84 24 3757 6969
Fax: +84 24 3757 0999

ciat@cgiar.org
www.ciat.cgiar.org

1. Logistics

Date: 11 – 15th June 2018

Location: Laboratory of Plant Protection Sanitary and Phytosanitary in General Directorate of Agriculture (GDA), Phnom Penh, Cambodia

Organizer: Nami Minato, PhD – Plant pathologist (CIAT)
Sok Sophearith, MSc – Research Associate - Cassava (CIAT)

Participants: 4 key trainees (2 from department of Plant Protection Sanitary and Phytosanitary (PPSP) and 2 from department of Industrial Crop (DoIC)) and those who are interested in

Name	Organization	Department	Email
Nami Minato (F)	CIAT	Cassava program	n.minato@cgiar.org
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Sor Sarika (F)	GDA	PPSP	sorsarika@yahoo.com

2. Justification

In Southeast Asia cassava production has recently been threatened by a complex of invasive pests and diseases, including a complex of mealybugs, several species of mites, and cassava witches' broom disease (Graziosi *et al.*, 2016). In addition to those threats, in 2015 cassava mosaic disease (CMD) was first reported in Cambodia, from a single commercial plantation testing positive for Sri Lankan cassava mosaic virus (SLCMV) infection in Ratanakiri province. This marked the first confirmation of CMD occurrence in Southeast Asia, with earlier reports confined to South Asia *i.e.* India and Sri Lanka. Drastic yield decline caused by CMD has been observed in Africa, while less is known about its impacts in Asia.

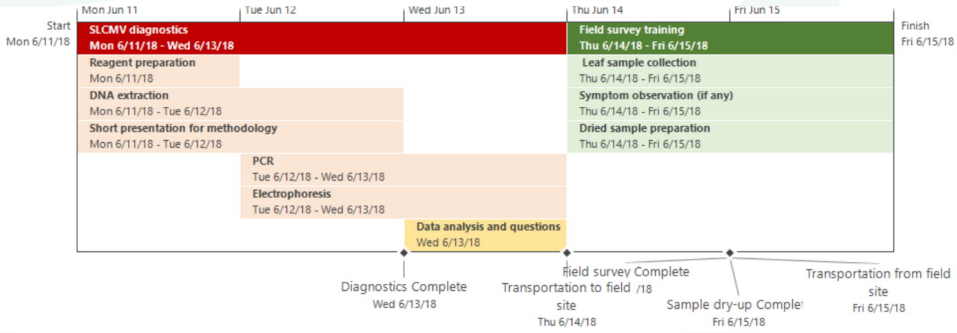
Monitoring and surveillance conducted by national researchers is one of the key elements to control the spread and to minimize the damage caused by pests and diseases in cassava production. In the case of SLCMV, no effective treatment measure is currently known. In 2016, a training event was carried out on cassava mosaic disease surveillance and sampling in Cambodia, in a collaboration with GDA and Provincial Department of Agriculture, Forestry, and Fishery (PDAFF) and CIAT, funded by the Australian Centre for International Agricultural Research (ACIAR) – Short research and development activity (SRA) "Developing an emergency response and long term management strategy for Cassava Mosaic Virus in Cambodia and Vietnam" (2016-2017).

To complement this previous training, a second workshop is scheduled for June 11-15, 2018. For the training a standardized protocol will be provided from CIAT, and in some cases translated into local language. Participants have gained a common understanding of (i) sampling methodology in fields and (ii)

molecular diagnostics techniques for confirming pathogen infection. Participants will acquire diagnostic skills for working with plant & insect samples collected systematically from fields, contributing to building a team working with common knowledge to generate epidemiological insights on emerging pests and diseases across the region.

3. Agenda

CIAT will organize a 5-day hands-on training event at GDA in Cambodia from June 11th to 15th 2018 (see the figure below). The first two days will be spent visiting fields in Kampong Cham and Tboung Khmum to learn monitoring and sample collection methodologies. Leaves will be dried up with silica gel after the collection. From the third to the fifth days we will work on SLCMV diagnostics in the laboratory of the Plant Protection Dept. in GDA. In our training, we will extract total DNA from samples using chemical buffers we will make in the lab (not expensive commercialized kits), which allows for more sustainable diagnostics in the region. Using these DNAs as a template we will run polymerase chain reaction (PCR) for amplifying (i) viral genes to detect virus infection, and (ii) plant house-keeping genes to confirm DNA quality. Results will be checked by electrophoresis, and on the last day data analytics will be run. On the first and second days of laboratory work, short presentations will be shown regarding additional critical information for CMD surveillance and diagnostics.



4. Expected outcomes

At the end of the training course, participants will be able to understand and gain knowledge and skills as follows:

- ☐ a common understanding of sampling methodology for diagnostics in fields
- ☐ molecular diagnostics techniques for detecting pathogens including
- ☐ DNA extraction buffer preparation
- ☐ Total DNA isolation
- ☐ PCR both on viral and plant genes
- ☐ Electrophoresis and data analysis

Those knowledge and skills on CMD diagnostics will allow participants to build a team working to generate epidemiological insights on emerging pests and diseases across the region in the near future.

5. Protocols for training *English version is attached as Annexes.

5-1. Field survey and sampling

[មុនពេលចាប់ផ្តើមយកសំណាក]

1. យកទីតាំង GPS

ប្រើ "ទីតាំងចាប់ទ" តំលើង app កម្មវិធី ក្នុង tablet របស់អ្នក។

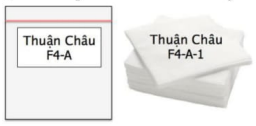
ចែកបញ្ជូន ទីតាំងក្នុង "Google drive" ដោយដាក់ឈ្មោះឲ្យ file ជាមួយឈ្មោះ ស្រុក និង លេខចំការ

(ឧទាហរណ៍៖ Koun Mom-F4)

2. រៀបចំសំភារៈ ជាមួយនឹងលេខកូដ

(i) ថង់កឹបមាត់ចំនួន៤ ដែលមាន លេខកូដចំការ និងលេខកូដ Transect, ដាក់ silica gel ពាក់កណ្តាលថង់

(ii) ក្រដាស ចំនួន១៦ ដែលមានលេខកូដ



ឈ្មោះស្រុក

លេខចំការ, លេខកូដត្រែនសិច, លេខដំណាំ

[នៅក្នុងចំការ]

3. ជ្រើសរើស **transect** ចំនួន៤ រួចជ្រើសរើសដើមដំឡូងមីចំនួន៤ដើម ពី **transect** និមួយៗ

ដើរទៅកាន់ដើមដំឡូងនិមួយៗ មើលជុំវិញដើមទាំងមូល រកមើលរោគសញ្ញានៃជម្ងឺ ម៉ូសូអ៊ិចៗប្រសិនបើមាន យកសំណាកដើមនោះ ហើយប្រសិនបើគ្មានរោគសញ្ញា យកសំណាកដើមដែលនៅចំពីមុខរបស់អ្នក។

4. ថតរូប

ថតពង្រីករូបនៃស្លឹកខ្ចីដែលអ្នកយក

ដាក់ឈ្មោះឲ្យ file រូបថត ជាមួយឈ្មោះស្រុក លេខចំការ ត្រែនសិច លេខដើមដំឡូងមី

(ឧទាហរណ៍៖កូនមំ - ចំការ៤- A – 1)

5. យកសំណាកស្លឹកដែលខ្ចី

យក ចំនួន៣ស្លឹកដែលរីកនៅខាងលើគេ ឺ ច្រើនជាងនេះ(ខ្ចី ហើយតូច) យកទាំងធាង។

ពីដើមទាំងអស់ នៃដំឡូងមី

រុំស្លឹកដណ្តឹងឡូងមីជាមួយក្រដាស រួចដាក់ចូលក្នុងថង់ប្លាស្ទិកកឹបមាត់

6. រាប់ចំនួនរុយស និងយកសំណាក

ជ្រើសរើសដើសមដំឡូងមី ចំនួន១០ដើមជាប់ៗគ្នា(ដើម១ដល់១០) ពី ត្រែនសិច A និង B

រាប់ចំនួនរុយសពេញវ័យសរុប លើស្លឹក៥ខាងលើនៃដើម១ ដល់ដើម ១០

រាប់ចំនួនដឹកឌៀរបស់រុយស លើស្លឹកលេខ១០នៃដើមទី១ ដល់១០ និងជ្រើសរើស ចំណាត់ថ្នាក់នៃដឹកឌៀ

យក រុយសពេញវ័យ ពីដើមណាមួយនៃត្រែនសិច ហើយដាក់ចូលក្នុងបំពង់ចិទ

យកដឹកឌៀ ៥ ក្នុង១ត្រែនសិច ហើយដាក់ចូលក្នុងបំពង់មួយទៀត

7. បំពេញ បន្ថែមលម្អិតក្នុង field sheet

5-2. DNA extraction

ការចម្រាញ់ទាញយក DNA (កែប្រែផ្លាស់ប្តូរពីពិធីសារនៃក្រុមវីរុសសាស្ត្រ CIAT) Nami Minato

1 កិនស្លឹកឈើមួយបន្ទះ (រូបភាពទី 1) (ជាមួយឧស្ម័នអាសូតរាវ) នៅក្នុងបំពង់ចំណុះ 2 ម.លដោយប្រើប្រាស់អង្រែ ឬគ្រាប់ អង្កាំ។

- * មិនចាំបាច់ត្រូវថែមឧស្ម័នអាសូតរាវឡើយប្រសិនបើអ្នកប្រើប្រាស់ស្លឹកឈើស្ងួតទាំងស្រុងនោះ។
- * កិនយ៉ាងប្រុងប្រយ័ត្នរហូតទាល់តែវាក្លាយដូចជាម្សៅ (រូបភាពទី 2)។

ើយលាយច្របល់វាឲ្យសព្វល្អដោយរង្វិលកំណូច (យូរជាង 3 នាទី)។

3 បង្វិលក្នុងល្បឿន 9800 ជុំក្នុងមួយនាទី ឲ្យបាន 5 នាទី ស.ប (សីតុណ្ហភាពបន្ទប់៖ 22-24 អង្សាសេ)

4 ប្រមូលយក 800 មីក្រូលីត្រ នៃសារធាតុរាវដែលអណ្តែតពីលើកករសារធាតុរឹងដែលរងនៅខាងក្រោម

ើយបន្ទាប់មករក្សាវានៅលើទឹកកក។

ើយបង្វិលរយៈពេល 1 នាទី

- * សូមប្រើប្រាស់សារធាតុក្លរ៉ូហ្វូម (chloroform)

  ត្រជាក់ដែលត្រូវបានរក្សាទុកនៅក្នុងទូទឹកកកសីតុណ្ហភាព 4ºC

6 បង្វិលក្នុងល្បឿន 13 000 ជុំក្នុងមួយនាទី រយៈពេល 10 នាទី នៅសីតុណ្ហភាព 4ºC

ើយផ្លាស់ទីវាទៅកាន់បំពង់ដែលមានចំណុះ 1.5 ម.លថ្មី

- * កុំបឺត/ប្រមូលយកនៅក្នុងភាពអន្តរកាល ឬភាពសរីរាង្គ
- * រក្សាកាកសំណល់រាវដែលនៅសល់ទាំងអស់ឲ្យបានត្រឹមត្រូវ

ើយផ្គាប់ផ្ងារត្រលប់ចុះឡើង 2-3 ដង បន្ទាប់មករក្សាវានៅក្នុងទូបង្កកដែលមានសីតុណ្ហភាព -20ºC ឲ្យយូរជាង 30 នាទី។

្រើប្រាស់ isopropanol ត្រជាក់ដែលត្រូវបានរក្សានៅក្នុងទូទឹកកកសីតុណ្ហភាព 4ºC

- * អ្នកអាចបញ្ឈប់ទម្រង់ការនេះបាននៅត្រង់ចំណុចនេះហើយរក្សាទុកនៅក្នុងទូបង្កកនៅពេញរយៈពេលចុង សប្តាហ៍ពេលអ្នកគ្មានពេលវេលាដើម្បីបន្ត។

9 បង្វិលក្នុងល្បឿន 13 000 ជុំក្នុងមួយនាទី រយៈពេល 15 នាទី នៅសីតុណ្ហភាព 4ºC

10 បោះចោលសារធាតុរាវដែលអណ្តែតពីលើកករសារធាតុរឹងដែលរងនៅខាងក្រោម

- * អ្នកអាចឃើញគ្រាប់កករនៅផ្នែកខាងបាតក្រោម
- * រក្សាសំណល់សារធាតុរាវដែលអណ្តែតពីលើកករសារធាតុរឹងដែលរងនៅក្រោមឲ្យបានត្រឹមត្រូវ

11 បន្ថែម 1 ម.លនៃ EtOH (អេតាណុល) 70% សម្រាប់លាងជម្រះ

្រើប្រាស់អេតាណុលត្រជាក់ដែលត្រូវបានរក្សានៅក្នុងទូទឹកកកដែលមានសីតុណ្ហភាព 4ºC

12 បង្វិលក្នុងល្បឿន 13 000 ជុំក្នុងមួយនាទី រយៈពេល 10 នាទី នៅសីតុណ្ហភាព 4ºC

13 ការសិតវត្ថុរាវ (=បោះចោលសារធាតុរាវដែលអណ្តែតពីលើកករសារធាតុរឹងដែលរងនៅខាងក្រោម) ហើយទាំងប្រុងប្រយ័ត្នមិនបណ្តោយឲ្យគ្រាប់កករចេញមកក្រៅបាន

14 សម្ងួតនៅសីតុណ្ហភាពបន្ទប់ ប្រហែល 40 នាទី (រូបភាពទី 5)

15 រំលាយគ្រាប់កករនៅក្នុង DDW (ទឹកបិតយកអ៊ីយ៉ុងចេញអស់) (10-50 មីក្រូលីត្រ អាស្រ័យនៅលើកំហាប់របស់ DNA)

សូលុយស្យុងចម្រាញ់ទាញយក DNA (2 x CTAB ពីក្រុមវីរុសសាស្ត្រ CIAT)

<u>រូបមន្ត</u>

	ចំណុះ
CTAB	5 ក្រាម
PVP (Polyphenolpyrollidine) 40	5 ក្រាម
សូលុយស្យុងរក្សាលំនឹង pH Tris-HCL (pH 8.0) 1.0 ម៉ូល	25 ម.ល
NaCl 4 ម៉ូល	125 ម.ល
EDTA 0.5 ម៉ូល	12.5 ម.ល
DW (ទឹកបិតយកអ៊ីយ៉ុងចេញអស់)	រហូតដល់ 250 ម.ល

ហើយរក្សាទុកនៅក្នុងសីតុណ្ហភាពបន្ទប់

* បន្ថែម 0.2% beta-mercapto ethanol ភ្លាមៗនៅមុនពេលប្រើ

ខ.) បន្ថែម 10 មីក្រូលីត្រនៃ beta-mercapto ethanol ទៅលើ 5 ម.លនៃសូលុយស្យុងរក្សាលំនឹង pH

សូលុយស្យុងចម្រាញ់ទាញយក DNA

ដែលត្រូវបានចំហុយរំងាប់មេរោគដោយឆ្នាំងចំហុយចំហាយទឹកមានសម្ពាធ។

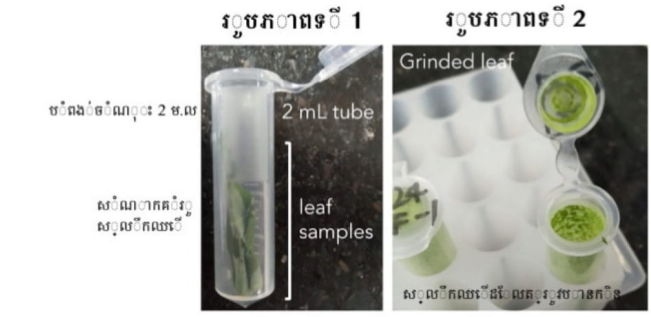


Figure 1

Figure 2

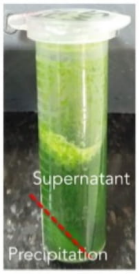


Figure 3

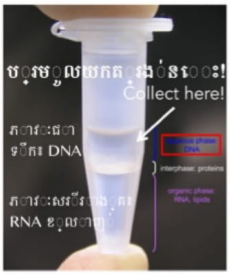


Figure 4

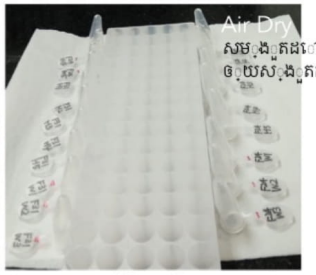


Figure 5

5-3. PCR

Sequences of primers used in this training are as follows:

Name	Sequence (5´-3´)	Target	Length of amplicon	Reference
SLCMVdur_F	TAGCTGCCCTGTGTTGGAC	Replicase, SLCMV	1,056 bp	Duraisamy *et al.*, 2012, Mol Biotechnol
SLCMVdur_Rv	TGAGAAACCCACGATTCAGA			
RBCL-F535	CTTTCCAAGGCCCGCCTCA	RubiscoL, plant	171 bp	Nassuth et al., 2000; Alabi et al., 2008, J Virological Methods
RBCL-R705	CATCATCTTTGGTAAAATCAAGTCCA			

(1) Setting up for PCR

Reagents to be added into 0.2mL tube:

	(x1)	(x)
DW	10.5 µL	µL
Primer_F (10µM)	0.5 µL	µL
Primer_Rv (10µM)	0.5 µL	µL
2x Green Master Mix	12.5 µL	µL
DNA template	1.0 µL	µL
Total volume	**25.0 µL**	µL

*Make sure to have a reaction control (eg. DW as template).

(2) PCR amplification

Reaction conditions for SLCMVdur_F/Rv:

94℃	2:00	
94℃	1:00	40 cycles
63℃	1:00	
72℃	1:00	
72℃	10:00	
4-16℃	∞	

Reaction conditions for RBCL_F535/R705:

94℃	2:00	
94℃	0:30	35 cycles
54℃	0:45	
72℃	1:00	
72℃	5:00	
4-16℃	∞	

*PCR product can be stored at 4℃ fridge or -20℃ freezer.

5-4. Electrophoresis

(1) Preparation

Agarose Gel (1%(w/v))

Eg.) for 50mL gel: Agarose 0.5g, 1x TAE buffer 50mL => Dissolve with heating in microwave
=> Add Redsafe (x20,000) 2.5μL

1x TAE buffer

50x TAE buffer (Stock solution)	
Trizma base	242 g
Acetic acid	57.1 mL
0.5M EDTA disodium salt solution	100 mL
DW	up to 1000mL
Total	1000mL

*No need to be autoclaved
*Store at room temperature
*For 100mL of 1x TAE buffer= 2mL of 50x TAE + 98mL of DW

(2) Electrophoresis and visualization of DNA

1. Load 1kb ladder in the well
2. Load samples in the wells in the desired order
3. Connect the power cord to power supply; Set the voltage 50-135V
4. Confirm that loaded samples start going down in the gel
5. Switch off when the tracking dye reaches ¾ of the gel
6. Transfer the gel into a tray; Observe under UV light inside the dark box