

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

**Progress Report**

**SRSFC Project #2006-01**

**Research Proposal**

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**Objectives:**

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

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. The MPU at NCSU maintains a repository of blackberry and raspberry cultivars grown in the Southeastern U.S., and is the source of micropropagated, virus-indexed, true-to-type certified planting stocks.

## **Methodologies and Results:**

### **Tissue Culture Protocols 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 properly 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, the explants 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 should be 0.5 mm or less in length. Meristems are transferred into 20 x 150 Belco 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.

#### **MS PGR free liquid medium**

<b><u>Component</u></b>	<b><u>Conc/L</u></b>
Macronutrients (1/2 MS)	50 ml
Micronutrients (1/2 MS)	5 ml
Iron	2.5 ml
Myo-inositol	50 mg
Thiamine HCL	1 ml
Sucrose	30 g

Bring to final volume.

Adjust pH to 5.75 prior to autoclaving.

Place filter paper bridges in tubes before pouring the medium; pour 1- ml/tube.

## Stage II - Proliferation

Two formulations are currently used for *in vitro* culture of *Rubus* genotypes in the MPU. MS is used for blackberry proliferation and MS or Anderson's Raspberry Medium are used for raspberry proliferation (Fig. 1) 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.

### MS basal salt medium

<u>Component</u>	<u>Conc./L</u>
Macronutrients & Micronutrients MS	4.4 g
Sodium phosphate monobasic	170 mg
Myo-inositol	100 mg
Thiamine HCL	2 ml
Adenine Hemisulfate	80 mg
Sucrose	30 g
<i>Hormones</i>	
Benzyladenine (1 mg/L)	10 ml
IBA (0.1 mg/L)	1 ml
GA <sub>3</sub> (0.1 mg/L)	1 ml

Bring to final volume.

Adjust pH to 5.75 before autoclaving.

Add 7 g of Agar.

### Anderson's Raspberry Medium

<u>Component</u>	<u>Conc./L</u>
M&S Nitrates	2.5 ml
M&S Sulfates	10 ml
Anderson's Halides	10 ml
Anderson's B, Mo	10 ml
M & S Iron	20 ml
M & S Vitamins	10 ml
NaH <sub>2</sub> PO <sub>4</sub>	170 mg
Adenine Sulfate	80 mg
Myo-inositol	100 mg
Thiamine HCL	2 ml
Sucrose	30 g
<i>Hormones</i>	
Benzyladenine (1 mg/L)	10 ml
IBA (0.1 mg/L)	1 ml
GA <sub>3</sub> (0.1 mg/L)	1 ml

Bring to final volume.  
Adjust pH to 5.7 before autoclaving.  
Add 7 g of Agar.

### **Stage III – Rooting**

Blackberry plantlets are transferred to MS medium without hormones or with 10 $\mu$ M IBA for one week and then to no hormone medium for 3 weeks (Fig. 2). Some of the genotypes may require longer rooting periods (some blackberry cultivars can root directly in the greenhouse).

In case of raspberry plantlets activated charcoal is essential for rooting. IBA is essential for good rooting and plantlet growth of black raspberries. It is essential for growth of red raspberries, but not for root initiation (Anderson, 1987). The following rooting formulation has produced good results with raspberry genotypes produced in the MPU:

### **Anderson's Raspberry Medium**

<b><u>Component</u></b>	<b><u>Conc./L</u></b>
M&S Nitrates	2.5 ml
M&S Sulfates	10 ml
Anderson's Halides	10 ml
Anderson's B, Mo	10 ml
M & S Iron	20 ml
M & S Vitamins	10 ml
NaH <sub>2</sub> PO <sub>4</sub>	170 mg
Adenine Sulfate	80 mg
Myo-inositol	100 mg
Thiamine HCL	2 ml
Sucrose	30 g
<i>Hormones</i>	
IBA (0.1 mg/L)	1 ml
Activated charcoal	600 mg

Bring to final volume.  
Adjust pH to 5.7 before autoclaving.  
Add 7 g of Agar.

### **Stage IV - Hardening**

TC plantlets 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**

The MPU is currently using the protocol developed by Reed et al. (2005) for cold storage of *Rubus* with good results. 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.

*Rubus* genotypes currently maintained in cold storage in the MPU repository are: raspberry Pocahontas (Fig. 3); blackberry Apache, Arapaho, Chester, Chickasaw, Choctaw (Fig. 4), Illini Hardy, Kiowa, Navaho, Ouachita, Prime Jim, Shawnee, NC 533, NC 534, and NC 535.

The following *Rubus* genotypes from NCSU were established in TC and have been maintained in the MPU repository: (1) primocane fruiting blackberry selections NC 533, NC 534, NC 535 (Fig. 5), NC 537, NC 538, and NC 539; (2) thornless blackberry selection NC 430; (3) raspberry selections NC 451 and NC 344; and (4) Tupy, thorny blackberry cultivar from Brazil.

In 2005-2006 the MPU has produced 600 Mandarin plants and 130 Pocahontas plants for field evaluation for trueness-to-type by the NCSU Bramble Breeding Program and their collaborators. Also, small numbers of plants of several NCSU blackberry and raspberry advanced selections were produced for field evaluations in 2006-2007. Protocols for virus-indexing of raspberry are being developed in the MPU, as well as the certification standards. Micropropagated, virus-tested and true-to-type blackberry plants from the MPU are available to researchers and certified nurseries for propagation.

### **Conclusions:**

The MPU has established 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.

The MPU serves as the repository for commercial blackberry cultivars grown in the Southeastern U.S., advanced blackberry and raspberry selections from the NCSU Bramble Breeding Program and two raspberry cultivars: Mandarin and Pocahontas that are currently being evaluated for commercial production.

### **Impact Statement:**

Knowledge of how to efficiently propagate blackberry and raspberry genotypes *in vitro* and in the nursery is vital to the prosperity of the bramble industry in the region. The use of micropropagated, virus-indexed and true-to-type planting stocks is expected to increase plant productivity and longevity, as well as the overall fruit quality. The MPU at NCSU maintains a repository for bramble, strawberry, blueberry and sweetpotato cultivars grown in the Southeastern U.S. It also serves a source of Nuclear Stock and Foundation plants for blackberry, strawberry and sweetpotato nurseries in North Carolina.

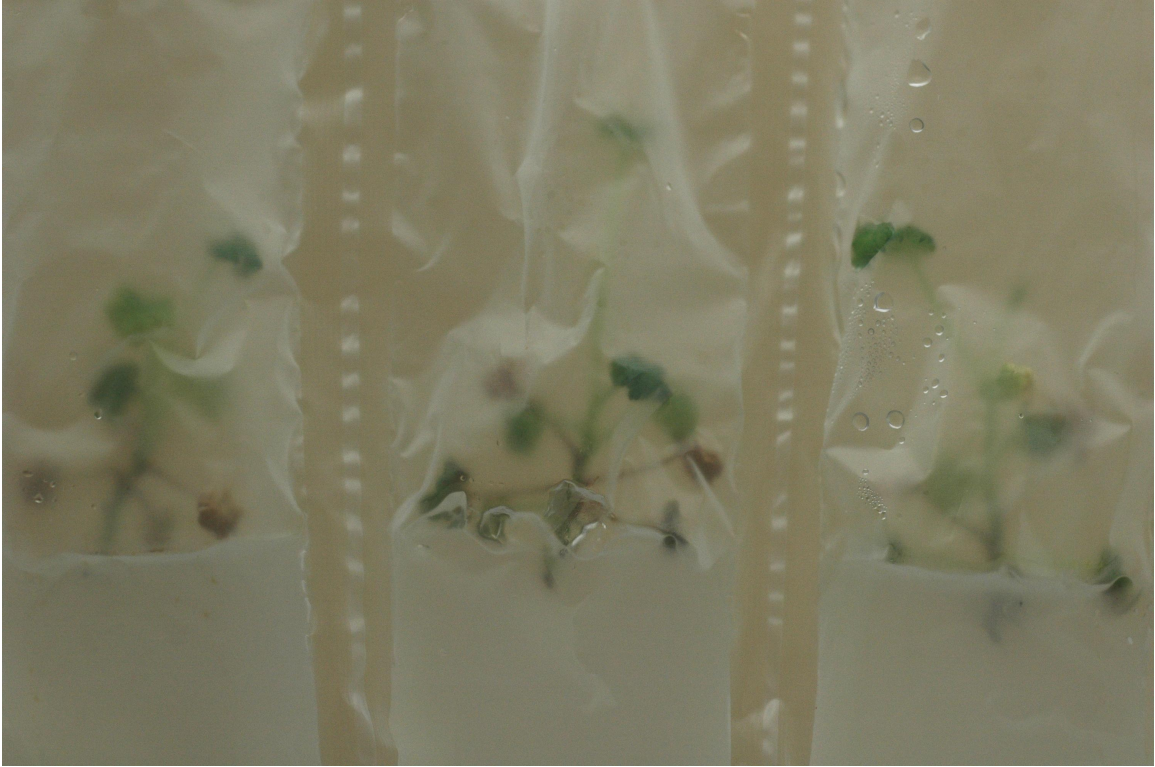


**Fig. 1. Mandarin explant in proliferation medium in the MPU.**

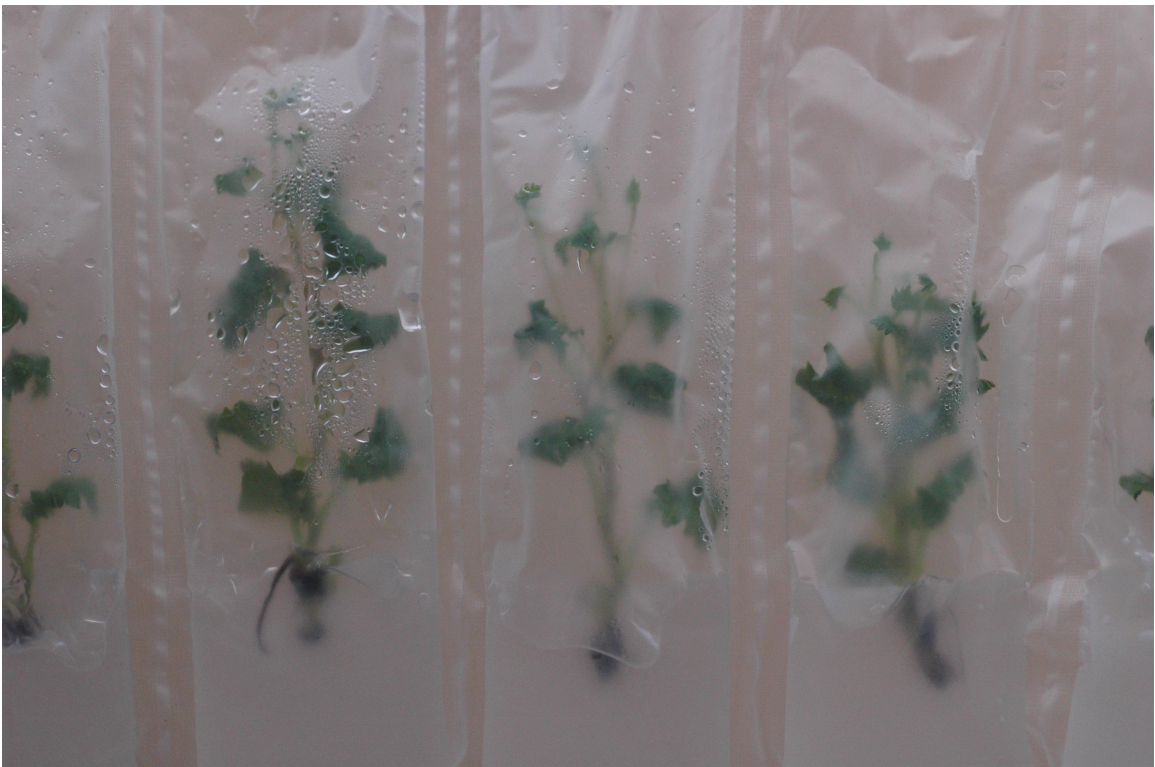


**Fig. 2. Blackberry NC 533 explants in rooting medium in the MPU.**





**Fig. 3. Raspberry Pocahontas in cold storage in the MPU.**



**Fig. 4. Blackberry Choctaw in cold storage in the MPU.**



**Fig. 5. Micropropagated blackberry selection NC 534 in the MPU greenhouse at NCSU.**

**References:**

Anderson, W.C.1980.Tissue Culture Propagation of Red and Black Raspberries, *Rubus idaeus* and *R. occidentalis*. *Acta Horticulturae*. 112:13-20.

Reed, B.M., K.M. Hunter, Y. Chan and S. Gupta.2006.Medium and Long-term Storage of **Rubus** Germplasm. *Acta Horticulturae*. In Press.