COOPERATIVE EXTENSION
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Transitioning Tissue Culture Banana Seedlings from Lab to Field

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Tissue or micropropagation is an artificial method for multiplying plants in a short duration of time using the tissue or cell culture technique in a controlled environment. This method was selected to produce genetically identical, pathogen-free banana plants in Hawaii. Disease-free does not equal disease-resistance to Banana Bunchy Top Virus (BBTV). **Through our program, plants produced via the micropropagation are verified to be clean of BBTV and ready to be planted.**
There are many benefits for the utilization of micro-propagation, including:

Figure 1: Rooted banana plantlets ready to be moved into the nursery.

- An alternative method of vegetative propagation (macro-propagation) for mass propagation
- Production of plants in large numbers can be produced, in a short period of time and can be maintained in small spaces. This helps especially in the case of germplasm shortage.
- New plantlets are genetically identical to its parent material
- The micro-propagation method produces disease-free plants (considering the stock materials have been tested to ensure all propagated seedlings are disease-free as well).
- Fast international exchange of plant material with a minimized risk of disease introduction. The time required for quarantine is lessened by this method.
- The micropropagation technique is also useful for seed production in certain crops when there's a limited number of seeds for conventional propagation techniques.

Micropropagation has many advantages over conventional techniques. However, it's known to be costly due to the need for sterile laboratory conditions and trained technicians. One micropropagated plant in Hawaii can cost $5-10 a plant. Due to the decline in banana production due to BBTV, we wanted to produce and distribute a large quantity of disease-free banana plantlets in a timely manner, based on the local industry needs/request, Dwarf Brazilian Apple Banana was selected for this project. **Disease-free plant materials are different from disease-resistant plant materials. The disease-free plant materials can be infected if not protected from the pathogen. However, disease-resistance plant materials may resist or avoid the infection**.

1) Producing Banana Tissue Culture Plantlets:

The process started by obtaining a clean stock (3-5 ft tall) of banana plants and testing each plant individually for the Banana Bunchy Top Virus (BBTV) using Agdia (http://agdia.com) BBTV testing kit (Figure 2). Selection of stock or parent plants is critical. Do not use plants previously produced from tissue culture. This is important to minimize the chance of producing off-type banana seedlings. The number of seedlings produced from each mother plant and the time involved to produce tissue culture seedlings vary between banana varieties. Banana seedlings were tissued cultured by Hawaii Agricultural Research Center in Kunia, Oahu. The newly produced banana plantlets, which lack a protective layer of wax, were potted and placed under a plastic sheeting to reduce moisture loss (Figure 3-5).

Figure 2: BBTV testing kit and results (one line means negative for BBTV).

Figure 3: Banana plantlets from tissue culture.

Figure 4: Potted banana plantlets.

Figure 5: Plastic sheet been placed over the new potted plantlets.

2) Preparing Banana plantlets for Transplant:

The protective plastic sheet is removed after two weeks and a small amount of a slow-release fertilizer (synthetic or organic) is added to ensure a continuous supply of nutrients. The use of slow-release fertilizer may prevent burn of the small plantlets. After a month, the young plantlets can be placed under partial shade (25-50%) in a nursery to harden them and prepare the plants for the field conditions. Plants benefit from natural sunlight which aids in the photosynthesis process. Continuous monitoring and control of aphids and other pests are important as the plantlets continue to grow in the nursery. After 3 months, the plantlets will be ready to be transplanted into the field.

Figure 6: Banana plantlets under partial shade and over-head irrigation system.

3) Managing Bananas after Transplant:

After transplanting the young banana plants into the field, it is crucial to continue BBTV management efforts which includes field monitoring, aphid management, nutrient management, etc. In addition to obtaining and planting new tissue culture plantlets, farmers are harvesting non-infected banana keikis from the original mat and planting them in new fields (Figure 7 & 8). Implementing a rotation plan by moving clean keikis to a new field will reduce the chance of the entire mat being infected with the virus. The rotation will also help in minimize the number of plants per mat which could help with black leaf streak management. As mentioned earlier, disease-free plants are not disease-resistant. So, the plants may get infected if not managed properly. Utilizing an integrated pest management (IPM) approach to manage BBTV is important.

Figure 7: Keeping banana field clean and less dense.

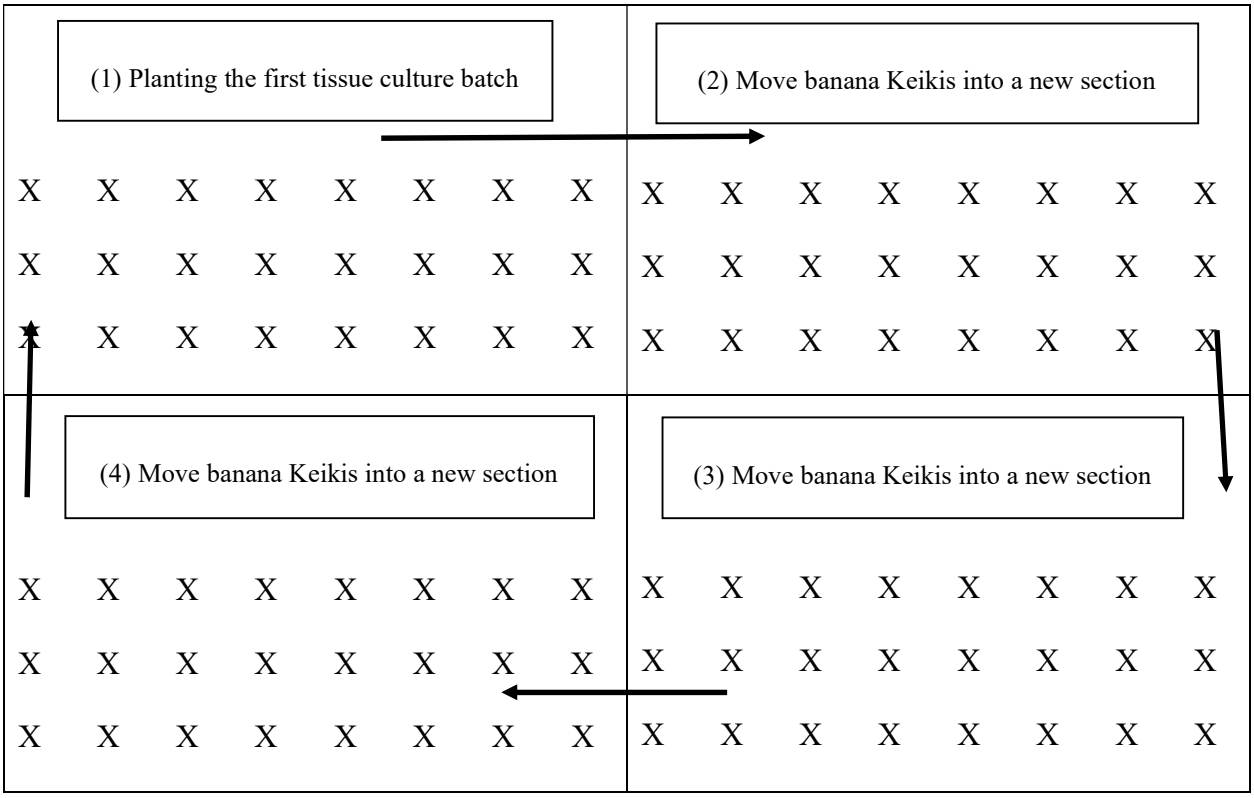

Figure 8: Example of a crop rotation to move banana keikis into new field to control and prevent infection with BBTV.

4) Implementation of a Crop Protection Insecticide Rotation Program:

The use of crop protection chemicals may help to prevent and delay BBTV infection in a field setting. The banana aphid (*Pentalonia nigronervosa*) is the insect vector for BBTV. The use of crop protection chemicals in a rotation will prevent the banana aphid from developing resistance to the chemicals and allow chemicals to be used over a longer duration of time. Below is a list of approved synthetic and organic pesticides (Table 1 & 2) to manage banana aphids on banana. Plants which exhibit signs of BBTV should be removed immediately and destroyed. Consult your local Extension Agent for BBTV verification. Only clean, disease-free plantlets should be used for re-planting.

Table 1: Examples of Currently Licensed Synthetic Insecticides Approved for Use in Banana in Hawaii.

Product Name	**IRAC Group**	**Active Ingredients (A.I.)**	**EPA No.**	**Rate per acre**	
				From	**To**
Admire Pro	4A	Imidacloprid	264-827	14.0 fl oz Soil, 2.8 fl oz Foliar	
Sivanto Prime	4D	Flupyradifurone	264-1141	10.5 fl oz	14.0 fl oz
Movento	23	Spirotetramat	264-1050	16.0 fl oz	
Evergreen Pro 60-6	3A	Pyrethrins+Piperonyl butoxide	1021-1770	2.0 fl oz	12.6 fl oz
Tersus	3A	Pyrethrins	1021-2616	4.5 fl oz	16.3 fl oz

Table 2: Examples of Currently Licensed Organic Insecticides Approved for Use in Banana in Hawaii.

Product Name	**IRAC Group**	**Active Ingredients (A.I.)**	**EPA No.**	**Rate per acre**	
				From	**To**
M-Pede	N/A	Potassium salts of fatty acids	10163-324	1% v/v	2% v/v
Neemix 4.5	UN	Azadirachtin	70051-9	5.0 fl oz	7.0 fl oz
PyGanic EC 5.0	3A	Pyrethrins	1021-1772	4.5 fl oz	15.61 fl oz
Trilogy	UNE	Neem oil	70051-2	1% v/v	2% v/v
Debug Turbo	UN+UNE	Azadirachtin+Neem oil	70310-5	32.0 fl oz	104.0 fl oz

5) Symptoms of BBTV in the Field:

The virus is transmitted by the banana aphid and is also spread through infected planting material. Once infected, plants do not recover, which shows the importance of preventive measures. The typical symptoms of BBTV are very distinctive and readily distinguished from those caused by other viruses of banana. The most distinctive symptoms are:

- Infected plants exhibit a rosetted or ‘bunchy top’ appearance;
- Yellowing and curling of leaf margins on BBTV-infected banana;
- Stunted plants arising from infected suckers, and
- Dark green 'dot-dash' patterns in minor leaf veins forming 'hooks' where they enter the edge of the midrib.

Figure 9: Infected banana with BBTV.
