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Annual report

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1 Progress summary

The overall aim of the project "Establishing sustainable solutions to cassava disease in Mainland Southeast Asia" is to enhance smallholder livelihoods and economic development in Mainland SEA by improving the resilience of cassava production systems and value chains by addressing the rapidly evolving disease constraints. The project involves a multi-pronged strategy that includes breeding, pest and disease surveillance, agronomy, seed systems interventions, and engagement with government institutions and agribusiness.

Despite the challenges of the current COVID-19 situation, the project has made significant progress in the past 12 months. This includes the implementation of successful surveillance and awareness raising campaigns; developing recommendations for rapid multiplication of the least susceptible existing elite varieties; evaluation and release of the first CMD resistant varieties in Vietnam; introduction of new breeding techniques; capacity building in tissue culture and diagnostics; development of rapid multiplication systems; and establishing a range of public-private partnerships to bring the research outputs to scale.

Economic analysis, business models and platforms

The 2020-21 season continued to see cassava market to be influenced by a range of internal and external factors impacting supply and demand, prices, trade flows and utilisation[1]. The strong market prices in 2020-21 is impacting the spread of the disease as a result of area expansion occurring in 2021-22 crop. This has led to the movement of stem cuttings into new farmland (eg. conversion of sugarcane to cassava) and also the cross-border movement of stems in new frontiers, particularly in Laos.

The price differential between the Vietnamese and Thai market also reorienting trade-flows, particularly within Cambodia. At the same time the relatively high price of chips compared to fresh roots has impacted the supply of roots available for starch processing, pushing out the hinterland in which factories need to source raw material. This has impacted both the movement of stems and the relationship between value chain actors. This is a good illustration of why different public-private partnership models are required in different location to ensure market changes don't lead to stranded investments.

The COVID pandemic has not significantly impacted overall demand for cassava-based products, with reductions in some segments offset by growing demand in China. As local COVID cases increased towards the end of the 2020-21 harvesting season, additional logistic costs at borders were resulting in lower farmer prices. More seriously, there is a growing impact on cassava producers in rural communities as many households see reductions in non-farm income, with migration back to the village and across country borders increasing. It is anticipated by industry that this will lead to early harvesting of roots in the 2021-22 season as household become in urgent need for cash. Early harvesting potentially will create additional demand for stems from outside the farm for the 2022-23 crop, if replanting cannot occur within the window of viability for harvested stems. This may see further movement of disease in the absence of a clean seed source and distribution system.

Data continue to be monitored and analysis presented in various forums to different stakeholders. The project continues to supporting the development of the 'Cassava Lighthouse[2]' to archive data collected during previous ACIAR cassava projects and provide access to data collected during the project. There are ongoing efforts to establish a geo-referenced app for real time reporting of root and chip prices. This will also be used to

[1] *Summary of key indicators in Appendix 1 and in* Market update and outlook January 2021

[2] *The Cassava Lighthouse can be accessed at: https://cassavalighthousetest.ciat.cgiar.org/*

monitor prices of stems and advertise availability of new varieties or disease-free planting material.

The economic impact of cassava disease continues to accumulate rapidly. Estimates of infected production area from partner include **474,800 ha in Cambodia** (205,000ha in 2020), **72,400 ha in Vietnam** (57,000ha in 2020), **105,777ha in Thailand** (56,000ha in 2020), and **600 ha in Lao PDR** (10ha in 2020). This represents about 24% of the total cassava area in those countries (up from 14% in 2020).

Plot level analysis is ongoing, drawing on results from objective 4. To date, this has highlighted the urgent need for a source of clean planting material (stems) to mitigate the impact. The economic impact of CMD on yields is highly variable based on the disease pressure and other management practices. The difference in losses will influence farmer adoption decision of different management practices and changing over varieties (see results in Objective 2 and Objective 4).

Analysis of the impacts on household incomes has been delayed due to postponement of household surveys. Factories in highly impacted areas are operating well below capacity, particularly in Tay Ninh which relies heavily on Cambodian feedstock. An industry survey planned for 2020-21 has also been delayed due to COVID. The ability to get information through a phone survey are being evaluated, otherwise key informant will continue to be used. There has been a resurgence in the trade value into Vietnam from Cambodia as roots are extracted from deeper into Cambodia as a result of higher price (see Appendix 2).

Willingness to Pay (WTP) for planting material methods were designed but priority implementation in Cambodia and Vietnam remains postponed due to travel restrictions. In Laos the experimental auctions were conducted between November 2020 and March 2021. Data analysis is ongoing, but initial findings show that farmers do bid significantly higher and for a larger number of bundles for clean seed. Bids varied significantly between provinces but were similar between districts and villages within a given province. Average bids for one bundle of clean stems varied from 5000 kip in Bolikhamxay (0.5 USD) to 20,000 kip (2 USD) in Champassak and Attapeu, with a grand mean across all sites of 13,000 kip (1.3 USD). These findings help us to understand local stem prices, willingness to pay for clean seed, and diversity in demand and volumes by location. Reports from Tay Ninh in Vietnam indicate that traders are preparing to charge $4.5-5.3 USD for the new CMD resistant varieties (HN5). Prices are currently around $2.2-2.6 USD per bundle for infected stems of recently released elite (but not resistant) varieties (KM505). This highlights the need for the study to be conducted in different locations as soon as possible.

In addition to the auctions, a follow-up was conducted using the reference price of 13,000 kip for farmers to place bids in Laos' two worst SLCMD-affected districts in Attapeu Province. With the collaboration of Khousub import/export, 700 bundles of cassava stems were transported from a cassava mosaic-free district and sold to farmers whose fields were affected by SLCMV. The sale stem price was lowered to 10,000 kip per bundle, equivalent to the supply price (purchase plus transport), and all stems were sold to 32 distinct buyers in a single day. This demonstrated a viable and sustainable model for dealing with small outbreaks within supply zones.

Economic analysis of rapid multiplication procedures is ongoing. Initial breakeven and returns-on-investment analysis is helping target areas for technology refinement, and where public sector support is required. The 'Future Stems' site at NAFRI has been launched[3] and is a useful site to generate multi-stakeholder collaboration. This has seen the establishment of additional multiplication hubs in Champasak (with a private company) and Salavan

[3] *Cassava program in Asia brings together stakeholders in Lao PDR for 2 days of training and the official launch of the country's first cassava clean stem multiplication facility | Alliance Bioversity International - CIAT (alliancebioversityciat.org)*

Province (with an association supported by a development project). Rapid multiplication hubs have now also been established in Cambodia (GDA research station) and Vietnam (HLARC Research station) with more under development.

Platforms in Lao PDR at this stage are informal and include donor projects (USDA-Winrock; SDC-Helvetas; LuxDev) and private sector partners. Several awareness raising and training activities have been conducted with multi-project support. In Cambodia the project maintains a working relationship with CAVAC and other donor projects and looking to expand the rapid multiplication system through these networks. In Vietnam there is less relevant donor projects and partnerships will be strengthened with private sector in the coming months now that two resistant varieties have been approved. Market leaders have been identified and construction of rapid multiplication tunnels is planned with both large starch processors and individual traders.

Screening and breeding

In the past year, significant progress has been made in Component 2. Given the urgency of the situation, many activities were supported by national partners and through RTB to ensure the 2019-2020 cassava season was not missed. This has paid dividends for activities in the 2020-21 season. The several sources[4] of CMD resistance introduced to the region from CIAT-Colombia, IITA-Nigeria, & Hawaii-NextGen have all been progressing through the breeding pipeline. The collaboration with CTCRI is still being negotiated.

The 48 asymptomatic clones from initial screening (2019-2020) were planted in advanced yield trials in Tay Ninh and Dong Nai in May 2020 to confirm the CMD resistance and evaluate the agronomic traits. In these trials, 9 CIAT clones and 3 IITA clones showing both good CMD resistance and agronomy traits and were selected and advanced to regional yield trials in 7 locations in Vietnam. The best introduced CMD resistant clones from CIAT and IITA produced 30% more starch yield than KU50 (10.5 vs. 7.9 ton/ha) under medium or high CMD pressure, providing the first generation of CMD resistant varieties for the cassava farmers in SEA (see Appendix 4). Based on these results MARD has approved the multiplication and distribution of two varieties for cultivation in the South East region of Vietnam, where the disease pressure is the highest. The best performing clones are currently being evaluated in regional yield trial (2021-2022 season), the best clones will be determined for variety release. They are also undergoing in-vitro multiplication for shipment to Laos and Cambodia.

HLARC has imported 4,964 seeds crossed for CMD resistance in Hawaii supported by the NextGen Cassava Project. Approximately 600 genotypes are being evaluated in the field and 70% show CMD resistance. The resistant clones will be harvested and advanced to the next-stage evaluation in September 2021. NAFRI imported 4,246 seeds with around 1000 germinating and 622 currently in the field for evaluation for agronomic traits. We also validated the *CMD2* markers in Asian germplasm. Two linked *CMD2* markers will be used for marker-assisted selection in variety development.

CMD resistant clones were used as progenitors in crossing nurseries of HLARC and AGI, and more than 9,000 seeds were produced for new variety development. New crosses nurseries were developed by AGI and HLARC to generate new seeds for developing the next generation of CMD resistant varieties. Flower inducing technology developed by CIAT and IITA was implemented in Vietnam to promote flowering of erect clones and shorten the days required for the first set of flowers. The implementation of this technology will help to shorten the duration of breeding cycle and increase genetic diversity, in turn, increase genetic gains of the breeding programs.

[4] *Presentation on introduced sources of resistance can be accessed here: CMD sources of resistance for Asia*

Screening for CWBD resistance has been delayed due to COVID-19 and reduced operations at the CIAT genebank. The screening protocol for Cassava Witches Broom was tested and is at the finalizing stage in Laos. Two sets of the core collections were sent to Laos and Vietnam (170 and 134, respectively). These accessions will be evaluated in the field for their resistance to CMD and Cassava Witches Broom in the 2021-2022 season.

Samples for DNA extraction and fingerprinting are to collected during the household survey in Vietnam, Laos and Cambodia in the coming 6 months should COVID travel allow.

Diagnostics and surveillance

Travel restrictions related to COVID-19 have impact the completion of some planned activities in Objective 3. In the second semester of 2020 the teams continue to use the Basic Surveillance Protocol (BSP) with data integrated with previous developed standards for data organization implemented in the PestDisPlace[5] platform. Field surveys have been delayed in 2021 due to travel restrictions in Southeast Asia. Some colleagues in the region got infected and we experience more delays in the movement of samples to CIAT's labs in Colombia.

Awareness raising campaigns have been successful at gaining early reports of new outbreaks, especially in Laos where CMD had only just been reported in mid-2020. Project staff in collaboration the CLEAN[6] project organised a meeting and field visit for all southern Provinces DAFO and PAFO after the initial report, resulting new areas identified in Attapeu Province by government officials soon after. The main cause of outbreaks continues to be movement of infected stems across border in large volume by traders and companies, highlighting the importance of engagement with industry and government quarantine. Some small outbreaks have been caused by farmers moving stems across borders. A new CMD spot in central Laos has been detected in June 2021 (Savannaket Province). An additional outbreak was also confirmed in early July 2021 in Vientiane Province. PPC has confirmed the presence of the virus and has sent samples to CIAT-Colombia for complete genome analysis of the virus. Once we get rid of redundant sequences or mis annotated sequences, we share the curated maps with partners. PPC has also been able to arrange eradication an continues to monitor the situation.

The CTAB protocol for DNA extraction and virus diagnostics in cassava developed at CIAT has been sent for publication. Evaluation of Dipsticks for quick DNA extraction and virus diagnostics has been validated for rapid detection of the virus in CMD-infected symptomatic plants but is not efficient for asymptomatic infections (most likely due to lower titres of the virus in these plants). We continue updating genomic surveillance data to monitor for the appearance of new virus variants a raw tree is available at: https://nextstrain.org/community/pestdisplace/CMDASIA1?c=virus&r=location (Appendix 6 Figure 1).

The work of Cassava Witches broom is advancing, but requires new information. Inoculation of CWB disease, via grafting of lateral chip buds, is underway in Laos Plant Protection Center (PPC). We have reviewed all DNA sequence data available for CWB phytoplasma in public databases, and confirmed the association of Asian CWBD with phytoplasmas of at least two different genetic clusters (Subgroup I and Subgroup II), occurring in Thailand, Cambodia, Vietnam and Laos (shown in red in Appendix 6 - Figure 2). This analysis is now informing the design when designing of PCR-based diagnostic kits. A review on CWBD, based on the genetic analysis of phytoplasma sequences is underway and should be submitted this year.

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[5] PestDisPlace for cassava can be accessed at: https://pestdisplace.org/diseases/cassava

[6] USDA funded project implemented by DOA and Winrock International

Whiteflies: So far only samples from Laos (2020 surveys) have been analysed. Whitefly samples from Cambodia and Vietnam have been collected but could not be shipped to Colombia due to logistical issues. Whitefly identification was done via PCR and sequencing of the partial mitochondrial cytochrome oxidase gene (mtCOI). Sequencing was carried out using barcoding and the flongle protocol, as described by Oxford Nanopore technology (ONT). The results show the presence of two types of Asian whiteflies AsiaII-1 and Asia-6, with no specific regional distribution in the country. Next step is to compare the incidence of the whiteflies with the incidence of CMD in the southern provinces of Laos. Publication of these studies (genetic analysis and distribution of whiteflies in Laos), is underway and should be submitted this year.

LAMP diagnostics: Analysis in 2019-2020 found a number of reliable primer sets that are specific for SLCMV. One of these primers is now being tested as part of a complete CMV diagnostic assay in-field in SE-Asia by our project collaborators. The complete CMV diagnostic assay kits, (including all SOPs, instructional videos, reagents, dipsticks and Diagnostic Droid device need for >150 assays) have been sent to project collaborators in Thailand and Laos for in-field testing. COVID has impacted shipping between Australia and Laos, with the package yet to arrive, however Thai collaborators have begun to use the diagnostic kit.

Collaborators have collected and shipped additional CWBD samples to Australia for testing. It is proving difficult to detect CWBD due to their low level in the plant tissue and the limited amount of CWBD DNA sequence available to design primers against (only have access to the 16s rRNA sequence). In the coming months the complete CWBD genome will be sequenced to expedite primer development. Additional work is required on developing specific primer and testing different tissue locations for detection.

A strategic partnership was established in 2020 through a FAO-TCP to improve some of the physical capacity of partner institutions in Laos, Myanmar and Thailand and the use of the proposed BSP and CIAT's platform for data organization and sharing. In 2021, CIAT signed a Letter of Agreement with FAO RAP, to carry out an analysis for an Innovations Hub for coordinative surveillance and early warning for sustainable management of transboundary plant pests in Asia and the Pacific. The first step for this is to collet official information on the current situation on pests and diseases management, through a poll shared with APPPC country NPPOs officers (https://pdptest.ciat.cgiar.org/surveyFAO).

Agronomy and seed systems

Existing extension material from each country continues to been collated into one location to avoid duplication**7**. The project is using its social media presence and other networks to disseminate information. Additional efforts are ongoing together with development partners to produce videos on recognising CWBD and CMD. A video has been produced on constructing rapid multiplication tunnels with additional extension material is being developed on managing the rapid multiplication process. Links have been established with the ACIAR project SSS/2019/138.

CMD: Experiments in Cambodia demonstrate that clean stakes and positive selected stakes produce similar yield (~40t ha^{-1}) which is on an average 1.3 to 1.7-fold higher compared to stakes with symptoms. Furthermore, Asymptomatic branch of a symptomatic plants also yielded similarly as positive selected plants. Among the tested varieties, KU50 and Houbong80 prove less susceptible and produced similar yield (~40 t ha^{-1}) under the disease pressure at the research station at Chamkar Leu. In Vietnam, a similar trend was observed with clean and positive selected planting material yielded better compared to diseased

[7] *Extension material from other projects and initiates is being catalogued on the project website*

plating material. However, yield was severely depressed (~13 t ha^{-1} from clean stakes) due to high disease pressure which was not economically viable anymore to crop. This highlights the need for the multiplication of the two varieties from IITA as a medium-term solution, despite their relatively low starch yields under limited disease pressure.

CWBD: Fertiliser response was observed in respect to yield, however, we could not see any positive effect of fertiliser application on number of plants infected with CWBD by the end of the season. A repeat experiment in Vietnam (for CMD) and Laos (CWBD) have been established for 2021-22 season to confirm the results. Furthermore, an experiment to compare yield penalty between CMD and CWBD has been established in Cambodia.

Five IITA elite CMD resistant lines (TMEB419, IITA-TMS-IBA980581, IITA-TMS-IBA980505, IITA-TMS IBA972205, IITA-TMS-IBA920057) currently in regional yield trial 7 locations in Vietnam alongside CIAT clones and check varieties (see breeding section). Ongoing negotiations are occurring with ICAR-CTCRI for access to germplasm. The yields from these introduced varieties needs to be competitive against clean planting material from existing Asian elite varieties which will be tested in the coming season.

A total of 24 tunnels are operational in Laos (6 at NAFRI; 6 Lao Cassava Association; 10 Khonsup Import-Export). During 2020-21 season, from two tunnels 10,000 seedling were produced from 70 mother plants. Currently, multiplication of KU50 in two tunnels, Rayong11 in two tunnels, CIAT clone SM2775-2 in one, and five IITA varieties in one tunnel is on-going. A private sector partner has established 10 multiplication tunnels system in southern Laos (in Champasak) of which the project supported the construction of the first two. FutureStems (NAFRI) was officially opened by the Australian Ambassador and Vice Minister of Agriculture as part of a training activity and awareness raising campaign.

Four tunnels have been built at a research station under the management of GDA. Furthermore, at CARDI (new partner to the project), two tunnels have been built with support from CAVAC. CAVAC is also supporting the construction of tunnels in Stung Treng Province. Two tunnels have been established in HLARC in Southern Vietnam and a demonstration tunnel under construction at AGI in Hanoi (Photos in Appendix 9).

Invitro multiplication of IITA varieties: In Laos, a total of 300 and in Cambodia, a total of 540 plantlets were transplanted to screen house after invitro multiplication and most of them were transferred to field. A decision was made to harvest early and begin rapid multiplication to enable more locations in regional trials in 2022-23.

Results from on station experiments on optimum agronomic practices for multiplication purpose demonstrated that tuber yield of different plating material- Rayong11 from mature stems and Rayong11 from tunnel grown seedlings are similar which is about 30 t ha-1 for optimum density (10k plant ha-1) and high density (20k plant ha-1). However, starch content was about ~15% lower from tunnel grown seedlings. This could be due to variation in the field location and waterlogging during the growing season.

To estimate irrigation water use efficiency (IWUE) for multiplication purpose has been conclude in two locations- Laos and Vietnam; data are now at preliminary stage of analysis. Repeat experiment have been established in both locations to study the effect of different duration of crops of harvest.

2 Achievements against project activities and outputs/milestones

2.1 Achievements to date

Objective 1: Assess the opportunities, challenges and risk for the development of sustainable solutions for cassava disease management in mainland Southeast Asia

No.	Activity	Outputs/ milestones	Completion date	Comments
1.1	Understand the macro-level drivers for the development of the cassava industry and development of plausible market scenarios	Annual market update and revised market scenarios Reported in June each year	Ongoing	Market database maintained. Initiation of the 'Cassava Light house' for public access to datasets. Policies and pandemics: https://youtu.be/3IDkuB_x2Cg Market update January 2021
1.2	Assessment of the economic impact of cassava diseases on cassava producers and industry stakeholders.	Report June 2020 Publication submitted December 2020		**Plot level** ***CMD*** impacts from field trials in Cambodia analysed and combined with earlier data in paper to be submitted in July 2021. Data from Vietnam (Tay Ninh) Y1 trial has been analysed. Yr 2 trial has been established (Obj 4) and data will become available in 2022. The comparison between Cambodia and Vietnam show that different short-term management strategies will be required based on the disease pressure. It is expected that 2 IITA variety may have a role in high disease pressure areas, but less attractive in lower disease pressure areas. ***CWBD*** field trial in Laos – Y1 data analysed. Second year trial established. CWBD trial has been established in Cambodia to compare the impact of CWBD vs CMD with existing elite varieties given susceptibility of KU50 to CWBD **Household level** Survey has been delayed in Laos and Cambodia due to COVID. **Industry level** Trade data analysis has occurred and key informant interviews with a number of factories. Industry survey to be delayed.

1.3	Analysis of household decision making under production and market uncertainty, including on-farm management an market engagement for stems	Report June 2021 Publication submitted December 2021	Ongoing	**Household survey** has been developed. Implementation of the household survey has started in Vietnam but delayed due to COVID in Laos and Cambodia **Experimental auction** protocol has been developed. Initially planned to be carried out in Cambodia and Vietnam. Travel restriction resulted to the auctions initially implemented in Laos. Conducted between November 2020 and March 2021. Groups of 20 farmers bid on three different types of seed (product 1=farmer seed, 2=improved variety, 3=clean seed of improved variety) in 20 villages, for a total sample of 391 individuals
1.4	Characterisation of the cassava seed system and trader network	Report June 2020 Report Dec 2020 Report and maps Dec 2020 Publication submitted June 2021		Survey of traders postponed due to travel restriction
1.5	Develop innovative business models to strengthen the value chain for the production and movement of 'clean' planting material	Report June 2022 Application test and reported June 2023		Initial breakeven analysis has been conducted on current model. Intervention points to reduce costs have been identified. Analysis helping to develop public-private arrangements. Partnerships with private sector exist in Laos and are undergoing construction in Vietnam and Cambodia.
1.6	Map existing national and regional stakeholder networks, develop and strengthen multi-stakeholder national and regional platforms	Report June 2020 Report June 2021		**Thailand** – Continued engagement with TTSA and TTDI. **Vietnam** – Continued engagement with ViCaAs and several processors. Stakeholder field day and consultation planned for late 2021. **Cambodia** – collaboration with CAVAC; FAO. Opportunity with Khmer Enterprise indetified. **Lao PDR** –opening of "Future Stems" was postponed due to COVID. Relationship with the Lao Cassava Association; USDA-Winrock project; SDC-Helvetas Project; LuxDev; Several private sector partners.

1.7	Investigate alternative models for public-private funding to core activities at a local, national and regional scale	Report June 2023		Ongoing discussions with different partners.

PC = partner country, A = Australia

Objective 2: Enhance the capacity and collaboration between breeding programs in mainland Southeast Asia to develop new product profiles for commercially viable cassava varieties by identifying and incorporating known and novel sources of resistance to Cassava Mosaic Disease (CMD) and Cassava Witches Broom Disease (CWBD) into national breeding programs:

No.	Activity	Outputs/ milestones	Completion date	Comments
2.1	**Activity 2.1** Develop protocols and enhance capacity of national programs for the safe and effective transfer of genetic material between partners and countries, including receiving resistance material from Africa and India for evaluation in the region.	Protocols has been developed in Vietnam, Cambodia and Laos. They are able to receive and maintain in vitro plants from CIAT and IITA and other collaborators.	June 2021	Partners in Vietnam, Laos and Cambodia established the procedure of receive materials from other countries, which will facilitate the collaboration. AGI, Vietnam has good tissue culture lab and routinely receive germplasm from CIAT, and Laos and Cambodia, each received 500 KU50 in vitro plantlets in 2020 from CIAT and 100 x 5 IITA clones. Nineteen clones including 5 CMD resistant clones were shared with Sri Lanka. Ongoing discussion with ICAR for transfer of genetic resources to CIAT under the existing MOU.
2.2	**Activity 2.2** Introduce and evaluate selected botanical seeds from the CIAT-IITA Hawaii breeding program for resistance to CMD	HLARC has imported 4,964 seeds with CMD resistance from Hawaii. Approximately 600.genotypes are being evaluated in the field and 70% show CMD resistance.	June 2023	This is the first time for both AGI and HLARC to import cassava seeds. The procedure was established in both teams. These seeds were derived from the elite germplasm in CIAT and IITA. The best 30-100 genotypes will be selected in September 2021 for next-stage evaluation. NAFRI imported 4246 Seeds; 1000 germinate, 622 in the field. Issue with mites in the screen house impact survival of germinated plants.

2.3	**Activity 2.3** Conduct convention field breeding to develop new high yielding commercial acceptable varieties with resistance to CMD	• In total, 2,194 full-sib seeds and 7,400 half-sib seeds were produced from the crosses between CMD resistant clones and elite parents in SEA. All the seeds were planted for resistant variety development. • From 102 CIAT clones and 5 IITA varieties, 12 best CMD resistant clones were selected and planted in Regional Yield Trials at 6 locations in Vietnam. • New polycrossing and paired crossing nurseries with elite parents and CMD resistant clones were established in March 2021 by AGI and HLARC. CMD2 markers have been validated and will be used for marker-assisted selection.	June 2023 and continue	The seeds derived from the crosses between elite parents and CMD resistant clones will provide the next generation of CMD resistant variety with better productivity and agronomy traits. The introduced IITA variety, IBA980581 & IBA972205 has been accepted for official multiplication and distribution in south eastern Vietnam. The best introduced CMD resistant clones from CIAT and IITA produced 30% more starch yield than KU50 (10.5 vs. 7.9 ton/ha), providing the first generation of varieties for the cassava farmers in SEA. After evaluated in Regional Yield Trial in the 2021-2022 season, the best clones will be determined for variety release.
	Activity 2.4 Evaluate CIATs core collection, CMD resistant varieties from Africa and India, and advanced CIAT clones for resistance to Cassava Witches Broom Disease	The 134 and 170 accessions of the core collection were sent to Laos and Vietnam, respectively. These accessions will be evaluated in the field for their resistance to CMD and Cassava Witches Broom in the 2021-2022 season.	June 2023 and continue	Due to COVID-19, the accessibility of tissue culture lab is limited. The activities were slowed down. The third batch of ~100 accession will be sent to Laos and Vietnam in 2021. The screening protocol for cassava witches broom was tested and is at the finalizing stage in Laos. Field screening to be established in mid-2021.
	Activity 2.5 Analyse the current varietal composition of cassava cultivation in Cambodia, Lao PDR, and Thailand using DNA fingerprinting technology	• Distribution of released and landraces known throughout the region. Identifying areas with large area of highly susceptible varieties Informing seed system analysis	Dec 2020	Samples for DNA extraction are being collected during the household survey in Vietnam. Samples will also be collected in Laos and Cambodia.

PC = partner country, A = Australia

Objective 3: Develop and deploy diagnostic protocol, tools and information platforms fit for purpose in monitoring, surveillance, and certification applications.

No.	Activity	Outputs/ milestones	Completion date	Comments
3.1	**Activity 3.1** Conduct training and capacity building of plant protection institutes in key diagnostic tools, sampling design, and data management platforms	A standard and basic surveillance protocol (BSP) in major cassava growing region in SEA Training material developed for use within the region Training reports December 2019	Ongoing	Training material was developed as videos and protocols, adjusted after exchanging feedback from the teams doing field-sampling tests. Existing information products are being catalogued on the project website An initial protocol was implemented together with the group in Thailand, resulting in confirmation of CMD (no records of CWB) along the border between Thailand and Cambodia and the occurrence of a different isolate of the virus (the results have been published) Historical results of the use of the CTAB extraction protocol for virus diagnostics (part of the BSP), has been sent for publication as a technical support its implementation in SEA NPPOs. The next group to start the field sampling protocols (after online training), was Laos-PPC. First results already detected CMD in 4 out of 39 surveyed fields along 10 provinces. Peer-reviewed First report of CMD in Laos, is underway. The team in Vietnam has just finished the first survey in the Red River Delta and Northern Midland and Mountain provinces, and the results are being validated by the CIAT-HQ group.

3.2	**Activity 3.2** Design, implement and communicate regional surveillance activities for CMD and CWBD in Vietnam, Cambodia, Lao PDR, Myanmar and Thailand, with results shared in a common platform	Protocols for uploading and accessing data – Report Dec 2019 Generate and update maps with "confirmed/suspected/non-infected" data. Sampling in Sep-Nov Yr 2,3,4 Report in June	Ongoing	Implementation was delayed due to COVID19-related travel restrictions, but surveys have started in Laos and the North of Vietnam. Surveys in Thailand were carried out before COVID. Maps are being updated in near real-time in the PestDisPlace platform and symptoms recorded are confirmed by the CIAT-HQ team. Image data from all teams are accessible to each other are a good teaching resource used during online meetings to improve the recognition of symptoms from e.g. herbicide treatment. Significant percentages of SLCMV asymptomatic infections are being recorded by PCR. A second spot of CMD has been reported in southern Laos, province of Savannaket, The presence of the virus has been confirmed by PPC and samples have been sent to CIAT lab in Colombia for complete genome analysis
2.3	**Activity 3.3** Understand the distribution and diversity of whitefly populations throughout the cassava production regions of Vietnam, Cambodia, Lao PDR, Myanmar and Thailand	A first regional indexed collection of cassava whiteflies Sequence diversity of whitefly populations in SEA identified and characterized Online access to SEA Whitefly Distribution maps via PestDisPlace July 2021	Ongoing	The data collected so far includes data on adult whiteflies (WF) as recorded in the first extended leaf of 3-4 months old cassava plants. WF samples have been collected in Laos, Vietnam and Cambodia, and are stored in ethanol. We had experienced delays in obtaining import permits from the Colombian phytosanitary office (ICA), to run the first barcoding analyses. In the meantime, partners are preparing the labs for running the tests themselves. Only samples from Laos, have reached CIAT lab in Colombia. Genetic analysis of the COI region using Nanopore sequencing reveals the presence of two types of whiteflies in Laos: Asia II-1 and Asia II-6. This information will be uploaded into PestDisPlace Once again, the planned laboratory training activities have been postponed and we will have to rely on video training and testing of the samples first in CIAT-HQ labs.

	Activity 3.4 Evaluate new technologies for rapid field diagnostics with particular applications in seed systems	June 2020 June 2021		**SLCMV and CWBD-specific primer development** To ensure the specificity of the primers to target CMV, a set of rigorous tests were repeatedly performed using healthy or SLCMV-infected Cassava samples collected in Cambodia and Vietnam. Samples were also obtained from different parts of infected Cassava plants including: old leaves and young leaves, petioles, and stems. From this analysis, we appear to have a number of reliable primer sets that are specific for SLCMV. From this analysis, we appear to have a number of reliable primer sets that are specific for CMV. One of these primers is now being tested as part of a complete CMV diagnostic assay in-field in SE-Asia by our project collaborators. Complete CMV diagnostic assay kits, (including all SOPs, instructional videos, reagents, dipsticks and Diagnostic Droid device need for >150 assays) have been sent to project collaborators in Thailand and Laos for in-field testing, which has already been initiated. Collaborators collect and shipped CWBD samples to Australia for testing. Difficult to detect CWBD due to their low level in the plant tissue and the limited amount of CWBD DNA sequence availabe to design primers against (only have access to the 16s rRNA sequence). The complete CWBD genome will have been sequenced to expidite primer development. More work required on developing specific primer and testing different tissue locations for detection.

	Activity 3.5 Develop and validate protocols for screening and biological characterisation of cassava diseases, particularly CWBD	Testing chip bud grafting of CWB on cassava rootstocks	ongoing	Protocols and video training material developed and shared with our partners in Laos, showing the procedure for graft transmission of pathogens in cassava. Progress is not as expected, as symptoms take too long to develop under greenhouse conditions. Advances in the development of a LAMP test for molecular detection will accelerate the confirmation infection. At CIAT-HQ we have confirmed by PCR the association of a phytoplasma 16Sr-I with CWBD in Laos Comparison with available sequences indicate that there may be at least two different genetic subgroups of phytoplasmas associated with CWBD in Southeast Asia. Genetic analysis also distinguish these phytoplasmas with another genetic group reported in cassava in the Americas. These analysis will improve the design of routine diagnostics.
	Activity 3.6 Develop and evaluate the effectiveness of communication products and strategies utilising different public and private sector stakeholders			Communication products are being developed with development partners and distributed through government and private sector partners. Laos: https://youtu.be/yI2FeByiRV4 Cambodia: https://youtu.be/OcTRSCWXI9I Additional videos on CWBD in final editing

PC = partner country, A = Australia

Objective 4: Develop and evaluate economically sustainable cassava seed system models for the rapid dissemination of new varieties and clean planting material to farmers in different value chains and production contexts

No.	Activity	Outputs/ milestones	Completion date	Comments
4.1	**Activity 4.1** Developing communication products for effective field level management of cassava diseases (i.e. CMD, CWBD)	Publication & distribution of brochures, video cds, posters Training materials developed for use within the region	December 2020	Existing information products are being catalogued on the project website https://cassavadiseasesolutionsasia.net/thai/ https://cassavadiseasesolutionsasia.net/khmer/ Additional products are being developed involving development projects to avoid duplication and maintain a common message.

4.2	**Activity 4.2** Evaluation and on-farm demonstration of CMD resistant exotic cassava varieties from IITA (Africa) and ICAR-CTCRI (India) against clean available SEA varieties	Adapted CMD resistant cassava varieties identified on-farm from local and exotic germplasm from IITA and ICAR-CTCRI. Conduct training in improved cassava practices, demonstration trials, and participatory research methods, including public sector extension services (where present)	July 2021 (elite SEA cassava varieties) July 2023 (exotic cassava varieties)	To test the susceptibility of popular varieties to CMD and CWBD, experiments were conducted in Vietnam & Cambodia and Laos, respectively, during 2020-21 season. CMD: Experiments in Cambodia demonstrate that clean stakes and positive selected stakes produce similar yield (Figure 1). Furthermore, asymptomatic branch of a symptomatic plants yielded similarly as positive selected plants (Figure 3). In Vietnam, similar trend was observed, however yield was severely depressed due to high disease pressure (Figure 10). CWBD: Fertiliser response was observed in respect to yield, however, we could not see any benefit in number of plants infected with CWBD by the end of the season (Figure 7). A repeat experiment in Vietnam (for CMD) and Laos (CWBD) have been established for 2021-22 season to confirm the results. Furthermore, an experiment to compare yield loss between CMD and CWBD has been established in Cambodia. This includes using stems of different disease status. With and without fertiliser treatments are included in some trials to demonstrate the impact of good management. Key results from 2020-21 season and protocols for 2021-22 season are in the Appendices to this report. Due to travel restriction field days were postponed.

4.3	**Activity 4.3** Evaluation and comparison of rapid multiplication innovations in SEA context	Review document: Equipment and facilities for rapid multiplication innovation Semi-Autotrophic Hydroponics (SAH) technology /or Jiffy Pots established and equipped for rapid dispersal of planting materials into the seed system	July 2020 July 2021	Draft of the review document has been completed. From the recommendation of draft review document and due to difficulties in implementing SAH multiplication system, modified tunnel multiplication system have been established (see detail below). Due to COVID-19 international travel restrictions and local lockdown, progress in building the tunnels were interrupted. Vietnam: At HLARC two tunnels have been established for rapid multiplication and optimisation is in progress. Laos: Six tunnels at NAFRI are operational from the beginning of 2021. From first two tunnel 10k seeding have been (Table 1) produced and some of them were in use for experimentation and some have been distributed to development partners after maturing for 2021-22 season. Private partner have established 10 multiplication tunnel and all of them are operational. 6 tunnels with the Lao Cassava Association Cambodia: Four tunnels have been built at a research station under the management of GDA. Development partner CAVAC is in discussion to establish rapid multiplication tunnel system (i.e. six tunnels) in partnership with PDAFF at Stung Treng. Furthermore, at CARDI (new partner to the project), two tunnels have been built with support from CAVAC. Economic analysis is ongoing as modifications and performance improves. Multiplication of IITA varieties: In Laos, a total of 300 and in Cambodia, a total of 540 plantlets were transplanted to screen house after in vitro multiplication and most of them were transferred to field (Table 2). In Laos at NAFRI, after 9 months of growth in the field,10 plants of each IITA variety are now in tunnel for rapid multiplication. Check variety KU50 is also been transferred to the field from invitro multiplication to field and then into tunnels. In Cambodia at CARDI, tunnels are not fully operational, as soon as the facility are ready, rapid multiplication will start in the tunnels.

4.4	**Activity 4.4** Optimize agronomic practices (variety, density, fertilizer) for the economic production of both cassava roots and clean planting material	On-farm trials successfully established in different agro-ecological and value chain contexts. Training report	July: 2022 and 2023 July: 2022 and 2023	Results from on station experiments from all three countries on optimise the planting density, planting time and harvest time for multiplication and to get optimum yield have been analysed (Figures 5, 6, 9 & 11). There was no significant fresh tuber yield difference observed between different plating material sources- Rayong11 from mature stems and Rayong11 from tunnel grown seedlings (Figure 8). However, starch content was about ~15% lower from tunnel grown seedlings. Although, they were harvested in diffident time. Experiments to estimate irrigation water use efficiency (IWUE) for multiplication purpose were conducted in two locations- Laos and Vietnam- data are now at preliminary stage of analysis. Economic and trade-off analysis will occur after harvesting.

PC = partner country, A = Australia

2.2 Summary of achievements to date (for ACIAR website)

- Partner estimates of CMD infected area includes 474,800 ha in Cambodia, 72,400 ha in Vietnam, 105,777ha in Thailand, and 600 ha in Lao PDR. This represents about 24% of the total cassava area in those countries (up from 14% in 2020).
- Advanced yield trials confirmed the CMD resistance and agronomic performance of introduced varieties from IITA. MARD has authorised the multiplication and distribution of two varieties for use in the South East region of Vietnam.
- Imported seed from Hawaii (NextGen Cassava Project) are being evaluated in Vietnam and Laos. Flower inducing technology developed by CIAT and IITA was implemented in Vietnam. Over 9,000 seeds produced in HLARC and AGI crossing-block under evaluation in Vietnam.
- The CMD2 markers in Asian germplasm. Two linked CMD2 markers will be used for marker-assisted selection in variety development.
- Basic Surveillance Protocol (BSP) developed with data integrated with previous developed standards for data organization implemented in the PestDisPlace platform.
- A new protocol for DNA extraction and virus diagnostics in has been developed
- The complete CMV diagnostic assay kits (UQ) have been sent to project collaborators in Thailand and Laos for in-field testing.
- Varieties least susceptible to CMD identified for rapid multiplication and confirmed the ability for positive selection to mitigate yield loss under lower disease pressure.
- A total of 24 rapid tunnels are operational in Laos producing at a multiplication rate of between 120-140x.
- 6 tunnels have been established in Cambodia and 2 in Vietnam to date, with additional multiplication hubs underdevelopment
- Fresh root yield of plants established from plantlets from rapid multiplication is not significantly different from those established using traditional methods of stem cuttings adding to the viability of the seed system model.
- Willingness to Pay (WTP) for planting material methods were designed and to date implemented in Laos. A viable partnership model was demonstrated for transported of stems from a cassava mosaic-free district and sold to farmers whose fields were affected by SLCMV.

3 Impacts

3.1 Scientific impacts

- The project activities have contributed to the development of the RTB Seed System Toolbox. https://tools4seedsystems.org/tools/
- Combining two markers S12_7926132 and S14_4626854, provided the better prediction of CMD resistance. The genotypes with resistant alleles, T and A showed high resistant to CMD (red circle). MAS is being used to accelerate the variety development with CMD resistance in South East Asia. (Paper to be submitted in July 2021)
- The standard CTAB protocol for virus diagnostics has now been sent for publication[8]. This protocol, is part of the diagnostic component to be implemented in NPPO labs in the region.
- The Basic Surveillance Protocol (BSP) has been adopted by National Program. As a result, official scientific reports of CMD in Thailand and Laos, have been released[9][10]:
- We have standardized the use of Nanopore sequencing to support genomic surveillance of the SLCMV in Southeast Asia and this is the same technology we are now applying to the study the diversity of whiteflies in SEA[11]

3.2 Capacity impacts

The project has been contributing to both physical and human capacity development within National Programs. It has also been able to leverage further investment by other partners.

Physical:

- Physical capacity has been developed at NAFRI for rapid multiplication and agronomic trials. This includes the construction of a screen-house and tunnels jointly supported by the project and CIAT infrastructure fund. Improved fencing and irrigation equipment have been installed to ensure both security of field trials and enhance the efficiency of field multiplication.

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[8] *Jimenez J, Leiva AM, Olaya C, Acosta-Trujillo D, Cuellar WJ.* ***2021****. An optimized nucleic acid isolation protocol for virus detection in cassava (*Manihot esculenta *Crantz.).* MethodsX.

[9] *Chittarath K, Jimenez J, Vongphachanh P, Leiva AM, Sengsay S, Lopez-Alvarez D, Bounvilayvong T, Lourido D, Vorlachith V, Cuellar WJ.* ***2021****. First report of Cassava Mosaic Disease and Sri Lankan Cassava mosaic virus in Laos.* Plant Disease. *https://doi.org/10.1094/PDIS-09-20-1868-PDN*

[10] Siriwan W, Jimenez J, Hemniam N, Saokham K, Lopez-Alvarez D, Leiva AM, Martinez A, Mwanzia L, Becerra LA, Cuellar WJ. **2020**. Surveillance and diagnostics of the emergent Sri Lankan cassava mosaic virus in Southeast Asia. *Virus Research*. https://doi.org/10.1016/j.virusres.2020.197959

[11] *Leiva AM; Siriwan W; Lopez-Alvarez D; Barrantes I; Hemniam N; Saokham K; Cuellar WJ.* ***2020****. Nanopore-based complete genome sequence of a Sri Lankan cassava mosaic virus strain from Thailand.* Microbiology Resource Announcements. *https://doi.org/10.1128/MRA.01274-19*

- Rapid multiplication tunnels were established at GDA in Cambodia with project funds. CAVAC funds were leveraged for enhanced infrastructure at CARDI in Cambodia.
- Rapid multiplication tunnels have been established in HLARC and under construction in AGI. Red lights have been introduced into the crossing-blocks of both institutions

Human:

- Capacity in tissue culture of cassava has been built in Laos and enhanced in Cambodia through initial training in 2019 and ongoing virtual training and communication
- Capacity in surveillance and molecular diagnostics for CMD has been enhanced with project partners
- Conducting experimental auctions

3.3 Community impacts

3.3.1 Economic impacts

There have been no substantial direct economic impacts to date. Early results suggest significant impacts will be achieved in the future. With the area of infection increasing significantly in the past 12 months and root prices raising, the farm level impacts are very large. Using some conservative estimates of an estimated infected area around 600,000ha, a yield decline of 15t/ha, and a fresh root price of 60USD/t -the farm level losses due to CMD alone are likely approach $585 million this harvest season.

Results from Cambodia suggest that in areas of lower disease pressure switching variety and adopting 'positive selection' practices could mitigate the losses of CMD in the short term. For example, changing from a highly susceptible variety to a less susceptible variety (even if planted with symptomatic stems) could increase yields by 15.5t/ha ($1705/ha). If clean planting material could be sourced than the yield increased could be an additional 14.5/ha (1,595t/ha). This highlights the potential for seed system interventions given around 100-120 bundles of stems are required per ha, at the farm level there would be a significant return on investment from purchasing cleans stems if they were not available through positive selection.

In Tay Ninh where the disease pressure is higher, the current results suggest a potential benefit at the farm level of changing from existing varieties to the first released CMD resistant varieties may result in an additional $1400/ha USD at current prices. This would amount to around $67 million USD for the Province of Tay Ninh alone in farm level benefits. The additional benefits at the trader and processor level also added.

The surveillance activities in Laos and early response (eradication) is slowing the spread of the virus, limiting future losses.

3.3.2 Social impacts

There have been no significant social impacts to date.

3.3.3 Environmental impacts

There have been no significant positive or negative environmental impacts to date.

3.4 Communication and dissemination activities

The project seeks to build to the cassava community established during the ACIAR Cassava Value Chain and Livelihood Program. The projects online and social media presence includes:

- A project website: https://cassavadiseasesolutionsasia.net/
- A project Facebook Group: https://www.facebook.com/groups/2394808117512232
- A project twitter account: @CassDiseaseAsia
- A project SlideShare account
- A project Youtube account has been established

The Facebook group to date has 327 members of which 293 have been active in the last 60 days. The twitter handle has 232 followers. Activities are also promoted by the CIAT and RTB accounts.

The team presented at the Starch World Conference in January 2020 and February 2021. This is the largest industry meeting held each year.

Blogs and articles

https://blog.ciat.cgiar.org/ciat-and-partners-fight-cassava-diseases-in-southeast-asia-with-new-project/

Cassava program in Asia brings together stakeholders in Lao PDR for 2 days of training and the official launch of the country’s first cassava clean stem multiplication facility | Alliance Bioversity International - CIAT (alliancebioversityciat.org)

https://reachout.aciar.gov.au/protecting-incomegenerating-cassava-from-virus-duo

Collaborative research to save billion-dollar industry (aciar.gov.au)

Local media

Lao News - https://youtu.be/uQvEJw4GqF0

Vietnam News : Tây Ninh: Đẩy nhanh nhân giống mì kháng bệnh khảm lá cung cấp cho nông dân - Báo Tây Ninh Online (baotayninh.vn)

Vietnam News: https://youtu.be/TM1cXzqJF1E

Webinar Series

https://cassavadiseasesolutionsasia.net/webinar-series/

Webinar 1 - Keep the faith: Progress in developing commercially viable CMD resistant varieties for Asia
Recording can be accessed at: https://youtu.be/App3SENtxG8
9:00am – Introduction – Jonathan Newby (Project Coordinator
9:10am – Sources of CMD resistance in South East Asia – Dr. Xiaofei Zhang (Cassava Breeder CIAT, Colombia)
9:30am – CMD Breeding Activities in Thailand – Associate Professor Ed Sarobol (Emeritus Professor Kasetsart University, Thailand)
9:50 AGI – Update of CMD resistant screening in Vietnam – Dr Nguyễn Anh Vũ (Group Leader, AGI Hanoi)
Webinar 2 - Developing a sustainable cassava seed system through rapid multiplication technologies
Recording can be accessed at https://youtu.be/r7hh6VnWjZY
8:30am – Introduction – Dr. Jonathan Newby (Project Coordinator)
8:35am – Multiplication tunnels in Laos – Dr. Imran Malik (CIAT Cassava Physiologist)
8:55am – New rapid multiplication system in Thailand– Dr. Wannasiri Wannarat (Kasetsart University, Thailand)
Propagating cassava plants using aeroponic culture at Hung Loc Agricultural Research Center – Nhạn Phạm (HLARC)
Webinar 3: Enhanced regional diagnostic protocols, tools and information platforms fit for purpose in cassava systems of Southeast Asia
Recording can be accessed at https://youtu.be/_EtXu1NjmHc
8:30 – Introduction to the webinar– Dr Jonathan Newby (CIAT)
8:35 – Overview and update of Component 3 activities – Dr Wilmer Cuellar (CIAT)
8:50 – Status of cassava disease and update from Cambodia Dr Ny Vuthy (GDA)
9:05 – Status of cassava disease and update from Vietnam – Dr Nguyen Van Liem (PPRI)
9:20 – Status of cassava disease and update from Laos Ms Khonesavanh Chittarath (PPC)
9:35 – Status of cassava disease and update Thailand – presented on behalf of Dr Wanwisa Siriwan (KU)
9:55 – Update of the development of rapid field diagnostics for CMD and CWBD – Dr Mike Mason (UQ)
10:10 – Update and planning for research of whitefly (Bemisia tabaci) – Maria Isabel Gomez (CIAT)
Webinar 4: Status on assessment of CMD Resistant varieties in Vietnam and Thailand: Implications for short, medium and long-term plans
Recording can be accessed at https://youtu.be/jie_FFJuCtc
8:30 – Introduction – Dr. Jono Newby (CIAT)
8:40 – Presentation of results and analysis from Vietnam – Dr. Xiaofei Zhang (CIAT)
9:10 – Presentation of results and analysis from Thailand – Dr. Chalermpol Phumichai (KU)
9:25 – Presentation agronomic trials in Kampong Cham, Cambodia – Mr Sok Sophearith (CIAT)

4 Training activities

Due to the COVID restriction all major training events have been postponed or moved to online training and mentoring of individual teams and scientist.

- PCR diagnostics of SLCMV in PPC, Lao PDR (December 6th, 2019)
- Video training and teleconference on cassava chip bud grafting (May 12th, 2020)
- Video training and teleconference on field sampling for CMD, CWB and whiteflies (June 4th, 2020)
- Video training for transfer of tissue culture material to screen house (Laos and Cambodia)
- Use of the diagnostic droid

Training videos are being developed for key skills required for partners to complete activities.

5 Intellectual property

No significant IP has been generated to date.

6 Variations to future activities

Industry survey in Vietnam

There is a common interest in establishing a database of processors in the region for a number of modelling activities and engagement. Together with the CSIRO TRANSIT project (supported by both ACIAR and DFAT) a survey in Vietnam is being planned. This will not have budget implications for the project.

Screening of core collection in Vietnam

Screening of the cassava core collection for CMD resistance was initially planned to occur in Cambodia with the movement of stems from Rayong Research Centre (DOA Thailand) to a heavily infected area in Cambodia. Due to uncertainty over the phytosanitary status of the complete collection in Thailand, the plan is to now send in-vitro accession with certain status from the core collection in Cali to AGI for screening in TayNinh, Vietnam.

Cross-block established in Northern Vietnam

The original plan was for the breeding activities to be undertaken by HLARC with the establishment of a crossing block in Lam Dong Province in the Central Highlands. AGI will now also undertake breeding activities with a second crossing block established at the NOMAFSI station in Sonla Province in Northern Vietnam.

CARDI involvement

The activities in Cambodia depend on the safe movement of germplasm into Cambodia. Given that GDA does not have tissue culture facilities, a separate arrangement has been develop with CARDI using other funding sources. CARDI will remain involved in the project as an associate partner until the funding situation and certainty warrants a variation for formal inclusion.

7 Variations to personnel

CIAT – Dr Hernan Ceballos will retire and has been replaced in the project by Dr Xiaofei Zhang. Both scientist were able to visit partners together prior to COVID restriction and facilitate an introduction to partners and handover of activities.

Ms Cu Thi Le Thuy has become responsible to supporting and the implementation of breeding activities with Vietnamese partners under the supervision of Xiaofei Zhang and Augusto Becerra.

Mr Badit Mienmany was added to the CIAT team in Laos to maintain GIS databases and spatial modelling. He is also assisting in village meetings under objective 1.

Additional field assistants are being engaged in Vietnam, Laos and Cambodia to assist in the development of rapid multiplication systems and monitoring of outputs.

PPRI - Ms Hang Thi Le is a new member of the PPRI team. She will be responsibility for molecular biology.

NAFRI – Dr. Siviengkhek Phommalath, Deputry Director of the Maize and Cash Crop Research Center has been appointed as the project coordinator for NAFRI.

Mr Phunthasin Khanthavong is currently completing his PhD in Japan. Mr Saythong Oudthachit has taken over the day-to-day management of field activities at NAFRI related to Objective 4.

HLARC – Dr Nguyen Huu Hy has retired, but remains engaged in the project activities. Dr Pham Thi Nhan has taken over leadership of the project and has been promoted to Director of the Center.

GDA – Iv Phirun from GDA retired mid-year. The leadership of component 4 activities has been allocated to the new Deputy Director of the Industrial Crop Division Mr. Sao Chesda

8 Problems and opportunities

COVID-19 continues to be the main challenge faced by the project. International and domestic travel restriction have impact capacity building, implementation of some activities, and engagement with private sector partners. To date, the impact has been mainly incurred in Objective 1 with ongoing delays in surveys, interviews and group discussions that require face-to-face interaction with communities.

Travel restrictions not allowing staff from CIAT-Colombia to come to the Asia has impacted planned capacity building and collaborations in breeding, disease diagnostics, and rapid multiplication. Travel between countries is also not permitted, impacting team planning meetings and cross-site-visits between country teams. These impacts are being mitigated through frequent online conference calls, webinars and through CIAT National staff. However, some of the coordination and engagement between external projects and developing new private sector partners is more challenging.

COVID lockdowns in Colombia have also impacted the ability for the genebank in Colombia to send accessions to the region with the labs working on a skeleton staff to maintain the collection rather than facilitate shipments. Furthermore, the reduction in flights has greatly impacted the timeliness of courier deliveries. New shipping protocol have ben developed to increase the survival rate. Transport issues have also impacted other deliveries and the availability of inputs (fertiliser etc) in Laos.

The involvement of CATAS has also been impacted. It was planned for CATAS staff to spend time in the Laos office. This has continued to be to not be possible due to travel restriction.

The situation in Myanmar has resulted in the indefinite postponement of planned activities in the country and budget reallocated.

Opportunities

Several opportunities for partnerships have been developed and leveraging additional funding for the overall ‘program’ in line with Objective 1.7 on public-private partnerships.

1. FAO TCP for disease surveillance and diagnostics (Laos, Thailand and Myanmar)
2. Partnerships with several development projects in Laos is scaling out knowledge and innovations
3. Continued partnership with CAVAC in Cambodia, including supporting activities at CARDI aligned to the overall program implementation
4. Rapid multiplication activities in Laos have benefited from support from Mekong Timbers, a Forestry plantation company that utilizes micro-propagation. This has resulted in the purchase of new technology by external projects (USDA-Winrock) on recommendations of the project team.
5. A partnership was developed with the communication team of the LURAS project in Laos (SDC funded, Helvetas implemented) to co-produce awareness and extension videos. This has seen widespread dissemination through social media and TV.
6. CARDI has been added to the project as a funded project to ensure transfer and maintenance of new genetic material
7. There are new opportunities being developed with FAO-Cambodia on a TCP on cassava seed systems
8. There are new opportunities being developed with Khmer International in Cambodia

9 Budget

The total budget has not been significantly impacted by a decline in the AUD:USD exchange rate relative to the planned rate. Currently the AUD rate is slightly above the planned rate.

Delay in finalising initial contracts between ACIAR and CIAT and CIAT and partners slowed expenditure in the first 6 months with alternative resources used to start activities. This has resulted in significant carryover from the outset. The original budget distribution has been impacted by COVID-19 and the political situation in Myanmar. This has significantly reduced CIATs expenditure of travel funds, delayed onboarding some international staff, and delayed implementation of surveys. At the same time opportunities to expand partnerships and investment in rapid multiplication have become apparent.

As a result, a contract variation was developed during the reporting period/ The redistribution of funds moves operating and salary funds into later periods; and redistributes travel funds into additional operations. Funds earmarked for capacity building activities in Myanmar have been reallocated to include CARDI as a funded partner in Cambodia.

As travel continues to be interrupted there is expected to be further delays in the future in expenditure. It is likely that a non-cost extension may become required as the end of the project approaches. This will be evaluated in 12 months or after the mid-term review.

10 Appendices

Appendix 1: Annual Project Report Appendix One Publications list (See link on ACIAR website - Microsoft Excel document)

Appendix 2: Cassava market update

Appendix 3: Initial Results of Willingness to pay study in Laos

Appendix 4: Breeding results (2020-21)

Appendix 5: Breeding workplan (2021-22)

Appendix 6: Disease surveillance and diagnostics

Appendix 7: Agronomic results (2020-21)

Appendix 8: Agronomic workplan (2021-22)

Appendix 9: Photos of rapid multiplication systems in Laos, Cambodia and Vietnam

10.1 Appendix 2 - Cassava market update and outlook

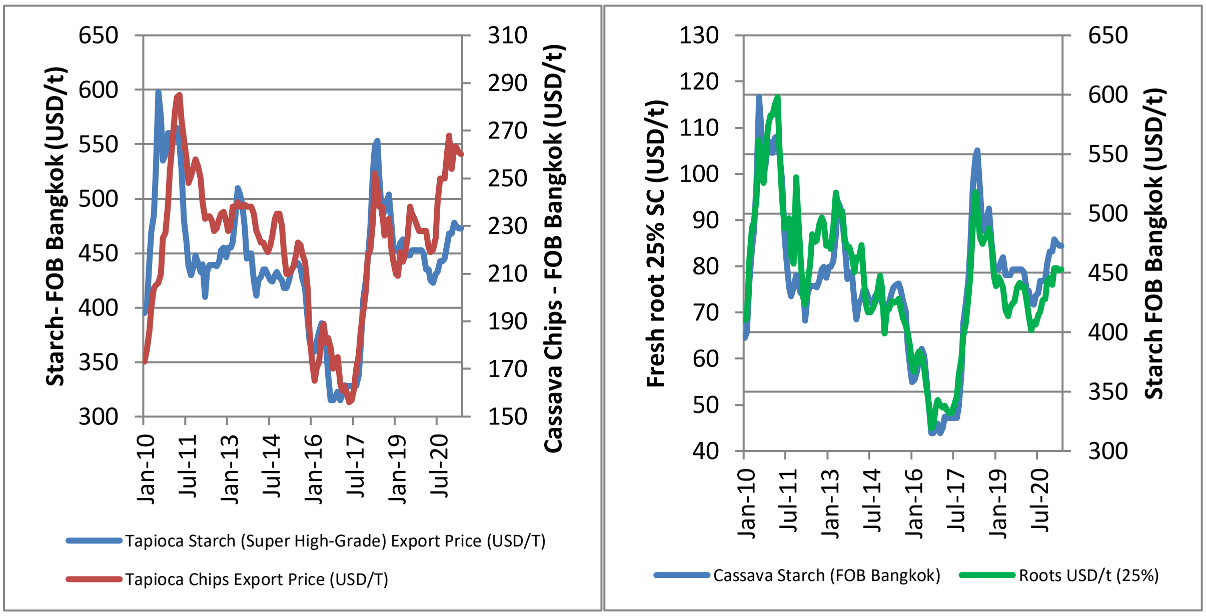


Figure 1a – Thai export prices of cassava starch and cassava dried. The price of dried chips has moved ahead of the starch price, putting pressure on supply for processors and changing how farmers engage with the market.

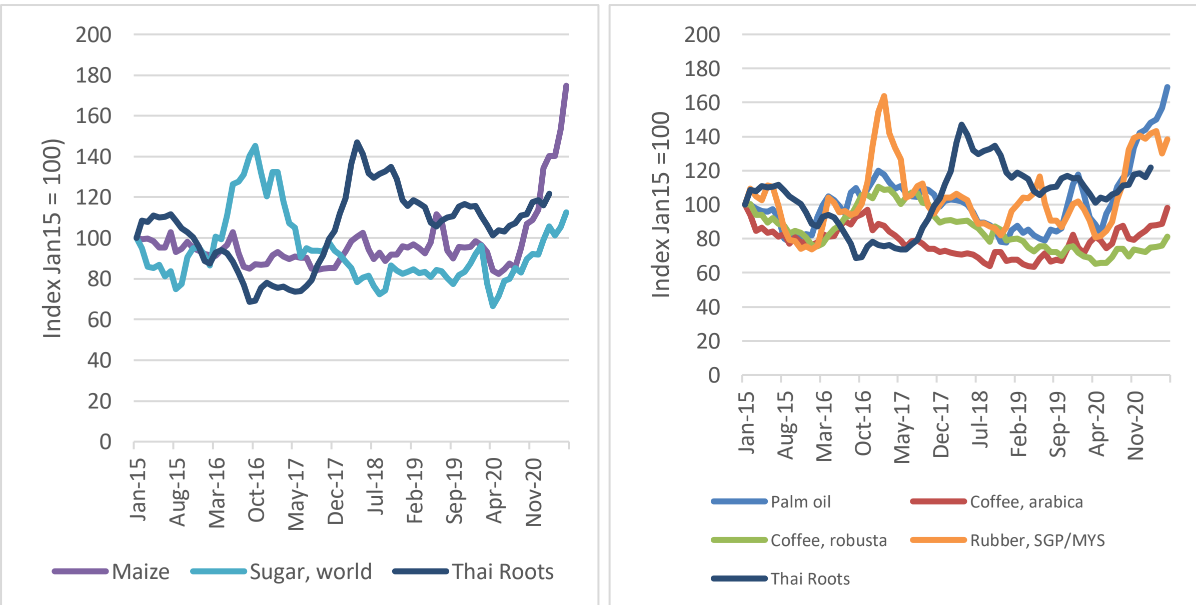


Figure 2a – Cassava prices relative to global maize and sugar prices. US maize prices have increased in recent months adding to upwards pressure on cassava prices. Figure 2b – Cassava prices relative to perennial tree crops indicating recent upswing in rubber and palm oil.

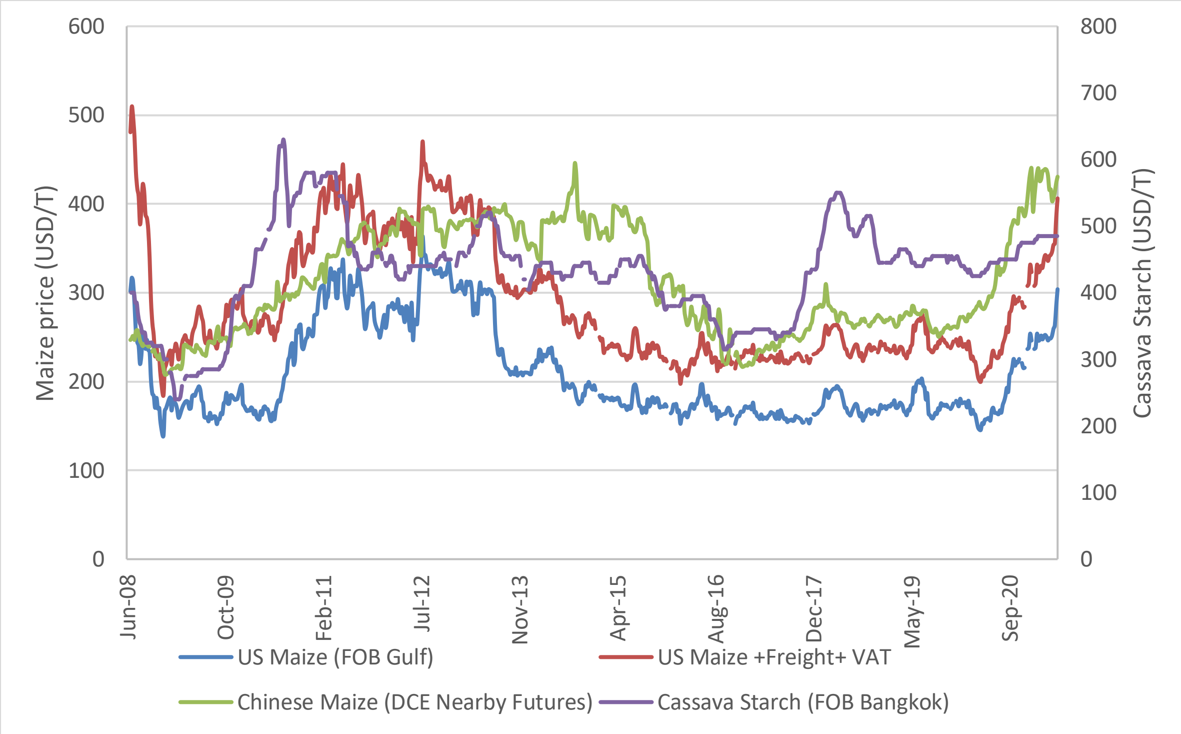


Figure 3- Chinese maize, US maize and cassava starch. Chinese demand for cassava has increased in part due to rising maize prices as stocks become depleted and recovery of feed demand after Swine Flu.

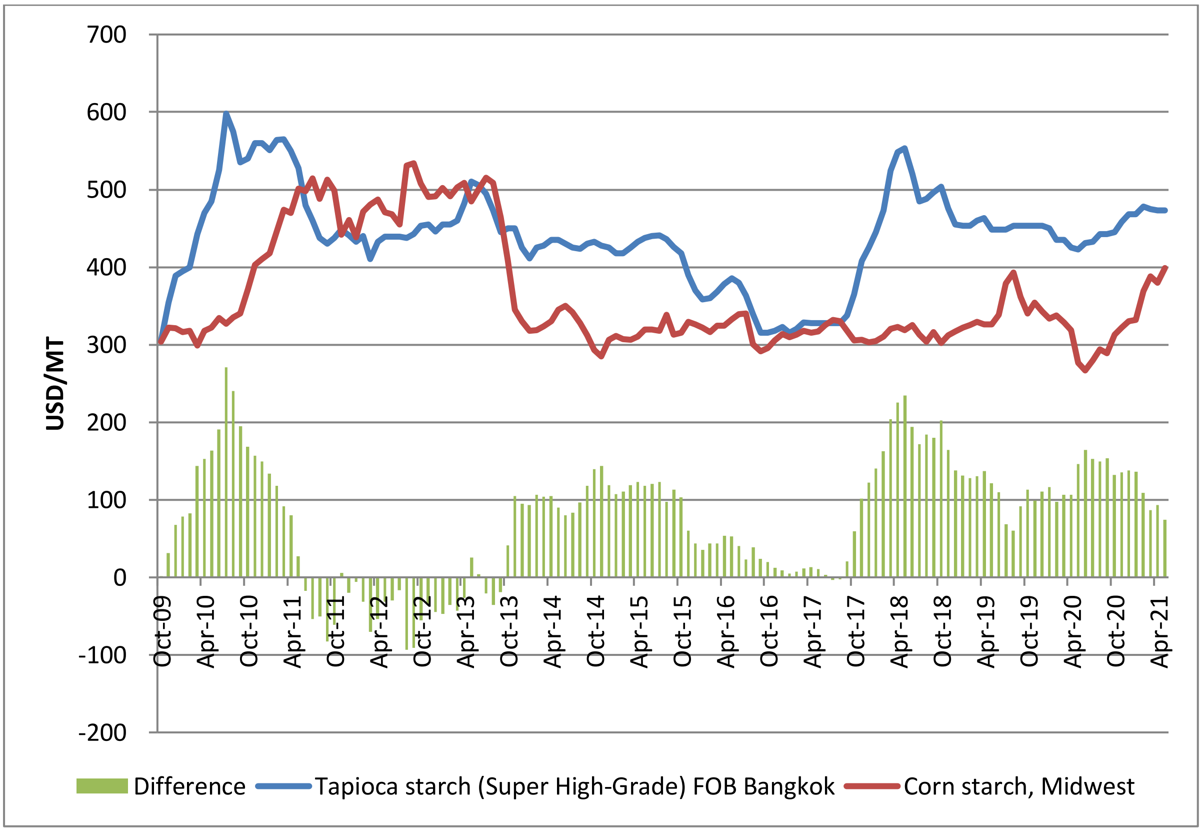


Figure 4 – Thai Cassava starch versus US corn starch. Cassava starch continues to trade at a premium of corn starch.

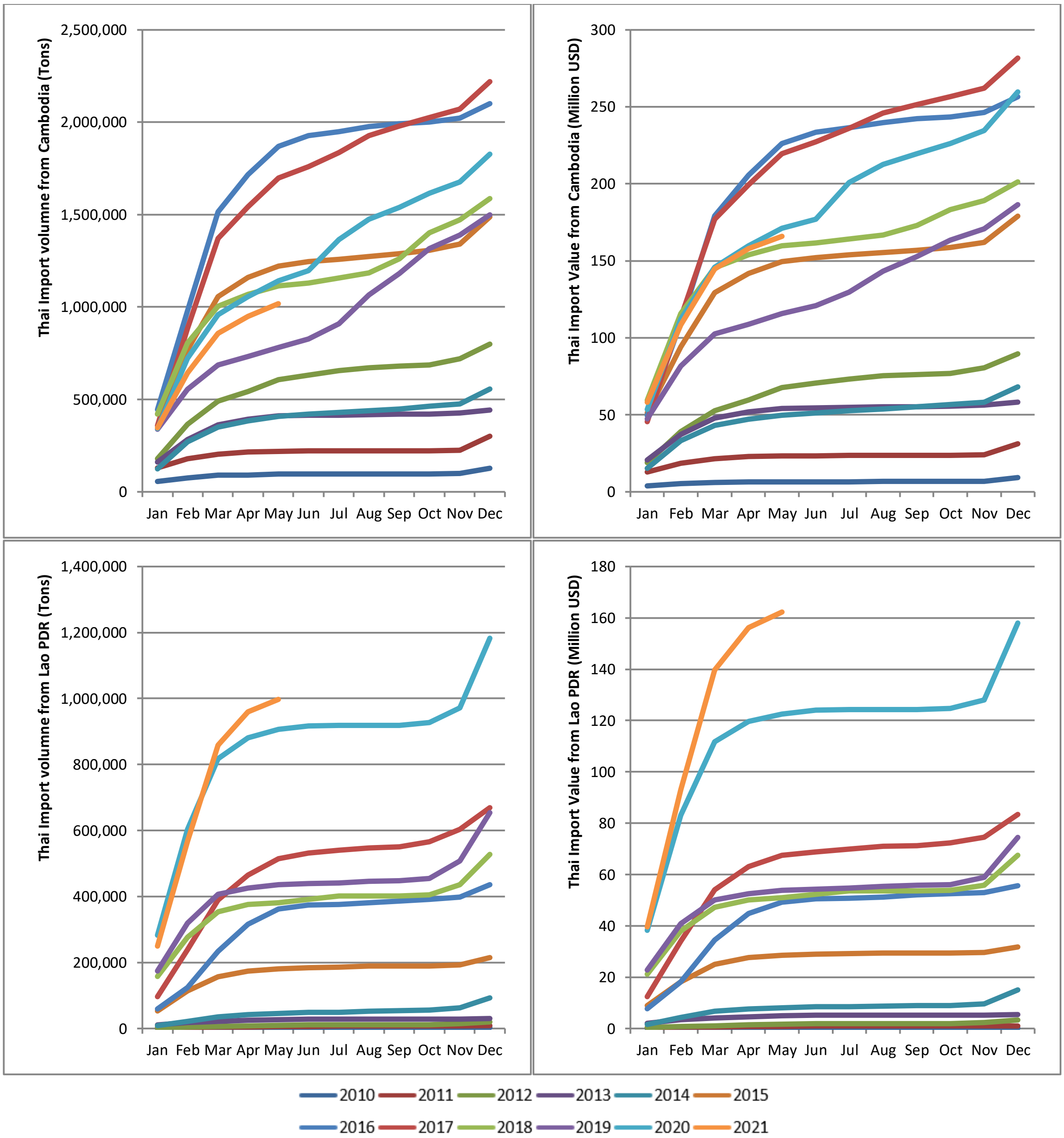


Figure 5 – Thai Imports of cassava (roots and chips) from Cambodia and Lao PDR by volume and value. Imports from Cambodia have declined as roots route to Vietnam due to price differential. Import value from Laos has surged making it one of the largest export commodities from Laos.

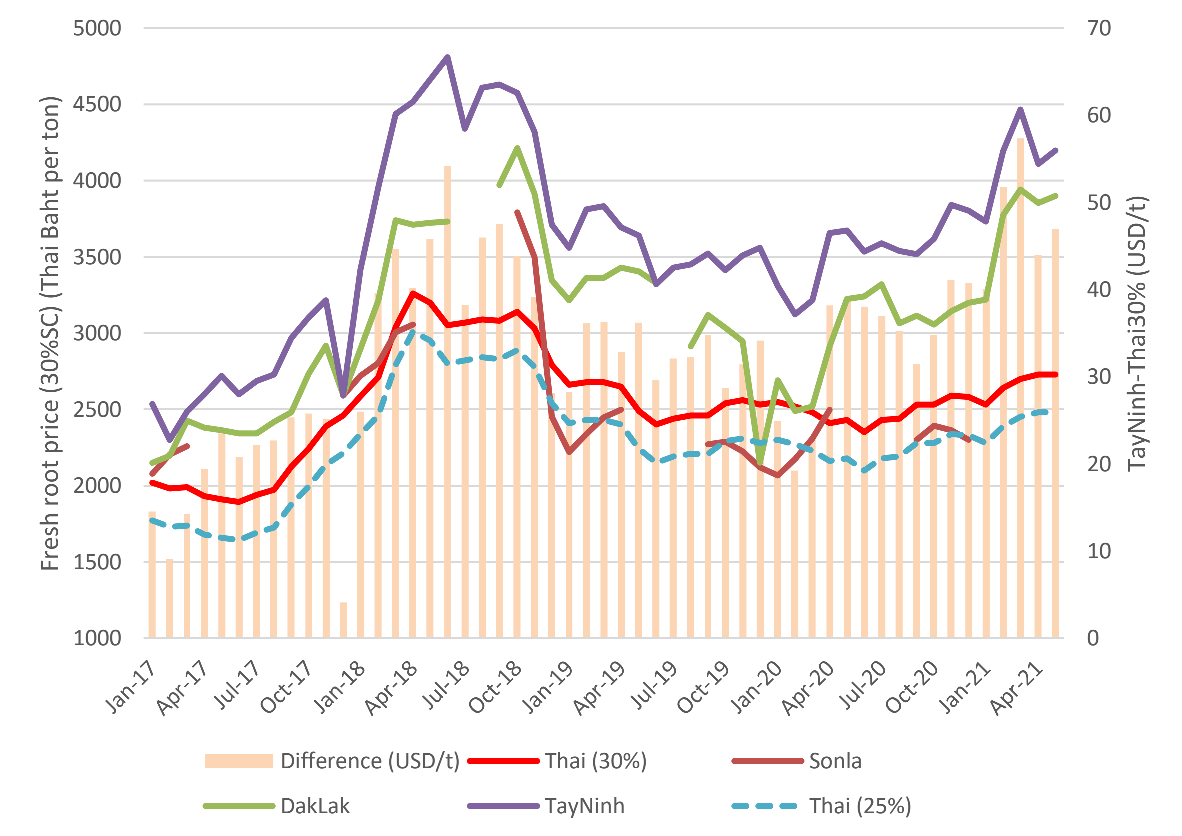


Figure 6 – Root price differential between Vietnam and Thai market. This has seen roots move to processing sector in TayNinh from throughout southern and central Vietnam and from deeper within Cambodia.

10.2 Appendix 3 - Accessing Farmer Willingness to Pay

Experimental auctions were conducted between November 2020 and March 2021. Groups of 20 farmers bid on three different types of seed (product 1=farmer seed, 2=improved variety, 3=clean seed of improved variety) in 20 villages, for a total sample of 391 individuals. Data analysis is ongoing, but initial findings include that farmers bid significantly higher and for a larger number of bundles for clean seed. While a single bidding strategy (low-middle price, low number of bundles) dominated the farmer seed, products 2 and 3 attracted higher bids, and for product 3 farmers adopted distinct strategies shown by groupings on the graph below (maintain volume and increase price; maintain price and increase volume).

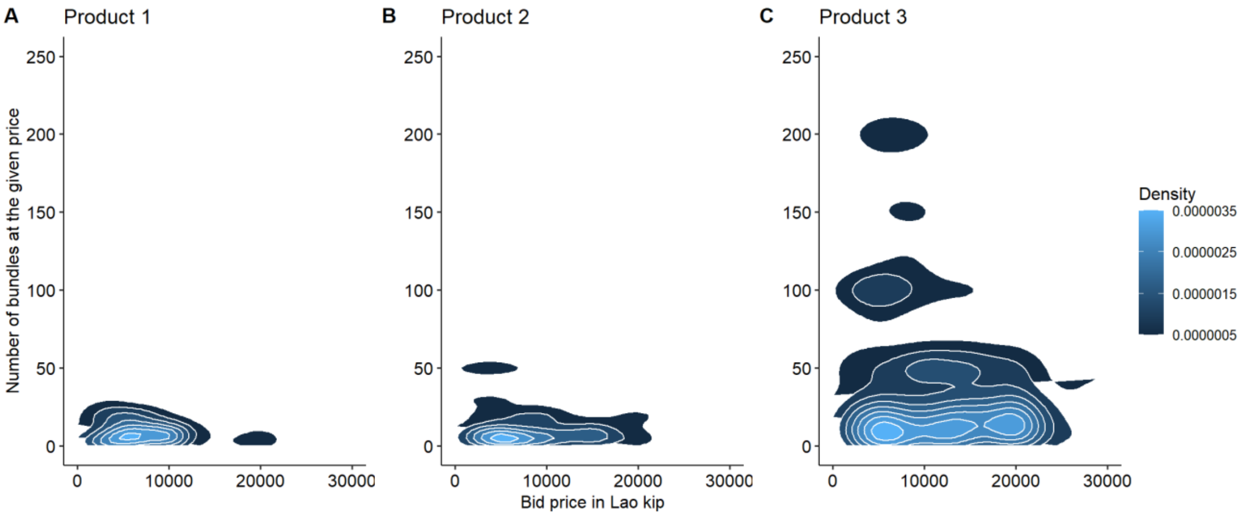


Figure 1: Density of bids over all villages, calculated using paired values of bid price and amount of stems desired at that price.

Bids varied significantly between provinces but were similar between districts and villages within a given province. Average bids for one bundle of clean stems varied from 5000 kip in Bolikhamxay (0.5 USD) to 20,000 kip (2 USD) in Champassak and Attapeu, with a grand mean across all sites of 13,000 kip (1.3 USD). These findings help us to understand local stem prices, willingness to pay for clean seed, and diversity in demand and volumes by location.

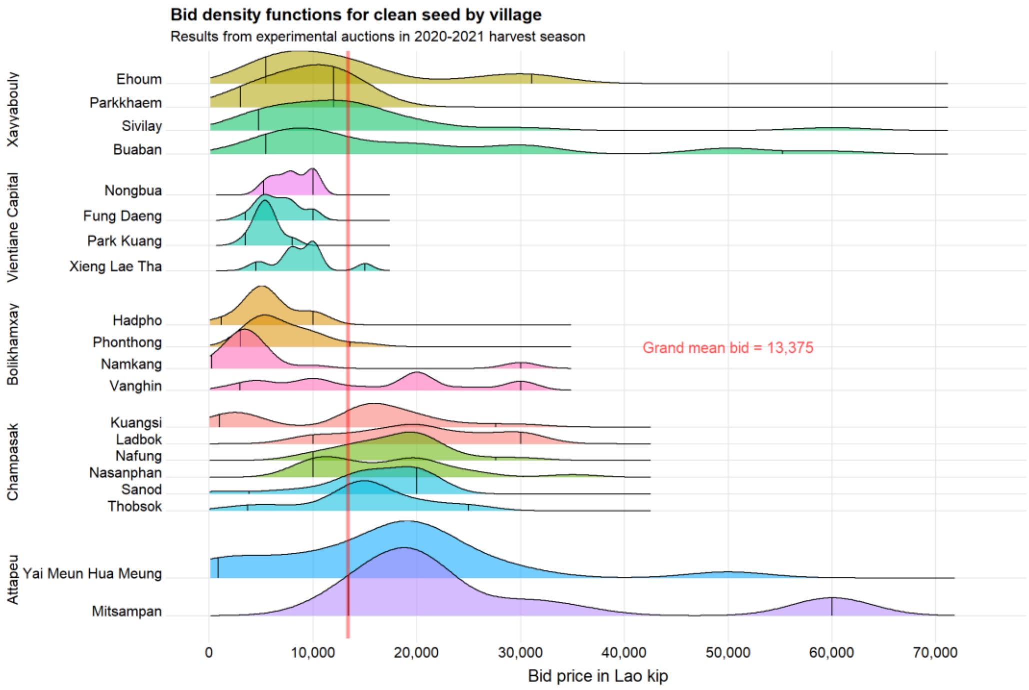


Figure 2: density of bids for clean seed grouped by province and by village. Different colors within a given province indicate different districts. Provinces are ordered North-South/top-bottom. Vertical lines in each density curve show 95% bid density bounded by 5% distribution tails, and red line indicated the grand mean over all samples.

In addition to the auctions, a follow-up was conducted using the reference price of 13,000 kip for farmers to place bids in Laos' two worst SLCMD-affected districts in Attapeu province. With the collaboration of Khousub import/export, 700 bundles of cassava stems were transported from a cassava mosaic-free district and sold to farmers whose fields were affected by SLCMV. The sale stem price was lowered to 10,000 kip per bundle, equivalent to the supply price, and all stems were sold to 32 distinct buyers in a single day. Numbers of bundles per participant were collected along with additional information to allow for follow-up with the recipients next season. The results of this activity help to confirm the results of the experimental work, and also to connect communities to a supply of uninfected stems, demonstrating the viability of the commercial model for clean stems at local prices.

10.3 Appendix 4 – Breeding results

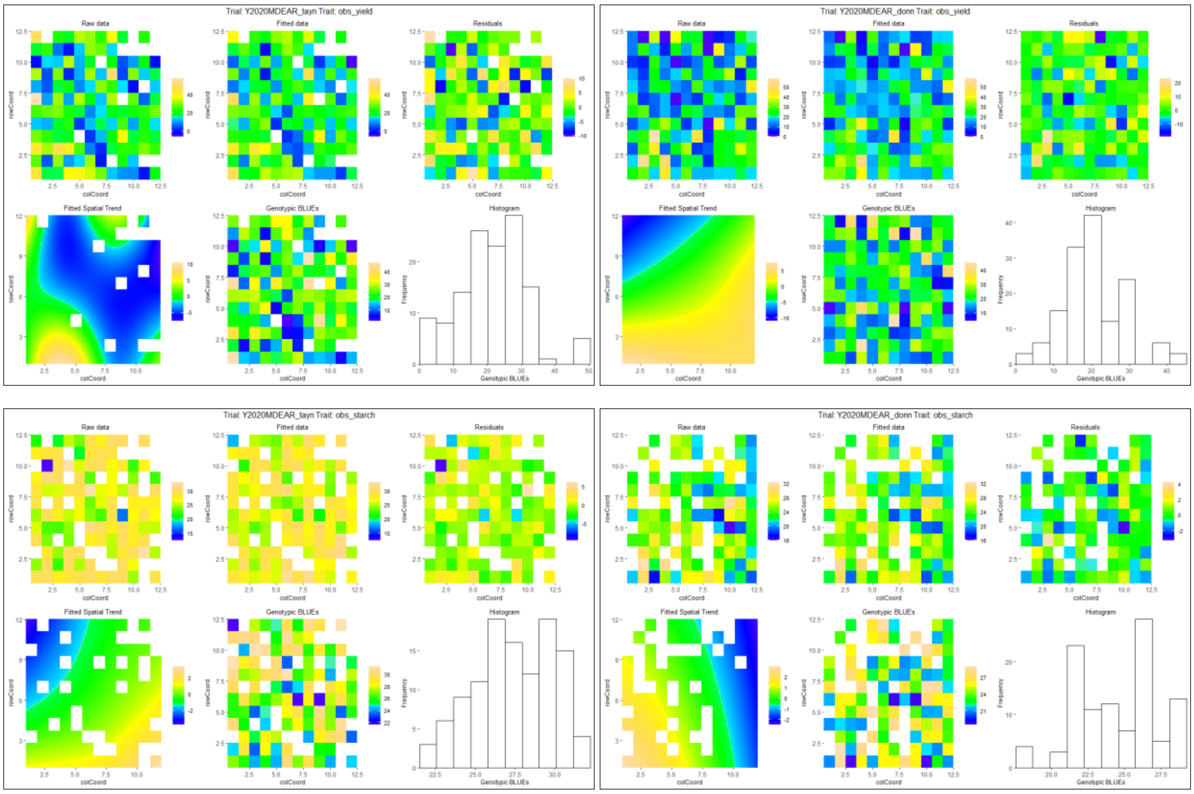


Figure 1 – Adjustment of the field spatial variation to obtain accurate estimation of the performance of the clones.

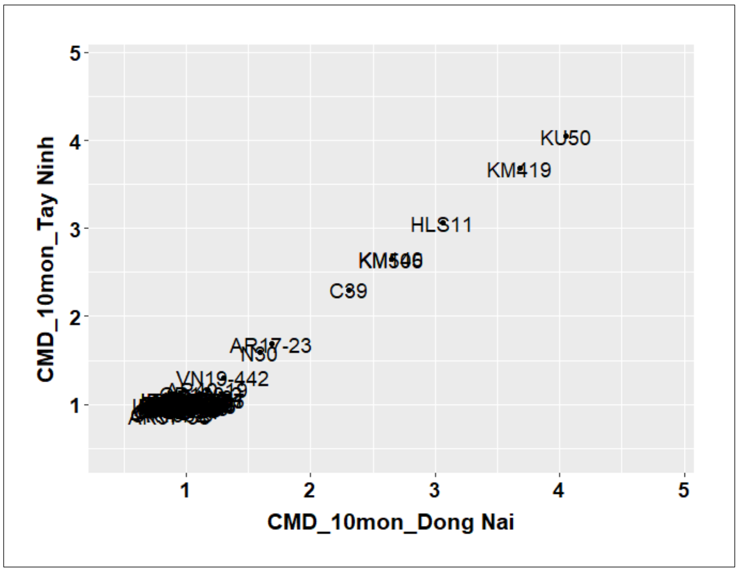


Figure 2 – The high correlation between the two locations in CMD severity at 10 months after planting.

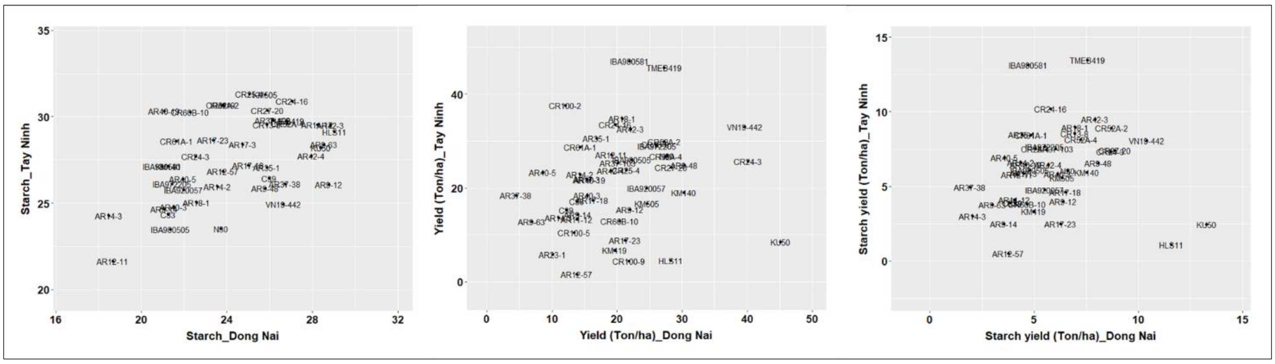


Figure 3 – The correlation between the two locations in starch content, yield, and starch yield.

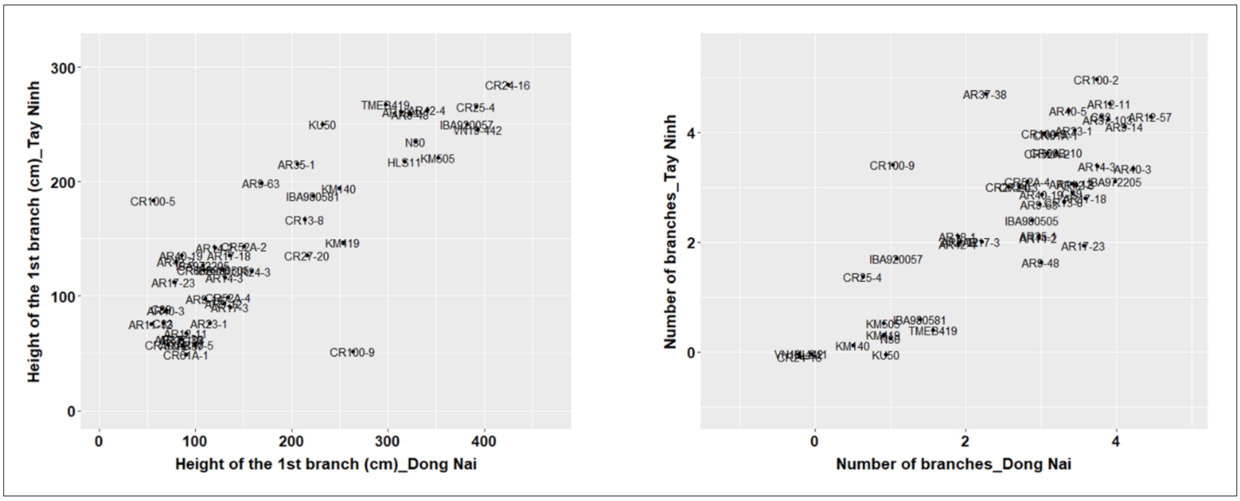


Figure 4 – The correlation between the two locations in height of the 1st branch and the number of branches.

Table 1 – The performance of the best resistant clones and check varieties

genotype	germination	CMD_10mon	height	height_1st_branch	branch_number	starch	yield	starch_yield
TMEB419	92.3	1.02	333.2	282.5	1.0	28.3	36.4	10.5
VN19-442	98.8	1.30	309.3	319.3	0.0	25.8	36.4	9.2
IBA980581	97.3	1.05	372.7	205.1	1.0	24.0	34.5	8.9
CR27-20	95.5	1.00	306.8	175.6	2.8	28.1	26.3	8.1
CR24-16	99.1	1.00	335.1	354.8	0.0	29.0	26.6	8.0
AR18-1	77.0	1.05	354.2	287.5	2.0	23.8	27.8	8.0
CR13-8	96.8	1.00	356.9	190.6	3.0	27.7	27.6	7.7
AR9-48	92.8	1.00	365.6	291.4	2.3	25.8	27.6	7.3
IBA972205	93.3	1.05	352.8	117.4	3.5	23.8	27.5	6.6
AR35-1	96.3	1.00	359.7	210.4	2.5	26.5	23.7	6.5
CR25-4	95.7	1.00	345.9	328.6	1.0	28.2	22.6	6.3
KM505	84.4	2.65	315.9	286.4	0.7	28.5	20.6	6.0
KM419	100.0	3.68	221.2	200.2	0.6	27.2	13.2	4.2
KU50	100.0	4.05	322.2	241.0	0.5	28.3	26.8	7.9
KM140	99.7	2.65	238.5	221.5	0.3	24.2	24.6	6.7
CR24-3	95.8	1.00	350.4	139.8	2.9	25.1	32.9	8.0
CR52A-4	100.00	1.00	332.2	116.7	3.0	28.2	27.1	7.7

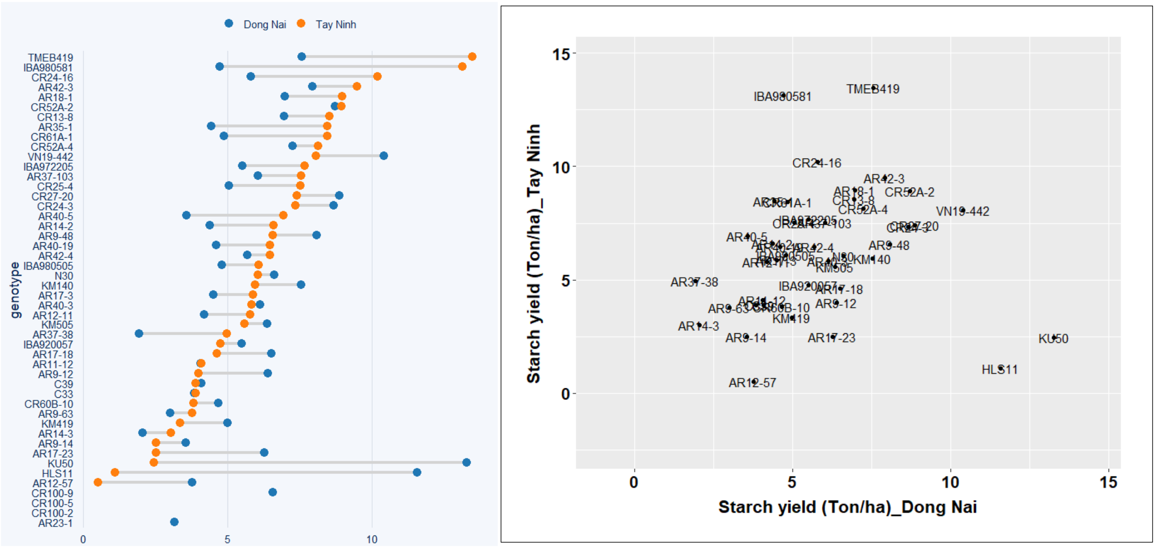


Figure 5 - The starch yield of the clones at the two locations, Tay Ninh and Dong Nai.

Table 2. Variation of CMD severity of VNM142 and CIAT102 populations in two growth seasons, 2018–2019 and 2019–2020.

Population	Trial	Trait	Mean	Median	Rang	V_g	V_e	H^2
VNM142	201801MDEAR	CMD_1.5MAP	1.56	1.43	1.00-4.00	0.10	0.21	0.50
		CMD_3MAP	1.87	1.75	1.00-4.00	0.18	0.22	0.63
		CMD_6MAP	2.23	2.20	1.00-4.00	0.27	0.19	0.75
		CMD_10MAP	2.82	2.86	1.19-4.00	0.40	0.19	0.82
	201901MDEAR*	CMD_10MAP	2.65	2.33	1.00-4.12	1.30	0.04	0.99
	201902MDEAR[#]	CMD_10MAP	2.83	2.63	1.70-4.10	0.62	0.11	0.94
CIAT102	201903MDEAR	CMD_3MAP	1.73	1.64	1.00-3.77	0.42	0.12	0.91

V_g, total genetic variance among unique clones; V_e, the variance of residue. The calculation of genetic variance was performed by using the mixed models by fitting replications and clones as random effects.

*the trials with 3 clones from VNM142 and four checks, HLS11, KM419, KU50 and C33. MDEAR, cassava mosaic disease advanced yield trial.

[#]the trials with 9 clones from VNM142 and three checks, HLS11, KM419 and KU50.

Table 3. The CMD score of the top resistant clones from VNM142 and the check clones.

Genotype/group	Clone	2018-2019	2019-2020
UNK-CI-2	VN19-442	1.5	2.3
CR63_PER262_TAI9	VN19-1432, VN19-1556	1.6	1.9
KM57_VNM8_Xanh Vinh Phu	VN19-1039, VN19-1050	1.6	1.9
UNQ-115	VN19-773	1.7	2.1
UNK-F	VN19-1184, VN19-1194	2.0	2.6
UNQ-44	VN19-320	1.7	1.8
UNK-AF-2	VN19-1805	1.8	NA
UNK-CH	VN19-390	1.9	2.2
KU50_KM94_TAI16	11 clone samples (e.g., VN19-1739	2.6	3.5
KM140	4 clone samples (e.g., VN19-2659)	3.6	NA
KM419	2 clone samples (e.g., VN19-2202)	3.0	4.0
C33	C33	NA	1.1

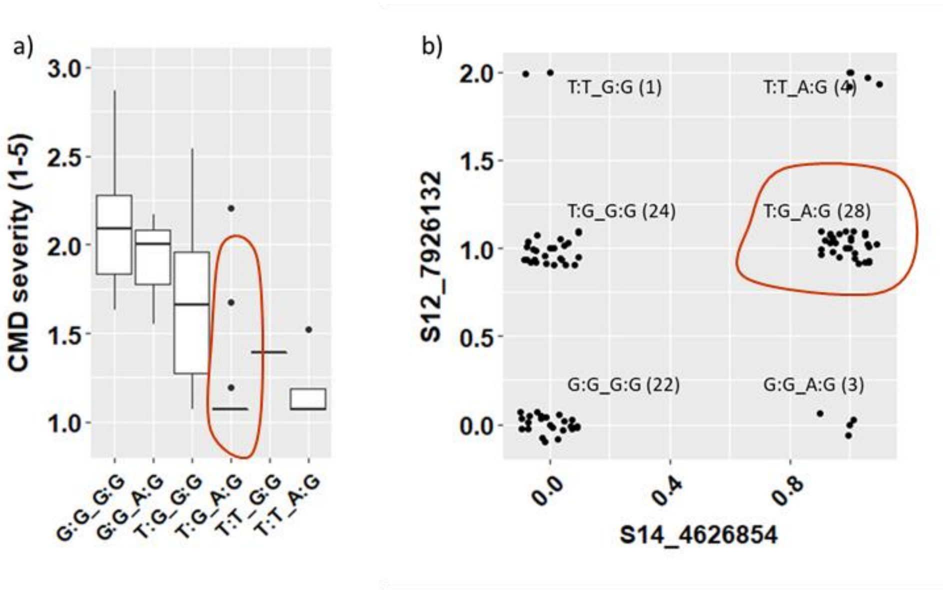


Figure - Validated the effect of *CMD2* markers in South East Asia

Combining two markers S12_7926132 and S14_4626854, provided the better prediction of CMD resistance. The genotypes with resistant alleles, T and A showed high resistant to CMD (red circle).

MAS is being used to accelerate the variety development with CMD resistance in South East Asia.

BỘ NÔNG NGHIỆP
VÀ PHÁT TRIỂN NÔNG THÔN
CỤC TRỒNG TRỌT

Số: 689 /TB-TT-CLT

CỘNG HOÀ XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập - Tự do - Hạnh phúc

Hà Nội, ngày 07 tháng 7 năm 2021

THÔNG BÁO
Về việc tiếp nhận hồ sơ tự công bố lưu hành giống cây trồng

Cục Trồng trọt thông báo:

1. Chấp nhận hồ sơ tự công bố lưu hành giống sắn HN3;

- Tên tổ chức đề nghị: Viện Di truyền Nông nghiệp (AGI);

- Địa chỉ: Km2 đường Phạm Văn Đồng - Bắc Từ Liêm - Hà Nội;

- Điện thoại: 024 37543198; Fax: 024 37543196; E-mail: info@agi.gov.vn.

- Nhóm tác giả: Nguyễn Anh Vũ[1], Nguyễn Hùng[1], Lê Ngọc Tuấn[1], Nguyễn Thị Hạnh[1], Đỗ Thị Trang[1], Đỗ Thị Như Quỳnh[1], Phạm Thị Hương, Nguyễn Hữu Phong[2], Lê Thị Kiều Trang[2], Nguyễn Văn Hồng[2], Peter Kulakow[3], Alfred G. O. Dixon[3], Francis Ogbe[3], Phạm Xuân Hội[1], Lê Huy Hàm[1];

- Cơ quan tác giả: [1]Viện Di truyền Nông nghiệp, [2]Chi cục Trồng trọt và Bảo vệ Thực vật tỉnh Tây Ninh, [3]Viện Nông nghiệp Nhiệt đới Quốc tế - IITA.

- Phạm vi lưu hành giống: vùng Đông Nam Bộ.

2. Văn bản kèm theo hồ sơ tự công bố lưu hành giống bao gồm:

- Bản tự công bố lưu hành giống sắn HN3 theo công văn số 124/VDT-KH ngày 14/5/2021 của Viện Di truyền Nông nghiệp;

- Bản công bố các thông tin về giống sắn HN3 theo công văn số 125/VDT-KH ngày 14/5/2021 của Viện Di truyền Nông nghiệp;

- Quy trình kỹ thuật canh tác giống sắn HN3 của Viện Di truyền Nông nghiệp biên soạn;

- Báo cáo kết quả tuyển chọn và khảo nghiệm giống sắn HN3 của Viện Di truyền Nông nghiệp.

3. Thông báo này được đăng tải trên cổng thông tin điện tử của Cục Trồng trọt từ ngày ký ban hành./.

Nơi nhận:
- Đơn vị có giống tự công bố lưu hành;
- Cổng thông tin điện tử Cục TT;
- Lưu: VT, CLT.

CỤC TRƯỞNG

CỤC TRỒNG TRỌT

Nguyễn Như Cường

MARD approval for release of TMS-IBA972205 to be released as ‘HN3’

BỘ NÔNG NGHIỆP
VÀ PHÁT TRIỂN NÔNG THÔN
CỤC TRỒNG TRỌT

Số: 405/TB-TT-CLT

CỘNG HOÀ XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập - Tự do - Hạnh phúc

Hà Nội, ngày 29 tháng 4 năm 2021

THÔNG BÁO
Về việc tiếp nhận hồ sơ tự công bố lưu hành giống cây trồng

Cục Trồng trọt thông báo:

1. Chấp nhận hồ sơ tự công bố lưu hành giống sắn HN5;

- Tên tổ chức đề nghị: Viện Di truyền Nông nghiệp (AGI);

- Địa chỉ: Km2, đường Phạm Văn Đồng, Bắc Từ Liêm, Hà Nội;

- Điện thoại: 024 37543198; Fax: 024 37543196; E-mail: info@agi.gov.vn.

- Nhóm tác giả: Nguyễn Anh Vũ[1], Nguyễn Hùng[1], Lê Ngọc Tuấn[1], Nguyễn Thị Hạnh[1], Đỗ Thị Trang[1], Đỗ Thị Như Quỳnh[1], Phạm Thị Hương, Nguyễn Hữu Phong[2], Lê Thị Kiều Trang[2], Nguyễn Văn Hồng[2], Peter Kulakow[3], Alfred G. O. Dixon[3], Francis Ogbe[3], Phạm Xuân Hội[1], Lê Huy Hàm[1];

- Cơ quan tác giả: [1]Viện Di truyền Nông nghiệp, [2]Chi cục Trồng trọt và Bảo vệ Thực vật tỉnh Tây Ninh, [3]Viện Nông nghiệp Nhiệt đới Quốc tế - IITA.

- Phạm vi lưu hành giống: vụ Đông Xuân tại vùng Đông Nam Bộ.

2. Văn bản kèm theo hồ sơ tự công bố lưu hành giống bao gồm:

- Bản tự công bố lưu hành giống sắn HN5 theo công văn số 79/VDT-KH ngày 02/4/2021 của Viện Di truyền Nông nghiệp;

- Bản công bố các thông tin về giống sắn HN5 theo công văn số 79/VDT-KH ngày 02/4/2021 của Viện Di truyền Nông nghiệp;

- Quy trình kỹ thuật canh tác giống sắn HN5 của Viện Di truyền Nông nghiệp biên soạn;

- Báo cáo kết quả tuyển chọn và khảo nghiệm giống sắn HN5 của Viện Di truyền Nông nghiệp.

3. Thông báo này được đăng tải trên cổng thông tin điện tử của Cục Trồng trọt từ ngày ký ban hành./.

Nơi nhận:
- Đơn vị có giống tự công bố lưu hành;
- Cổng thông tin điện tử Cục TT;
- Lưu: VT, CLT.

CỤC TRƯỞNG

CỤC TRỒNG TRỌT

Nguyễn Như Cường

MARD approval for release of TMS-IBA980581 to be released as 'HN5'

10.4 Appendix 5 – Breeding workplan

2021 Work Plan of the Objective 2 of ACIAR project

Xiaofei Zhang and Thuy Cu Thi Le

1. Activities in AGI

1.1 Germplasm introduction and evaluation

We will harvest the CIAT102 trial in March 2021, which was established in Tay Ninh in May 2020 using 48 resistant clones selected from the CIAT102 population. We will collect the agronomic traits, including dry matter, plant height, plant type, root yield, root number, et al. (Table 1). Combining the data of two yield trials (another in Dong Nai), we will make selections, and establish new regional trials in seven locations in Vietnam (**AGI: Son La, Thanh Hoa**; **HLARC: Tay Ninh, Phu Yen (established), Dong Nai, Dak Lak, Quang Ngai)**. We will use 20 plants per plot, 2 or 3 replications at each location, following the row-column design.

1.2 Screen the core collections

The first set of 160 clones will be planted in Tay Ninh in April 2021. We will use 10 plants per clone following augmented design. Five resistant checks and and three elite susceptible varieties will be planted in the trial.

For the other 359 clones, the genebank is checking their status and prepare for multiplication. The first set with 72 clones will be shipped to Vietnam and Laos in March. The other clones will arrive in August or September 2021.

1.3 Increase the genomic prediction training population

The training population will be increased in Hanoi before March 31, at least 5 plants per clone (Figure 1). Five CMD resistant clones and four local elite varieties will also be included in the trial. Stem cuttings will be harvested from the seed increase trial. At least 16 stem cuttings are expected from each clone in September 2021. In one month after planting, harvest leaf tissue to prepare the samples for DNA extraction in InterTek.

About 200 clones can be expected. AGI and CIAT will decide the list of clones for yield trials together. Yield trials will be established at two locations with high (Tay Ninh, September 2021) and low CMD pressure (Dong Nai, March 2022, raining season) in Southern Vietnam in September 2021. 4-plant plots with two replications will be used following the row-column design.

1.4 Initiate conventional breeding

1.4.1 Plant the F1 seedling trial

Harvest seeds from the 2020-2021 crossing nurseries and report the number of seeds from each half-sib or full-sib family. AGI share half of the seeds with CIAT. AGI will establish the F1 seedling trial with ~2,000 F1 plants.

1.4.2 Plant the new crossing nursery under red light

5-10 selected CMD resistant clones and 10-20 elite varieties will be used as progenitors.

Planting is expected in the end of Feb or early March 2021.

Son La, 800 m above sea level; When selecting progenitors, we need consider flowering behavior.

1.5 Manage the trial data using CassavaBase

After harvesting, the trials data from 1.1, 1.2 and 1.3 will be uploaded to CassavaBase.

2. Activities in HLARC

2.1 Germplasm introduction and evaluation

2.1.1 Seeds from Hawaii

From 600 plants, 30% infected by CMD. Harvest Select the best 30-100 plants (or clones) and evaluate them at the CMD hot sport for CMD resistance (grafting) and agronomic performance (Dong Nai). One trial in Dong Nai for agronomic traits.

2.1.2. CMD resistant clones from CIAT102

We will harvest the CIAT102 trials in March 2021, which was established in Dong Nai in May 2020 using 48 resistant clones selected from the CIAT102 population. We will collect the agronomic traits, including dry matter, plant height, plant type, root yield, root number, et al. Combining the data of two yield trials, we will make selections, and plant a new regional trials in at least 5 locations in Vietnam (**Tay Ninh, Phu Yen (planted), Dong Nai, Dak Lak, Quang Ngai**). We will use 20 plants per plot, 3 replications at each location, following the row-column design.

2.2 Conduct conventional breeding

2.2.1 Plant the F1 seedling trial

Harvest seeds from the 2020-2021 crossing nurseries and report the number of seeds from each half-sib or full-sib family. HLARC share half of the seeds with CIAT. HLARC will establish the F1 seedling trial with ~3,000 F1 plants.

HLARC made 1100 paired crosses, expected to havested about 3,000 seeds. In the open pollination nursery, we will only harvest seeds from best clones (elite or CMD resistant clones)

Several clones did not flower, either elite clones (Rayong 72) or CMD clones.

2.2.2 Plant the new crossing nursery under red light

5-10 selected CMD resistant clones and 10-20 elite varieties will be used as progenitors.

Select clones with best agronomic traits and good flowering behavior.

Note: Attention should be paid for not bringing CMD to Lam Dong.

2.3 Manage the trial data using CassavaBase

After harvesting, the trials data from 2.1.2 will be uploaded to CassavaBase.

3. Activities at CIAT

3.1 Breeding activities for population improvement

3.1.1 Plant the F1 seedling trial

CIAT will establish the F1 seedling trial with 1,000 (1,500 seeds) F1 plants from AGI and HLARC. Plant them in both Dong Nai and Da Lak. (cut one seedlings into two plants for two locations, Dong Nai and Da Lak).

3.1.2 Genotype the F1 selections

About 100 selected clones will be genotypic to predict the CMD resistance. The selected clones with CMD2 gene will be cycled back to the crossing nursery to produce seeds for the next cycle of population improvement.

3.1.3 Genotype the progenitors in the crossing nurseries in AGI and HLARC

Genotype the progenitors using ~96 markers for 1) quality control; 2) parentage analysis; 3) confirmation of the genotype of progenitors at the CMD2 locus. Thuy will use the freeze dryer in the lab in Ho Chi Minh City.

3.2 Implement genomic prediction

3.2.1 Genotype the training population and breeding population in Palmira

The training population with 400 clones and the F1 selections from Palmira, Colombia will be genotyped using 10x whole genome sequencing. The genome wide markers will be used for genomic prediction.

3.2.2 Establish yield trials of the training population

The training population will be evaluated at the north coast of Colombia at two locations with sub-humid and semi-arid lowland zones. We will use 5-plant plots with 2 replications at two locations following the row-column design.

3.3 validate the CMD markers for the CMD2 locus.

Finish the manuscript and submit for peer review. Use DNA markers to predict the CMD resistance.

4. Activities in Laos

4.1 Screen the core collections for resistance to witches' broom disease

The first set of 160 clones arrived in Laos. These clones will be maintained in the green house. The screening protocol is under development.

For the other 359 clones, the genebank is checking their status and prepare for multiplication. The first set with 72 clones will be shipped to Vietnam and Laos in March. The other clones will arrive in August or September 2021.

4.2 Evaluate the seeds from Hawaii

F1 seedling trials will be established to evaluate the performance and make selections for the next stage, clonal evaluation trials. For the selected clones, we can run the CMD2 markers to select for CMD resistance in 2022.

Table 1. List of agronomic traits to record from the yield trials.

Traits		
Germination	must have	3-6 weeks after planting
Vigor	must have	3-6 weeks after planting
CMD Scores	must have	before harvest (10MAP)
Plant height	must have	before harvest
#main stems	must have	before harvest
Height of the first branch	must have	before harvest
# branching events	must have	before harvest
Shape of plants	must have	before harvest
#storage root per plot (6 plants)	must have	at harvest
Root peduncle presence	take note if it ocurs	at harvest
Root shape	must have	at harvest
External color of storage roots	must have	at harvest
Root pulp color	must have	at harvest
Foliage weight	optional	
Fresh root yields	must have	at harvest
Starch contents	must have	at harvest
#rotten roots	take note if it ocurs	at harvest
#Lodging plants	must have	before harvest
number of plants harvested	must have	before harvest

For all traits, please record one number per plot based on the performance of the central 6 plants.

Collect data of all 3 replications

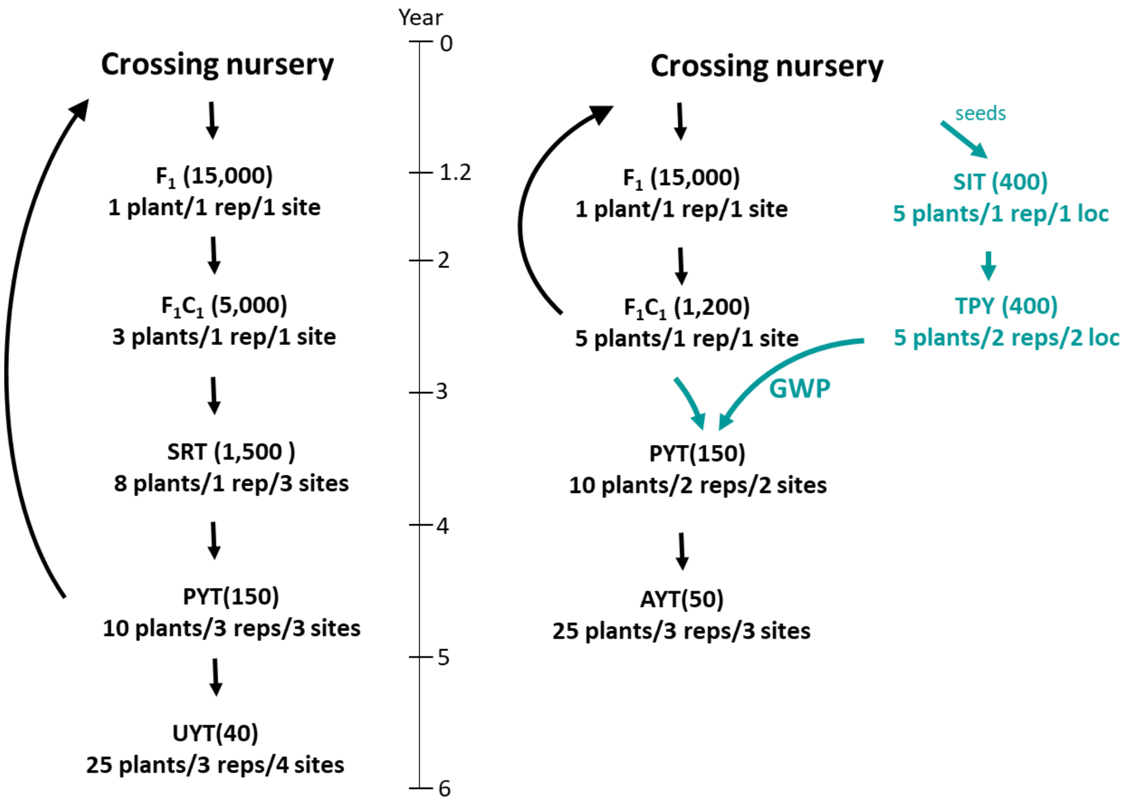


Figure 1. The cassava breeding schemes at CIAT. Starting in the 2010s, the seedling nursery (F1) was planted in the off season and grown for only six months. The F1C1 stage with three plants per genotype was added as the source of planting materials for the following single row trial (SRT), which could then be planted in three locations (red). The following stages are the preliminary (PYT), advanced (AYT) and uniform (UYT) yield trials with increasing number of plants per plot, but AYT was no longer grown in the 2010s. The development and implementation of the flower inducing technology shortened the duration of crossing nurseries in the 2020s. The F1 is grown for seven months, which allows F1C1s to have six plants per genotype and then PYT to grow in two locations. Selections for PYT will be made based on the total genetic value predicted using genome wide prediction (GWP). The F1C1 clones with the best predicted breeding value will be cycled back to crossing nurseries as progenitors for the next cycle of improvement (black upward arrow). The GWP training population is selected from the breeding population based the pedigree. Rapid multiplication (RM) is performed in green house to obtain five plantlets from each seedling. The plantlets are transplanted into the seed increase trial (SIT). Next spring, the training population yield trial (TPY) trials are established at two locations for phenotyping. The SIT and F1C1 clones are genotyped for genome wide prediction.

10.5 Appendix 6 – Disease surveillance and diagnostics

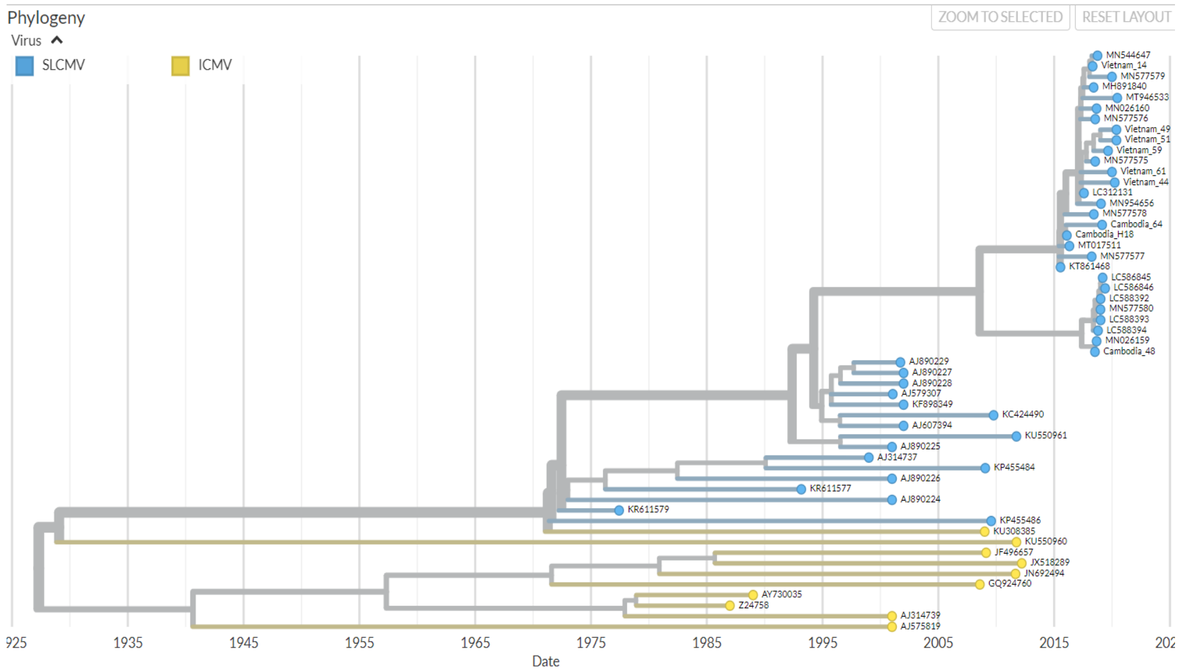


Figure 1. Current map showing the genetic distribution of SLCMV isolates detected in Southeast Asia (Upper right corner). At this moment the presence of a second genetic cluster of the virus has been confirmed in Thailand and Cambodia. The biological differences between both variants is still unknown.

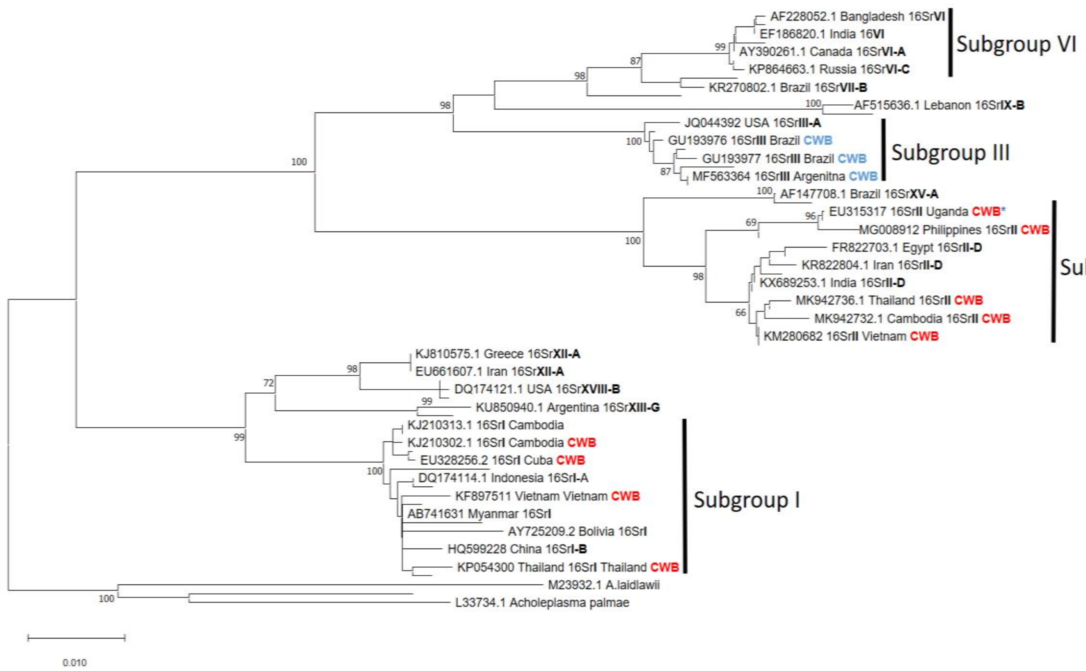


Figure 2. Phylogenetic analysis of CWBD-phytoplasma as reported in public databases. Noitice that phytoplasma associated to CWBD in Southeast Asia (Subgroups I and II) are genetically different from those reported in the Americas, which belong to Subgroup III (blue).

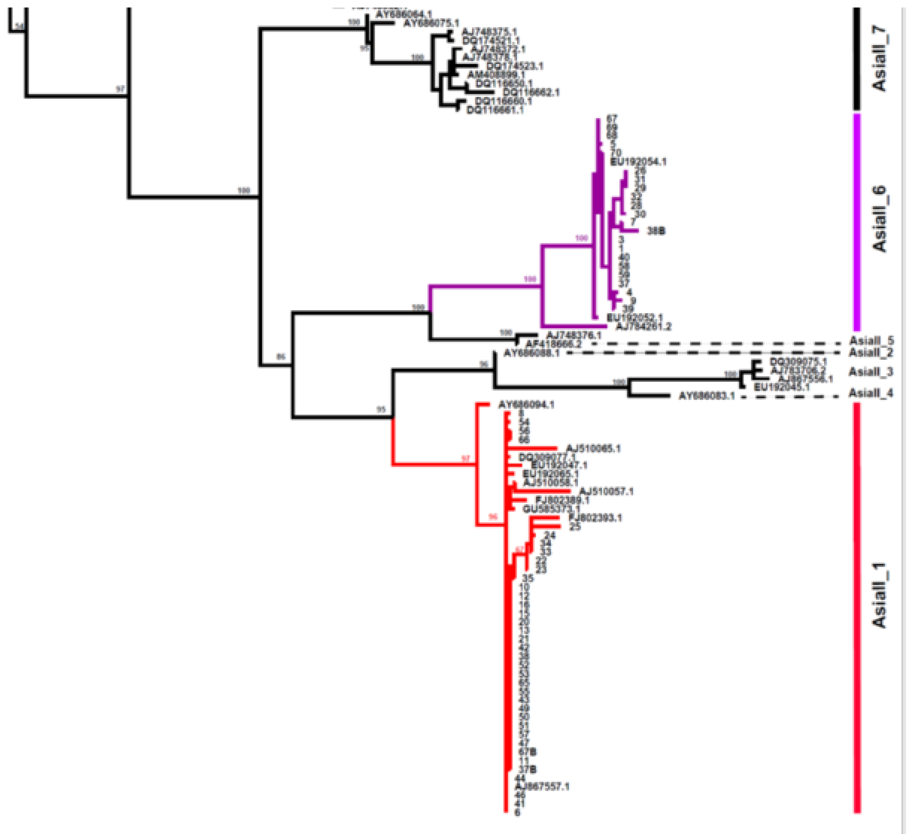


Figure 3. Genetic analysis of the whitefly COI gene from samples collected in Laos during the 2020 surveys. All collected whiteflies belonged to the Asia II-1 (red) and the Asia II-6 (purple) type.

10.6 Appendix 7 - Results of agronomy and seed system activities

Cambodia

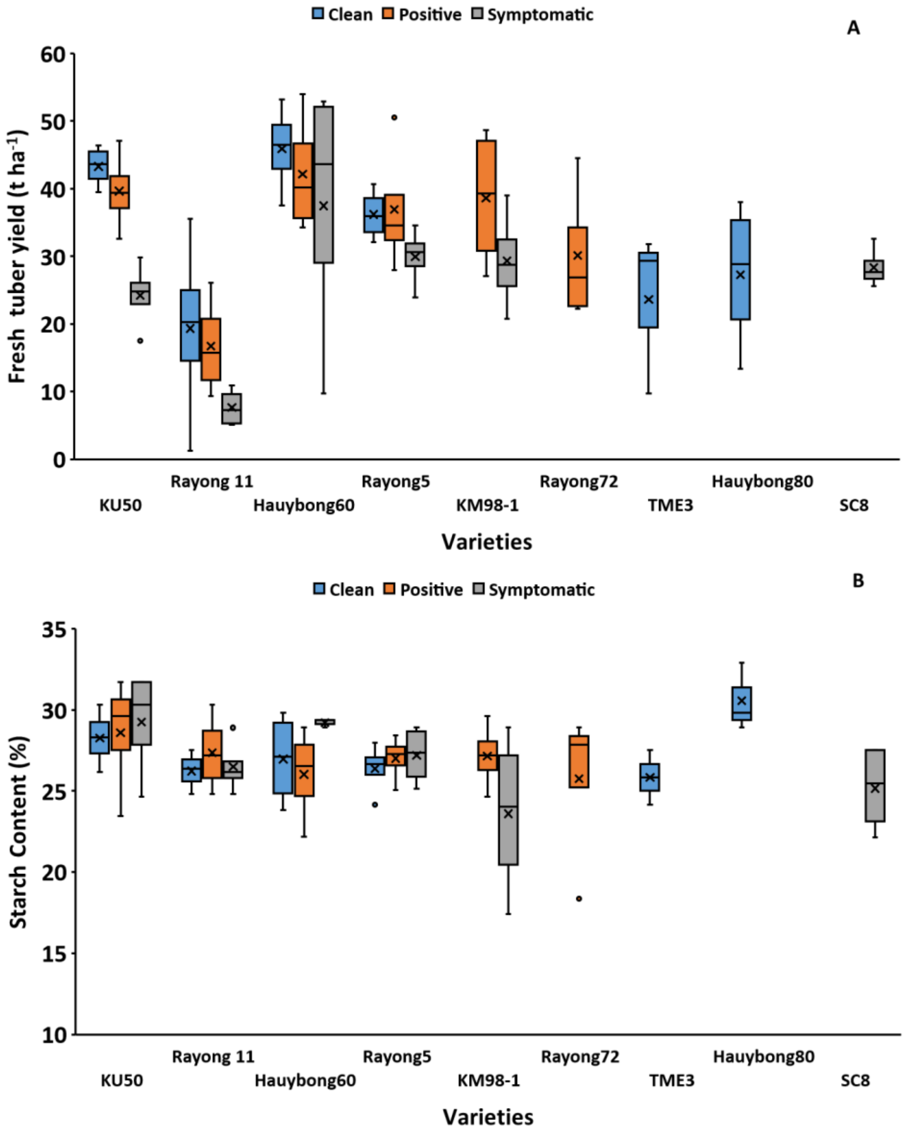


Figure 1: Fresh root yield (t ha^{-1}) (A), Starch content (%) (B) and number of plants with symptoms at the end of the season (C). There were six varieties, KU50, Royong11, Hauybon60, Rayong5, KM98-1 and SC8 with three different kind of planting stakes; collected from disease free area (clean), collected from diseased area without any symptoms i.e. positive selected stems (positive) and symptomatic stems (Symptomatic) were planted. Due to susceptibility of disease no clean planting materials were available for SC8 and KM98-1; and positive selection planting material of SC8 for planting during 2020-2021 season. TME3, planted in place of SC8 clean planting material and Rayong72 planted in place of positive section SC8; HB80 in place of KM98-1 clean planting material. Values are the means (n=4) (X), whisker indicates the range within a treatment.

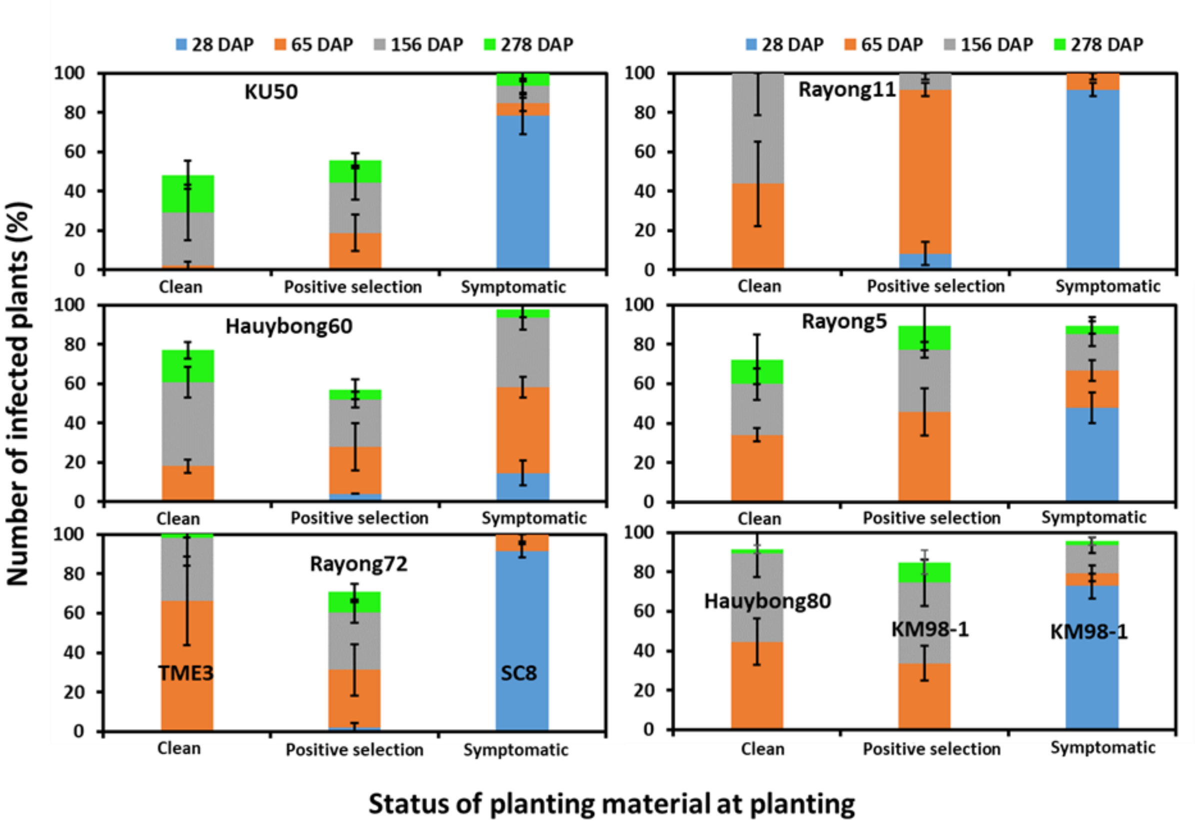


Figure 2 Number of symptomatic plants (%) per plot (cumulative) of cassava mosaic disease (CMD) after 26, 64, 156 and 278 days after planting (DAP) of 6 cassava varieties commonly grown in Southeast Asia. Three kind of stakes/seeds were planted, Disease free stakes (Clean), Positive selected stakes (i.e. without symptoms) from diseased field (Positive selection) and selected from symptomatic plants (Symptomatic). Bar represent means ± standard error (n = 4).

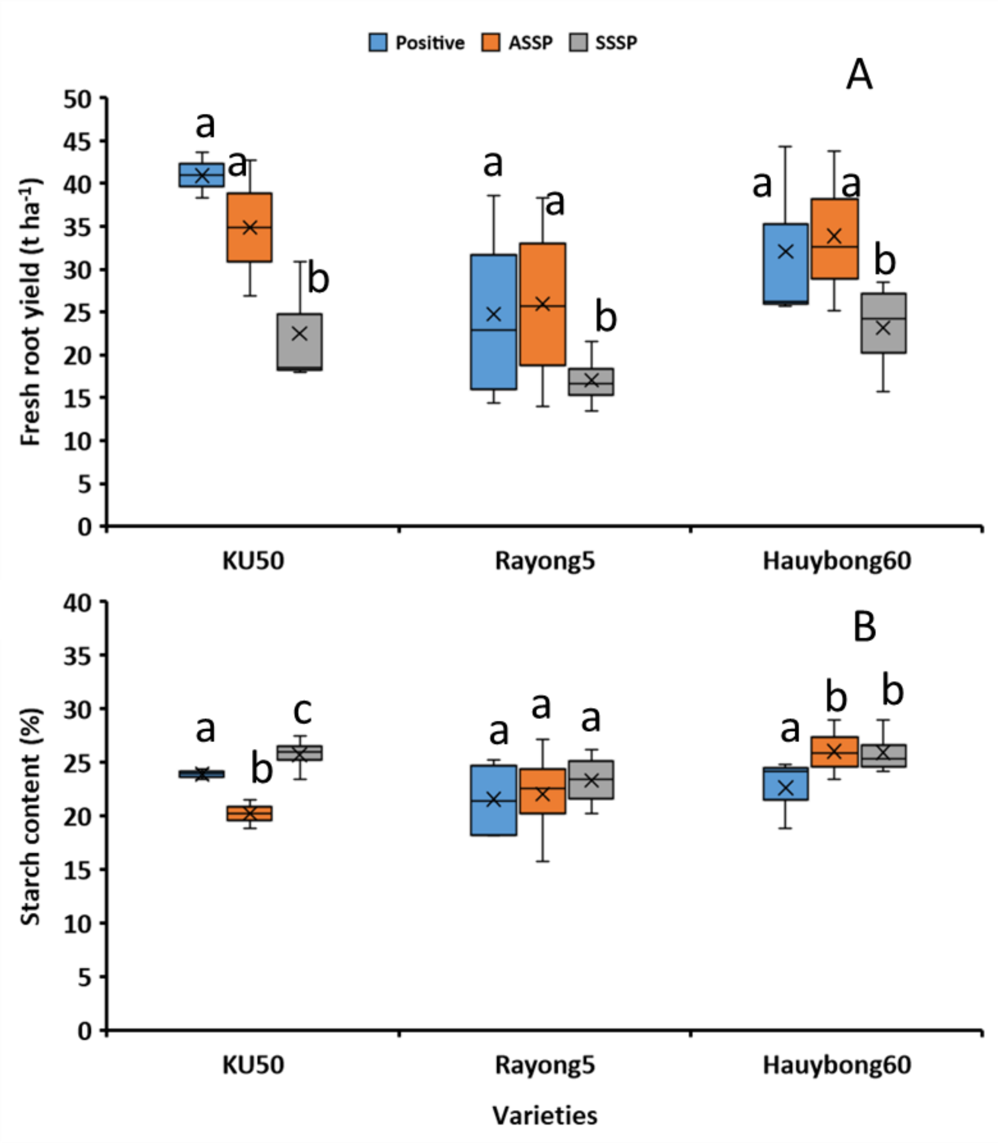


Figure 3: Fresh root yield (t ha-1) (A), Starch content (%) (B) and number of plants with symptoms at the end of the season (C). There were six varieties, KU50, Royong5, Hauybon60, with three different kind of planting stakes; collected from diseased area without any symptoms i.e. positive selected stems (blue), asymptomatic stems of a symptomatic plants (orange) and symptomatic stems (grey) were planted. Values are the means (n=4) (X), whisker indicates the range within a treatment.

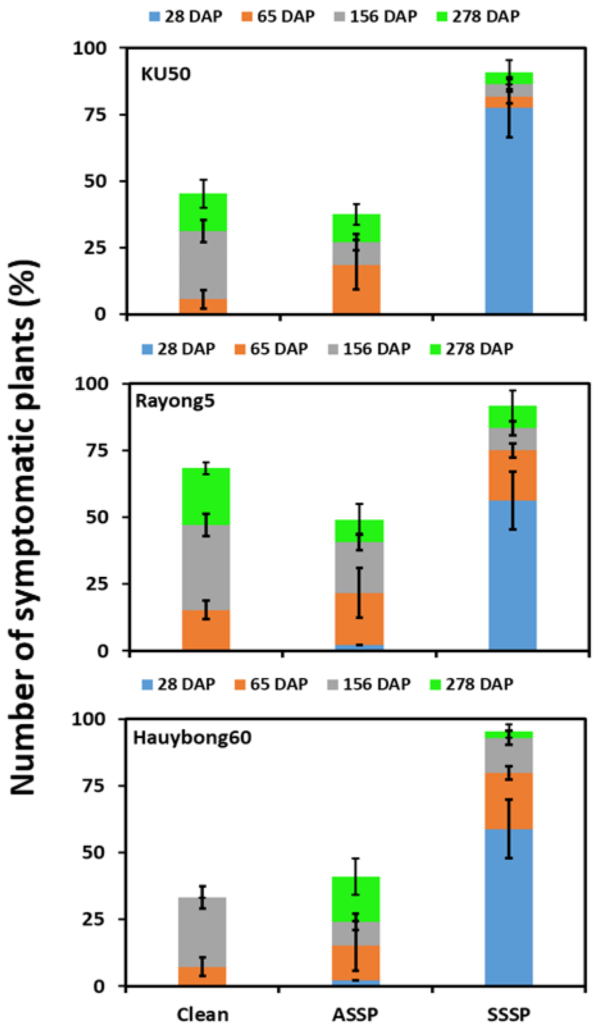


Figure 4: Number of symptomatic plants (%) per plot (cumulative) of cassava mosaic disease (CMD) after 26, 64, 156 and 278 days of planting (DAP) of 3 cassava varieties commonly grown in Southeast Asia. Three kind of stakes/seeds were planted, Disease free stakes (Clean), Asymptomatic stem from symptomatic plants (ASSP) and Symptomatic stems from symptomatic plants (SSSP). Bar represent means ± standard error (n = 4).

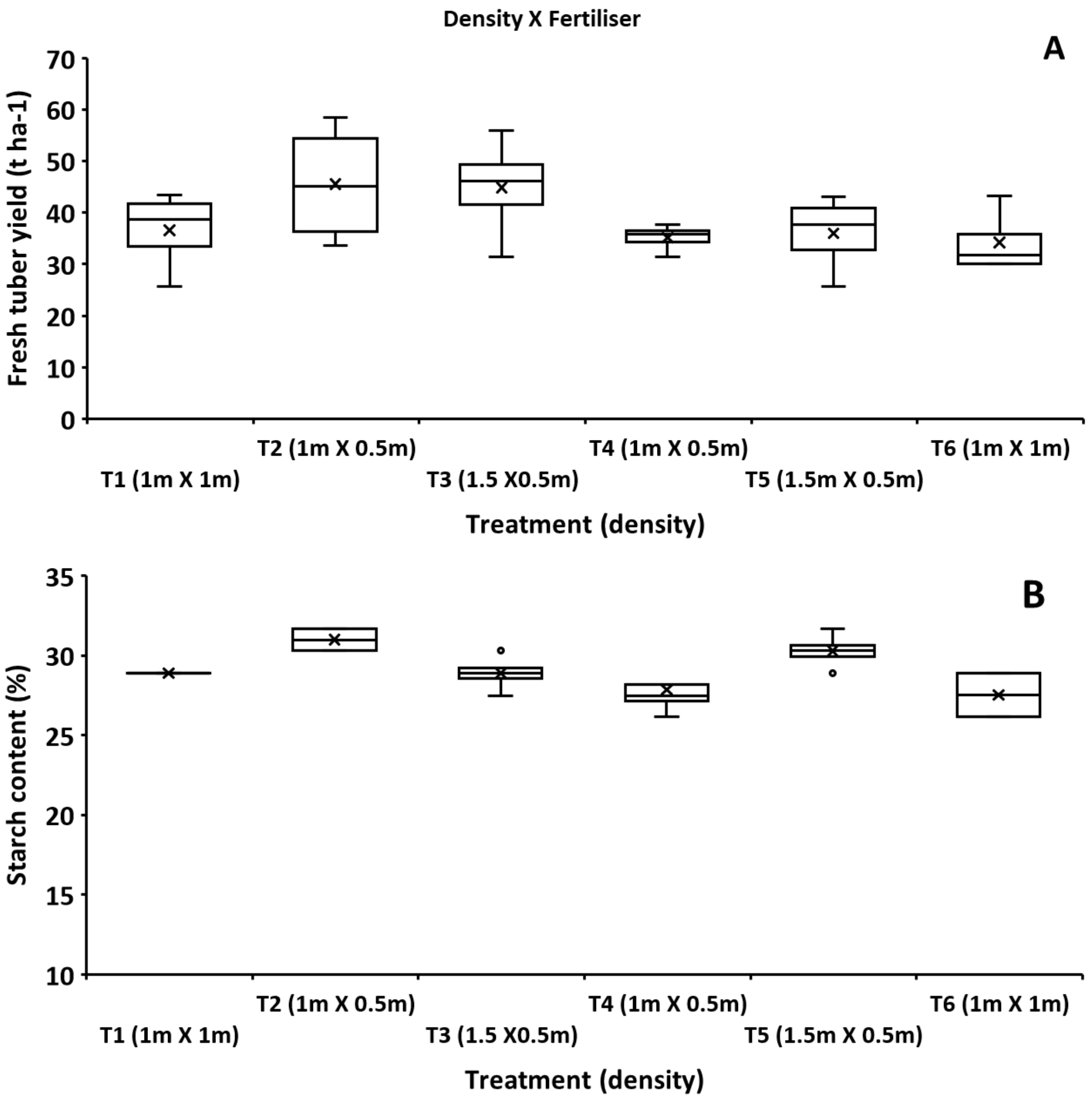


Figure 5: Fresh root yield (t ha^{-1}) (A) and Starch content (%) (B) of cassava roots where plants received six different fertiliser treatment NPK and planted in three different density. Density (1m X 1m- 10k ha^{-1}) Fertiliser (80-20-80) (T1), Density (1m X 0.5 m-20k ha^{-1}) Fertiliser (80-20-80) (T2), Density (1.5m X 0.5m-13.4K ha^{-1}) Fertiliser (80-20-80) (T3), Density (1m X 0.5m- 20k ha^{-1}) Fertiliser (160-40-160) (T4), Density (1.5m X 0.5m-13.4) Fertiliser (106.7-26.7-106.7) (T5), Density (1m X 1m- 10k ha^{-1}) No Fertiliser (0-0-0) (T6). Values are the means (n=3 to 4) (X) whisker indicates the range within a treatment, dot point are outlier.

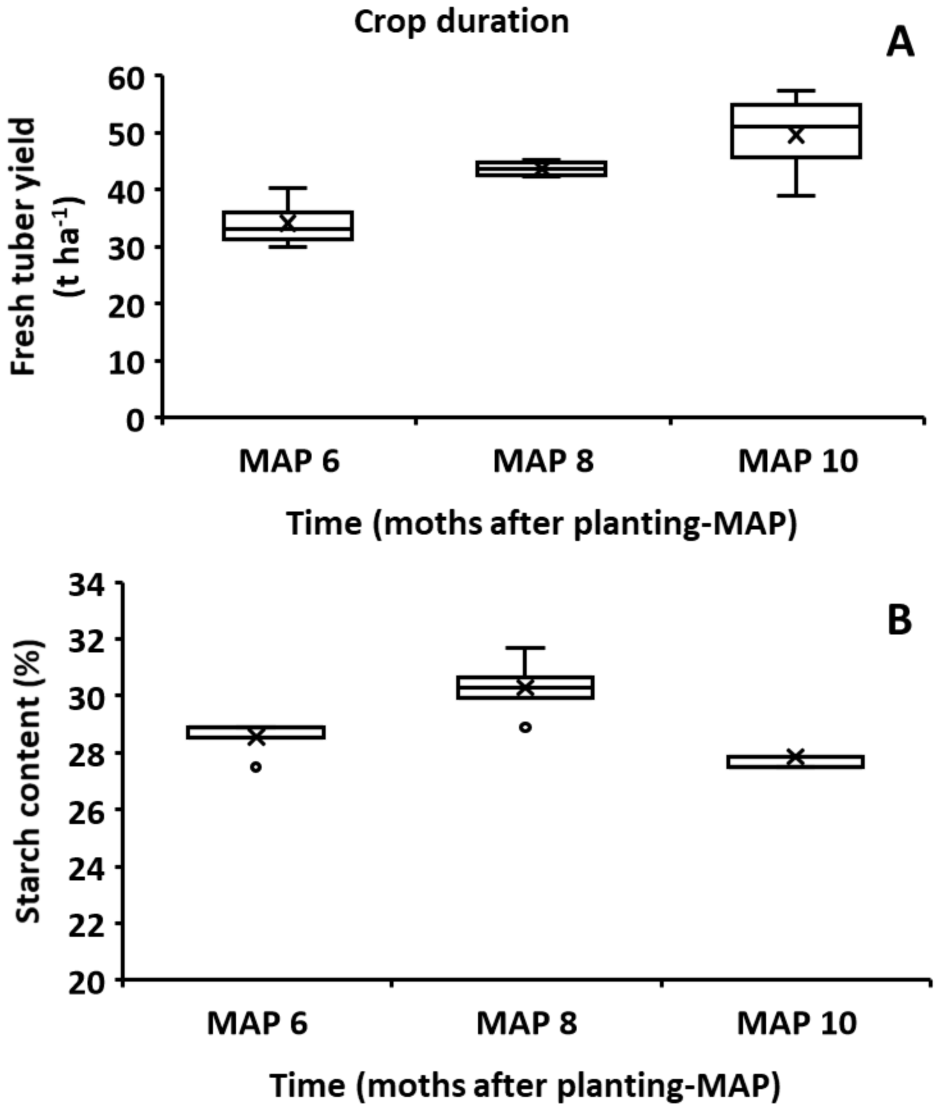


Figure 6: Fresh tuber yield (t ha^{-1}) (A) and Starch content (%) (B) of cassava plants after 6, 8 and 10 months of growth. Values are the means (n=4) (X) whisker indicates the range within a treatment.

Laos

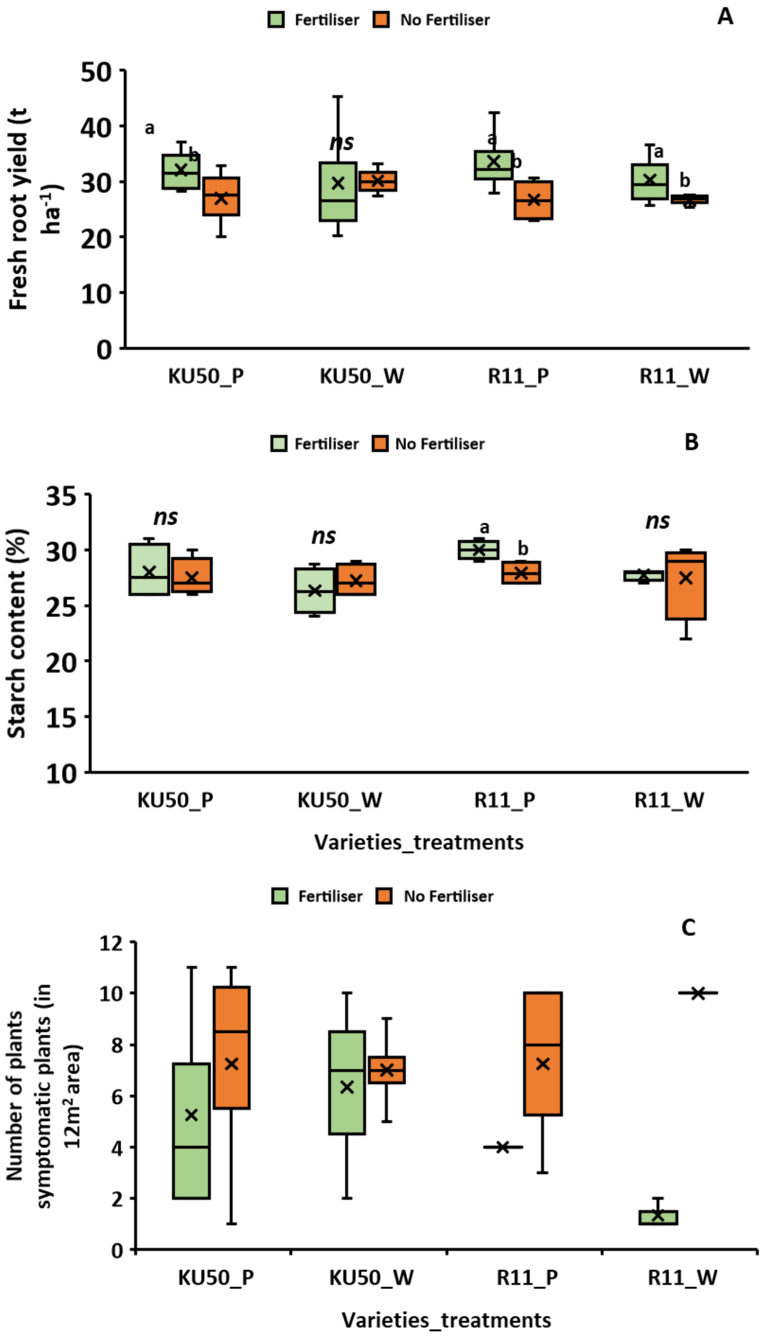
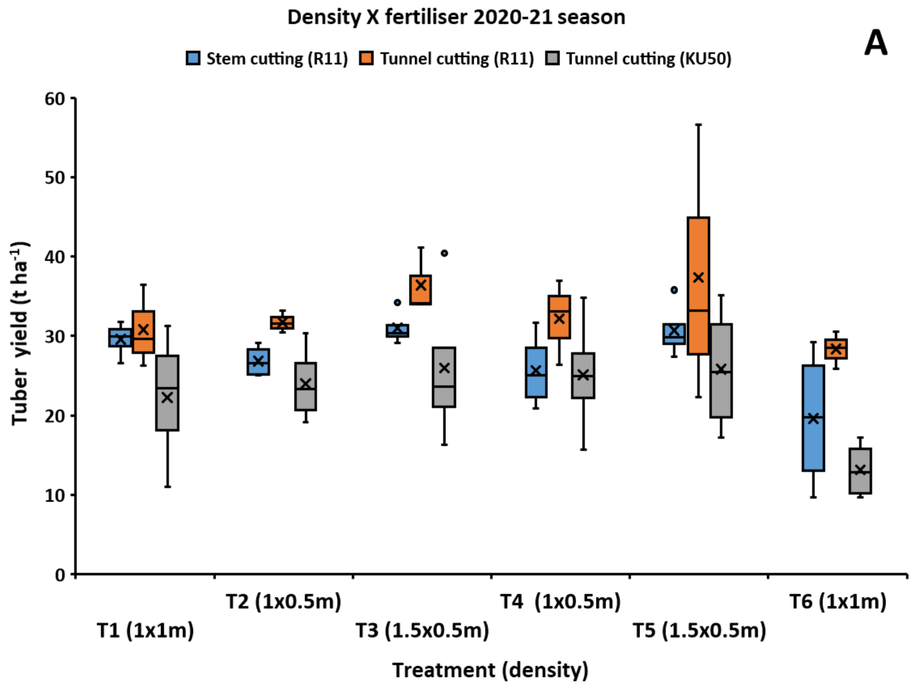


Figure 7: Fresh root yield (t ha-1) (A), Starch content (%) (B) and number of plants with symptoms at the end of the season (C). There were two varieties, KU50 and Rayong11, with two different kind of planting stakes from previous season, positive selected stems (P) and witches broom symptomatic stems (W) where plants received two fertiliser treatment, with fertiliser (green) and no fertiliser (orange). Values are the means (n=4) (X) whisker indicates the range within a treatment. Bars with different letters in a group are significantly different ($P < 0.05$).



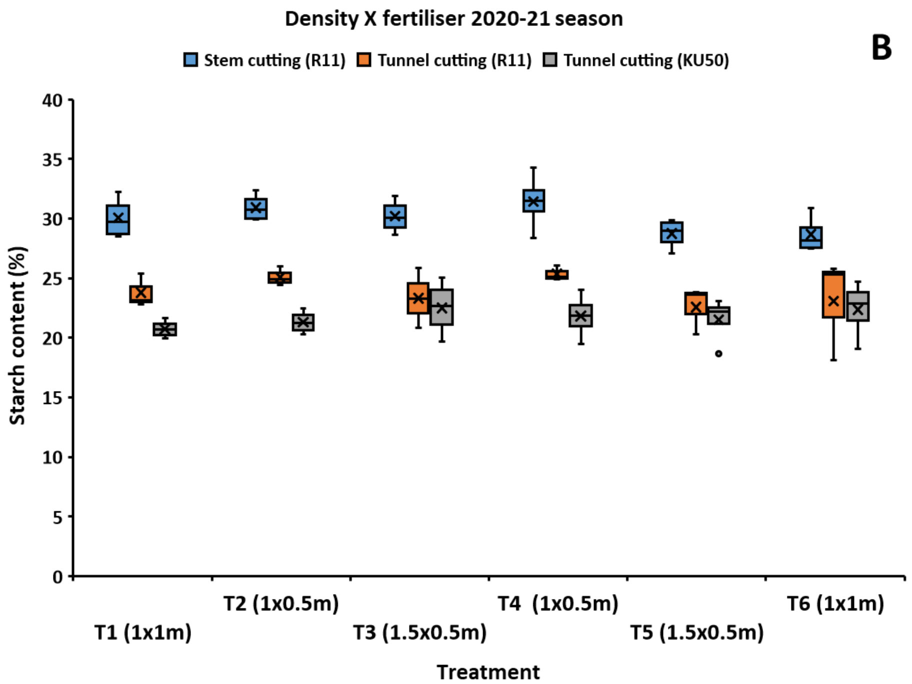


Figure 8: Fresh root yield (t ha^{-1}) (A) and Starch content (%) (B) of cassava roots where plants received six different fertiliser treatment NPK and planted in three different density. Density (1m X 1m- 10k ha^{-1}) Fertiliser (80-20-80) (T1), Density (1m X 0.5 m-20k ha^{-1}) Fertiliser (80-20-80) (T2), Density (1.5m X 0.5m-13.4K ha^{-1}) Fertiliser (80-20-80) (T3), Density (1m X 0.5m- 20k ha^{-1}) Fertiliser (160-40-160) (T4), Density (1.5m X 0.5m-13.4) Fertiliser (106.7-26.7-106.7) (T5), Density (1m X 1m- 10k ha^{-1}) No Fertiliser (0-0-0) (T6). There were three different plating material sources- Rayong11 from mature stems (Blue R11 stem), Rayong11 from tunnel grown seedlings (Orange, Tunnel cutting R11) and KU50 from tunnel grown seedlings (Grey, Tunnel cutting KU50). Values are the means (n=3 to 4) (X) whisker indicates the range within a treatment, dot point are outliers.

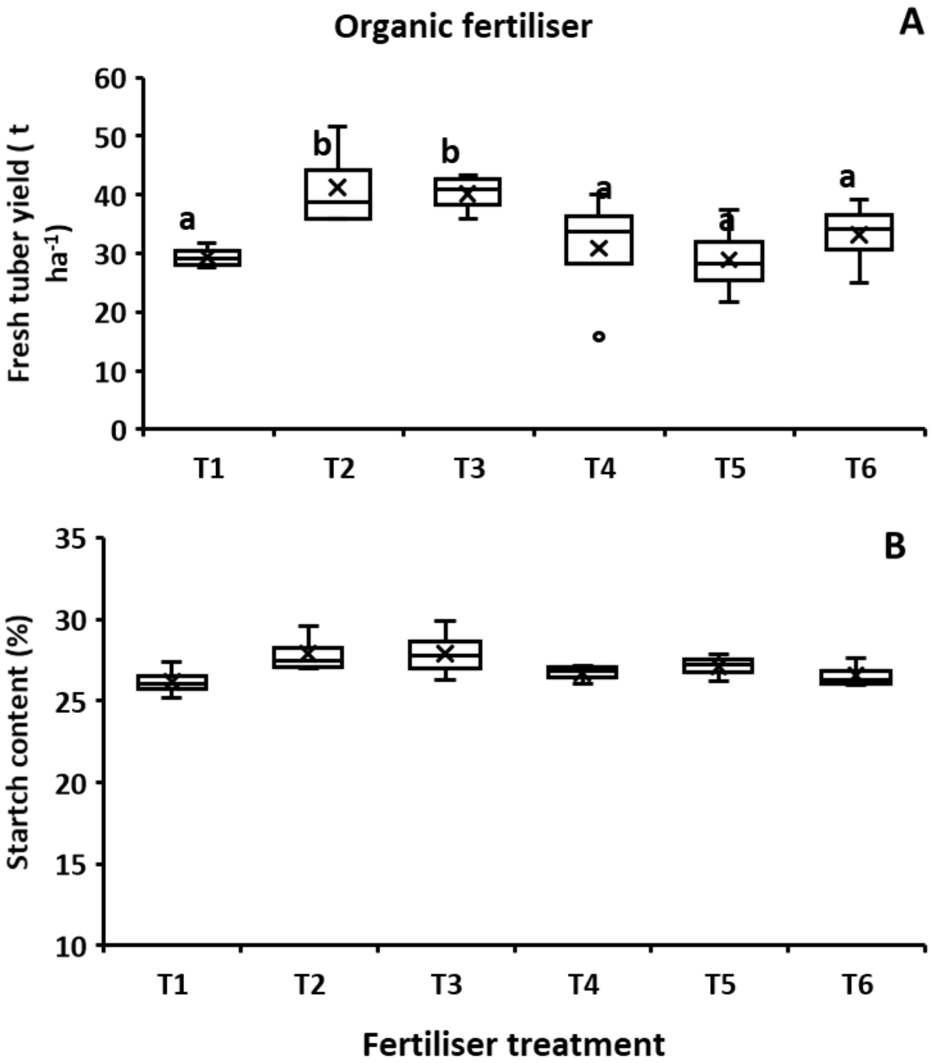


Figure 9: Fresh root yield (t ha^{-1}) (A) and Starch content (%) (B) of cassava roots where plants received different fertiliser treatment NPK and organic amendments- No fertiliser (T1), High rate fertiliser (80-20-80) (T2), low fertiliser rate (40-10-40) (T3), low fertiliser (40-10-40) rate + 1.5 t/ha of organic fertliser (T4), low organic fertiliser 1.5 t/ha (T5). Average yield (t ha^{-1}) indicated as (X) for T1- 29.4, T2-41.3, T3-40.2, T4-30.8, T5-29.0, T6-33.1. Values are the means (n=4) (X) whisker indicates the range within a treatment. Bars with different letters are significantly different (P < 0.05).

Table 1: Productivity of Tunnels. Approximately 35 plant (two long stem each plant) give about 700 and were horizontally planted on the sand bed cut into small pieces (two node cuttings). Viable sprouts are with 5 to 6 nodes, average height of KU50 sprouts were couple cm taller compared to Rayoung11. Multiplication rate from mother plants is 6-10x under traditional field multiplication. In tunnel multiplication it is 100-125x over the course of a season.

* Lost one batch to mealybugs, a= delayed by 7 day due to unavailability of substrate, b= delayed by 10 to 15 days due to delayed in irrigation system set up.

Variety	Number of seedlings per season per tunnel	No of viable sprout in each cutting	No of days to get new plantlets	No of days to transplant to field (from Tunnel)	Number of plants in the field	Transplantation field Success rate (%)
KU50	3840	768 ± 74	[a] 50 ± 4.6	[b] 96 ± 15	*2690	100
Rayong11	5040	840 ± 123	[a] 49 ± 3.0	[b] 95 ± 4	4210	100

Table 2: The tissue culture laboratory of Rice Research Centre (RRC), NAFRI, Vientiane, Laos and CARDI, Phnom Penh, Cambodia received five IITA varieties, TMEB419, IITA-TMS-IBA980581, IITA-TMS-IBA980505, IITA-TMS-IBA972205, IITA-TMS-IBA920057 as in vitro plantlet.

Germplasm ID	Number of plants transplanted to the field	
	Laos (RRC)	Cambodia (CARDI)
TMEB419 (HN1)	14	83
TMS-IBA920057 (HN2)	70	128
TMS-IBA972205 (HN3)	150	109
IITA-TMS-IBA980505 (HN4)	35	99
TMS-IBA980581(HN5)	31	121
KU50	240	

Vietnam

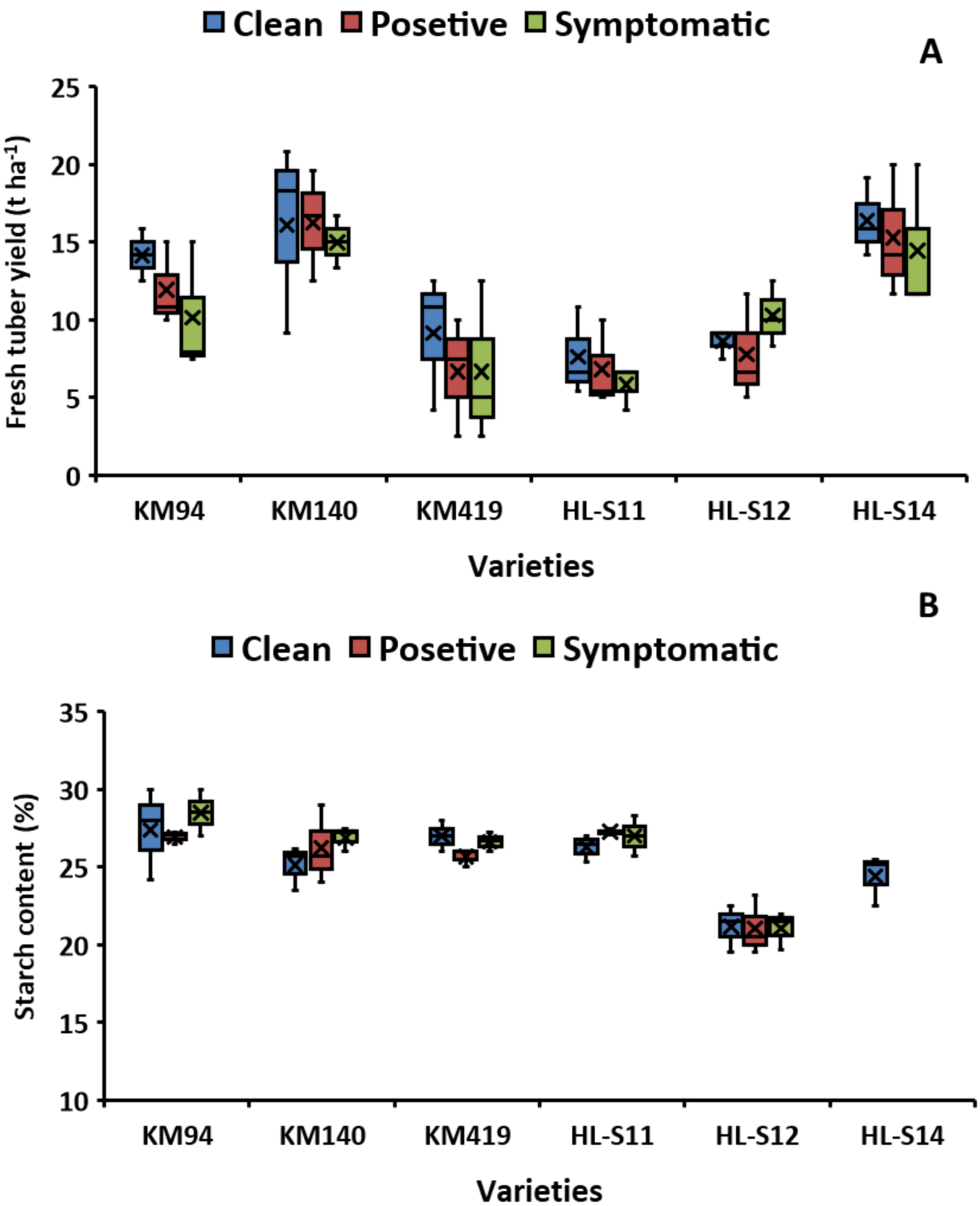


Figure 10: Fresh root yield (t ha^{-1}) (A) and Starch content (%) (B). There were six varieties, KM94 KM140 KM419 HL-S11 HL-S12 HL-S14 with three different kind of planting stakes; collected from disease free area (clean), collected from diseased area without any symptoms i.e. positive selected stems (positive) and symptomatic stems (Symptomatic) were planted. Values are the means (n=3) (X), whisker indicates the range within a treatment.

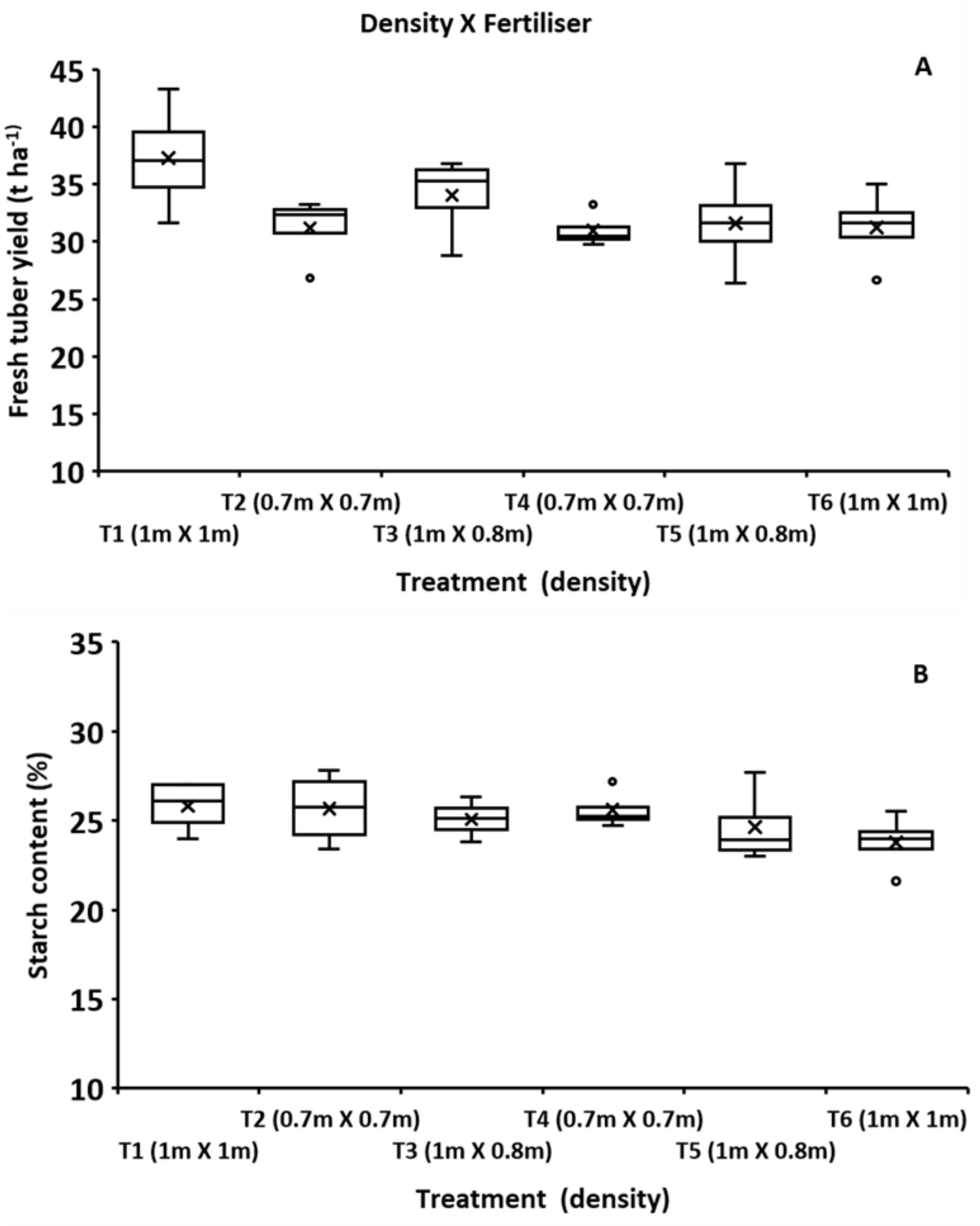


Figure 11: Fresh root yield (t ha^{-1}) (A) and Starch content (%) (B) of cassava roots where plants received six different fertiliser treatment NPK and planted in three different density. Density (1m X 1m- 10k ha^{-1}) Fertiliser (80-20-80) (T1), Density (0.7m X 0.7m-20.4k ha^{-1}) Fertiliser (80-20-80) (T2), Density (1m X 0.8m-12.5K ha^{-1}) Fertiliser (80-20-80) (T3), Density (0.7m X 0.7m- 20.4k ha^{-1}) Fertiliser (160-40-160) (T4), Density (1m X 0.8m-12.5K ha^{-1}) Fertiliser (100-25-100) (T5), Density (1m X 1m- 10k ha^{-1}) No Fertiliser (0-0-0) (T6). Values are the means (n= 3 to 4) (X) whisker indicates the range within a treatment, dot points are out layers.

10.7 Appendix 8 - Agronomy Protocol (2021-22)

Laos

1. Effects Planting Density on cassava yield and starch accumulation

Objective: To determine the optimum planting distance for seed production

Location: Naphok

Planting date: 14 May 2020

- Variety: Rayong 11
- Source: Positive selection from multiplication block 2019
- Design: Randomise block
- Replicate: 4

**Soil sampling: At the start of experiment, soil sample need to be collected from 5 spots of the field at 20 and 40 cm depth. Samples will be kept in sealed poly bags for analysis (i.e. ph, organic matter, NPK, (if possible Ca, Na, Mg, Zn)

Area:

- Plot: 5 x 6 m (spacing:1 x 1m), 5 x 6m(spacing:1 x 0.5m), and 5 x 7.5m(spacing:1.5 x 0.5m)
- Replication: 39 x 5 m =195m^2
- Experiment: 39 x 20 m = 780m^2

Spacing treatments: 6

A-1m x 1m = 10,000 plants/ha + 80N -20 P_2O_5 80 K_2O (in this treatment individual plants will receive for example 17.4g of urea, similarly for P_2O5 and K_2O)

B-1m x 0.5m = 20,000 plants/ha + 80N 20P_2O_5 80K_2O (in this treatment individual plants will receive half of amount compared to that of treatment A. urea 8.7g)

C-1.5m x 0.5m = 13,333 plants/ha + 80N 20P_2O_5 80K_2O (in this treatment individual plants will receive proportionately lower amount of fertilizer compared to treatment A. urea 13.4g)

D-1m x 0.5m = 20,000 plants/ha + 160N 40 P2O5 160 K2O (in this treatment individual plants will receive same amount of fertilizer as treatment A.)

E-1.5m x 0.5m = 13,333 plants/ha + 106.7N-26.7P2O5-106.7K2O (in this treatment individual plants will receive same amount of fertilizer as treatment A.)

F-1m x 1m = 10,000 plants/ha (No fertilizer application)

Fertiliser: Fertilizers will be applied at 1 month after planting after first weeding.

<u>Table of fertilizer to be applied per plant</u>

Density	**Spacing**	**Actual amount of Fertilizer to be added per plant (g)**				Recommendation/ha		
No. of plant	**m X m**	Treatment	Urea	P_2O_5(as TSP)	K2O(as KCl)	N	P_2O5	K_2O
10,000	1 X 1	A	17.4	4.35	13.33	80	20	80
20,000	1 X 0.5	B	8.7	2.37	6.66	80	20	80
13,333	1.5 X 0.5	C	13.4	3.26	10	80	20	80
20,000	1 X 0.5	D	17.4	4.35	13.33	160	40	160

13,333	1.5 X 0.5	E	17.4	4.35	13.33	106.7	26.7	106.7

Land preparation: Uniform with tractor

Planting method: Vertical 5-8 cm underground and buds facing up, replacing of missing hill at 2-3 week after planting

Stake length: 15-20 cm (5-7 buds)

Weed control: 4 times hand weeding at 1,2,3,4 months after planting when weed is small.

Harvest: At 9-10 months after planting, pull out the plants in effective plots, cut off roots and weigh fresh roots. Yield in t/ha = kg fresh roots x 10/no. plants harvested in effective plots. Measure starch content.

Stake requirements: 1120 stakes

2. The effects of water availability on cassava yield and starch accumulation.

Location: Naphok

Planting date: Three planting date 21 January, 23 March and 29 May 2020

- Variety : KU50 and Rayong 11
- Source: From ongoing experiment.
- Design : See below
- Replicate : 4

****Soil sampling**: At the start of experiment, soil sample need to be collected from 5 spots of the field at 20 and 40 cm depth. Samples will be kept in sealed poly bags for analysis (i.e. ph, organic matter, NPK, (if possible Ca, Na, Mg, Zn)

Area:

- Plot: length 44 m, plant spacing 0.8 X 0.8, 55 plants per row. 6 rows per treatment. There is a 4 m gap between each treatment and each replicate.

Fertiliser: Fertilizers will be applied at 1 month after planting.

Weeding: Plots will kept weed free, weeding will be carried out accordingly.

Irrigation: Irrigation need to apply as needed**.**

Data collection: Soil moisture data and precipitation data will be collected from the field with the nearby weather station and soil moisture meter.

Root, Stem and Leaves Biomass: The biomass (fresh weight) of the entire plant, roots, stem and leaves will be weighed immediately at the site of harvest with a weighing scale. During the growing period number of leaves and length of stem will be recorded at regular interval. Root starch content will be determined at each harvest.

3. Effect of CWBD on cassava root yield and starch content with different fertilizer treatment

Location: Naphok, at the old cassava garden where lot of cassava is currently growing with WB

Planting date: 29 May 2020

- Variety : KU50 and Rayong 11
- Source: Positive selection from ongoing experiment and infected planting materials are from the Cassava CWBD garden
- Design : Randomize block design
- Replicate : 3 but preferably 4

<u>Area:</u>

- Plot: plant spacing 1.0 X 1.0, 40m X 24 m

Treatment: With and without fertilizer.

Fertiliser: Fertilizers will be applied at 1 month after planting.

Weeding: Plots will kept weed free, weeding will be carried out accordingly.

Data collection:

1. Record infection rates in cassava plots after 30, 60 and 180 days after planting and at harvest.

2. Evaluate yield impacts between infected/symptomatic vs. non-infected cassava plants of multiple varieties (including fertilizer x disease trials)

Cambodia

1. Effects Planting Density on cassava yield and starch accumulation

Objective: To determine the optimum planting distance for seed production

Location: Chamkar Leu Up-land Farm (GDA station), Kampong Cham province

Planting date: 26 May 2020

- Variety : KU50 or Rayong 11
- Source: Positive selection from multiplication block 2019
- Design : Randomise block
- Replicate : 4

**Soil sampling: At the start of experiment, soil sample need to be collected from 5 spots of the field at 20 and 40 cm depth. Samples will be kept in sealed poly bags for analysis (i.e. ph, organic matter, NPK, (if possible Ca, Na, Mg, Zn)

Area:

- Plot: 5 x 6 m (spacing:1 x 1m) , 5 x 6m(spacing:1 x 0.5m), and 5 x 7.5m(spacing:1.5 x 0.5m)
- Replication: 39 x 5 m =195m^2
- Experiment: 39 x 20 m = 780m^2

Spacing treatments: 6

A-1m x 1m = 10,000 plants/ha + 80N -20 P2O5 80 K2O (in this treatment individual plants will receive for example 17.4g of urea, similarly for P2O5 and K2O)

B-1m x 0.5m = 20,000 plants/ha + 80N 20P2O5 80K2O (in this treatment individual plants will receive half of amount compared to that of treatment A. urea 8.7g)

C-1.5m x 0.5m = 13,333 plants/ha + 80N 20P2O5 80K2O (in this treatment individual plants will receive proportionately lower amount of fertilizer compared to treatment A. urea 13.4g)

D-1m x 0.5m = 20,000 plants/ha + 160N 40 P2O5 160 K2O (in this treatment individual plants will receive same amount of fertilizer as treatment A.)

E-1.5m x 0.5m = 13,333 plants/ha + 106.7N-26.7P2O5-106.7K2O (in this treatment individual plants will receive same amount of fertilizer as treatment A.)

F-1m x 1m = 10,000 plants/ha (No fertilizer application)

Fertiliser: Fertilizers will be applied at 1 month after planting after first weeding.

Table of fertilizer to be applied per plant

Density	**Spacing**	**Actual amount of Fertilizer to be added per plant (g)**				Recommendation/ha		
No. of plant	**m X m**	Treatment	Urea	P_2O_5(as TSP)	K2O(as KCl)	N	P_2O5	K_2O
10,000	1 X 1	A	17.4	4.35	13.33	80	20	80
20,000	1 X 0.5	B	8.7	2.37	6.66	80	20	80
13,333	1.5 X 0.5	C	13.4	3.26	10	80	20	80
20,000	1 X 0.5	D	17.4	4.35	13.33	160	40	160
13,333	1.5 X 0.5	E	17.4	4.35	13.33	106.7	26.7	106.7

Land preparation: Uniform with tractor

Planting method: Vertical 5-8 cm underground and buds facing up, replacing of missing hill at 2-3 week after planting

Stake length: 15-20 cm (5-7 buds)

Weed control: 4 times hand weeding at 1,2,3,4 months after planting when weed is small.

Harvest: At 9-10 months after planting, pull out the plants in effective plots, cut off roots and weigh fresh roots. Yield in t/ha = kg fresh roots x 10/no. plants harvested in effective plots. Measure starch content.

Stake requirements: 1120 stakes

2. Effect of harvesting date on cassava root yield and starch content

Objective: To determine the effect of time of planting and time of harvesting on the growth and yield of different cassava varieties and thus contribute to a better understanding of sustainable cassava production systems in specific agro-ecological areas.

Location: Chamkar Leu Up-land Farm (GDA station), Kampong Cham province

Planting date: 25 May 2020

- Variety: KU50
- Source : Positive selection from multiplication block in 2019
- Design: Split plot design
- Replicate: 4

Area:

- Plot: 5 x 6 m = 30m^2
- Replication: 18 x 5 m =90m^2
- Experiment: 18 x 20 m = 360m^2

Plant spacing: 1m x1m = 10,000 plants/ha

Harvest treatments:

A. Harvest end of November (6 months after planting)

B. Harvest end of January (8 months after planting)

C. Harvest end of March (10 months after planting)

Land preparation: Uniform with tractor

Fertiliser recommendation: 80N 20P_2O_5 80K_20 will be applied at 1 month after planting after first weeding

Variety: KU50

Planting method: Vertical 5-8 cm underground and buds facing up, replacing of missing hill at 2-3 week after planting

Stake length: 15-20 cm (5-7 buds)

Weed control: 4 times hand weeding at 1,2,3,4 months after planting when weed is small.

Harvest: At 9-10 months after planting, pull out the plants in effective plots, cut off roots and weigh fresh roots. Yield in t/ha = kg fresh roots x 10/no. plants harvested in effective plots and measure starch content for each plot.

Stake requirements: 360 stakes

3. Susceptibility of Cassava Varieties to Cassava Mosaic Disease Trial

Objective: To evaluate and compare yield penalty of cassava crop with infected planting material, positive selection materials and clean materials.

Location: Chamkar Leu upland farm, Kampong Cham province

Planting date: 24 May 2020

Variety:

Six common cassava varieties are used in the experiment.

Variety Name	Variety origin	Genetic background/ pedigree	Planting material origin
KU50	Thailand	R 1 x R 90	HuayBong Research Station, Thailand
Rayong 11	Thailand	R 5 x OMR 29-20-118	HuayBong Research Station, Thailand
SC8	China	CMR38-120-10	Chamkar Leu Station, Kampong Cham
HuayBong60	Thailand	R 5 x KU 50	Chamkar Leu Station, Kampong Cham
KM98-1	Vietnam	R 1x R 5	Chamkar Leu Station, Kampong Cham
Rayong 5	Thailand	27-77-10x R 3	Chamkar Leu Station, Kampong Cham

Experimental design and Treatments:

Design: Split-plot design with 4 replications (variety in main plots, type of planting materials in subplots).

Area: Plot: 15 x 6 m = 90m^2

Replication: 15 x 36 m =450m^2

Experiment: 60 x 36 m = 2160m^2

Effective plot size (subplot): 4 x 3m = 12m^2 = 12 plants

Planting distance: 1 m x 1 m

Planting method: Vertical: 8-10 cm underground with buds facing up.

Stake length: 15-20 cm

Stake requirements: 6 x 5 x 4 = 120 stakes/test type of stems

Weed control: 4 hand weeding, as necessary (i.e 4-5 weeks, 8-9 week, 12-13 weeks, 16-17 weeks after planting)

Fertilizer recommendation: N80 20P_2O_5 80K_2O will be applied at 1 month after planting after 1st weeding.

Harvest: at 10 months after planting

At harvest measure yield (t/ha) and starch content of roots in each treatment.

Yield: kg of fresh roots x 10/12m^2 = ……tons/ha

Vietnam

1. Effects Planting Density on cassava yield and starch accumulation

Objective: To determine the optimum planting distance for seed production

Location: Hong Loc, HLARC

Planting date: 28 April 2020

- Variety: VN150-442
- Source: Positive selection from multiplication block 2019
- Design: Randomise block
- Replicate: 4

**Soil sampling: At the start of experiment, soil sample need to be collected from 5 spots of the field at 20 and 40 cm depth. Samples will be kept in sealed poly bags for analysis (i.e. ph, organic matter, NPK, (if possible Ca, Na, Mg, Zn)

Area:

- Plot: 5 x 6 m (spacing:1 x 1m), 5 x 6 .3 m (spacing: 0.7 x 0.7 m), and 5 x 6.4 m (spacing: 1.0 x 0.8 m)
- Replication: 39 x 5 m =195m^2
- Experiment: 39 x 20 m = 780m^2

Spacing treatments: 6

A-1m x 1m = 10,000 plants/ha + 80N -20 P_2O_5 80 K2O (in this treatment individual plants will receive for example 17.4g of urea, similarly for P_2O_5 and K_2O)

B-0.7 m x 0.7m = 20,408 plants/ha + 80N 20P_2O_5 80K_2O (in this treatment individual plants will urea 8.52 g)

C-1.5m x 0.8 m = 12,500 plants/ha + 80N 20P_2O_5 80K_2O (in this treatment individual plants will urea 13.9g)

D-1m x 0.5m = 20,408 plants/ha + 160N 40 P_2O_5 160 K_2O (in this treatment individual plants will receive same amount of fertilizer as treatment A.)

E-1.5m x 0.5m = 13,333 plants/ha + 100N-25P_2O_5-100K_2O (in this treatment individual plants will receive same amount of fertilizer as treatment A.)

F-1m x 1m = 10,000 plants/ha (No fertilizer application)

Fertiliser: Fertilizers will be applied at 1 month after planting after first weeding.

<u>Table of fertilizer to be applied per plant</u>

Density	**Spacing**	**Actual amount of Fertilizer to be added per plant (g)**				Recommendation/ha		
No. of plant	**m X m**	Treatment	Urea	Superphosphate	K_2O (as KCl)	N	P_2O5	K_2O
10,000	1 X 1	A	17.4	12.5	13.33	80	20	80
20,408	0.7 X 0.7	B	8.52	6.13	6.53	80	20	80
12,500	1.0 X 0.8	C	13.91	10.0	10.67	80	20	80
20,408	0.7 X 0.7	D	17.04	12.25	13.07	160	40	160
12,500	1.0 X 0.8	E	17.40	12.50	13.33	100	25	100
10,000	1 X 1	F	0	0	0	0	0	0

Land preparation: Uniform with tractor

Planting method: Vertical 5-8 cm underground and buds facing up, replacing of missing hill at 2-3 week after planting

Stake length: 15-20 cm (5-7 buds)

Weed control: 4 times hand weeding at 1,2,3,4 months after planting when weed is small.

Harvest: At 9-10 months after planting, pull out the plants in effective plots, cut off roots and weigh fresh roots. Yield in t/ha = kg fresh roots x 10/no. plants harvested in effective plots. Measure starch content.

Stake requirements: 1120 stakes

2. The effects of water availability on cassava yield and starch accumulation.

Location: Hong Loc, HLARC

Planting date: Three planting date April, June, and August 2020

- Variety : KM94 and D7
- Source: From ongoing experiment.
- Design : See below
- Replicate : 4

****Soil sampling**: At the start of experiment, soil sample need to be collected from 5 spots of the field at 20 and 40 cm depth. Samples will be kept in sealed poly bags for analysis (i.e. ph, organic matter, NPK, (if possible Ca, Na, Mg, Zn)

Area:

- Plot: length 44 m, plant spacing 0.8 X 0.8, 55 plants per row. 6 rows per treatment. There is a 4 m gap between each treatment and each replicate.

Fertiliser: Fertilizers will be applied at 1 month after planting.

Weeding: Plots will kept weed free, weeding will be carried out accordingly.

Irrigation: Irrigation need to apply as needed**.**

Data collection: Soil moisture data and precipitation data will be collected from the field with the nearby weather station and soil moisture meter.

Root, Stem and Leaves Biomass: The biomass (fresh weight) of the entire plant, roots, stem and leaves will be weighed immediately at the site of harvest with a weighing scale. During the growing period number of leaves and length of stem will be recorded at regular interval. Root starch content will be determined at each harvest.

3. Susceptibility of Cassava Varieties to Cassava Mosaic Disease Trial

Objective: To evaluate and compare yield penalty of cassava crop with infected planting material, positive selection materials and clean materials.

Location: Tay Ninh, Tan Chua discrict

Planting date: 18 May 2020

Variety: Six varieties will be included-KM94, KM140, KM419, HLS11, HL 14 and HL 12.

Experimental design and Treatments:

Design: Split-plot design with 4 replications (variety in main plots, type of planting materials in subplots).

Area: Plot: 15 x 6 m = 90m^2

Replication: 15 x 36 m =450m^2

Experiment: 60 x 36 m = 2160m^2

Effective plot size (subplot): 4 x 3m = 12m^2 = 12 plants

Planting distance: 1 m x 1 m

Planting method: Vertical: 8-10 cm underground with buds facing up.

Stake length: 15-20 cm

Stake requirements: 6 x 5 x 4 = 120 stakes/test type of stems

Weed control: 4 hand weeding, as necessary (i.e 4-5 weeks, 8-9 week, 12-13 weeks, 16-17 weeks after planting)

Data collection:

1. Record infection rates in cassava plots after 30, 60, 90 and 270 days after planting.

2. Evaluate yield impacts between infected/symptomatic vs. non-infected cassava plants of multiple varieties.

4. Evaluate the performance of imported CMD resistant varieties from Africa and India against 'clean' elite existing varieties in Asia

A total of 42 cassava varieties (i.e. 38 CIAT and 4 IITA) were in *invitro* culture at AGI and transferred to Hung Loc to evaluate varieties. These materials were planted on 2 Jun 2020 at HLARC.

10.8 Appendix 9 – Photos of rapid multiplication systems in Laos, Cambodia and Vietnam

Photos 1 – FutureStems: NAFRI Maize and Cash Crop Research Center

Photo 2 - Construction of 10 tunnels in partnership with Khonsup Import-Export Company, Champasak Province

Photo 3A -Tunnels at Chamkar Leu Research Station (GDA) in Kampong Cham, Cambodia; 3B) Tunnels at HLARC in DongNai southern Vietnam.

Photos 4 - Rapid multiplication system at CARDI, Phnom Penh, Cambodia