INTRODUCTION

High quality and certified planting materials are important to start a farm as it will give less management problem and give higher profit to the growers. Producing planting materials is another industry that cannot be neglected. The basis of the production relies on the knowledge in plant propagation.

OBJECTIVE

1. To know the propagation of planting materials through seeds and vegetative
2. To discuss the factors influence the seed germination
3. To discuss the factors influence regeneration of vegetative propagation
4. To know the grafting methods to produce selected plant variety
5. To know the process of planting materials production through tissue culture
Topic 1: Seed production

Important Content

Plants are planted specially for seed production → Hybrid seeds

Activities:
1) Seed harvesting
2) Seed processing
3) Seed storage
4) Seed quality control
5) Seed certification

Seed source:
1. Commercial – seed for vegetables, flowers, turf grass, maize
2. Collected from shade trees, shrubs and herbs
   - Plants which grow or planted at the road side or in the jungle
3. Fruit processing industry

Seed viability:
- The ability for a seed to germinate
- Viability period different according to species/type of seed and environment during storage (temperature and humidity)
  - hot and humid, seed deteriorate faster
  - dry and cool, longer viability period
- Low viability:
  - Incomplete seed formation
  - Seed injury during harvest
  - Improper way of processing or storing

Viability test/Germination test
1. Direct germination
2. Excised embryo
3. Tetrazolium test (TZ test)
4. X-ray analysis
Topic 2: Seed propagation

Important Content

Type of seed

1. **Recalcitrant/short-lived seed**
   - live for a few days or months
   - kept only in moist environment for a short period
   - cannot be dried until the moisture contain less than 30 – 40 %
   e.g. mango, citrus, durian, rambutan, mangosteen, cocoa, coffee and palm

2. **Orthodox seeds/medium-lived seeds**
   - viable for 2-15 years
   - can be dried to low moisture contain (5%)
   - most of the seed for vegetable, flower, turf grass, grain
     e.g. chili, tomato, marigold, zinnia, melon
   - keep in < 12% moisture content and <10°C

3. **Long-lived seeds**
   - live more than 15 years
   - seed contain hard testa, impermeable to water
     e.g. weed seeds
   - Indian lotus (*Nelumbo nucifera*) able to germinate after 1000 years

Seed Dormancy

- Condition within the seed (anatomy/physiology) prevent the seed from germinate although under favorable environment condition
- “Quiescent seed” (non dormant seed) – not germinate under unfavorable environment condition
- **Primary Dormancy**: Dormancy after harvest due to the condition in the seed
  A. **Seed Coat Dormancy**
  B. **Chemical Dormancy**
  C. **Morphological Dormancy**
  D. **Physiological Dormancy**
E. Double (Combinational) Dormancy

- **Secondary Dormancy**: Dormancy due to unfavorable environment condition/Controlled by environmental factors
  
e.g. ~ temperature too high or low
  ~ long dark period (skotodormancy)
  ~ long light period (photodormancy)
  ~ water deficit (water stress)
  ~ oxygen deficit (anoxia)

**Treatment to Break Dormancy**
1. Mechanical Scarification
2. Hot Water Scarification
3. Acid Scarification
4. High Temperature Scarification
5. Warm Moist Scarification
6. Stratification
7. Hormone

**Topic 3: Vegetative propagation**

**Important Content**

Factors Affecting Regeneration of Plant from Stem Cutting

1. **Selection of cuttings**
   a) Physiological state of stock plant
      - N and CHO
        • Plant CHO status
        • Plant nutrient contain
      - Environment: water stress
   b) Etiolation / blanching
      - small leaves, less chlorophyll, shoots elongated
- more IAA  
- high rooting cofactors  
- anatomical changes  
- more parenchyma cells, thin cell wall  

c) Girdling  
- Inhibit CHO movement, reduce basipetal transport CHO, IAA cofactors  
- Promote rooting  

II. **Juvenile factor (age of stock plant)**  
- Physiological age of tree  
- Pruning ~ lengthen the juvenile state  
- Rooting and age  
- Root inhibitors and cofactors  

III. **Types of stem cutting**  
- clonal difference  
- Parts/branch position  
  - Topophysis – effect of the position or age on the type of vegetative growth after rooting/grafting  
  - Orthotropic (upright shoots) – grow vertically  
  - Plagiotropic (lateral shoots) – grow horizontally  
  - Periphysis – long term persistence of position effects  
  - Cyclophysis – long-term persistence of age effects  
    e.g. cuttings from adult phase ~ adult  
    cuttings from juvenile phase ~ juvenile  
- crown position  
- Differences between stem parts  
  Hardwood – basal part of the stem  
    Low rooting %; high CHO  
  Softwood - High rooting %; high auxin co-factors
Environment condition during rooting

- **Water**
  - reduce transpiration, increase humidity
  - environment vapor pressure = vapor pressure in leaf intercell
  - use intermittent mist or mist system
    - causing nutrient leach
      - e.g. CHO, GA, auxin, N,P,K, Ca, Mg

- **Temperature**
  - air temperature and temperature of rooting media (21°C-27°C)

- **Light**
  - Intensity, PPFD (Photosynthetic Photon Flux Density),
    - energy unit 400-700nm µmol m⁻²s⁻¹
  - Photosynthesis

- **Rooting media**
  - Function of media
    - hold cuttings
    - provide moisture / water
    - aeration
  - Influence of media
    - peat –pH (< 7.0), acidic
  - Type or morphology of roots
    - 100% sand –brittle root

Cuttings care

- Prevent from desiccation (drying)
- Harvest cuttings in the morning, soak / wrap in plastic bag
- Wounding

Why rooting enhancer are needed?

After wounding

- More callus and root formation on wounded areas
  - auxin & CHO increased
  - increase respiration
- Ethylene emission enhance rooting
Cuttings take in more H₂O from media
Wounding removed anatomy barrier for rooting

Genetic variation in vegetative propagation
- Mutations in somatic cells, permanent changes
  ~ ‘bud-sports’ or ‘bud-mutations’
- Chimera
  e.g variegated citrus, kekwa, coleus,
  sanseviera, dahlia, hydrangea
  ~ Fruits – citrus (Washington Navel), apple

Origin and Structure of Chimera
- Shoot apex
- Meristem layer
  ~ tunica (L-I, L-II), corpus (L-III)
- L-I ~ anticlinal division
- L-II ~ periclinal division
- L-III ~ random division
Topic 4: Grafting

Important Content

- Grafting (cantuman)
- Budding / budgrafting (cantuman tunas)
- Scion
  - upper part of a graft ~ produce stem / branch
- Stock, root stock (pokok penanti)
  - lower part of a graft ~ form root system

Reason to graft

- Other methods (vegetative and seed) difficult or slow
- Advantageous
  ~ Supply assured
  ~ Purity of clone assured
  ~ Save cost
- Obtained the benefits from the positive characteristics of root stock
- To get a better tree form and better growth
- Shorten the flowering and fruiting period
- Various varieties/clones on a tree

Mango – Chok Anan (MA 224), Malgoa (MA 200), Apple Mango (MA 194), MAHA 65 (MA 165), ‘Nam Doc Mai’ (MA 223), Harumanis (MA 128)

Bougainvillea – various colours

Callus

- Mass of parenchyma cells from wounded tissues
- In graft union
- Origin from cells of scion and root stock
- Parenchyma cells (callus) from scion and root stock form interlock

Formation of Graft Union

- Cambium tissues in scion contact with cambium tissues in root stock
• Callus formation. Cambium of root stock & scion produce parenchyma cells (callus) to fill the gaps between scion & root stock
• Parenchyma cells from callus differentiated to form new cambium cells that connected to the original cambium layer in scion and root stock
• Formation of new vascular tissue (xylem & phloem) by newly formed cambium
  ~ Connection of vascular system between root stock and scion

Vascular Cambium
• Plant tissues in between phloem and xylem
• Meristemic cells able to differentiate and form new cells

Factors affected the healing of grafting
• Incompatibility (Ketidakserasian) - Due to genetic differences between the root stock and scion
  • ~ graft failure
  • ~ % of success very low
  • ~ success graft only last for a short period of time
• Environmental condition during and after grafting - temperature, humidity, and oxygen affecting the callus formation
• Grafting technique - poor grafting method ~ slow recovering of grafting
• Contaminations
  • viruses, diseases and pest

** In general, the more closer the botanical relationship between the plants to be grafted, the higher the successful rate

Grafting for dicotyledon (angiosperm) and gymnosperm
• In between the same clone, Example: D24 grafted to D24
• Same genus, different species
• Graft between genera in the same family - chances to success reduced (some success)

Symptoms of Incompatibility
• Grafting failed (leaves drying and fall)
- Plant wilt
- Grafted plant detached from grafted area

Root Stock and Scion Relationship
- Effect of root stock on scion
  ~ Size control (dwarfing)
  ~ Change of plant behavior
  ~ Production of flower, fruiting and yield
  ~ Disease resistance
- Effect of scion on root stock
  ~ vigor – main effect

Grafting Techniques
- For a success graft
  ~ Root stock and scion compatible
  ~ Cambium of root stock and scion connected
  ~ Suitable physiological condition of root stock and scion
  ~ Avoid wounded parts from drying
  ~ Good maintenance after grafting

Handling Scion
- Collect in the morning
- Vigor, disease free
- Graft as soon as possible
- Storing the scion:
  ~ Avoid expose to light
  ~ Do not keep in air condition room for long period

Types of Grafting
Topworking/topgrafting
- Perform on matured / old tree
- To change the clone / cultivar
- Flower / harvest earlier compared to replanting
- Use suitable grafting or budding methods
Whip & Tongue Graft
- Woody ornamental plant
- For small branch (6-13mm diameter)
- Root stock and scion are in the same size
- Fast healing, easy to success

Wedge grafting
- Commonly practice
- Suitable for most fruit tree and ornamentals
- Easy to success
- Durian, ciku, manggis, nangka, duku etc.

Saddle graft
- Scion and root stock same diameter (< 2.5 cm)

Cleft graft
- Scion properly aligned in cleft
- Wax graft union

Bark graft
- Flap of bark opened to receive scion
- Wax all exposed surfaces
- Tuck bark flap back into place to secure scions
Side graft

- Many variations
  - Stem diameter around 2.5 cm
  - Root stock bigger, scion at the side
  - Ornamental plant
- Stub graft

- Side tongue graft

- Side-veneer graft

- Splice grafting
Double-working (intermediate stock/interstock)

- More than 2 types of plant
- Different type of plant grafted in between root stock and scion
- To overcome incompatibility
- To obtain good characteristic that do not exist in root stock and scion

Approach grafting

- Ornamental plants
- Fruit trees ~ langsat, dokong
- Usually stock/scion planted in pot
Types of Budding

- Budding, Bud grafting (Cantuman mata tunas)
- Use 1 eye bud + bark (kulit kayu) – periderm, cortex, phloem, cambium
- Small stem (< 2.5 cm diameter)
- Fruit trees, roses
- Save grafting materials

Patch Budding

- For plants with thick bark (rubber tree, fruit trees)

T-budding

- Shield budding
- Thin bark layer
- Fruit trees, roses

Inverted T-budding

- Prevent water from collected on grafted area

Chip budding

- Bark layer + wood
Bud wood

- Stock plant – source of bud wood
- Size of stock plant - Depends on the seedlings production, types of plant, clone
- Maintenance of stock plant
  - Fertilization, pest and disease control, weeding
  - Pruning
- Handling:
  - Collect in the morning
  - Soak in water
  - Graft as soon as possible
  - If storing and transportation are needed
  - wrap in damp paper or gunny (guni)
  - place in plastic bag
Topic 5: Tissue culture

Important Content

- The culture of a small portion of plants (embryo, seeds, leaves, stem, shoot, roots, callus, cells) in a certain medium under a sterile condition
- Known as: Micropropagation or In-vitro Culture

Usage of Tissue Culture/ Micropropagation

- Mass production of plantlets
  ~ especially for those that are slow in propagating: Orchids & Palms
  ~ for new cultivar: can meet the high demand in a short time
- Production of plantlets that are free from pathogens (virus, bacteria, fungus)
- All year round production of plantlets

Disadvantages of Micropropagation

- High cost
  - Specialized equipments
  - Man power
- Requires special skills
- Contamination
  - High profit lost in short time
- Variation – The probability of producing plantlets that are different from parents
Types of Vegetative regeneration in Tissue Culture

1. **Meristem Culture**
   - The use of a small portion of meristem tip (0.25-1 mm) as explant
   - Can produce plantlets that are free from virus
   - Fruits (banana), ornamentals (orchids, carnation)

2. **Axillary bud culture** (bud culture)
   - Buds (2mm – 2cm) as explant
   - Growth of axillary buds promoted through control of concentration and ratio of Auxin & Cytokinin (arrest the elongation of shoots and promote the growth of axillary buds)

3. **Adventitious Bud Culture**
   - The formation of adventitious bud through:
     - ‘direct initiation’ – directly from explant
     - ‘indirect initiation’ – from callus cells that formed at wounded explant
   - Explant that can be used:
     ~ Leaf (African violet)
     ~ Bud (orchids, ferns)
     ~ Cotyledon (Conifer)

4. **Organogenesis from callus culture**
   - Formation of adventitious bud &/or roots from a mass of callus cells
   - Callus formed from explant in culture-due to wounding and hormone treatment
   - Explant from various plant organs: seeds, roots, leaves, fruits, stem
   - For tomato, corn, tobacco etc.

5. **Somatic Embryogenesis**
   - Formation of embryo (embryogenesis) from vegetative cells in culture
   - Two ways:
     i) Formation of embryo directly from explant (direct)
     ii) Formation of embryo from the callus that formed from explant (indirect)
• Explant consists of:
  - Cells/Callus that are related to reproductive organs;
  e.g.: - nucelus tissue
  - immature ovule tissue

**Tissue Culture Media**

• Ingredients to sustain the growth
• Ingredients are different according to plants and growing stage
• Components in the media:

  ~ Non-organic materials (mineral salts)
  • Provide macro elements (N,P,K,Ca,Mg) and micro elements (B, Co, Cu, 
    Mn, Zn, Fe)
    - Example: NH$_4$NO$_3$, KNO$_3$, CaCl$_2$, MgSO$_4$, ZnSO$_4$ etc.

  ~ Energy source / carbon
  • Sucrose (2-5%) suitable for most plant
  • Glucose – for some monocot species

  ~ Vitamins and hormones / PGR
  • Vitamin : thiamin, nicotinic acid, pyridoxine, inositol
  • Hormones :
    - Most important– auxin and cytokinin regulate the organ formation)
      Auxin : NAA, IBA, IAA, 2-4-D
      Cytokinins : BA (benzyladenine), Kinetin, 2iP(N$_6$-isopentenyl-adenine),
      Zeatin

  ~ Undefined additives (Natural complexes)
  • Materials which added to enhance the growth in culture
  • Composition → Unknown
  • Example: coconut water (10-20% volume), banana, tomato
Contamination

- Fungus contamination
- Bacteria contamination

Browning (not contamination)

Other Ingredients which can be Used in Culture Media

- Citric / ascorbic acid (antioxidant)
- Activated charcoal
  - to absorb toxic materials in the media
  - (Phenolic compound and carboxylic which excreted from explant)
  - disadvantage – absorb hormones and vitamins
- Anti-contaminants
  - prevent / reduce contamination
  - e.g.: fungicides, anti-biotic (bactericides)

Culture Media

- Solid
  - Use agar
    - e.g. Gelrite
  - Concentration (0.6% - 1%)
  - Not interact with other materials in the media

- Liquid
  - Without agar

pH of the Media

- pH 4.5 – 6.5
- pH < 4.0 – media unable to solidify
  - affect the growth of plantlet

Sterilization

A few sterilization methods:

- Autoclave
  - For media and equipments
- 121 °C
- 15 psi (1.2 x 10⁵ Pa)

- Microwave oven
- Open flame
- Chemical

Chemicals Used to sterile plant materials (explant), equipments and working area

- Calcium hypochlorite (9-10%) 5-30 min
- Sodium hypochlorite (Clorox) (5.25% NaOCl), 5-30 min
- Alcohol (ethyl, methyl, isopropyl) (70-90%)
- Hydrogen peroxide (10-15%) 5-15 min

** Effectiveness depends on concentration and duration

Stages in Tissue Culture Propagation

1. Culture establishment
   
   Objectives:
   
   I. to obtain plant culture for shoot multiplication
   
   II. Control callus and organ formation through manipulation of auxin & cytokinins concentrations

   Might involve:
   
   - Promote the growth of axillary shoots
   - Promote adventitious shoots initiation
   - Promote callus initiation on tissue / explant in culture
   - Choosing suitable media

   Example:

   For axillary shoot formation, use :-
   
   Cytokinins concentration as low as 0.5 – 1mg/L
   
   Low auxin concentration (0.01- 0.1 mg/L) or without auxin

2. Shoot multiplication
   
   Objective:

   to increase the number of plant structure / organ for propagation purposes
• Shoots grow from explant being dethatched and transplanted to new culture media (subculture)

3. **Acclimatization (Preparation for transplant)**

   **Objective:**
   to prepare the plantlets for transplanted from culture media to planting media
   
   **Might involved:**
   • In-vitro rooting~ shoots rooted during culture stage
   • Ex-vitro rooting (out side culture) shoots taken out from culture media and root in rooting media under high humidity
   • Enhance the shoots to become more hardy and resistance to drier condition after transplant (increase sucrose and light intensity)

4. **Transplant to planting media**

   • Rooted shoots being transplanted to planting media and protected from drying (shade and high humidity are given)

**Factors Affecting Successfulness of Tissue Culture**

• **Genetic**
  ~ Plant species that affect the successfulness

• **Microorganisms**
  ~ Contamination of fungus, bacteria, etc.
  ~ explants, equipments, working area need to be sterile
  ~ Types, age, healthiness, vigorous, juvenile, etc.

• **Media and hormones**
  ~ Concentration and ratio of auxin dan cytokinins

• **Surrounding condition of culture**
  ~ Light intensity, temperature