Protocol of Regenerating Vegetable crops

World Vegetable Center

Genetic Resources and Seed Unit

Yung-kuang Huang

Germplasm Conservation

Registration - Accession number, passport data

Regeneration – Growth, harvest, store

Characterization – Morphologic

Documentation – Passport, character, Inventory, Distribution

Distribution – Send for utilization

Acquisition of Germplasm

- **Obtained from research institute**
  - **Seed amount limit (30-50seeds)**
- **Collected from farmer who growing the traditional cultivar**
  - **Seed vigor(quality) may not stable**
- **Collected in wild habitat or border field**
  - **Need taxonomy identification**

Why Regeneration

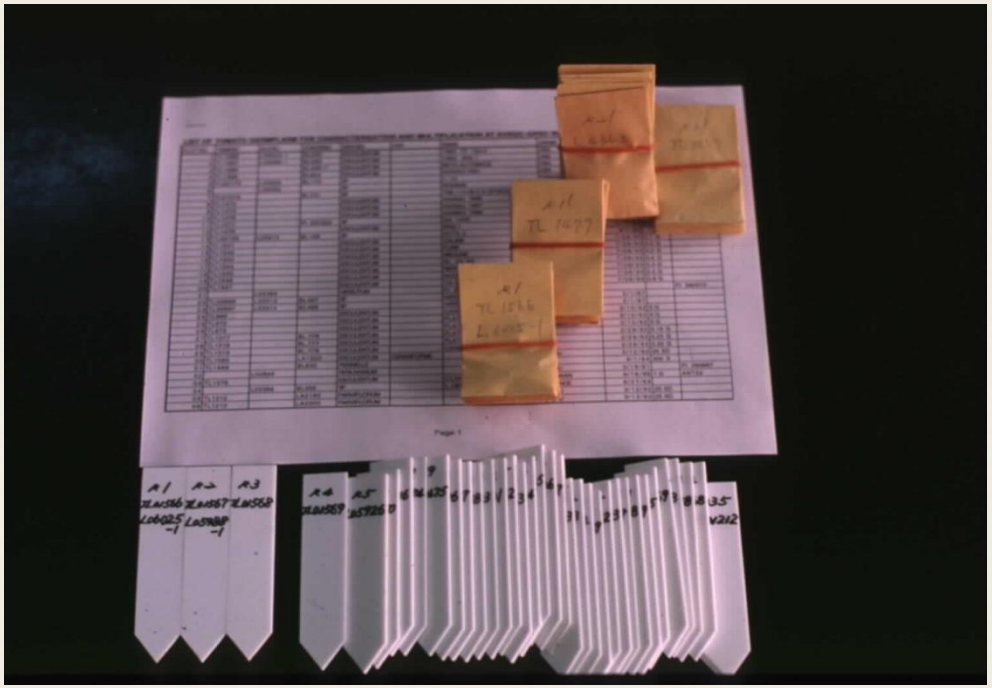
Original seed: limit number, poor quality

Cannot use on any purpose, cannot store for long

Idea statue of germplasm material

1. **Good quality : >85% germination rate**
2. **Enough quantity : >20 $^{+}$ distribution samples**
   Long-term preservation
3. **Long storage life : Can stored for >50 years**

Regeneration protocol(1)



Preparation :

List of selected germplasm

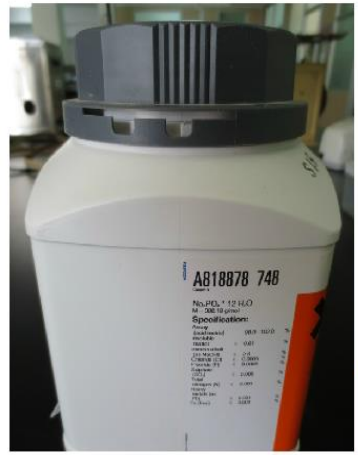
Seed of accessions to be regenerated

Label for both seedling nursery and field growing

Regeneration protocol(1-1)

- **What germplasm should be put in highly priority?**
- **Back log materials for regeneration especially the ones be acquired for long ago.**
- **Materials loosing its viability.**
- **Been asked but can not distributed because no available inventory.**

Regeneration protocol (2)



Seed treatment

To prevent the infection of seed-born disease

Coating seed with fungicide – protect seeds from damage of microorganism,

Regeneration protocol (2-1)

- Seed treatment:
- TSP (Trisodium phosphate) – Remove virus particle from seed surface
- Hot water treatment - priming
- Coating seed – with fungicide, fertilizer to make an artificial seed.
- Machinery injury on seed coat

Regeneration protocol (3)

Preparing medium of seedling nursery

Mixture of soil, fertilizer,

Regeneration protocol (3-1)

- **Good medium of seedling nursery**
  - **Free from soil born disease such as fungus and bacterial**
  - **Free from weed seed**
  - **Good on permeable**
  - **Good on retention capacity**
  - **Good on drainage**
  - **Enough fertilizer for growth of seedling**

Regeneration protocol (4)

Filling medium in the plugs (or nursery cup)

Regeneration protocol (5)

Sowing seeds – one seed seed (or 2-3seeds if seed vigor not ideal) in one cell (cup)

Regeneration protocol (5-1)

- Seed bags and label must double check before seeds are sown.
- Seed should be berried into the medium for 2-3 time thickness of seed.
- Watering carefully right after sown.
- Shading but with full light is necessary for seedling protection.

Regeneration protocol (6)

Seedlings in nursery until 3-4 true leaves appear

Watering, thin down (if necessary), spraying (fungicide or insecticide), Fertilizing (liquid one) and purifying

Regeneration protocol (6-1)

- Plastic or nylon cage for protect young seedling from outside impact can help resulting good seedlings.
- Transfer seedling within accession to one seedling per cell of plug
- Watering should dependent on weather to keep medium in a proper moisture.
- In case weed appear, should remove immediately.

Pollinating of Angiosperms flowering plants

- Self-pollination (ex. Soybean)
- Wind-pollinated (ex. Corn, rice)
- Water-pollination (ex. Aquatic)
- Entomophilous (ex. Crucifer, Cucumber
- Other biotic (bird, bat)

Pollinating of Angiosperms flowering plants

- **Self-pollination**

  - Open field is alright

- **Wind-pollinated**

  - Need facility to block or reduce wind speed

- **Entomophilous**

  - Net cages are needed for prevent pollinator making out-crossing happen or bagging before flowering and after pollinated.

Pollination behavior of some vegetable crops

Crop	Species	Pollinat. type	Pollination mechanism	Method used
Amaranth	*Amaranthus* spp.	CP	Wind	Isolation 1000 m; bagging; net cage
Beet	*Beta vulgaris*	CP	Wind	Isolation 2000 m
Black gram	*Vigna mungo*	SP		
Bottle gourd	*Lagenaria siceraria*	CP, monoecious	Insects	Bagging & hand pollination; net c.
Brown mustard	*Brassica juncea*	Mainly SP; 4-14% CP	Insects	Isolation 1000 m; bagging; net cage
Cabbage	*Brassica oleracea* var. *capitata*	CP	Insects	Isolation 1000 m; Net cage w/ pollinator
Carrot	*Daucus carota*	CP; protandrous	Insects	Isolation 1000 m; Net cage w/ pollinator
Cauliflower	*Brassica oleracea* var. *botrytis*	Mainly CP	Insects	Isolation 1000 m; bagging; net cage
Chickpea	*Cicer arietinum*	SP		
Common bean	*Phaseolus vulgaris*	Mainly SP	Insects	Isolation 100 m; bagging; net cage

Pollination behavior of some vegetable crops (2)

Crop	Species	Pollinat. type	Pollination mechanism	Method used
Cowpea	*Vigna unguiculata*	Mainly SP		
Cucumber	*Cucumis sativus*	CP; monoecious	Insects	Isolation 1000 m; bagging & hand pollination; net cage
Eggplant	*Solanum melongena*	Partial SP; 0-8% nat. outcrossing (AVRDC)	Insects	Net cage; supple-mentary hand pollination
Endive	*Cichorium endiva*	SP		Isolation 600 m
Faba bean	*Vicia faba*	Mainly SP; 4-8% outcrossing	Insects	Isolation 1000 m; bagging; net cage
Grass pea	*Lathyrus sativus*	SP; significant levels of CP		Bagging; Net cage
Hyacinth bean	*Dolichos lablab*	Partially CP;	Insects	Isolation 500 m;
Lentil	*Lens culinaris*	SP		
Lettuce	*Lactuca sativa*	Mainly SP; 1-6% outcrossing	Insects	Isolation 100 m; bagging; net cage

Pollination behavior of some vegetable crops (3)

Crop	Species	Pollinat. type	Pollination mechanism	Method used
Lima bean	*Phaseolus lunatus*	Mainly SP; up to 18% outcrossing	Insects	Isolation; net cage
Melon	*Cucumis melo*	CP	Insects	Isolation 1000 m
Mungbean	*Vigna radiata*	SP		
Okra	*Abelmoschus esculentus*	Partial SP; out-crossing 4-19%	Insects	Isolation 500 m; bagging; net cage
Onion	*Allium cepa*	Mainly CP; protandrous	Insects	Isolation 600 m; net cage w/ pollinator
Garden pea	*Pisum sativum*	Mainly SP		Isolation 100 m
Chili, sweet pepper	*Capsicum annuum*	Often CP	Insects	Isolation 500 m; bagging; net cage
Pigeonpea	*Cajanus cajan*	Normally SP; nat. outcrossing 5-40%	Insects	Isolation 500 m; bagging, net cage
Pumpkin	*Cucurbita moschata*	CP; monoecious	Insects	Isolation 1000 m; bagging & hand pollination; net cage

Pollination behavior of some vegetable crops (4)

Crop	Species	Pollinat. type	Pollination mechanism	Method used
Radish	*Raphanus sativus*	CP; self-incompatible	Insects	Isolation 600 m; net cage w/ pollinator
Safflower	*Carthamus tinctorius*	SP		
Sesame	*Sesamum indicum*	Mainly SP; up to 5% CP	Insects	
Soybean	*Glycine max*	SP		
Spinach	*Spinacea oleracea*	CP; dioecious	Wind	Isolation 2000 m; net cage
Sword bean	*Canavalia gladiata*	Mainly SP		Isolation 100 m
Tomato	*Solanum lycopersicum*	Normally SP; some species self-incompatible		Isolation 50 m; net cage w/ suppl. pollination, if necessary
Watermelon	*Citrullus lanatus*	CP; monoecious	Insects	Isolation 1000 m; bagging & hand pollination; net cage

Regeneration protocol (7)

Construction of field net cage :

Digging holes in the prepared field

Setting up skeleton of net cages

Regeneration protocol (7-1)

- Advantage of field net cage:
- Plant growth normally as in production condition
- More fruit can be set and produce more seed
- Disadvantage of grow materials in field:
- Facility cost is high
- Need move to different field yearly for prevent soil born disease

Regeneration protocol (8)

Covered with PE plastic mulch on planting plot

Regeneration protocol (8-1)

- **Mulch can:**

- Reduce water evaporation of soil

- Reduce impact of heavy rain

- Block growth of weeds

- Reflect solar heat and keep soil temperature cooler.

Regeneration protocol (9)

Sewing nylon net to suitable size

Regeneration protocol (9-1)

- 32strings/in nylon net are used in regeneration field of GRSU/World Vegetable Center

Advantage:

- Permeable is acceptable
- Can block most pollinating insects

Disadvantage:

- Very small insect such as aphid, white fly can not be stopped.

Regeneration protocol (10)

Put and fix door on the skeleton of cage for easier in and out when working on management or morphological characterization

Regeneration protocol (11)

Covering nylon net on skeleton of cages

Fixing nylon net with soil

Regeneration protocol (11-1)

- A whole piece net won't have gap to let insect slipped in the net cage.
- Not tightly fixed on skeleton provide some extra elasticity of net cage to tolerate strong wind.

Regeneration protocol (12)

Complete the construction of field net cage

Should be down at same time with seedling nursing

Regeneration protocol (13)

Make holes on the mulch for planting seedling in the hole

Labels of plots need be settled as well

Regeneration protocol (14-1a)

Transporting seedlings from seedling nursery to growing field.

Be very careful of the young and still weak seedling while moving them.

Regeneration protocol (14-1b)

- Labels in field must be settled down in advance
- Double check of accession number or variety should be done before this movement

Regeneration protocol (14-2a)

Transplanting

Regeneration protocol (14-2b)

- Check again to make should all materials be planted in right cage and right plot.
- Transplant the plantlet in right way to let the plant can grow smoothly and normally.
- Some chemical may need be used for prevent impact from pest(especially the early stage).

Regeneration protocol (15)

Watering for transplanted plantlet

Gentle and carefully watering soil surround the plantlet

Regeneration protocol (15-1)

- **Immediate watering can help the plant recover and stand erectly quickly.**
- **Suitable moisture supply is the first and most important component for plant growing fine.**

Regeneration protocol (16)

Staking the young plants benefits the growth of soft main stems

Regeneration protocol (16-1)

- **Staking the plant with pole can:**

- Support the soft main stem growing up

- Let the plants growing not too spread

- Let plant vein can clime up and easer for management

- Support plant for not lodging when there are heavy fruiting.

Regeneration protocol (17)

Regeneration

Spraying pesticide to control diseases and pest when necessary

Germplasm regeneration can not afford fail

Regeneration protocol (17-1)

- **Be aware the environment protection when using chemical (pesticide and herbicide), fallow the instruction of chemical expert is very important.**
- **Protect your worker when spreading chemical, hat, masks, gloves, boots and frock are necessary.**

Regeneration protocol (18)

Handy Pollination for better fruit and seed setting

Regeneration protocol (18-1)

- Dioecious or dioecious flower crops (ex. Most gourd) need handy pollination.
- Some hermaphroditic crop may also need handy pollination because the special flower structure or growing habit (ex. Cultivar eggplant)

Regeneration protocol (19A)

Release pollinator into net cage to help pollination on some self-incompatibility crops (ex. Part of *brassica* crops)

Regeneration protocol (19A-1)

- **Most common used pollinators are bees and flies**
- **Put bee hive into net cage (close gate of bee hive for 24 hours before re-open in cage)**
- **Bees are used in pollinating Cruciferae, Cucurbitaceae and Solanaceae crops.**
- **Put pupaes of fly into net cage and wait for their eclosion.**
- **Flies are used in *Allium* crops.**
- **The adult flies should be eliminate after pollination completed.**

Regeneration protocol (19B)

Bagging before and after pollination if not planted in net cage.

(protected young fruit from fruit fly)

Won't be necessary if grow in net cage.

Regeneration protocol (20)

Harvesting pumpkin and bottle gourd fruits and store for late mature

Other gourd no need late mature

Pods of legume need dried under shade for easier seed extraction

Regeneration protocol (21-1)

Regeneration

Seed extraction :

Open the fruit and apart seeds from fresh

Regeneration protocol (21-2)

Seed extraction :

Washing seeds out from fruits utricles

Regeneration protocol (21-3)

Seed extraction :

Washed and separating seeds from fruit utricles

Regeneration protocol (22)

Pure seeds were extracted

Regeneration protocol (23)

Put extracted seeds in nylon bag with label and centrifuge to remove the water on seed surface

Regeneration protocol (23-1)

- **While washing wet seeds such as pumpkin, bitter gourd, tomato and chili pepper a plastic label writing identical number with permanent pen should always stay with seed.**
- **When extract dry seeds such as Crucifers, beans, amaranths and kang-kong a label (thick paper or plastic) of identity should keep together with seed.**

Regeneration protocol (24A)

Using a grinding machine on broken chili fruits can avoid touch pungency by hand and save man power

Regeneration protocol (24B)

There is a layer of pectin on tomato seed need be removed before or after seed was extracted.

Regeneration protocol (24-1)

- **The pectin on tomato seed will let seed stick tightly together after be dried.**
- **Broken tomato fruits and put in a seal container for 2-3 days fermentation before washing seed out can remove the pectin safely.**
- **Add some chemical into seed lot after extracted can remove the pectin as well be need to be careful the concentration and treatment time.**

Regeneration protocol (24C)






Seed in dry state fruit (ex. Pod) should be dried then make pod crack for separating seed from pod

Regeneration protocol (24-2)

- The most priority work after seed was extracted is drying seed in terms of orthodox seed.
- One of most ideal condition of storage seed is the seed moisture content between 4 to 7%.
- The seed moisture content is 30-50% (wet extracted seed) or 18-25% (dry extracted seed) both are too high for preserve safely.

Regeneration protocol (25 - 26)

Air dry under shade

1-2 days that make SMC down to 12-15%

Seed cleaning

- Remove foreign material and broken or immature seed.

Regeneration protocol (27 - 28)

Advance Drying

Let seed moisture content reduce to target % (eg. 4-7%)

Packing

Keep dried seed won't re-absorb moisture from air

What is next?

- How to store the seed.

SEED QUALITY & PRESERVATION

Thank you for your attention

Y. K. HUANG

GRSU/WORLDVEG