

## Standard Progress Report: 2<sup>nd</sup> QTR-2012

### Ministry of Agriculture and Forestry

#### Training on Disease-Free Citrus Nursery Management and Mother Tree Propagation

26th April to 3rd May 2012, Bangkok, THAILAND

##### Objectives

1. To study and acquaint with diseases diagnostic techniques for maintenance of disease free nursery stocks for mass propagation
2. To register the local cultivars of citrus and to establish national citrus repository (NCR) in the country

##### Material and Methods

The training was organized by National Citrus Program in collaboration with the Plant Pathology and Research group under Plant Protection Research and Development Office, Department of Agriculture, Department of agriculture, Thailand for 10 days. The training program comprised of theory, practical and field training. The laboratory session included PCR Detection of HLB. Enzyme-Linked Immunosorbent Essay (ELISA) test CTV, Iodine tests, Strip tests, Shoot tip grafting.

##### Polymerized Chain Reaction (PCR) detection of HLB

PCR involves two steps: Leaf sample were collected based on visual observation of symptoms expression for different diseases. The yellowing and mottled young leaves' mid ribs were used for DNA extraction as per the protocol of Doyle and Doyle 1990 with slight modification.

##### ELISA for CTV Detection by DAS-ELISA with Polyclonal antibody

ELISA technique was used for detection of CTV. The symptomatic CTV leaves (vein corking & leaf cupping) were collected and brought to the laboratory. The leaves' midribs were grounded in Extraction Buffer with mortar. The protocol given in the ELISA kits were followed for detection.

##### Iodine Tests for HLB

Recent anatomical studies have shown that there is an increase of starch in HLB infected leaves by six folds over healthy leaves (Etxeberria et al., 2011). Therefore, when iodine reacts with starch, it produces a dark grey to blue color. This technique was used in identification of citrus HLB and it was found 90% in congruent with PCR tests. The technique is ideal for our condition as it is not only cost effective but also relatively easy with considerable precision. We have used the iodine kits for detection of HLB.

### **Strip Tests for CTV**

Strip test is also an important tool for detection of CTV in citrus. The CTV suspected citrus leaves were chopped and grounded with pestle in extraction buffer and then transferred to Eppendorf tube. The strip is then immersed in the Eppendorf tube containing grounded samples in buffer.

### **Shoot Tip Grafting (STG)**

STG is micro grafting of shoot tips for obtaining virus free citrus foundation stocks. This is based on the fact that meristem of infected auxiliary bud is generally free from viruses. Thus seedlings obtained through this method are reliably free and used to recover pathogen free saplings from infected parental sources.

### **Field practical sessions**

1. Demonstration of media preparation & composting
2. Practical training on budding and disease indexing
3. Visit to citrus nursery and certification process
4. Sample collection from farmers fields

### **Practical training on budding and disease indexing**

Budding is the most common method of propagation used in citrus. The training team was also demonstrated with budding. Budding is also useful in disease indexing. Many indicator plants have been identified for HLB. The table below shows the indicator plants of HLB for different citrus groups.

### **Visit to citrus nursery and certification process**

The training team was taken to the citrus repository at the Bangkok Campus. The repository consisted of 32 trees (2 trees/cultivars) which are maintained in foundation block after fully indexing before registration. About 800 mother trees registered and certified mother trees are maintained in a mother block. From this registered and certified mother trees block, the plant materials are supplied to approved citrus nurseries (government and private).

### **Sample collection from farmers' field**

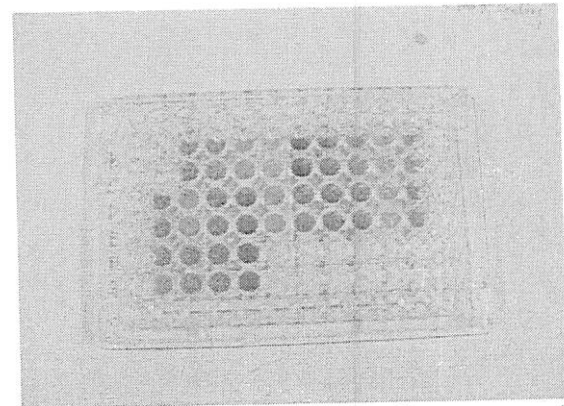
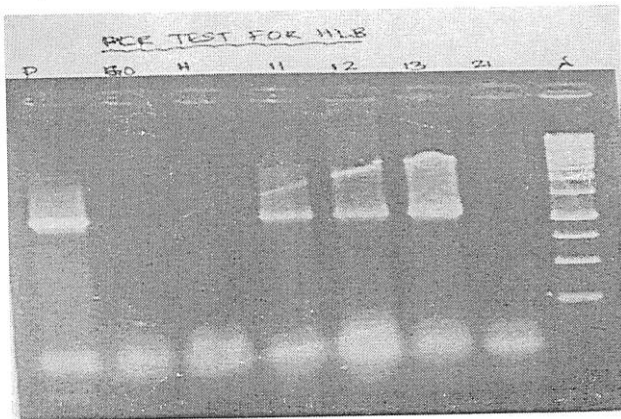
Thailand is one of the countries hard hit by the greening disease. The training team visited the citrus orchard in Petchburi province where the citrus cultivation has been badly affected. Currently, the lime varieties being susceptible to HLB have huge demand in the market and the lime variety is still grown in this province which was visited by the team. The intercropping with banana and other fruits species was a common practice being adopted by the farmers in that province. Main purpose is crop diversification and minimization of risk.

## Way Forward / Recommendations

1. There is immediate need for the establishment of national citrus repository with fully equipped molecular and phytosanitary laboratory.
2. Registration of all the citrus varieties existing within the country.
3. Rescuing of cultivars from HLB using shoot tip grafting technique.
4. The training team found necessary to thoroughly inspect citrus nurseries (both private and government) and put in place a strong procedure for certification.
5. There is need for additional infrastructures (internal irrigation systems) and few more green houses.
6. The need was also found to improve the current green house and peripheral constructions of ant-drain double door dark room and a water tape with constant water source.
7. There is also a need for permanent deployment of 3 nursery men, 2 field supervisor and a laboratory technician.
8. Introduction of indicator plants (Madam vinous and acid lime)

## Conclusion

The citrus is one of the important crops and it is threatened worldwide by a disease called Citrus HLB. It is imperative to conserve and rescue the rich genetic resource of our local citrus varieties beside up scaling citrus yield and production. The conservation can be achieved only through maintenance of these varieties in the repository and availing a clean material for commercial plantation. Thus, registration and certification of citrus trees is must. The certification process requires assurance for materials to be free from pathogens. The diagnostic technique includes PCR



test for HLB, ELISA for CTV, Iodine test and

indexing for HLB using indicator plants.

PCR Results obtained for the 5 unknown samples      ELISA Reaction results obtained from different unknown samples



Results obtained: Double line (positive) & single line (Negative) for CTV. Indexing with indicator plants

## **Awareness workshop / training on Farmers Group and Cooperatives for RNR Extension officers of Bumthang, Trongsa & Zhemgang Dzongkhag**

### **I. Awareness program for RNR Extension**

A day long awareness program on Farmers Group and Cooperatives was conducted for RNR Extension Agents of Bumthang, Trongsa & Zhemgang Dzongkhag on 18<sup>th</sup>, 20<sup>th</sup> & 22<sup>nd</sup> June, 2012 respectively. This kind of program is conducted to implement the Capacity Building Masterplan developed for the farmers and Cooperatives. The resources persons were Mr. Yonten Gyamtsho, CMO, DAMC and Mr. Tenzin, CDD, DAMC .

### **II. Objective**

Bhutan is just entering the cooperate world on the recognition of its importance at the government level but most of the population at large are not aware of its importance. Keeping this in mind, the Department of Agricultural Marketing and Cooperatives developed a capacity building master plan. DAMC is dedicated towards providing awareness and capacity building training nationwide. Since, the Gewog RNR extension agents are the real promoter of Farmers group and Cooperatives at the grass root level, it was found important to make those promoters aware and give a better understanding on the concept of Farmers Group and Cooperatives.

### **III. Awareness program at Bumthang**

On 18<sup>th</sup> June, 2012, the awareness program on Farmers Group and Cooperatives was imparted to 17 RNR Gewog Extension Agents in Bumthang Dzongkhag. The following are the list of participants form different RNR sectors that participated in the program.

1. Agriculture	6
2. Livestock	6
3. Forest	5

During the program, the following important topics were covered/imparted to the participants:

- A. Generalities of Farmers' Groups and Cooperatives
- B. Group Formation and Development Strategy
- C. Defining By-Laws and Constitutions
- D. Act and Regulations
- E. Benefits of Registration
- F. Linkages and Roles of Stakeholders
- G. Registration Procedure

And the following mode of training were used to impart the program

1. Chart
2. Power point
3. White board
4. Group works and

5. discussions

#### **IV. Awareness program at Trongsa**

On 20<sup>th</sup> June, 2012, the awareness program on Farmers Group and Cooperatives was imparted to 20 RNR Gewog Extension Agents in Trongsa Dzongkhag. The following are the list of participants from different RNR sectors that participated in the program.

- |                |   |
|----------------|---|
| 1. Agriculture | 7 |
| 2. Livestock   | 5 |
| 3. Forest      | 6 |

Besides the Gewog RNR sector, the following sector heads also attended the program

1. Dzongkhag Agriculture Officer
2. Dzongkhag Forest Officer

During the program, the following important topics were covered/imparted to the participants:

- H. Generalities of Farmers' Groups and Cooperatives
- I. Group Formation and Development Strategy
- J. Defining By-Laws and Constitutions
- K. Act and Regulations
- L. Benefits of Registration
- M. Linkages and Roles of Stakeholders
- N. Registration Procedure

And the following mode of training were used to impart the program

1. Chart
2. Power point
3. White board
4. Group works and
5. discussions

#### **V. Awareness program at Trongsa**

On 22<sup>nd</sup> June, 2012, the awareness program on Farmers Group and Cooperatives was imparted to 22 RNR Gewog Extension Agents in Zhemgang Dzongkhag. The following are the list of participants from different RNR sectors that participated in the program.

- |                |    |
|----------------|----|
| 1. Agriculture | 4  |
| 2. Livestock   | 10 |
| 3. Forest      | 8  |

During the program, the following important topics were covered/imparted to the participants:

1. Generalities of Farmers' Groups and Cooperatives
2. Group Formation and Development Strategy
3. Defining By-Laws and Constitutions
4. Act and Regulations
5. Benefits of Registration
6. Linkages and Roles of Stakeholders
7. Registration Procedure

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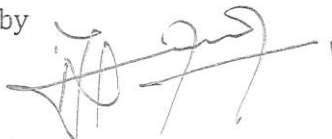
## **VI. Expected Outcome**

With such awareness program imparted, the Gewog RNR extensions are expected to form a quality Farmers group and Cooperatives in line with the Cooperative (Amendment) Act of Bhutan, 2009 and Cooperative Rules and Regulations, 2010. Besides, it is also expected to create awareness on Farmers Group and Cooperatives to farmers in their respective Gewogs. In turn, it is expected that many farming communities unite and form Farmers Group and Cooperatives which will realize the MOAF's objective of commercializing the farming activities.

## **VII. Conclusion**

With the registration of 105 Farmers Group and Cooperatives 16 Cooperatives under the Cooperative (Amendment) Act of Bhutan, 2009 the DAMC hopes to register more Farmers Group and Cooperatives by imparting such awareness program.

Submitted by



**Implementing partner**

