REGENERATION OF TRUE-TO-TYPE STRAWBERRY PLANTING MATERIALS

Milagros R. Dumaslan
Lorelie R. Ollayan
Winsley B. Saytoc, Jr.

RATIONALE

- Tissue culture is an important area of biotechnology that can be used to improve the productivity of planting materials
- Enhanced availability of identified planting stocks with desired traits.
- It can produce true to type selected genotypes using in vitro culture propagation technique.

RATIONALE:

- This technique provides a rapid reliable system for the propagation of large number of genetically uniform disease free plantlets.
- Indexing should be done through the use of biological indexing using the variety Fragaia vesca as an indicator plant to check the different viruses present from the plant.
- To regenerate and produce quality planting materials, tissue culture should be done in order to supply the needs of growers for quality and disease free planting materials.

OBJECTIVE:

General Objective:

The aim of this study is to regenerate and produce virus free planting materials of strawberry through tissue culture.

OBJECTIVE:

Specific Objectives:

- Regenerate and clean old planting materials of different strawberry varieties used by the farmers through the use of meristem.
- Biological Indexing of tissue cultured plantlets through the use of graft indexing using the Fragaria vesca as indicator plant
- Mass produce the indexed plantlets that is free from virus and supply the needs of the farmer

PROCEDURE/METHODOLOGY

Multiplication stage

Rooting stage

Establishment stage

Seeds of Fragaria vesca (indicator plant) and meristem of Strawberry (Sweet Charlie and Festival variety) were inoculated under the laboratory condition using the Modified MS with the addition of 1 ppm of BAP and GA3

Acclimatization stage

Potted plantlets ready for grafting

Grafted indicator plant with tissue cultured strawberry

LABORATORY CONDITION

- Runner tips of the old mother plant of Strawberry variety were gathered from the farmers' field were cultured through the use of meristem technique and seed of Fragaria vesca were inoculated in a fresh culture media and maintain in the culture room with a temperature of 10-24°C.
- The cultures of Strawberry varieties and Fragaria vesca variety were transferred in a proliferation stage. Cultures were subdivided every month until 6 sub culture for shoot multiplication. These cultures were inoculated in a modified MS with the addition of growth regulator which is the Benzyl Amino Purine (BAP) and Gibberellic Acid (GA3).

LABORATORY CONDITION

- After proliferation stage, cultures were transferred into elongation and rooting stage with the same media with the addition of Indole Butyric Acid (IBA) and Activated Charcoal (AC).
- Cultures produced roots were transferred to the greenhouse for acclimatization wherein the cultures were adopt in a normal condition

GREEN HOUSE CONDITION

- Acclimatized and hardened tissue cultured strawberry plantlets inside the greenhouse for one to two months. Then, transfer the acclimatized plantlets to a plastic bag. Strawberry plantlets were maintained in the greenhouse until they produced two or more fully expanded leaves.
- Tissue cultured strawberry varieties were grafted into the indicator plant (Fragaria vesca) to test the cultures if they are free from virus and diseases.

BIOLOGICAL INDEXING THROUGH LEAF GRAFTING

Prune the middle leaf of the indicator plant and the Tissue Cultured Strawberry

Slit the petiole of the indicator plant and insert the trifoliate leaf of tissue cultured strawberry

Tie with parafilm the grafted trifoliate leaf

Grafted indicator plant with tissue cultured strawberry

Indicator plants were grafted with the same size leaves of the tissue cultured Strawberry variety to check if they are free from diseases and viruses

PROCEDURE AND METHOD OF GRAFT INDEXING

1. Prune the indicator plants to two young actively growing trifoliate leaves.
2. Using a scalpel blade, remove the center leaflet of each leaf and make a 1-2 cm vertical split down the middle of the petiole.
3. Remove young actively growing trifoliate leaves from the imported plant to be tested and trim away the two outside leaflets, leaving only the center leaflet and the petiole. Cut the petiole into a wedge shape, trim away about half of the leaflet blade and insert in the split of the indicator plant petiole. Bind the graft firmly with self-adhesive medical tape (e.g. stericrepe) or similar.

PROCEDURE AND METHOD OF GRAFT INDEXING

4. A single plant of each indicator cultivar must be left ungrafted as a negative control each day that grafting is performed.
5. Hold the grafted plants in a mist bed or create a humid atmosphere by covering with a plastic bag or tub for about 1-2 weeks until a graft union has formed.
6. Approximately two weeks after grafting, check that the graft union has successfully healed and that the inoculum is still alive. All graft inoculations must have survived; the graft inoculation must be repeated if the inoculum is dead.
7. Examine grafted plants regularly for symptoms over a 3 month period.

After indexing, mass propagation of quality planting materials will be done in the tissue culture laboratory to cater the needs of the farmers.

DIFFERENT VIRUS AND DISEASES OF STRAWBERRY THAT CAN BE DETECTED BY THE INDICATOR FRAGARIA VESCA *

Fragaria indicator cultivars for graft indexing	***Fragaria indicator cultivar***
Viruses	
Strawberry chlorotic fleck virus	*Fragaria vesca 'EMB' or 'EMK'*
Strawberry latent C virus	*Fragaria vesca 'EMC' or 'UC-5'*
Strawberry mild yellow edge-associated virus	Two of the following indicators: *Fragaria vesca 'UC-4' or 'UC-5' or 'Alpine'*
Strawberry pseudo mild yellow edge virus	*Fragaria vesca 'UC-4' or 'Alpine' or Fragaria virginiana 'UC-12'*
Strawberry vein banding virus	Two of the following indicators: *Fragaria vesca 'UC-5' or 'UC-6' or 'Alpine' or Fragaria virginiana 'UC-12'*
Diseases of unknown etiology	
Strawberry feather leaf disease	*Fragaria vesca 'UC-1' or 'UC-4' or 'Alpine'*
Strawberry lethal decline disease	*Fragaria vesca 'Alpine'*

* Fragaria Post-Entry Quarantine Testing Manual · 6 October 2008

Strawberry crinkle virus

Strawberry chlorotic virus

Strawberry vein banding virus

RESULT AND DISCUSSION:

- Since, there is no available anti sera to use for indexing the tissue cultured plantlets, thus, biological indexing is use as an alternative method of detecting virus of tissue cultured plants using the *Fragaria vesca* as an indicator plant.
- Result showed that, three months after grafted the indicator plants with different tissue cultured strawberry varieties (Whitney, Early Bright, Sweet Charlie (Texas, Argentina), Fern x Festival, Korean variety, Hawaiian variety and Japanese variety) was observed that there were no signs and symptoms of disease and virus appeared in the indicator plants. Tested different tissue cultured strawberry varieties are now planted as mother plant for runner production and will be used for variety trial in the different elevation.
- Meristem is only the technique use to regenerate the old strawberry stock plant.

RECOMMENDATION

- Meristem is the technique use to regenerate the old stock strawberry planting materials using by the farmers.

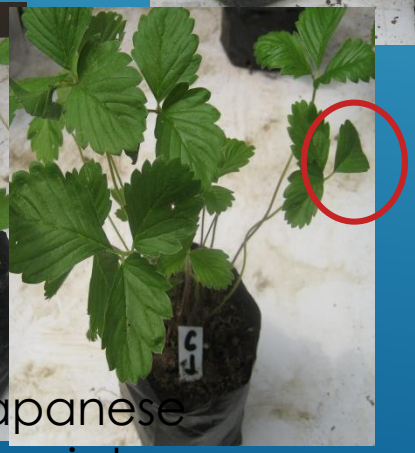
- Indicator plant *fragaria vesca* should be maintain in the tissue culture laboratory for biological indexing of tissue cultured strawberry varieties to test the strawberry plants that are free from viruses.

Argentina

Texas

Whitney

Early Bright

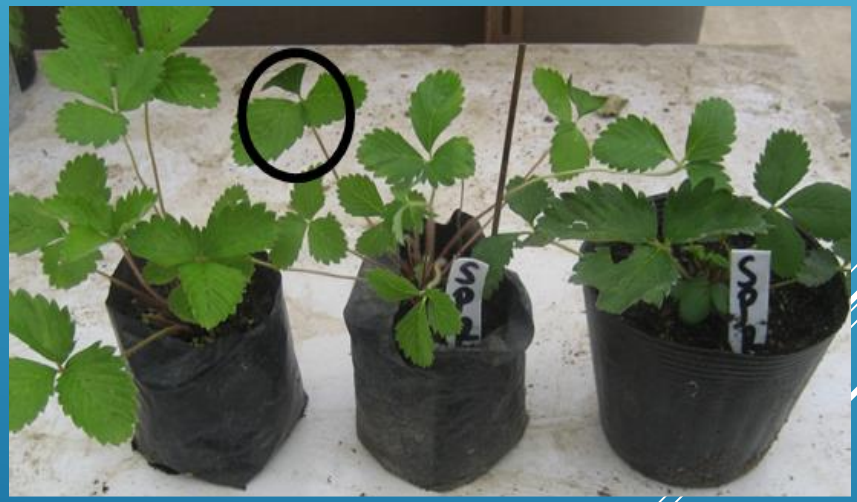


Japanese variety

Fern x Festival

Hawaiian variety

Korean variety



Summer Princess

THANK YOU VERY MUCH FOR LISTENING