

Title: Enhancement of Bramble Production in the Southeastern U.S. Through Micropropagation, Virus-Indexing, and Field Evaluation for Trueness-to-Type

Final Report

SRSFC Project #2009-09

Research Proposal

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OBJECTIVES:

- 1) To develop protocols for *in vitro* propagation of blackberry and raspberry cultivars grown in the southeastern U.S.
- 2) To maintain virus-indexed *in-vitro* Nuclear Stock plants of selected cultivars and advanced selections in the repository in Micropropagation Unit and Repository (MPUR) at NCSU.
- 3) To evaluate micropropagated, virus-indexed Nuclear Stock plants for trueness-to-type.

JUSTIFICATION:

Raspberries are potentially a very high-value crop, but they are also one of the most difficult crops to grow in North Carolina and the Southeastern U.S. In North Carolina, commercial raspberry production is concentrated in the western part of the state, in the mountains and in the foothills. Raspberries are not commonly grown in the piedmont and coastal plain areas because most raspberry cultivars are poorly suited to warm

temperatures in those areas. New raspberry cultivars that are better adapted to the climate in these areas are being developed by NCSU breeding program.

Propagation of raspberry plants by stem cuttings is difficult and inefficient, and most breeding programs are using meristem-tip culture techniques and apical meristems to produce *in-vitro* plants. Also, meristem-tip culture has been used successfully for elimination of plant viruses and other plant pathogens that are commonly found in vegetatively propagated blackberry and raspberry plants and have been known to cause economically important diseases. Differences in the genetic make-up of blackberry and raspberry cultivars and advanced selections require certain modifications of standard tissue culture media and growth conditions to produce viable *in-vitro* plants. MPUR at NCSU produces and maintains in the repository blackberry and raspberry cultivars grown in the Southeastern U.S., and is the source of micropropagated, virus-indexed, true-to-type Nuclear Stock plants.

METHODOLOGIES

Tissue Culture Protocol for *Rubus*

A. Collection of plant material

Blackberry or raspberry primocanes are collected from pot-grown greenhouse plants and/or field plants using sterile razor blades, placed in plastic bags, labeled and stored at 4 C. Shoot tips are trimmed to 1-1.5 cm, placed in a beaker and washed in running water for 5 min and then surface sterilized for 10 min in 100 ml of a solution consisting of 0.79% w/v Sodium Hypochlorite and five drops of Tween-20. It is necessary to stir plant material constantly to facilitate surface contact. Shoot tips are transferred and held in deionized sterile water until meristeming.

B. *In vitro* culture

Stage I – Establishment

After surface sterilization, shoot tips are transferred to a Bioflow laminar vertical flow cabinet and rinsed three times in sterile deionized water for 5 minutes each. Shoot tips are trimmed of all but 1-2 primordial leaves enclosing the dome. Apical meristems consisting of a dome and 1-2 primordial leaves are 0.1-0.5 mm long. Meristems are transferred into 20 x 150 Bellco culture tubes containing 10 ml of Murashige and Skoog (MS) PGR-free liquid initiation medium and placed on a bridge made of filter paper. Meristems are maintained for five to seven days in this medium under bright white fluorescent lights at 24-27 C and the 16-h photoperiod. The same medium is used for establishment of blackberry and raspberry explants.

Stage II - Proliferation

Two formulations are currently used for *in vitro* culture of *Rubus* genotypes. MS is used for blackberry proliferation and MS or Anderson's Raspberry Medium are used for raspberry proliferation depending on the genotype. Meristems are transferred to either MS or Anderson's Raspberry Medium in Magenta boxes (50ml/box), placed under the same conditions as during the establishment stage and sub-cultured every three to four weeks. For those explants which do not multiply but are healthy the BA should be

increased to 2 mg/L. Those which fail to multiply on MS may do better on Anderson's medium.

Stage III – Rooting

The explants are transferred to MS medium without hormones or with 10µM IBA for one week and then to no hormone medium for 3 weeks. Some of the genotypes may require longer rooting periods (some blackberry cultivars can root directly in the greenhouse). For raspberry plants activated charcoal is essential for rooting. IBA is essential for good rooting and plant growth of black and red raspberries but not for root initiation (Anderson, 1987).

Stage IV - Hardening

TC plants are transferred into 4" pots or 38-cell trays containing MetroMix and placed under intermittent mist for 2 wks. Plants are then maintained for 6-8 weeks under greenhouse conditions at 24-27 C and 9-14 hr photoperiod before transplanting in the field. Plants in the greenhouse are fertilized once a week with 20-20-20 liquid fertilizer.

C. Cold Storage

Blackberry and raspberry plantlets (2-3 cm long) are maintained in sealed tissue culture bags, one plant per section (15 x 150 mm) of a five-section bag (AgriStar Inc., Conroe, TX, USA). Each section contains 10 ml of MS or Anderson's medium without hormones, 3.5 g of Agar and 1.75 g Phytigel/L. Culture bags are maintained in the growth room for 1 week then cold acclimatized with alternating temperatures and a short photoperiod and low radiance [22 C with 8 hr photoperiod ($10\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and -1 C for 16 hours in the dark] for one week before cold storage. Plantlets are stored at 4 C and the 12 hr photoperiod 4-6 months.

RESULTS

In 2009, NC344, NC548 and NC612 were established *in-vitro* and will be virus-indexed in 2010.

Table 1. Blackberry and raspberry plants produced in 2009 for field evaluation trials

Date	Cultivar/advanced selection	Quantity	Unit type
5/20/09	Nantahala	146	plant
5/20/09	Mandarin	47	plant
9/18/09	NC536 #6M	35	plant
9/18/09	NC536 #5M	226	plant
9/18/09	Nantahala	127	plant
Under the mist	NC621 #1M	160	plant
10/30/09	RUB1113.001	3	plant
10/30/09	RUB440.001	3	plant
10/30/09	RUB1240.002	2	plant
10/30/09	RUB215.001	14	plant

Table 2. Blackberry and raspberry plants tested for viruses by dsRNA in 2009

Cultivar/advanced selection	Meristem #	Result
Nantahala	#1M	Negative
Mandarin	#1M	Negative
Prime Jim	#1M	Negative
NC621	#1M	Negative
NC537*	#11M	Positive
NC430*	#10M	Positive

*plants will be heat treated, meristemmed and tested for viruses in 2010.

CONCLUSIONS:

MPUR has developed protocols for blackberry and raspberry *in vitro* propagation and storage that have been used successfully for genotypes adapted to growing conditions in the Southeastern U.S. MPUR serves as the repository for commercial blackberry and raspberry cultivars grown in the Southeastern U.S., and advanced selections from NCSU breeding program.

IMPACT STATEMENT:

Knowledge of how to efficiently propagate blackberry and raspberry genotypes *in-vitro* and in the nursery is vital to the prosperity of bramble industry in the Southeastern U.S. The use of micropropagated, virus-indexed and true-to-type planting stocks is expected to increase plant productivity and lifespan, as well as the overall fruit quality. MPUR at NCSU produces, maintains and distributes blackberry, raspberry, strawberry and blueberry cultivars grown in the Southeastern U.S.