Foundation Plant Services

Guide to the Strawberry Clean Plant Program

CONTENTS:

MERISTEM PLANT PRODUCTION	2
STRAWBERRY VIRUSES	5
WHY TISSUE CULTURE IS USED	5
DISEASE TESTING	6
DNA FINGERPRINTING	8
REFERENCES	9
ORDERING	10
UC DAVIS LICENSING PROGRAM	10



Foundation Plant Services University of California One Shields Avenue Davis, California 95616-8600

Phone: (530) 752-3590 Fax: (530) 752-2132 Email: fps@ucdavis.edu Web: http://fps.ucdavis.edu

October 2008

UCDAVIS

For over 50 years, the Department of Plant Sciences Pomology Program at the Davis campus of the University of California (UC) has been the home of one of the most successful strawberry breeding programs in the world. Cultivars developed by the Plant Sciences Department are patented and licensed by UC Davis Technology Transfer Services, and maintained at and distributed by Foundation Plant Services (FPS).

Foundation Plant

Services

Maintenance and distribution involves annual disease and identity testing, production and distribution of tissue culture meristem tip explants, and distribution of conventionally propagated plants produced off-site by special agreement. FPS tests and maintains advanced selections produced by UC strawberry breeders, ensuring the smooth release of disease-tested propagating stock when new cultivars are introduced. Virus testing and therapy services are also available for domestic non-UC cultivars.

This publication describes the University of California strawberry licensing program and the process and procedures for establishing and maintaining healthy Foundation strawberry planting stock.

ABOUT FOUNDATION PLANT SERVICES

Foundation Plant Services is a self-supporting department in the College of Agricultural and Environmental Sciences at the University of California, Davis. The FPS mission is to produce, test, maintain and distribute disease-tested plant propagation material; to provide importation, quarantine, virus testing and virus elimination services; to coordinate release of UC-patented plant cultivars; and to link researchers, nurseries and growers.

FPS has grape, strawberry, fruit and nut tree, sweet potato and rose programs; each is funded by the industry it serves. FPS is accredited by the California Department of Food and Agriculture registration and certification programs for grapevines, strawberries, and fruit and nut trees. Foundation level stock of all University of California-patented strawberry, grape, fruit tree and nut tree cultivars is grown and distributed by FPS to licensees.

Every year, visitors from around the world come to FPS to view the state-of-the-art facilities and inquire about the clean plant programs. An advisory committee comprised of members from UC, nurseries, growers and the California Strawberry Commission provides the guidance and financial support that makes the strawberry program a strong and vital part of California agriculture.

Guide to the Strawberry Clean Plant Program at Foundation Plant Services

Mother plants are heat treated at 37° C for three to four weeks in

a heat chamber.

Production of Meristem Plants

A 'meristem plant' is the term used in industry to refer to a plant grown in tissue culture from a meristem tip. The main reasons for producing meristem plants are to eliminate pathogens from planting material and to reinvigorate plants. Meristem plants produce many more daughter plants than are produced by conventionally-propagated plants.

A 'meristem tip' consists of meristem tissue plus one or two leaf primordia, and is approximately 0.4 to 0.5 mm in size. Meristem tissue is found in young growing points where cells are dividing but have not yet differentiated into leaves, stems or other organs.

The following steps describe the process of producing strawberry 'meristem plants.' The process—from mother plant to meristem plant in potting soil—takes approximately one year.

1. Mother plants

The original strawberry plant material for a UC cultivar is provided by the UC strawberry breeder before release, and is tested repeatedly over several years before becoming a mother plant (also known as nuclear stock). Mother plants are propagated conventionally by runner propagation throughout the year.

2. Annual Disease Testing

To maintain their certification status, mother plants of each cultivar are inspected and tested annually for viruses using strawberry biological indicators and herbaceous hosts, in accordance with the California Department of Food & Agriculture's (CDFA) Registration & Certification Program. They are also tested for viruses and other pathogens by polymerase chain reaction (PCR).

3. DNA Identification

The cultivar identity of each mother plant from which meristem tips are excised is verified using DNA 'fingerprinting' marker technology for quality control (details on page 8). Leaves are sampled prior to heat treatment.

4. Heat Treatment

Mother plants with attached daughter plants are placed into a heat treatment chamber for a minimum of 3 weeks at a constant 37°C, 70% relative humidity and a 16-hour daylength. A strong plant with well-established roots is required to survive this treatment.

5. Tissue Culture

Daughter plants are harvested and meristem tips are excised from terminal and axillary buds on the crowns. Surface sterilization is not necessary if care is taken when removing the outer leaves. Each meristem tip is excised aseptically, in a laminar airflow transfer hood using a scalpel and forceps under an 8–100x zoom stereoscope. The scalpel is flame sterilized frequently. Meristem shoot tips are gently placed on MSI medium (half-strength Murashige and Skoog salts and vitamins, 2 % sucrose, 1mg/l IAA, 0.6% agar pH 5.8) on which they will usually form shoots and roots.

Mother plants are maintained in a greenhouse with double doors,

Mother plants are maintained in a greenhouse with double doors, a footbath and insect mesh over intake vents. Entry is restricted to a limited number of authorized people.



Leaf samples of each mother plant are dried for DNA fingerprinting to verify identity.





Daughter plants after heat treatment.



Viewing through a stereoscope, meristem tips are aseptically dissected from daughter plant buds.



After roots form and the explant is approximately one cm. tall, the meristem plant is transferred to a maintenance medium without growth hormones (Murashige and Skoog salts and vitamins, 3% sucrose, 0.2% Phytagel®, pH 5.8). The plants are transferred to fresh medium every four to eight weeks.

Plants destined for out-of-state or foreign countries are usually shipped *in vitro* (in tubes). Nurseries have the option of buying plants *in vitro* or established in soil if they can arrange to pick them up at FPS. Plants can also be stored in MS in tubes wrapped with Parafilm® at 4° C for one to two years.

On MSI medium, we find that plants produce no or very little callus tissue. These strategies minimize the risk of generating an off-type plant. Similar conclusions were reached on a similar medium by Sobczykiewicz (1979). Occasionally, plants with leaf variegations or white-streak chimeras are observed in tubes or soil and are discarded.

The percent of meristem tips that successfully grow depends on cultivar and technician dexterity among other factors. Success averages 30 to 50%, although the range is as low as 10% for some cultivars such as 'Seascape,' and can reach 90– 100% for other cultivars such as 'Camarosa' and 'Albion.'

As daughter plants typically each have three to six meristem shoot tips, a mother plant that has 30 attached daughter plants can have more than 100 meristem shoot tips. The FPS program minimizes the possibility of producing a variant type plant by producing only a single meristem plant from a meristem shoot tip; each mother plant can therefore be expected to yield approximately 100 meristem plants.

Meristem tips and a flower bud on an apical tip. An excised meristem tip is approximately 0.4 mm and consists of the meristematic dome and one or two leaf primordia.



Left: Meristem tips are placed on tissue culture medium for 3 to 5 months, at which point they can be shipped, stored or transferred to soil.

Right: Meristem plants at FPS in a growth chamber, where the environment is set at 23°C, 70% humidity and a 16-hour daylength.



6. Acclimation

Plants for in-state orders are transferred from tubes to 2-inch



pots with sterile potting mix and then gradually acclimated to ambient humidity in a humid chamber. The acclimation is critical and takes two to three weeks.

7. Greenhouse

Meristem plants are transplanted to 4-inch pots and moved to a greenhouse. They are inspected by the California Department of Food and Agriculture prior to being picked up by customers.





Strawberry Viruses

Strawberries are affected by over 30 viruses and phytoplasmas, many of which can greatly reduce yield, rapidly spread in the field, and may not cause obvious symptoms. Strawberry mottle virus (SMoV), Strawberry crinkle virus (SCV) and Strawberry mild yellow edge virus (SMYEV) are some of the most common viruses of strawberries.

Some severe strains of SMoV can reduce yield as much as 30%. Often, modern cultivars are symptomless when infected with just one virus but in a mixed infection with more than one virus, a decline disorder can develop in which the leaves turn red, and plants weaken and may die (Martin and Tzanetakis, 2006).

Symptoms of virus infection in susceptible indicator strawberry species can be very mild or severe, and include leaf distortion crinkling, and flecks or a mottled pattern. SMoV, SCV and SMYEV are aphid-transmitted; other viruses, including strawberry pallidosis associated virus (SPaV) and beet pseudo-yellows virus (BPYV), are whitefly-transmitted; and another group, including tomato ringspot virus (ToRSV), strawberry latent ringspot virus (SLRSV), and Arabis mosaic virus (ArMV), are nematode-transmitted.

One of the best ways to minimize loss due to virus infection is to plant healthy stock that has been certified by the California Department of Food & Agriculture or a similar government agency.



In a commercial field, the Ventana plant on the left shows decline symptoms due to infection by several viruses. Photo courtesy of R. R. Martin.



Leaf distortion and chlorotic flecks are some of the symptoms of virus infection, revealed here in a leaf of the indicator plant *Fragaria virginiana*.

WHY TISSUE CULTURE IS USED FOR STRAWBERRY CLEAN STOCK

At FPS, tissue culture is used to eliminate viruses and other pathogens and to reinvigorate strawberry plants for increased runner production in nurseries. Multiplication of plants in tissue culture is avoided to minimize chances of variant type plants.

The use of tissue culture for the elimination of strawberry viruses was first reported in the 1960s and is used by certification programs worldwide. It is a common technique used successfully in many crop plants. Combined with heat treatment, tissue culture has been very successful in producing healthy strawberry planting stock. For many years at FPS, all of the plants produced from virus-positive mother plants have tested negative for virus after treatment.

Research and experience has shown that, even when viruses are not detected, a plant that has been through heat treatment and tissue culture is more vigorous and can produce many more daughter plants than conventionally propagated plants. One explanation is that this is because the plant regains juvenile traits which are thought to have a more vegetative rather than a fruiting growth habit. Runner productivity of tissue culture derived plants can be expected to be at least 50% more than conventionally propagated plants; reports range from no increase to 400% more (Swartz, et al. 1981; Scott et al. 1985; Merkle 1993).

One 'meristem plant' from the FPS program can bear between 300 to 800 daughter plants in one season in a screened house, depending on cultivar and growing conditions; up to 2000 daughter plants from one 'meristem plant' has been reported. This is in contrast to conventional runner propagation, in which each plant produces approximately 100 daughter plants in a season (C. Gaines, personal communication).

Multiplication of strawberry plants in tissue culture has mixed acceptance and is not used at FPS. Its advantages are reduced generation time and reduced exposure to insects and disease (Moisander et al. 2006). However, problems with variant types, especially a hyperflowering trait, have been documented (Jemmali 1995). These problems can be addressed by restricting the number of subcultures, reduced hormones in media, and fruiting trials of plants produced in tissue culture. (Martinelli 1992, Boxus et al. 2000).

Disease Testing

Each cultivar and advanced breeder selection is tested annually for over 15 pathogens (see list on opposite page). Three standard methods are used to detect pathogens: graft indexing, herbaceous host indexing and polymerase chain reaction (PCR). These tests complement and serve as checks for each other. The graft and herbaceous host indexes are capable of screening large numbers of plants relatively inexpensively for the presence of a wide range of viruses. PCR is rapid, highly sensitive, and can identify which virus is present but requires expensive equipment and reagents, specific primers, and highly skilled technicians.

Graft index -

Graft indexing is currently the only test required for the CDFA Registration and Certification Program. Each plant to be tested, referred to as the candidate plant, is grafted into two virus-sensitive strawberry species used as indicator plants for strawberry viruses: *Fragaria vesca* (clone 'UC4' or 'UC5') and *F. virginiana* (clone 'UC10' or 'UC11').





The leaf blade of the candidate plant is trimmed and the petiole is cut into a wedge. The center leaflet on an indicator plant is removed and the petiole split to receive the leaflet from the candidate plant. Three grafts are placed on each indicator plant. The petiole is wrapped and the indicator plant is kept under mist to allow grafts to heal. If the candidate plant is virusinfected, symptoms appear on the new growth of the indicator plant in approximately one month.

Herbaceous host index.

Herbaceous host indexing is employed to test for the presence of sap-transmissible viruses. Four virus-sensitive herbaceous species—*Chenopodium amaranticolor, C. quinoa, Cucumis sativus* and *Nicotiana clevelandii*—are used.



Young leaves of candidate plants are ground in buffer. Then, this plant extract is rubbed onto indicator plants pre-dusted with carborundum. This transmits virus particles that may be present in the candidate plant to the indicator plant.



Indicator plants are incubated in a greenhouse. If the candidate plant is infected, symptoms appear in indicator plants in approximately 10–14 days.

Polymerase chain reaction (PCR) tests

Candidate plants are PCR tested for viruses, phytoplasmas and *Xanthomonas fragariae*. As more is known about the molecular biology of pathogens, and primers become available, tests are added to the program.

Small tubes, each containing one plant sample and PCR test reagents for one pathogen, are put in a thermocycler for three hours.

Reaction products are loaded into an agarose gel for gel electrophoresis lasting about one hour.

The gel is stained with a UV-sensitive stain and photographed with UV light.

If the candidate plant is infected, a bright band appears where stain binds to virus-specific nucleic acid. Each column represents one virus test for one plant sample. PCR test results can be obtained in one day.







PCR gel results show a bright band (marked with white circle) for a sample testing positive for Strawberry crinkle virus (SCV).

Strawberry Pathogen Testing at FPS

Pathogen	Pathogen Type	Detection Test(s)
Aphelenchoides besseyi (Strawberry crimp nematode)	Nematode	Visual inspection
Arabis mosaic virus (strawberry strain) (ArMV)	Virus	PCR, Herbaceous host index
Phytophthora fragariae var. fragariae (Red stele root rot)	Fungus	Visual inspection of in-vitro meristem plants
Phytoplasmas	Phytoplasma	PCR
Raspberry ringspot virus (RpRSV)	Virus	Herbaceous host index
Strawberry crinkle virus (SCV)	Virus	PCR, Graft index
Strawberry feather leaf virus	Virus	Graft index
Strawberry latent "C" virus (SLCV)	Virus	Graft index
Strawberry latent ringspot virus (SLRSV)	Virus	PCR, Herbaceous host index
Strawberry leaf roll virus	Virus	Graft index
Strawberry mild yellow edge virus (SMYEV)	Virus	PCR, Graft index
Strawberry mottle virus (SMV)	Virus	PCR, Graft index
Strawberry pallidosis virus	Virus	PCR, Graft index
Strawberry vein banding virus (SVBV)	Virus	PCR, Herbaceous host index
Tobacco necrosis virus (TNV)	Virus	Herbaceous host index
Tobacco ringspot virus (TRSV)	Virus	Herbaceous host index
Tobacco streak (TSV) = Strawberry necrotic shock virus (SNSV)	Virus	PCR, Graft index
Tomato black ring virus (TBRV)	Virus	Herbaceous host index
Tomato bushy stunt virus (TBSV)	Virus	Herbaceous host index
Tomato ringspot virus (ToRSV)	Virus	Herbaceous host index
Xanthamonas fragariae (angular leafspot)	Bacterium	PCR

Strawberry DNA Fingerprinting for Identity

New strawberry cultivars are regularly introduced to the strawberry industry, with no single cultivar dominating the market. More than 70 percent of the strawberries produced in California are from UC Davis cultivars. Strawberry growers rely on UC Davis Foundation Plant Services (FPS) to provide nurseries with virus-tested, correctly identified planting stock of the newest and best UC-patented strawberry cultivars. Historically, there has been no objective screening test to accurately distinguish cultivars. In response to industry and consumer needs, FPS scientists developed a DNA "fingerprinting" methodology to correctly identify strawberry cultivars.

In the fingerprinting process, DNA is extracted from fresh strawberry leaves, then specific marker DNA is amplified using polymerase chain reaction. The amplified marker DNA for each strawberry cultivar is unique, just like a human fingerprint. By analyzing the marker DNA and comparing the results to a newly developed database of strawberry DNA fingerprints, researchers can determine a strawberry plant's varietal identity.

Simple Sequence Repeats (SSRs) are areas in the DNA of an organism in which the same small nucleotide sequence, usually consisting of two to six base pairs, are repeated many times. SSRs are abundant in many organisms, are widely used for genetic studies, and several, in combination, can distinguish closely related cultivars within a species. SSRs are also called microsatellites.

All mother plants that are used to produce meristem plants are DNA tested to confirm correct cultivar identification. An efficient and reliable DNA fingerprinting scheme using Simple Sequence Repeats (SSRs) was developed by FPS in cooperation with the USDA and made publicly available. Strawberry plant samples can now be sent to private labs to verify their identity. Nurseries are encouraged to check their stock regularly for varietal correctness.

Other biochemical and molecular marker systems have been used for strawberry cultivar identification, including isozymes (Bell and Simpson, 1994), Randomly Amplified Polymorphic DNA (RAPD) markers (Degani et al. 1998; Garcia et al. 2002; Hancock et al. 1994), Amplified Fragment Length Polymorphism (AFLP) markers (Miroslaw et al. 2002), and Inter Simple Sequence Repeat (ISSR) markers (Arnau et al. 2002). Each of these systems has technical limitations; all have known problems with reproducibility of data among labs.

Simple Sequence Repeats have been widely used for cultivar identification in grape, walnut and other clonally propagated crops (Dangl et al. 2001; Dangl et al. 2005), but SSR markers take considerable time and expertise to develop (Lewers et al. 2005). Application of this technology to strawberry is particularly difficult because strawberries are octaploid, with eight copies of each gene, and because inbreeding can lead to cultivars with multiple copies of the same allele at a single locus.



DNA is extracted from plant tissue, which is first ground to a fine powder. Dried leaves are routinely used, however any tissue will work: fresh or dried leaves, flowers, fruit, calyx, roots or stems.

Since SSR technology can detect only the presence or absence of an allele, but not copy number, there are often several genetic combinations that could lead to a particular profile.

This SSR marker system can distinguish between closely related UC-patented strawberry cultivars. Previously published markers were used to reduce development time; amplified fragments are scored as dominant markers. Because primer binding is highly specific, this system is both highly accurate and reproducible by other laboratories.

The basic procedure is to collect, dry and extract DNA from young leaves, although other tissues can also be used. The DNA is amplified using one optimal PCR condition. The PCR products are loaded into the genetic analyzer which results in a pattern of peaks that are scored as present or absent. The process is fast (about two days are required), accurate and very reproducible. The disadvantages are that it requires highly skilled technicians and expensive reagents and equipment.



Samples are placed into the genetic analyzer, which records the time required for fluorescent-labeled marker DNA to travel through the capillary tubes, past a laser and camera.



Results from five different strawberry samples; the red peaks in each sample are size standards. The length of the marker DNA is determined using a size standard to convert travel time into fragment lengths; smaller fragments travel faster than larger ones. Strawberry cultivars are differentiated by comparing the lengths of their amplified markers.

Sixteen previously published primer pairs from strawberry were tested and described in A New System for Strawberry Cultivar Identification Developed at Foundation Plant Services (FPS). (Dangl, G.S. et al. 2007) This and other publications are available at http://fps.ucdavis.edu. Six primer pairs amplified consistently as well as produced variable peak sizes over our study set of 45 commonly grown strawberry cultivars and breeder's selections. One primer pair, ARSFL-09, had the most peaks and generated unique profiles for 29 of the 45 accessions. The remaining 16 fell into eight profile groups of two cultivars each, which were easily resolved with any one of three other primer pairs. For added confidence, strawberry samples at FPS are routinely tested at all six selected markers.

Although the octaploid nature of strawberry complicates interpretation, this system can reliably identify strawberry cultivars at an early developmental stage and distinguish between closely related UC-patented cultivars. This process is used routinely for DNA identity verification of all FPS strawberry mother plants. Furthermore, the SSR technology developed at Foundation Plant Services is available to the public, and private labs are encouraged to use the system.

References

- Arnau, G., J. Lallemand and M. Bourgoin. 2002. Fast and reliable strawberry cultivar identification using inter simple sequence repeat (ISSR) amplification. Euphytica 129:69-79.
- Belkengren, R.O. and Miller, P.W. 1962. Culture of apical meristems of *Fra-garia vesca* strawberry plants as a method of excluding latent A virus. Plant Dis. Rep. 46:119-121.
- Bell, J.A. and D.W. Simpson. 1994. The use of isoenzyme polymorphisms as an aid for cultivar identification in strawberry. Euphytica 77:113-117.
- Boxus, P., A. Jemmali, J.M.Terzi and O. Arezki. 2000. Drift in genetic stability in micropropagation: the case of strawberry. Acta Hort. 530:155-161.
- Converse, R.H. (ed.) 1987. Virus and virus-like diseases of *Fragaria* (strawberry. Pages 1-100 in: Virus Diseases of Small Fruits. United States Department of Agriculture, Agricultural Handbook No. 631, 277 pp.
- Dangl, G.S., M.L. Mendum, B.H. Prins, M.A. Walker, C.P. Meredith and C.J. Simon. 2001. Simple sequence repeat analysis of a clonally propagated species: A tool for managing a grape germplasm collection. Genome 44:432-438.
- Dangl, G.S., K. Woeste, M.K. Aradhya, A. Koehmstedt, C. Simon, D. Potter, C.A. Leslie and G. McGranahan. 2005. Characterization of fourteen microsatellite markers for genetic analysis and cultivar identification of walnut. J. Amer. Soc. Hort. Sci. 130(3):348-354
- Dangl, G.S., E.W. Lee, S.T. Sim and D.A. Golino. 2007. A New System for Strawberry Cultivar Identification Developed at Foundation Plant Services (FPS). Proceedings of the 6th North American Strawberry Growers Conference and North American Strawberry Symposium, Ventura CA, pp. 118-121.
- Jemmali, A., P. Boxus, C. Kevers and T. Gaspar. 1995. Carry-over of morphological and biochemical characteristics associated with hyperflowering of micropropagated strawberries. Plant Physiol. 147:435-440.
- Martin, R.R. and I.E. Tzanetakis. 2006. Characterization and Recent Advances in Detection of Strawberry Viruses. Plant Disease 90:384-396.
- Martinelli, A. 1992. 'Micropropagation of Strawberry (*Fragaria* spp.) in Biotechnology in Agriculture and Forestry Vol. 18 High-Tech and Micropropagation II ed. Y.P.S. Bajaj. Springer-Verlag. Berlin. Pp. 354-370.
- Merkle, S. 1993. Yield and other quantitative characters of strawberry plants micropropagated on media with different phytohormone contents. Acta Hort. 348:403-413.
- Miller, P.W. and R.O. Belkengren. 1963. Elimination of yellow edge, crinkle and vein banding viruses and certain other virus complexes from strawberries by excision and culturing of apical meristems. Plant Dis. Rep. 47: 298-300.
- Moisander. J. and M. Herrington. 2006. Effect of micro-propagation on the health status of strawberry planting material for commercial production of strawberry runners for Queensland. Acta Hort. 708:271-273
- Scott, D.H., G.J. Galletta and H.J. Swartz. 1985. Tissue culture as an aid in the propagation of 'Tribute' everbearing strawberry. Advances in Strawberry Production 4:59-60.
- Sobczykiewiecz, D. 1979. Heat treatment and meristem culture for the production of virus-free strawberry plants. Acta Hort. 95:79-82.
- Strand, L. (ed.) 2008. Integrated Pest Management for Strawberries, 2nd Edition. University of California Agricultural and Natural Resources Publication 3351. 174 pp.
- Swartz, H.J., G.J. Galletta, R.H. Zimmerman. 1981. Field Performance and Phenotypic Stability of Tissue Culture-propagated Strawberries. J. Amer. Soc. Hort. Sci. 106(5):667-673.



Ordering Information

It requires approximately one year from ordering plants to receiving them. A license agreement must be obtained, and depending on the destination, import permits, phytosanitary certificates, and checking for quarantine compliance testing may also be needed. Plants are normally produced in the fall for spring sales. Other delivery times are available as existing inventory allows. Plants are custom made to order; however, there may be a few extra plants available. The general steps are listed below. Order forms and more information are available on the FPS website. Please contact FPS to discuss your individual needs.

- 1. Obtain license to propagate. Most strawberry cultivars at FPS are patented by the University of California (UC) and can be supplied only to those who are licensed by UC Davis Technology Transfer Services. To request a license, please contact Mr. Michael Carriere by email at *mdcarriere@ucdavis.edu* or by phone at (530) 754-8462. The application process requires approximately four to six months. UC cultivars that are off-patent can be distributed without restriction in the United States, but may still have restrictions outside the U.S.
- 2. Submit signed order form to FPS with the appropriate pre-payment. Plant orders are due on July 1 for receipt the following Spring. Please indicate the approximate date you prefer to receive your plants. All attempts will be made to meet your preferred date. Meristem plants are available to be picked up in tissue culture tubes (*in vitro*) or in potting medium ("in soil") in 4-inch pots. Please specify "*in vitro*" or "in soil." Strawberries shipped to other states and countries are exclusively *in vitro*.
- 3. For materials destined outside the U.S., obtain import permits and check quarantine regulations.
- 4. FPS will contact you close to the date the plants will be ready to arrange for pick up or shipping.
- 5. Please contact FPS if you notice any problems upon receipt of your plants.

The UC Davis Strawberry Licensing Program

The strawberry improvement program located at the University of California, Davis focuses on cultivar development for the California strawberry industry. At UC Davis, strawberry cultivars are developed for the cool coastal Mediterranean and arid subtropical regions of California. However, due to climatic similarity with other strawberry growing regions, UC Davis cultivars are popular worldwide and have become the basis of a global fresh-market strawberry industry. UC Davis strawberry cultivars represent 70%-75% of the production of the US\$1.6 billion California strawberry industry and 50%-60% of worldwide commercial production. Due to the significant market-presence of the cultivars, the University of California has implemented a worldwide technology transfer and licensing program for its strawberry cultivars. This article summarizes the successful, intellectual property (IP)-based UC Davis strawberry licensing program.

The licensing program is active in the United States, Europe, Asia, Africa, South America, and Australia. In the United States, newly developed strawberry cultivars are protected under U.S. plant patents administered by the U.S. Patent and Trademark Office (PTO). A U.S. plant patent is available for asexually propagated plant species, while plant variety protection certificates administered by the U.S. Department of Agriculture (USDA) are reserved for the protection of sexually propagated species.

In areas outside the United States, the most common form of IP is UPOV-compliant Plant Breeders' Rights. UPOV, the Union for the Protection of New Varieties of Plants, has set forth standards for plant-based IP. In many parts of the world, IP applied to plants is a new legal construct, and UP-OV-compliant protection is unavailable. As a result, the UC Davis licensing program and its licensees are active in expanding the scope of protection for plants in these areas.

In the United States and Canada, varieties are licensed on a nonexclusive basis directly to plant nurseries. Nurseries are licensed the right to propagate plants and to sell the propagated daughter plants to fruit growers. Strawberry growers annually replant fruiting fields, so a royalty is collected annually. Royalties are assessed on a per-1,000 plants basis rather than on the basis of sales.

Outside of the United States and Canada, the licensing program relies on business partners as an intermediary in support of the strawberry licensing program. These partners, referred to as master licensees, are provided with exclusive rights within a defined territory. The master licensee is granted the right to issue nonexclusive sublicense agreements to nurseries within the territory. In exchange for this exclusive right, the master licensee supports IP development and provides enforcement of IP rights, including access to the local court system, as required. Additional responsibilities of the master licensee are market development, technical support, and the transfer of production know-how. In addition to being the local eyes and ears of UC Davis' licensing program, the master licensee facilitates testing and evaluation of promising new cultivars. In exchange for the services provided by the master licensee, UC Davis agrees to share a percentage of collected royalties.

A three-tier royalty rate structure is utilized worldwide. California growers of UC Davis cultivars pay the lowest rate. Growers in the United States outside of California pay an intermediate rate. Finally, growers outside of the United States and Canada pay the highest rate, a percentage of which is shared with the master licensee. In addition to the royalty component described



above, a research fee is collected to directly support new cultivar development. The research fee of US\$1.00/1000 plants entitles the licensee to a lower royalty rate.

The structure of the strawberry licensing program is driven in part by UC Davis' role as a public institution in the state of California. In addition to the reduced royalty described above, nurseries and fruit growers in California are given preferential treatment through the licensing program. For example, California-based nurseries (licensees) are the only nurseries in the worldwide licensing program that have access to all licensed markets. The sales territories of non-California nurseries are limited to a defined region. Also, after the initial release of a new strawberry cultivar, its use is restricted to California for the first two years. This policy is designed to benefit fruit growers in the state who are concerned about competition in their own markets from UC Davis cultivars grown abroad.

UC Davis cultivar strawberry plants are shipped worldwide from California nurseries. To facilitate monitoring of worldwide strawberry plant shipments, an electronic, internet-based system has been implemented with the goal of providing real-time shipping information for UC Davis and its master licensees worldwide. Licensed nurseries electronically declare sales before shipment. This pre-shipping electronic notification enables master licensees to assess a proposed sale based on the intended use of the plant material and the licensing status of the recipient. The system is expected to reduce the occurrence of out-of-compliance shipments and provide the supplying nursery with assurance that its shipments are consistent with UC Davis' licensing structure worldwide. The worldwide demand for UC Davis' strawberry cultivars translates to a significant volume of sales and attendant royalty income. Gross annual income for the strawberry licensing program has averaged US\$4.5 million in recent years. The largest non-U.S. markets, by country, are Spain, Mexico, Morocco, and Australia (from largest to smallest, within this group). In addition to royalty income, total research fee

> collection now totals US\$650,000 annually and represents the lion's share of funding for the strawberry breeding program at UC Davis.

> The ultimate success of the licensing program is tied to the continued development of competitive cultivars. The UC Davis strawberry breeding team is highly skilled, and funding for the endeavor is stable. As a result, the strawberry breeding and licensing programs are positioned for success.

The strawberry licensing program of the University of California provides a clear example of how intellectual property protection by a public sector institution enables the global dissemination of innovative results by providing an economic stimulus to those who adopt the technology. It also allows those who benefit most directly from the technology to help sustain financially the program that serves them.



UC Breeder Selections

Every year, UC strawberry breeders submit to FPS the advanced breeder selections with potential to become new cultivars. These selections are treated using heat and tissue culture techniques to eliminate virus they may have been infected with through exposure to field conditions. The meristem plant for each selection is returned to the breeder for further trials, and a runner from it is held at FPS. This plant is propagated by conventional runner propagation and tested for disease annually.

When a breeder decides to release a selection as a new cultivar, FPS staff increases it by conventional runner propagation and moves it to the mother plant greenhouse. By the time a breeder's selection is released as a new cultivar, it has been at FPS for several years and undergone repeated testing for disease. *Guide to the Strawberry Clean Plant Program* is a publication of Foundation Plant Services, a department in the College of Agricultural and Environmental Sciences at the University of California, Davis.

We would like to thank Michael Carriere, Business Development and Intellectual Property Manager, for contributing material about the UC Davis strawberry licensing program.



Director: Dr. Deborah Golino FPS Plant Pathologist: Dr. Adib Rowhani Plant Introduction and Distribution Manager: Cheryl Covert Strawberry Production Manager: Susan T. Sim Plant Identification Lab Manager: Gerald Dangl Greenhouse Manager: Tracey Echeverria Edited by Susan T. Sim

Design and photography: Bev Ferguson

UCDAVIS

0791

Foundation Plant Services One Shields Avenue University of California Davis, CA 95616-8600



Foundaton Plant Services takes pride in the quality of its plant materials and service to customers. From left: Susan T. Sim, Waclawa Pudlo, Tracey Echeverria, Tracy Pinkelton, Josh Chase and Cheryl Covert.