Sampling strategy

Dharani Dhar Burra
Statistician and Data manager
CIAT Asia team
Hanoi

- District level sampling
- Within plot sampling for virus detection
- Within plot sampling for adult whiteflies and nymphal counts

District level sampling

- 15 largest cassava producing districts in both the countries (For Vietnam – production area - 2013 data*, for Cambodia – production density - 2011 data**)

- 15 sampling points in each district – Sampling points should be equidistantly placed along the main motorable road (Sseruwagi *et al.*, 2004)

- **Extra** : 15 sampling points in Koun Mom district in Cambodia (First report of the disease; Wang et al, 2016)

*** In-house census data**

**** From the FAO/CelAgrid report titled "National Assessment of Cambodia's main crop and fodder resources", February 2012**

Sampling points

Country	Province	District
Vietnam	Gia Lai	Huyện Chư Prông
Vietnam	Tây Ninh	Huyện Dương Minh Châu
Vietnam	Kon Tum	Huyện Đắk Tô
Vietnam	Đăk Nông	Huyện Đăk G'long
Vietnam	Bình Thuận	Huyện Bắc Bình
Vietnam	Đồng Nai	Huyện Xuân Lộc
Vietnam	Sơn La	Thuận Châu
Vietnam	Kon Tum	Huyện Sa Thầy
Vietnam	Yên Bái	Huyện Văn Yên
Vietnam	Gia Lai	Huyện Krông Pa
Vietnam	Phú Yên	Huyện Sông Hinh
Vietnam	Tây Ninh	Huyện Châu Thành
Vietnam	Bình Thuận	Huyện Hàm Tân
Vietnam	Tây Ninh	Huyện Tân Biên
Vietnam	Tây Ninh	Huyện Tân Châu
Cambodia		Kampong Cham
Cambodia	Battambang	Sampov Luon
Cambodia		Kracheh
Cambodia	Pailin	Sala Krau
Cambodia	Tboung Khmum	Dambae
Cambodia	Banteay Meanchey	Phnum Proek
Cambodia		Svay Rieng
Cambodia		Pailin
Cambodia	Banteay Meanchey	Malai
Cambodia		Steung Treng
Cambodia	Battambang	Kamrieng
Cambodia	Svay Rieng	Bavet
Cambodia	Tboung Khmum	Memot
Cambodia	Svay Rieng	Chantrea
Cambodia	Svay Rieng	Svay Chrum

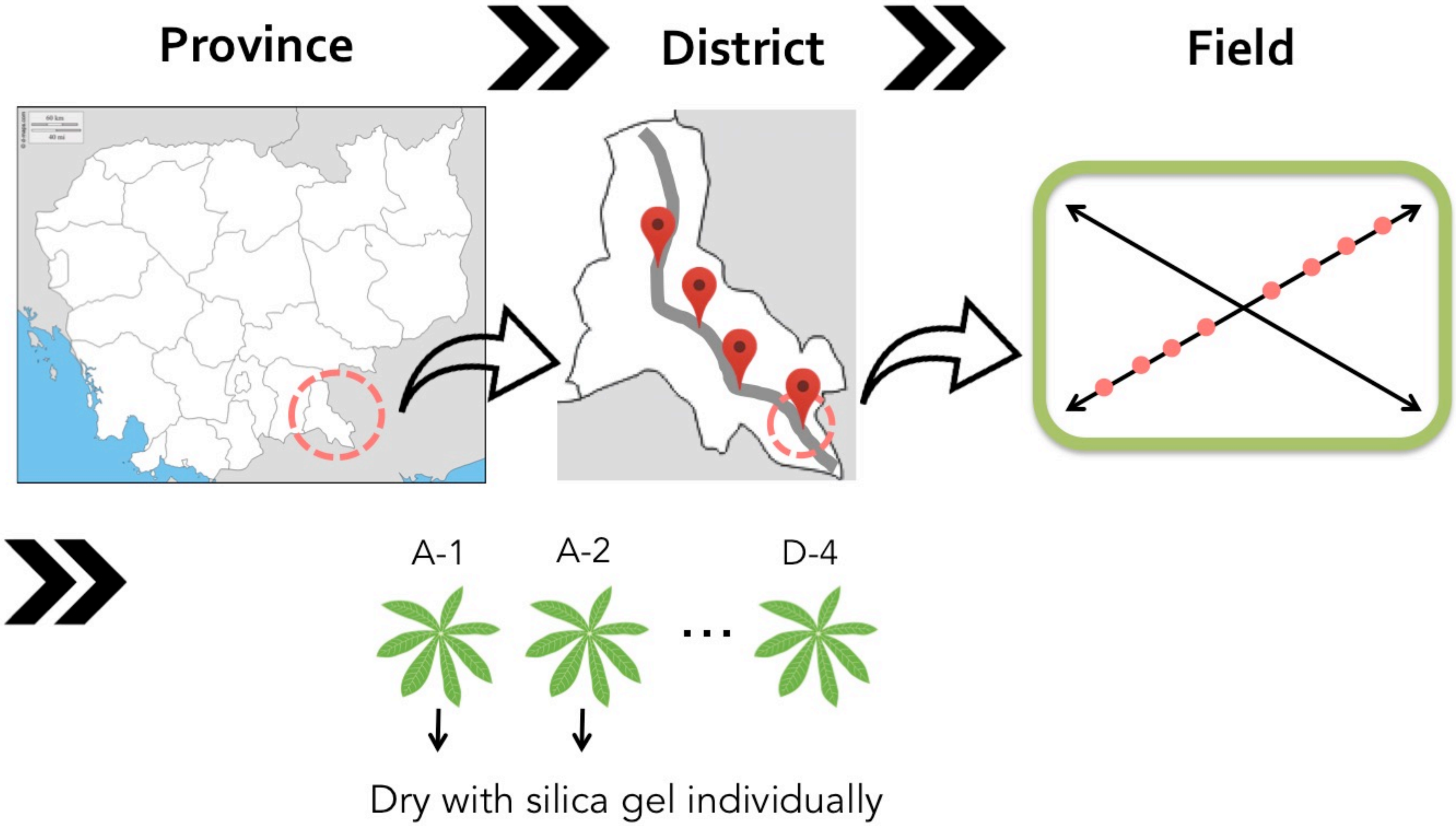
Within plot sampling

- An X transect will be identified across each plot. Mark the GPS coordinates
- On each transect, 8 equidistantly placed plants will be selected for sampling. **16 plants/plot**
- Top leaf from each plant will be sampled, marked, photographed and stored individually on silica gel. **16 leaf samples/plot** (see sampling document for details)

Within plot sampling – Virus detection

- Each leaf sample will be powdered individually, and DNA will be extracted from each sample
- Before grinding, care has to be taken to remove adult whiteflies (if any) on the sample
- Equal volumes of DNA extracted from plants adjacently placed along the transect will be pooled, and will be used as a template to test both universal and specific PCR primers.
- If the pooled sample tests positive with either of the primers, then both primers will be tested on individual samples that make up the pooled sample.
- **Positive control**: DNA from SLCMV infected plant (from Sri Lanka)/ SLCMV clone pSL7 (from Japan)

Overview of material sampling



Province

District

Field

A-1

A-2

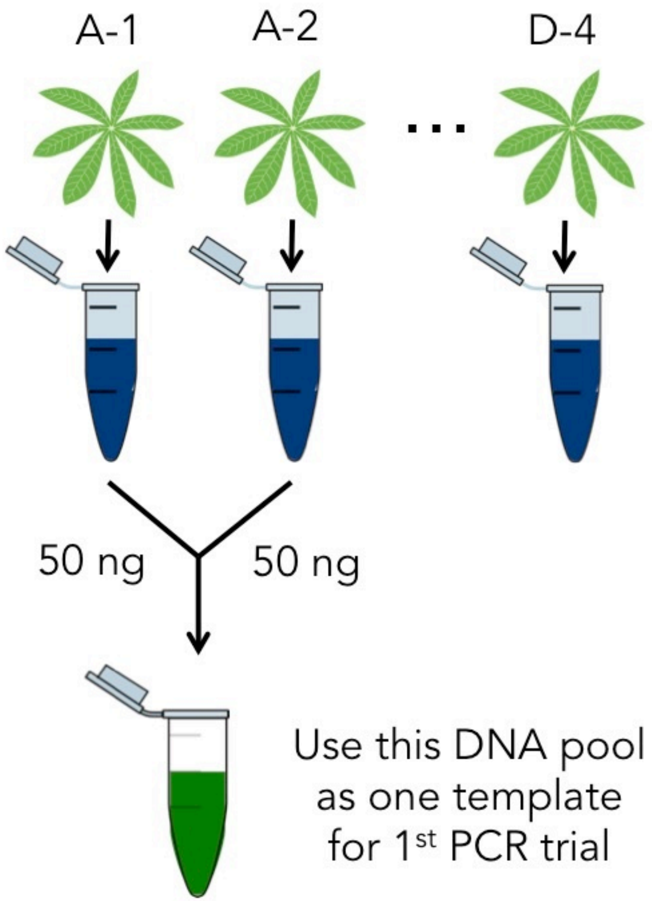
…

D-4

Dry with silica gel individually

Overview of virus detection

DNA extraction



Use this DNA pool
as one template
for 1st PCR trial

PCR

1st PCR trial

☐ Primer sets: 1) Universal primers on Geminivirus**
2) SLCMV-specific primers

☐ Templates : DNA pools

Make the cost half

If we get any positives

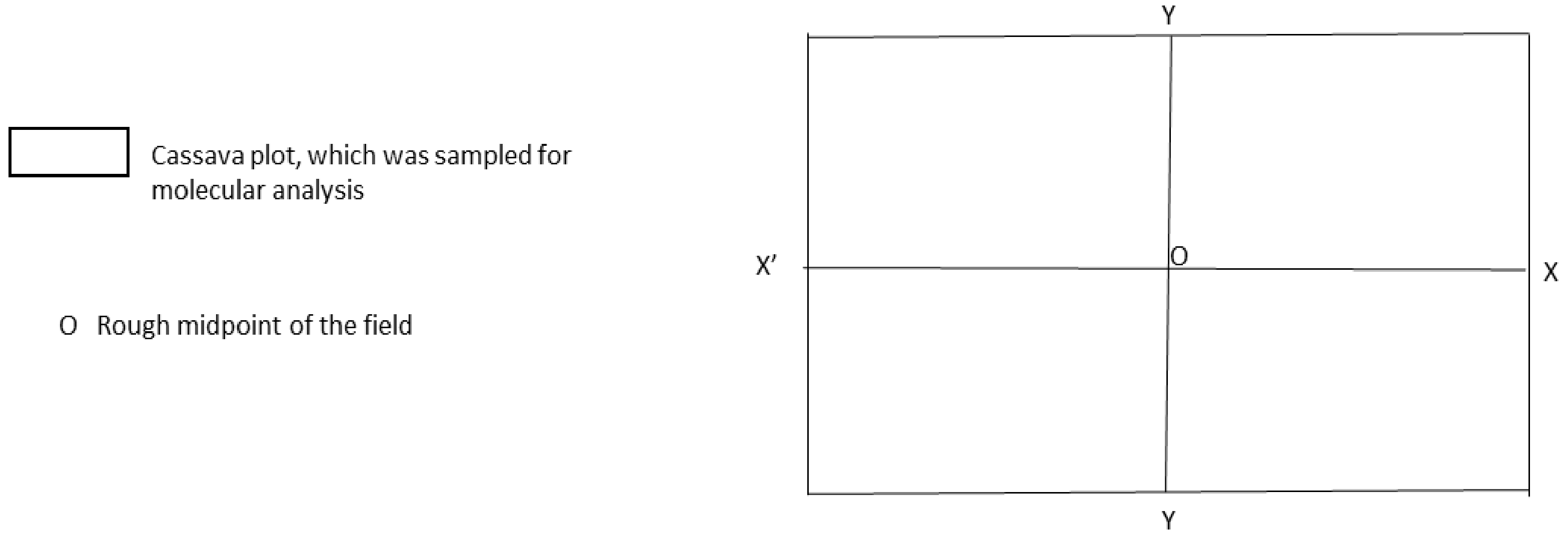
2nd PCR trial

☐ Primer set : SLCMV-specific primers

☐ Templates : individual extracted DNAs

**SLCMV belongs to family *Geminiviridae*, genus *Begomovirus*.

Within plot sampling – Excess sampling



- 8 equidistantly placed plants on Y-Y transect and 8 equidistantly placed plants on X-X' transect. **16 plants per plot** (see sampling document for further details)

Sampling for whitefly

- Will be performed on plots chosen for molecular sampling

- Specifically, every 3rd plot used for molecular analysis sampling will be selected. **5 plots/district**

- Along the transect used for the molecular analysis, 10 consecutive plants placed on either end of each transect will be chosen. **20 plants/plot**

- On each plant, 5th and 10th leaf from the top will be checked for the presence of nymphs

- The numbers will be roughly estimated and each leaf on each plant will be classified into either of the 5 groups, based on nymph counts.

- In the coding sheet, mention field ID, transect number, plant number (between 1 to 5), leaf number (either 5th or 10th) and the number of nymphs counted on the leaf

- 10 adults will be collected on each end of the transect using **aspirators. 20 adults/plot**

- Adults have to be stored in **epitubes, with 95% ethanol** – each epitube is labelled with the field ID and the transect number (1-4 for 2 ends of 2 transects in each plot).