PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Improvement of basic seed potato production in Myanmar

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1. Introduction

1.1 Avant propos

Myanmar grows about 600,000 ton potato on 40,000 ha (yield 15 ton/ha, consumption 10 kg per capita. Production and consumption grow rapidly. For growers it is a real cash crop in which they invest seed, labour, land and chemicals. In 'winter' there is an irrigated valley crop, in the hills there is a minor early monsoon and a major late monsoon crop. There is no official seed system. Farmers keep some late monsoon crop yield for the early monsoon crop. All seed for the winter and late monsoon crop is bought from traders that buy all ware crops, grade it, sell the large ones immediately and sell the small ones after a few months when they sprout. Growers cannot keep the seed for 8 months at ambient temperatures hence the need to buy only 4 months old seed from traders. Disadvantages: varietal mixture, uncertain health. Diseases (late blight fungus attacks the leaves and brown rot bacteria the tubers) are the main problems with potato in Myanmar.

The CDN potato project aims at the introduction of healthy brown rot free seed of late blight resistant varieties. The approach and results are shown in this report. The numerical examples are mainly meant to illustrate the potential of the technique once perfected and carried out on routine base: producing plantlets in-vitro -> mini-tubers from these n a screen house in sterile soil -> G1 in first field generation and so on. Due to two crucial hygiene errors (mini-tubers not produced in sterile soil and seed cut without disinfecting the knives) seed of a late blight resistant variety (L121) distributed to growers in 2013 was heavily infected with brown rot. This has set back this part of the program. Newly adopted protocols within the framework of the 2014 project with Wageningen UR are aimed at overcoming this. The program now (late 2014) is well on its way with L11 cleaned and being built up, with more varieties in the pipeline, seed stores constructed and procedures, protocols in place. Continuation will assure impact on potato production provided seed production is taken over by specialized growers/entrepreneurs and disentanglement of seed from ware potato trade. This merits a specific future project that meanwhile assures a subtle landing of the current one.

1.2 Project rationale and organization

The CDN seed potato project at Heho (Shan state) was visited by Wageningen researchers and technician in November 2013, April and September/2014. The objectives were:

1. To analyze the potato cropping system and find solutions for problems encountered in the basic seed production program.
2. To assist the Myanmar CDN staff to restart the rapid in-vitro multiplication of three potato varieties at the Agricultural Research Station in Heho.
3. To create awareness of the necessary security of healthy mini-tuber production through sterilization of the soil (steaming) in the mini-tuber production facility at the research station.
4. To guide the initiation, construction and proper use of:
   a. Insect proof screen house,
   b. Soil sterilizing equipment for steaming,
   c. Tables containing sterilized (steamed) soil to raise mini-tubers as the best starting material for larger scale seed potato production.
5. To introduce testing procedures of improved seed material in farmers' fields to compare it's performance against traditional seed.

Wageningen UR staff involved:
- Dr. Anton Haverkort, Plant Research International, Agrosystems Research (2 missions of 1 week each, Nov 2013, Spring 2014),
- Maarten Holdinga, Plant Research International, BioInteractions, (2 missions of four weeks each),
- Romke Wustman (MSc.), Applied Plant Research (1 mission one week),
- Dr. Annette Pronk, Plant Research International, Agrosystems Research (project manager).

Myanmar staff involved:
- Saw Eh Law Shaw of the Consortium of Dutch NCO's (project leader),
- Maung Maung Htun of the Consortium of Dutch NCO's (project manager),
- Saw Eddie of the Consortium of Dutch NCO's (agriculture facilitator),
- Saw Nycin Htun Farm Manager of Agricultural Research Station at Heho (Shan State),
- Daw Aye cho cho Soe tissue lab facilitator, Agricultural Research Station at Heho.

1.3 Description of seed potato system in Myanmar (November 2013)

Informal system
- There is no seed potato industry in Myanmar, small tubers are traded as seed.
- Farmers sell small tubers at harvest in October – November for 350 Kyats/viss (1 viss ≈ 1.63 kg) where bigger tubers are sold for 550 Ks/viss. In January, the small sprouted "seeds" are then sold for 600 Ks/viss and are often more expensive than ware potatoes.
- The multiplication rate of seed potatoes of small upland growers at Heho (rule of thumb): you plant 1 viss and harvest 5 viss (= multiplication rate of 5, note in Netherlands this is 20).
- General practice for seed potatoes:
  Seed for the early monsoon crop is purchased from Wholesale at Aungban (≈ 600 Ks/viss);
  Seed for the late monsoon crop is kept from their own paddy fields which were harvested in April;
  Seed for the January planting is saved from the early monsoon August harvest or purchased from higher upland production locations (NonTaye in case of Heho).

Quality issues
- Bacterial wilt (BW, caused by bacteria Ralstonia solanacearum).
- Root knot nematode.
- Virus degeneration was not mentioned, probably not know/recognized as problem.
- When bought quality and origin not sure.
- Old and young seed often mixed.
- Mixture of varieties Up-to-Date and Kufri Joti.

Figure 1 *An obvious example of blemish of seed tuber: root knot nematode.*

Conclusions on the seed potato market (farmers' perspective):

1. Discussion with Potato Growers Association at Heho (120 members): we need a governmental or a NGO or a Private seed farm.
2. Obvious blemish of seed tuber: root knot nematode seed issue is felt as the most important potato production constraint.
3. As seed quality is not known before planting, it is considered a gamble.
4. Bacterial wilt and root knot nematodes are the main concerns (most likely due to ignorance of viruses, these are not considered to be a problem).
5. There is a need for an official seed production farm and qualitied technical assistance for seed production.

1.4 Basic seed production – in-vitro based – in Myanmar

Heho Research Farm

- 2004-2006: there were 2 Korean volunteers setting up rapid multiplication in-vitro lab,
- There was no adequate transfer of technology but through national capacity in 2009 rapid multiplication was restarted with the then available varieties L11 of CIP and Up-to-Date. Emphasis was on L11 as that variety yielded higher due to late blight resistance.
- Note: Yezin University has an in-vitro lab of potato and currently has L11 and Up-to-Date available in stock.
- Current (2013) the capacity at Heho Research Farm to grow mini-tubers:
  - Take apices if 3 mm s.
  - 12 such containers = 96 plants transferred to tray with soil medium ex-,
  - Here hardened for 30 days.
  - Then transferred to soil medium, which is NON STERILIZED SOIL. As a result, in 2013 many bacterial wilted plants were observed in the screen house.
  - When these plants are growing, 40 x 3-leaf cuttings are taken per plant growing (mother plant produce 1100 tubers per viss, so very small tubers) the cuttings (40 per plant) produce 4 mini-tubers each.
  - Theoretically: 2500 vitro plantlets produce per year 40 cuttings each (100,000 plants) and each plant produces 4 mini-tubers => 400,000 mini-tubers (when everything runs at full capacity this system can produce 800,000 mini's per year).
  - 400,000 mini-tubers => yields enough for 10 hectares to plant G1 => yields enough for 100 ha to plant G2 => yields enough for 1000 ha G3 and so on.
  - The farm has tried other varieties than L11 and Up-to-Date in field trials, such as Chinese Yushwi, Cooperation 88, V(ietnam)4, V14, V16 from CIP and 55 other clones non retained.

Figure 2 *Mother in-vitro plants at Heho research farm (left) and mini-tubers screen house (right, pre-improvement, 2013).*

1.5 CDN potato project, progress and problems

In late 2013, the CDN had two agronomists and a financial person employed at Tangji to implement a potato project. It started with CDN funding but in 2012 a 3 year EU supplemental grant came through, specifically for basic seed production based on in-vitro multiplication techniques:

- 2012:
  May planting tests of three varieties L11, Up-to-Date and Kufri Jioty on two farms.
  August planting test of four varieties on two farms. Seed kept for the 2013 April/May planting.
- 2013:
  January irrigated season mass multiplication of L11 of Heho Research farm in paddy fields. After harvest in April about 1/3rd of the tubers were cut in smaller pieces without disinfecting the knives. Result: bacterial wilt infection was severely aggravated.
  April: early monsoon season was planted with the best performing seed from November 2012 trails (L11) at 10 farms in 10 villages.
  August: the bacterial wilt infected L11 seed given to the 10 farmers at 10 villages in April, was distributed to 101 growers in 11 villages. This had disastrous results, as the seeds were heavily infected by bacterial wilt.

The main reasons that severely aggravated the infection of bacterial wilt were:

1. The use of infected soil for growing mini-tubers in the screen house,
2. Cutting the seeds without disinfecting the knives between every cut made.

Figure 3 *Oozing sticky eyes and ringed oozings (left) and symptom of bacterial wilt (right) caused by the bacteria Ralstonia solanacearum.*

Conclusions regarding in-vitro and mini-tuber propagation:

- Aiming at 1 variety L11 only and discarding other sources proved risky.
- Comparison of healthy Up-to-Date in-vitro mini-tubers with traditional seed was not done.
- Massive seed cutting by knife in prone area's is not advised.
- Having only one in-vitro lab is risky because of continuity of knowledge and technical capabilities. Currently rapid multiplication is stopped at the Heho Farm because of the bacterial wilt dilemma.

Figure 4 *There is in-vitro rapid multiplication experience available at Yezin Agricultural University.*

1.6 Recommendations for implementation in 2014

(This to be developed in the 2014 project, not treated exhaustively here)

- Stop multiplication of L11 by CDN, growers and Heho Research Farm. Tell farmers not to use L11 as seed and remove volunteer plants in the next season. This variety may be susceptible but is infected in the seed production stage.
- Restart rapid multiplication with L11, Up-to-Date and Kufri Jioty.
- Invest in an insect proof screen house, in soil sterilization and in tables to grow mini-tubers in.
- Absolutely necessary security system (water tight) for mini-tuber production by sterilizing (steaming) the soil where mini-tubers are produced in (tables with 20 cm soil); make screen house 100% insect proof.
- Raise interest of business people/entrepreneurs from the start. There is a lot of money to be made (very profitable) with improved seed, if not the whole exercise is futile.
- Introduce more varieties with the aid of CIP (BP1 from South Africa, best varieties from China, Mexico, Rwanda, …).
- Find highest possible sites for seed multiplication 6000 ft. anywhere, and descend from there.
- Compare G1 seed of existing varieties with that of at least two conventional sources to find out if seed health is an issue at all. This is a very important aspect of the whole exercise: demonstrate to the project and growers the benefits. Or not and draw conclusions from there. Experiments as OFT (on farm trials).
- Assist growers in forming formal links (ware and seed growers), buying “seed” being just the small ones of ware production has (too) many uncertainties.
- Introduce concept of positive and negative selection and train growers.
- Farmers who receive improved seed – once proven it is superior – could specialize,
- Make use of expertise of other labs (Yezin Agricultural University, Tangji vitro lab along with banana).

1.7 Objectives of the 2014 project

(Terms of Reference Expert Missions to CDI potato project in Myanmar)

The EU funded potato project in Myanmar in 2014, will end its first phase. A second phase is currently being considered. Following the expert mission in November 2013 by Wageningen UR potato specialist Dr. Anton J. Haverkort (Rapid appraisal of the Myanmar potato industry, Opportunities for seed production, attached) the following activities should be carried out by Dr. Haverkort and an as yet to be identified hands-on practical potato specialist:

- Assist the Myanmar/CDN staff to restart of the rapid in-vitro multiplication with the 3 varieties L11, Up-to-Date and Kufri Jioty.
- Create awareness for the absolutely necessary security for healthy mini-tuber production by sterilizing (steaming) soil where mini-tubers are produced in (tables with 20 cm soil); make the screen house 100% insect proof and that no seed cutting takes place at any stage of seed production.
- Guide the initiation, construction and proper use of
  1) insect proof screen house.
  2) soil sterilizing equipment for steaming.
  3) tables to raise mini-tubers rather than in soil-on-soil and.
  4) the establishment of a small cold store (refrigerated at 4°C) to store for 8 - 9 month maximum.
- Assist the Myanmar/CDN staff to raise interest of Myanmar business people/entrepreneurs from the beginning. There is money to be made with improved seed so after a few years no more NGO nor governmental interference should be needed. This process needs to be guided.
- Introduce more varieties with the aid of CIP (BP1 from South Africa, best varieties from China, Mexico, Rwanda, …), act as a liaise, the go-between and assist the 'in the field evaluation' of such material.
- Assist the Myanmar/CDN staff and the agricultural office in the identification of sites with the lowest incidence of bacterial wilt, that is the highest possible sites for seed multiplication 6000 ft. anywhere and descend from there.
- Guide the construction and use of seed storage in Diffused Light Stores, just 3 - 4 layers high, screened fully to keep insects out. This at the Heho Research Farm level and with seed growers.
- Assist the Myanmar/CDN staff to carry out on farm trials: G1 seed of existing varieties on farm compared with that of at least two conventional sources to find out if seed health is an issue at all. This is a most important aspect of the whole exercise: demonstrate to the project and growers the benefits.
- Assist growers in forming formal links (ware and seed growers), buying "seed" being just the small ones of ware production has (too) many uncertainties.
- Introduce with the Myanmar/CDN staff the concept of positive and negative selection and train growers in the three different planting seasons and sites (paddy and upland).
- Assist Myanmar/DDN staff in encouraging some farmers receiving improved seed – once proven it is superior – to specialize as seed growers based on basic seed from in-vitro.

To achieve this, the following stays are foreseen:

1. During the January – April paddy crop a mission by the senior expert to introduce the practical hands-on expert and make a joint work plan; the practical expert will then stay on for another three weeks to carry out above mentioned tasks in the Shan State based at Taunggyi (materialized in April 2014).

2. During the early monsoon season (May - August) the practical expert will be in the Shan State for another 4 weeks accompanied by the senior expert during the last week to complete the job (materialized in September 2014).

2. Principles and protocols for healthy potato plant production in Myanmar

2.1 Protocol for rapid propagation methods

2.1.1 The in-vitro laboratory

General schedule for rapid propagation stages to go in 26 weeks form one seed tuber to 1,000 seed tubers.

The following stages are passed:

- Introduce healthy stock, preferably in-vitro plantlets from acknowledged institutions.
- In flow cabinet preparation of the glass tubes for the shoot tips and undeveloped buds.
- Transfer of material (in-vitro plantlet of sprout shoot tip) to in-vitro into flow cabinet.
- If needed, that is when diseases and bacterial infections are suspected: after three to four weeks the plants are sufficiently large for testing with M-5-virus test kit and with Bacterial Wilt kit.
- If tests are negative, bring the young bud nuts into vitro.
- After 4 or 5 weeks plantlets are checked for presence of bacteria and fungi. Plantlets are then cut into 5 or more pieces with each node having a growing point. The nodes with a growing point are placed separately in a glass tube; one growing point in one glass tube.
- After 5 weeks again the nodes are grown into plantlets and cut again. Depending of the size of the glass or plastic pot you can put in between 6 or 8 cuttings per container.
- After 5 weeks the plants are ready to be transferred to the soil.

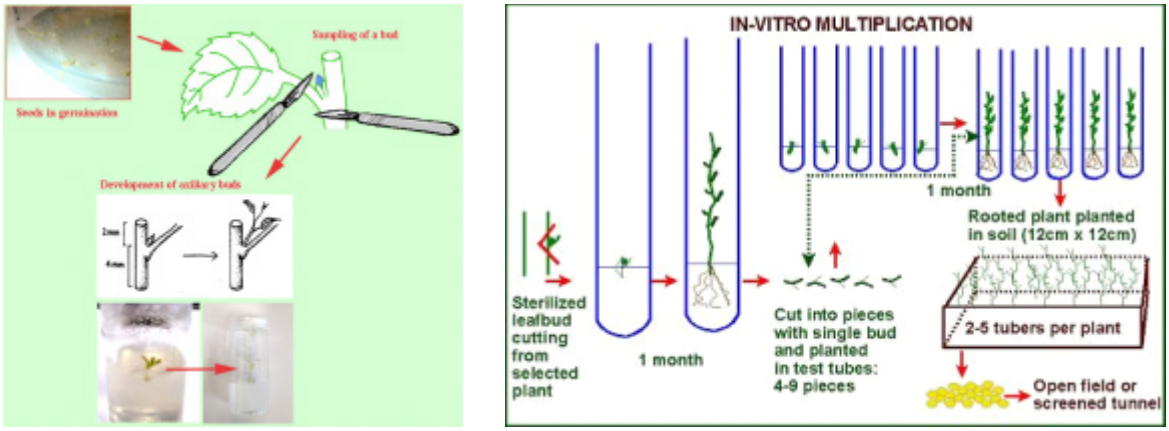


Figure 5 *An overview of a scheme of cutting buds from plants (left) and different steps of in-vitro multiplication (right).*

In principle in 26 weeks the following scheme is feasible:

- One in-vitro plantlet from clean and disease free tuber but preferable from other lab: → one sprout.
- 5 weeks growing: one plant cutting into: → 5 new plants.
- 4 - 5 weeks growing: 5 plants cut each plant into 5 to 8 pieces: → 25 to 40 plants.
- 5 weeks growing: 25 to 40 plants are ready to be planted into the steamed/sterilized soil in the screen house.
- 10 to 15 weeks, depending on the growing conditions in the screen house: harvest of mini-seed tubers of Stage G0. After harvest of the G0, multiplication can be as fast as:
- 25 plants with 4/6 tubers → 100 to 150 tubers.

or, when conditions are very good:

- 40 plants with 4/6 tubers 160 to 240 tubers.

All of this depends on the variety, climate in the growing chamber, climate in the screen house and on the soil conditions, assuming it is sterilized/steamed properly, and the soil is loose (that the soil is not too compact for rooting) by using the right mixture of components and fertilizer.

Figure 6 *In-vitro procedures: cutting of in-vitro plantlets.*

2.1.2 Obtention of initial clean stock for in-vitro propagation

The best and preferred method to obtaining in-vitro plants is to obtain them from recognized laboratories (e.g. CIP or CPRI, India) as the guarantee on clean stock is high. However, the procedures described below can be used to obtain clean stock if the protocol is followed into great detail, but the risks of failure are higher than obtaining clean stock.

The first step to obtain initial clean stock for rapid multiplication in the tissue lab (in-vitro lab) is to obtain virus and disease free healthy tubers or plants. For each variety the material is brought into the in-vitro lab, making sure that these are free of viruses and bacteria's. The second step is to break the dormancy if needed. The third step is the

Virus and bacteria free plant material

To obtain virus and bacteria free plant material the following procedure must be followed:

- Identify healthy plants which produce clean tubers in the field (during the cultivation):
  - Go into the field during the growing season and put labels around good looking, disease free plants.
  - Inspect these plants several times during the growing season to see if they stay healthy and disease free.
  - Harvest the labeled plants when tubers are of desired size, big enough, manually and one by one.
  - Inspect each plant during harvest to see if the tubers are clean and disease free. If not, dispose of the entire plant before mixing with the healthy, already harvested plants. Dispose of all tubers of the infected plant, even if some tubers look healthy).
- Use the healthy and disease free looking tubers for cuttings of sprouts or eyes (Figure 7) or store the tubers until you are ready for cuttings in the Diffused Light storage buildings (Figure 14). Note: not more than two 3 to 4 layers of potatoes stored above each other in fully netted buildings.

Figure 7 *Example of eye cutting.*

Dormancy of tubers

The healthy and disease free tubers collected in the field may still be dormant at the moment cuttings need to be made. If the dormancy period is completed naturally, eyes can be cut. Place the cut eye in sterilized (steamed) soil to let it grown. These eyes need to be checked on a regular basis visually to see if the plant is healthy. Then the test kids M-virus 5 and Bacterial Wilt are used for testing. If negative (meaning plants are healthy) the young buds are removed and brought to the in-vitro laboratory to be multiplied.

If the dormancy period is not completed the dormancy can be broken by placing the cut eyes in a solution with gibberellins as described below.

Procedure to break the dormancy of the seed potato eyes:

- Select healthy seed tuber plants in the field crop. Mark these healthy looking plants so that you can follow this plant during the field period and check whether it will stay healthy. When the tubers are sufficiently big, harvest them. Use these tubers for eye cutting.

- Make a stock solution of gibberellin as follows:
  Take a GA3 tablet of 1 gram,
  Dissolve the tablet in 1 ml of 96% ethanol.
  Your stock solution is ready.

- Dilute the stock solution: dissolve 1 ml of the stock solution in 1000 ml water.
  This is your solution to break the dormancy of the cut tuber eyes.

- Put the cut eyes in the solution for 10 minutes.

- After these 10 minutes, plant the eyes in the steamed soil and follow the procedure described for the dormancy broken eyes.

Continued protocol for eyes (dormancy broken) and cuttings

- Tag (label) each seed lot in the propagation cycle by cultivar name, propagation date and make sure this is logged in the note book.
- Write the cultivar name and date on the tag (label), and keep track in the note book of all activities concerning in-vitro work.
- Rinse the material for 30 seconds in a 70% ethanol solution.
- Sterilize the plant material for 20 minutes in a 1.5% hypochlorite solution.
- Rinse 3 times 5 - 10 minutes with sterilized water to remove all the hypochlorite. Demi water can be used. Cut off the white parts (caused by sterilisation) from the plants. Transfer each tip, bud growing point sprout, separately into tubes with MS 30 medium (Figure 5, right). Close the tube with para film. Bring the tubes to the growing chamber.
- Check in the first week regularly if the material remains clean. Tubes which are infected by fungi need to be thrown away.
- After 4 to 5 weeks the healthy plants are cut into five pieces with 1 bud per piece. Use clean, disinfected knifes.
- These pieces are transferred to new, clean tubes, closed with para film and placed in the growing chamber.
- After another 4 to 5 weeks the second cycle of cutting (multiplication) of the plants starts. The plants are cut and the buds are put in a set of 12 to 20 plants in a plastic box, individually planted. Bring them to the growing chamber.
- After another 4 or 5 weeks of growing the in-vitro plants have reached the stage for being transferred to the sterilized (steamed) soil.

To keep a stock in the in-vitro lab of the healthy varieties the media MS 20 or MS 30 are used, depending on the potato variety. The stock must be refreshed every 3 months, which is registered in the not book. Keep good track of the health of the plantlets as this is the basic stock for multiplication. Preferably, bring stock of varieties to other tissue labs in Yezin or Yangon to spread the risks. They can use the same procedure to keep them in stock. This way there is always a back-up of your stock available if something goes wrong at Heho Multiplication Farm.

Obtaining in-vitro plans from recognized laboratories (e.g. CIP of CPRI (India)) is a much preferred method with much less risk than the procedures described above.

2.1.3 Procedures in the laboratory

Climatic conditions in the in-vitro lab for growing cultures: The climate condition of the grow chamber are essential for the optimal growth of the in-vitro plantlets. For multiplication, the plantlets need a day length of 16 hours followed by 8 hours darkness per 24 hours. The light intensity must be at least 1,500 lux and the ideal temperature is between 23°C and 25°C during the day and night.
Conditions during preparation: When potato plantlets are brought into the in-vitro working area, it is essential that the area is clean and sterilized from the beginning, using70 - 96% alcohol. All tables and working tools are sterilized before using. Lab coat and latex hand gloves are worn to avoid any pollution by bacteria and fungi from the clothing and hands.

Media used by Heho Multiplication Farm

I. Initial media
MS 30 + 30 gr sugar +5 gr agar / 1 l water
MS 30 + 5 mg GA3 + 30 gr sugar + 5 agar / 1 litre water

II. Stock solution for multiplication:
MS 30 + 1 mg Kinetin + 30 gr sugar + 5 agar / 1 litre water
½ MS 30 + 60 gr sugar + 5 gr agar / 1 l water
MS 30 + 60 gr sugar + 5 gr agar / 1 l water

III. Rooting media: MS 30
MS 30 5 gr + 5 mg auxine + 40 gr sugar + 5 gr agar / 1 litre water

Standard media used at Wageningen University and Research Centrum (Department of Plant Breeding and Biodiversity), the Netherlands

I. Stock solution: MS 10

MS medium includes vitamins	4.4 g
Saccharose	10 g
Dissolve in 1 litre MQ	
pH = 5.8	
Micro agar	8 g
Autoclave 15 min. at 121°C	

II. Stock solution used when growth rate should be reduced: MS 20

MS medium includes vitamins	4.4 g
Saccharose	20 g
Dissolve in 1 litre MQ	
pH = 5.8	
Micro agar	8 g
Autoclave 15 min. at 121°C	

III. Standard stock solution for multiplication: MS 30

MS medium includes vitamins	4.4 g
Saccharose	30 g
Dissolve in 1 litre MQ	
pH = 5.8	
Micro agar	8 g
Autoclave 15 min. at 121°C	

IV. Stock solution to stimulate rooting: B5 - 30

Gamborg B5 medium	3.2 g
Saccharose	30 g
Dissolve in 1 litre MQ	
pH = 5.8	
Micro agar	8 g
Autoclave 15 min. at 121°C	

Figure 8 *Media or stock solutions are kept in glass jars in the growing chamber.*

2.2 Protocol of planting in-vitro material in the screen house

2.2.1 A 100% insect free screen house

It is essential that the screen house is totally insect free and remains totally insect free. Several measures need to be taken to construct a 100% insect free screen house, which are listed below. Some additional demands are listed as well to guarantee the young plants grow well. Furthermore, to keep the screen house 100% insect free, an user protocol must be followed. It is essential that a log book is kept on all the activities in the screen house. That includes the entrance of employees, their activities and the handling of the young plants.

The construction of a 100% insect free screen house
An insect free screen house is constructed by the application of nettings to keep aphids, trips and other sucking insects out. The mesh size of the netting must be 0.16 mm x 0.16 mm. The screen house needs two entrances to function as a sluice. Both entrances are made of the mesh netting with the appropriate mesh size to ensure it is totally aphid proof.
The immediate surroundings of the screen house must be cleaned and kept free from host plant weeds. No potato crop residues may be found in the area around the screen house.
The screen house floor must be made of concrete or covered with fine gravel to prevent weed growing. This is necessary as some weeds are host plant for insects. It also provides a good solid foundation for the growing tablets.
The screen house must be equipped with sunshade screens to protect the young plants from direct sunlight exposure. The roof of the screen house must be waterproof as rain may damage the young plants.
The planting substrate or soil of the tablets for the in-vitro plants. The substrate of soil used to plant the young plants in should be in perfect condition.
First of all, it means that the substrate or soil is sterilized/steamed for at least 1 hour at 100°C and therefor free of any fungal or bacterial contamination. After steaming/sterilizing the substrate/soil must be left for week in a clean and dry storage place near the screen house facility, in such a way that reinfection of fungal and bacterial contaminations cannot occur. After this week of rest, put the substrate/soil on the growing tablets. Secondly, the substrate/soil must be loose for easy rooting and tuber formation, must have the right composition of nutrients and must have a good physical composition. River sand may be needed to provide better aeration. A preferable composition of the substrate/soil is: 1/3 sand,1/3 compost and 1/3 peat. This substrate provides good rooting

ability's and sufficient opportunity for optimal growth. Put the right dose of fertilization mixtures into the substrate/soil before planting but after the substrate/soil is steamed.

2.2.2 The user protocol to ensure a 100% insect free environment in the screen house

When entering the screen house, the outside door and the inside door MAY NOT be opened at the same time, to prevent the insects to fly into the screen house. Opening the outside door is only allowed when the inside door is closed. Visa versa, the inside door may only be opened when the outside door is closed. When entered and closed the outside door: check the sluice for before the inside door is opened.
Placement of aphids traps in the screen house (Figure 9). Yellow aphid traps are placed inside the screen house and one additional trap is placed at the outside close to the entrance of the screen house. The plates must be monitored daily to keep track on the number of aphids in the closed vicinity of the screen house entrance. When the number of aphids increases an insecticide spray in the screen house is needed.
The use of chemicals to keep the in-vitro plants in good condition and free of insects and fungi. It is essential to keep the plants free of viruses, fungi's and bacteria, which can be transmitted by insects. When chemicals need to be used for the elimination of insects, viruses, fungi's or bacteria, use different chemicals/chemical groups to avoid resistant development against the chemicals used. Records are made in the log book on what was used, including the dose of pesticide and the proportion of water.

Figure 9 *A yellow trap for aphid trapping.*

Activities in the screen house
Planting the in-vitro plants into the soil. To pant the plantlets into the soil, they are taken out of the medium carefully. It is best to plant the in-vitro plantlets separately, one by one, in the in-vitro box. First, they are flushed carefully with water to remove the adherent agar. When planting the in-vitro plantlets, make sure they are planted sufficiently deep. This means that the proportion between leaves and roots should be 40% (upper part)/60% (lower part) as is shown in Figure 10. The buds on the stem are now covered in the substrate/soil and will grow to form tubers. If these buds are in the air, no tubers will be formed, so plant the plantlets deep enough to stimulate tuber forming. Water the plants frequently and enough in such a way that the leaves are wetted as well and provide shade during the first days (at least a week) inside the screen house to prevent the young leaves from sun burn.
Each activity for each batch is recorded in the note book. Use is made of tags on the plants. Use clean tags and write down date and cultivar name clearly.

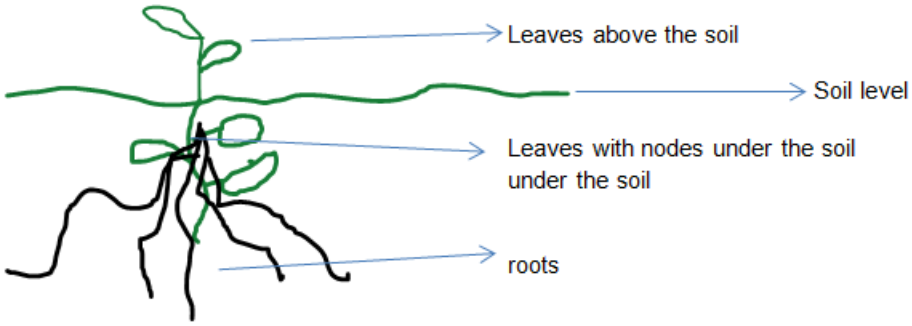


Figure 10 *An example how to plant in-vitro plants: they must be planted deep enough so buds on the stems are in the soil.*

2.2.3 Planting a new series of in-vitro material in the screen house

When starting a new cycle of planting of in-vitro plants ensure that the substrate/soil is treated as describe in the paragraph above (The construction of a 100% insect free screen house). In case of any uncertainty or possibility of reinfection, the substrate/soil has to be steamed again to avoid problems with diseases. It is a MUST that the substrate is absolutely FREE of any contamination.
The maximum allowable number of plantings in the same soil is 2; but only when the substrate/soil is totally free from diseases. If any fear of contamination exists, the substrate/soil needs to be steamed again. In addition, it is vital to be sure all the seed tubers are harvested. Otherwise risk of contamination (variety mixing) exists.
Before starting the planting, a schedule must be made. The schedule contains the planting combined with the activities of the in-vitro lab concerning production of new plants. Because of the warm climate in February and March: do not plant in-vitro plantlets in the screen house in these months. Use this period for steaming the substrate/soil, cleaning the tables and the screen house both inside and outside, check the netting of the screen house and repair if needed, and prepare for the new planting cycle.

2.3 Requirements for the screen house with in-vitro plants

The screen house is 100% insect free

For the screen house it is essential that it is 100% insect free. This can be assured by using nettings that keeps out the aphids, trips and other sucking insects. The size of the mesh should be 0.16 mm x 0.16 mm. The screen house also needs a sluice consisting of two entrances. Both entrances are made of gauze of the above specified size. The floor must be of concrete and free from weeds. This is because weeds are always attractive for insects. Also, a good foundation is needed for the growth tables. Then, use screens to protect the young plants from sunshine. The roof of the greenhouse must be waterproof as rain may damage the young plants. Care should be taken so that the immediate environment of the screen house is free of weeds/host plants and that there are no remaining potato plants in the area around outside the screen house.

Step by step

- Have a log book presented in the screen house and have all activities, entries and handling in the screen house recorded meticulously.
- Before entering the screen house be sure you are clean, meaning your clothing and shoes are free of all dirt. If you have been working in the field, DO NOT go into the screen house unless you carefully cleaned yourself of all attached dirt. Keep all materials needed in the screen house and DO NOT use them anywhere else, just let them in the screen house.

- The irrigation water of the plants should be clean. Surface water is often not clean, can contain bacteria for example Ralstonia (Wilt). Therefore, avoid surface water and install a separate water system, preferably from a deep tube well. Check the water for contamination like bacteria frequently (once a year).
- Restrict the entry to the in-vitro multiplication screen house to a few qualitied employees. Use the logbook, that they have to sign when entering and leaving the screen house. In addition, register all the activities in the screen house of all labor. In this logbook make notes on the chemicals used for spraying against insects and fungi.
- When sprays against aphids need to be applied, do not spray during the day time as temperatures are too high and the sunlight is too strong. Spray in early morning or late afternoon before sunset. The insecticide is much more effective when used at these times as the crop will stay wet for a longer period. Fungicides need to be sprayed in the early morning or early evening as well. When pesticides are applied, put a sign on the door to forbid entrance for at least several hours (Figure 11).

Figure 11 *A sign to prevent people to enter the screen house when pesticides are applied.*

2.4 Current situation and situation after implementing recommendations

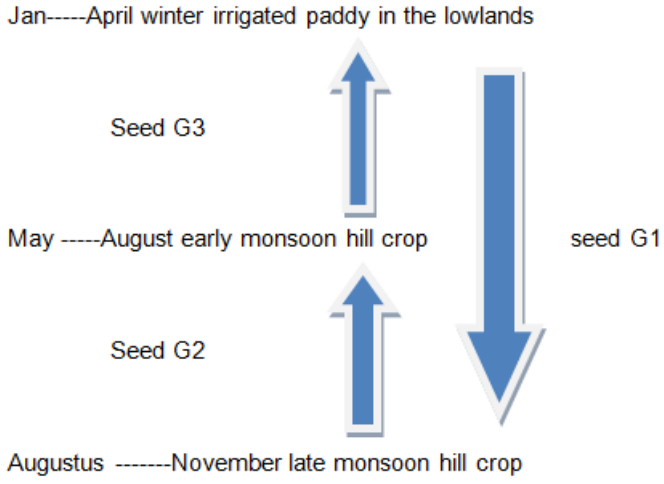
At the moment (2014), the screen house has 12 tables for the production of G0 mini-tubers. Each table can accommodate 160 plantlets. They are planted in four rows of each 40 plantlets. The total production is therefore 1,920 plantlets on twelve tables. If the tissue lab production is running well, two cycles per year can be made.

Figure 12 *Pictures of soil sterilization equipment (steamer) established in 2014.*

Figure 13 *Pictures of mini-tuber rearing tables at Heho (established in 2014).*

2.5 Field stages

Cropping sequence
Seed production from mini-tubers (G0) to produce G1, G2 and G3 in subsequent three field stages in the Myanmar situation where three potato crops are grown per year on average yields 1.5 multiplication per year. A generation of potato is 4 month in the field and subsequently storage for 4 month preferably in Diffused Light storage buildings. Schematically:



Or quantitatively the time line is shown in Table 1.

Table 1 *The time line of seed production in 4 years from 1 in-vitro plant.*

Month	Multiplication age	Yield in the field (kg)
0	1 in-vitro plant in the screen house, G0	-
12	G1	0.3
20	G2	3
28	G3	30
36	G4	300
42	G5	3000
48	G6	30000

2.6 Some relevant practices when growing seed potato

1. When the seed tubers are harvested one has to decide which tubers are wanted to use for planting next time (Figure 14, left). These tubers should be graded and sorted (removal of diseased and disordered tubers).
2. When storing in the new Diffuse Light Storages the seed tubers should have a better condition for planting than when stored in the dark. Store them at a maximum of two layers thick (Figure 14, right). So all the tubers get the same amount of light during the storage. They will have better sprouts: shorter, greener and stronger. During storage check the seed tubers regularly for insect (tuber moth) damage.
3. It is essential to locate the right conditions for seed tuber production. The best locations are the fields which have the highest altitude. The higher the altitude the better, there the problems with Aphids which infect and spread the viruses are less. The farmers that are involved in this project point at the area War Gyi Myaung as the area where the conditions are better because of the higher altitude (above 4,500 feet) and better soil conditions. They told that the area around Heho Farm is not the most suitable for seed tuber production. Therefore CDN should look for suitable fields and farmers for seed tuber production of the varieties.
4. Before starting to plant check seed tubers for infected tubers by late blight or other tuber diseases for example Brown rot, Fusarium dry rot, Silver scurf.
5. Use the right size of tubers (between 30 and 70 grams). When planting seed tubers with the objective of producing a next generation: never cut the seed.
6. When planting time has come, take care of good planting soil and the right amount of fertilizer, especially an overdose of nitrogen is costly and makes the crop more vulnerable to diseases like late blight.

Figure 14 *Manual harvest of potato crop (left) and the Diffused Light storage building (right).*

7. Compare newly introduced seed tubers (e.g. G1) to farmers' own seed comparison of new varieties with growers' own variety: introduce on-farm trials. Attention is paid to:
   - Assuring plots with innovation is marked at 4 corners and assure that the adjacent plot with grower's practice are more or less same size and marked with sticks at 4 corners as well,
   - Length and width accurately measured of both plots,
   - At harvest: count number of plants, number of tubers and weigh the tubers (netted bag on spring balance),
   - Carry out similar on-farm trials (same treatments) on at least 4 farms,
   - Treat each farm trial as a replicate when applying statistics (ANOVA).
8. When the plants start growing be aware of diseased plants (Figure 15) and remove any plant that shows viruses (mosaics), brown rot (wilting) or off types (different variety) and place the collected material in a pit to be buried immediately. For this we use the term Roguing. Not only the green parts of the plant are remove but also the mother tuber and <u>all</u> the already newly grown tubers. They are immediately put in a plastic bag that is carried along when checking the crop. Keep the bag closed when diseased/inappropriate material is collected. Roguing is a very important activity to keep quality seed. The employees/farmers need to be trained to identify the infected plants at an early stage to minimize spread of the infections and because diseases may not be identified as the crop matures. Roguing must be done at least 2 times during the growing cycle before the crop canopy is closed/fully grown.

Famer's field, traditional tchniques

Traditional 50 plants	Innovation1 50 plants

On farm trial single factor (clean seed

Traditional 50 plants	G1 seed 50 plants

Variety trial in farmer's field….

Traditional variety (mixture)
New variety A
New variety B
New variety C

Figure 15 *Bacterial wilt (middle left) and Potato Virus Y (middle right, leaf reverse side) and mosaic virus (bottom, left) and a suspicious looking plant (bottom, right). All these plants need to be rogued.*

9. Late blight will survive in tubers and on stems of the potato (Figure 15). In most cases Late blight will grow out of the infected tubers into the plant or a field is infected by spores from neighbouring fields. The ideal temperature for late blight development is between 15 and 25°C with a high humidity. This disease needs preventive sprays to minimize yield losses and rotten tubers.

Figure 16 *Symptoms of late blight (Phytophthora infestans) infected foliage (left) and tuber (right).*

10. When harvest starts, the diffused light stores need to be cleaned thoroughly. This includes the removal of old potatoes, soil and insects. Prior to storing the newly harvested crop, remove the old mother tubers and all tubers which are damaged during harvest or look infected with diseases. When not all infected tubers are removed properly, they are a source of diseases and increase losses during storage.

3. Hazard analysis at all critical control points of basic seed potato production in Myanmar

Table 1 shows all critical control points, the aim of the intervention, risks associated with the intervention (hazards) and acts (rules) to avoid risks. These rules must be observed strictly.

Table 2 *An overview of the critical points, the aim of intervention, the risks associated with the intervention and actions to avoid risks.*

Nr	Subject	Aim	Risks	Risk avoidance acts
1	Selection of visually healthy mother tubers in field crops	Creating new in-vitro stock of healthy (new) varieties	Introduction of diseases from the start and losing generations	Use more than 1 location of origin and of multiplication (better introduce mini plants from a recognized lab)
2	Stimulate sprouting	Have material ready in time	Sprouting not successful	Breaking dormancy with gibberellic acid
3	First step in-vitro production of plantlets	Rapid multiplication	1. Contamination 2. Power cuts 3. Too few technicians	1. Disinfection 2. Back-up generator 3. Train 3 technicians
4	Disease diagnostics	Assuring material is healthy	Lack of test kits and experience	Introduce kits and training, team with other crops
5	Maintenance of healthy stock	Keeping stock for future use	1. Power cuts 2. Too high temperatures	1. Back up of generator 2. Daylight can be used, assure ventilation
6	Routine in-vitro rapid multiplication	Assure flow of plantlets to screen house	Contamination	Disinfection of laminar flow chamber and tools, separate room for this activity
7	Transfer of plantlets from tissue laboratory screen house	Production of mini-tubers in screen house	1. Bad rooting 2. Contamination	1. Watering, shading, soil disinfection (steaming) and assuring insect proof screens 2. Insecticide application based on yellow trap results
8	Stem cutting from plantlets in screen house	More rapid multiplication than in-vitro only	Bad rooting, contamination	See point 7: additionally disinfect tools between each cut
9	Screen house construction	Insect proof environment	Holes allowing insects in	Application of appropriate mesh, double door entrance
10	Soil disinfection for screen house	Avoid bacterial infection	Contamination from inside soil and external sources	Steaming of soil: placing it on tables, not on house-bottom. Avoidance of introduction from outside. Leave equipment inside screen house, explore other energy sources beside wood
11	Insect free screen house	Avoidance of vectors of diseases	Presence of insect s(aphids, white flies)	Beside keeping screened: yellow trap for aphids, when present apply insecticide.
12	mini-tuber production	Small tubers from greenhouse for G1, 2 and 4 field stages	Insufficient numbers and size at desired time	Carefully planning flow of in-vitro plantlets, cuttings in screen house and plants producing mini-tubers taking variety and need for field (G1,2,3) into account
12	Record keeping	Assuring regular flow and tracing and tracking	Records lost	Make back-ups on paper and on computer, ensure two persons keep the records separately (paper and computer)
	Harvest of mini-tubers	Reaping hardened mini-tubers	Premature harvest reduces storability	Harvest after complete foliage death (if in hurry remove foliage 2 weeks before harvest
13	Storing of mini-tubers	Allowing sprouting	Losses due to moths	Store in Diffuse light store (up to 6 months) or

		and distribution	or rotting	in cold store (up to 10 months) at 4 degrees
14	Planting of mini-tubers and G1,2,3 in field	Production of next generation seed	Mixtures. Field is contaminated, low emergence	Label and separate seed lots and plots. Select fields known to be brown rot free, no ground keepers, high altitude, well sprouted
15	Crop management	Obtaining high yields of good health	Inadequate fertilization and disease control.	Use decision support systems for NPK application and control of late blight and insect pests. Assurance pesticides still perform well
16	Roguing	Removal of diseased plants	Too late removal, symptoms not recognized	Inspection once a week, follow training for diagnostics of virus, bacterial and fungal diseases
17	Harvest	Reaping well sized and hardened tubers	Too small or too large, skin is loose	Harvest not too early nor too late, check tuber size; defoliation two weeks before harvest
18	Transport from field	Delivery to store	Damage to tubers, contamination	Gentle treated bags, no dropping just gently laying bags down, emptying gently
	Grading and sorting	Assuring right size an unblemished tubers are stored	Storage of wrongly sized and diseased tubers stored	Grading on field which (large) tuber size will go for consumption and which seed sized tubers with blemishes/rot are discarded
19	Storing of seed tubers	Allowing sprouting and distribution	Losses due to moths or rotting	Store in Diffuse light store (up to 6 months)
20	Subsequent multiplications	Obtention of G2 and G3 seed	Too rapid degeneration	Assure disease free field selection (high, no groundkeepers) and adequate roguing, tack and trace field history

4. Suggestions for further developments

Within the framework of the project results described in this report in 2014 a relative small effort took place to remediate problems encountered in the basic seed production chain in Myanmar. In brief these serious problems consisted of:

- Insufficient level of knowledge with the technicians involved in the various stages of the basic seed chain
- Lack of diagnostic tools to detect and recognize tuber borne diseases
- Lack of hygiene of the screen house soil
- Lack of insulation of the screen house
- Storage of mini-tubers in ambient conditions with losses due to insects
- Seed was cut without disinfecting knifes in between cuts
- No on-farm trials took place to adequately compare improved seed with farmer's practice

During the involvement of the Wageningen UR scientists and technicians the points above were addressed to a greater or lesser extent. Training sessions took place, disease detection kits were introduced, soil disinfection apparatus and procedures were introduced, the screen house was upgraded, diffused light stores use for mini-tubers was discussed, seed cutting of seed used for further multiplication was declared prohibited and procedures of how to carry out on-farm trials were demonstrated.

This by far does mean that the program now is self-reliant. In order to build upon the achievements to date (late 2014) and obtain real impact on the potato sector the following actions (most of them were touched upon in this first phase) are needed with additional input from CDN and or other interested parties:

A. Hardware

- Restructure the in-vitro lab to as the flow cabinet needs to be separated from the plantlets in the in-vitro laboratory
- Install a standby generator or solar energy generation system to back up the in-vitro laboratory
- Contracted supply of test kits for bacteria and viruses
- A generator as back-up when electricity fails
- Complete overhaul of the screen house
- Inquiry into the use of alternative energy for the soil steaming facility
- Inquiry into the establishment of either a hydroponic or aeroponics system for mini-seed tuber production once this pilot of in soil medium production is operating smoothly
- Improvement of both Heho Farm based diffused light storages for mini-tuber and field tuber storage
- A small cold store unit to store up to 2 tonnes of mini-tubers for periods up to 9 months allowing a better planning of the deployment of mini-tubers.

B. Training and guidance

1. Training of personnel employed at the Heho Agricultural research Station specifically in relation to:
- disease and pest diagnostics in the field and with diagnostic kits,
- hygienic procedures in cutting in-vitro plants and in screen house management,
- logistics of the planning process from in-vitro introduction to the lab all the way to 1 or two multiplication steps on farms (numbers of plantlets, mini-tubers, G1, G2, G3) per variety,
- train the trainers of farmers to improve their practices.

2. Training of seed potato growers to:
- enhance their roguing abilities (negative selection),
- teach them seed plot techniques (from positive selection, introduced G1) separate seed from table production,

- show how on-farm trials are carried out with improved varieties and other cultural practices (seed age, pre-sprouting in diffuse light, fertilization, crop protection),
- improved seed storage and pre-sprouting,
- become specialized seed potato entrepreneurs.

C. Institutional support

- Training schedule of all involved,
- Embedding potato laboratory techniques in national efforts (other crops, increase critical mass),
- Value chain development: seed - ware trade - processing (grading, sorting, production, packing) - trade – retail with all stakeholders involved,
- Upscaling from this pilot to larger amounts and from the few growers involved to many growers.