The Clean Plant Program

Catalina Moyer, Natalia A. Peres and Vance M. Whitaker

Summary

The goal of the Clean Plant Program is to generate clean planting stock of new cultivars from the UF strawberry breeding program and to make this stock available to nursery and fruit growers as quickly as possible. This report summarizes the methods that are utilized to achieve this goal.

Introduction

Cultivars developed by the University of Florida Strawberry Breeding Program are licensed to strawberry nurseries by the Florida Strawberry Growers Association (FSGA). These cultivars are bred to provide the traits needed by the Florida industry. Yet, if a new cultivar has the performance that Florida growers need, but clean planting stock is not available in a timely manner, the benefits of the cultivar to the industry will be limited. Strawberries are susceptible to many pathogens, and it is important to create stock of new cultivars long before the commercial release of the cultivar, given a minimum three-year propagation cycle from mother stock to commercial fruiting plants. The Clean Plant Program is a division of the breeding program that generates the pathogen-tested planting stock that nurseries and fruit growers require.

Tissue Culture

Every year approximately 12 advanced breeding selections go through a tissue culture of meristems to eliminate viruses and other pathogens that may have been acquired during field trials. Potted plants of the selections are grown in a greenhouse over the summer that is well-separated from other greenhouses. Rapidly-growing runners are harvested and processed for meristem culture (Fig. 1). High temperatures in the greenhouse during the summer are favorable for reducing virus replication in the meristems.

Figure 1. Strawberry runners used for meristem culture.

The youngest runner tips are surface disinfected and placed on a clean bench. Under a stereoscope and with a scalpel, thin slices of the runner are cut horizontally until the apical meristem is reached, scooped out and placed in a 13 mm test tube with MS media (Murashige and Skoog salts, vitamins, sugar, agar and growth regulators) (Fig. 2). When meristems develop shoots they are subcultured in multiplication media which contains a low amount of hormones, just enough to induce shoot formation and 30-40 plantlets. Since our goal is not the mass propagation of in-vitro plantlets, hormones are used judiciously and in-vitro multiplication is limited.

Figure 2. Meristem excision under a clean bench.

During multiplication, shoots are transferred into fresh media every four weeks (Fig. 3). They are also tested for bacteria and fungi using four different types of media that provide the right conditions for their growth. The number of subcultures during multiplication are limited to 10 to reduce the risk of somaclonal variation which can produce off-types.

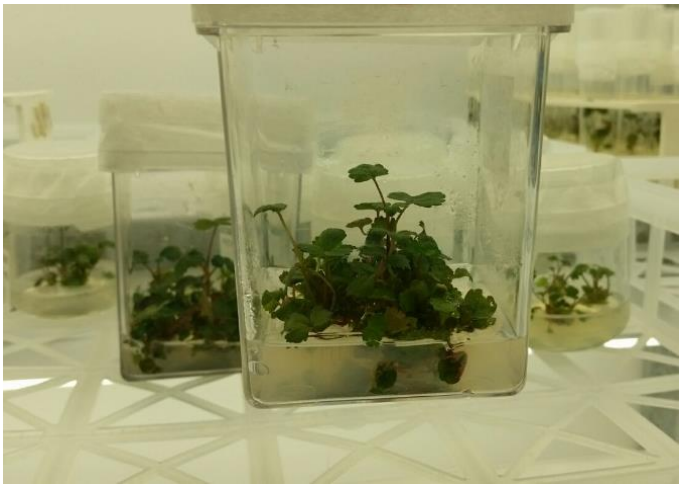

Figure 2. Strawberry shoots during multiplication.

Shoots that test negative for bacteria and fungi are divided, and clumps of plantlets are sub-cultured into rooting medium where they grow taller and form roots before they are taken out of in-vitro conditions (Fig. 3).

Figure 3. Strawberry in-vitro plants, from meristem (left) to fully developed plantlet (right).

Acclimatization and Virus Testing

Rooted plants in test tubes are placed in a growth room in cell trays filled with soilless peat-based media inside a humid chamber (Fig. 6). These plantlets need to be slowly acclimatized for a successful transition from in-vitro to ambient conditions. The plants develop in the growth room for a minimum of 8 weeks before virus testing procedures begin.

Figure 4. Acclimatization of meristem plants, from test tubes to plants in soilless media.

Testing for viruses occurs multiple times throughout the process and using different techniques. At first, the advance breeding selections are tested when plants are actively growing and producing runners. Before harvesting runners for

meristem culture, leaf samples from these plants are collected and sent to testing facilities for a multi-virus screen using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). Only plants that test negative are used for meristem culture. Secondly, leaf samples from meristem plants that have been acclimatized are collected for an ELISA test performed at GCREC. Meristem plants with negative results are sent to a commercial nursery where they undergo the certification program of the California Department of Food and Agriculture (CDFA). At CDFA plants are tested for multiple viruses by grafting onto biological indicators, as well as by PCR.

Verification Trials

Meristem plants are propagated via runners and placed in verification trials (Fig. 5) at GCREC, where performance is evaluated for multiple years. The strawberry breeder and the breeding staff regularly monitor the trials to observe any diseases or other problems. DNA markers are employed along with visual observations to confirm that the plants are true-to-type, meaning that there are no mix-ups or mislabeling of cultivars. Only the meristem sources without defects are released to nurseries for further multiplication.

Figure 5. A verification trial at UF/IFAS GCREC.

Stock Distribution

Meristem plants of new cultivars are distributed to multiple nurseries under transfer agreements and/or licensing contracts. The propagation cycle at the nursery level takes a minimum of three years, as the mother plants coming from tissue culture are juvenile and thus more vegetative and slower to flower and fruit than third-generation plants. Vegetative characteristics are an advantage in the nursery where runner production, not fruit production, is the goal. A single meristem plant can produce about one million plants in a three-year cycle or 20 million plants in a four-year cycle depending on nursery conditions and practices.

Thank You

The Clean Plant Program is an important bridge between the breeding program, the strawberry nursery industry and the Florida strawberry industry. We would like to thank the Florida Strawberry Research and Education Foundation for their ongoing support for this effort.

Contact

Dr. Vance M. Whitaker
UF/IFAS Gulf Coast Research and Education Center
P: 813.419.6608
E: vwhitaker@ufl.edu
http://gcrec.ifas.ufl.edu/faculty/dr-vance-m-whitaker/