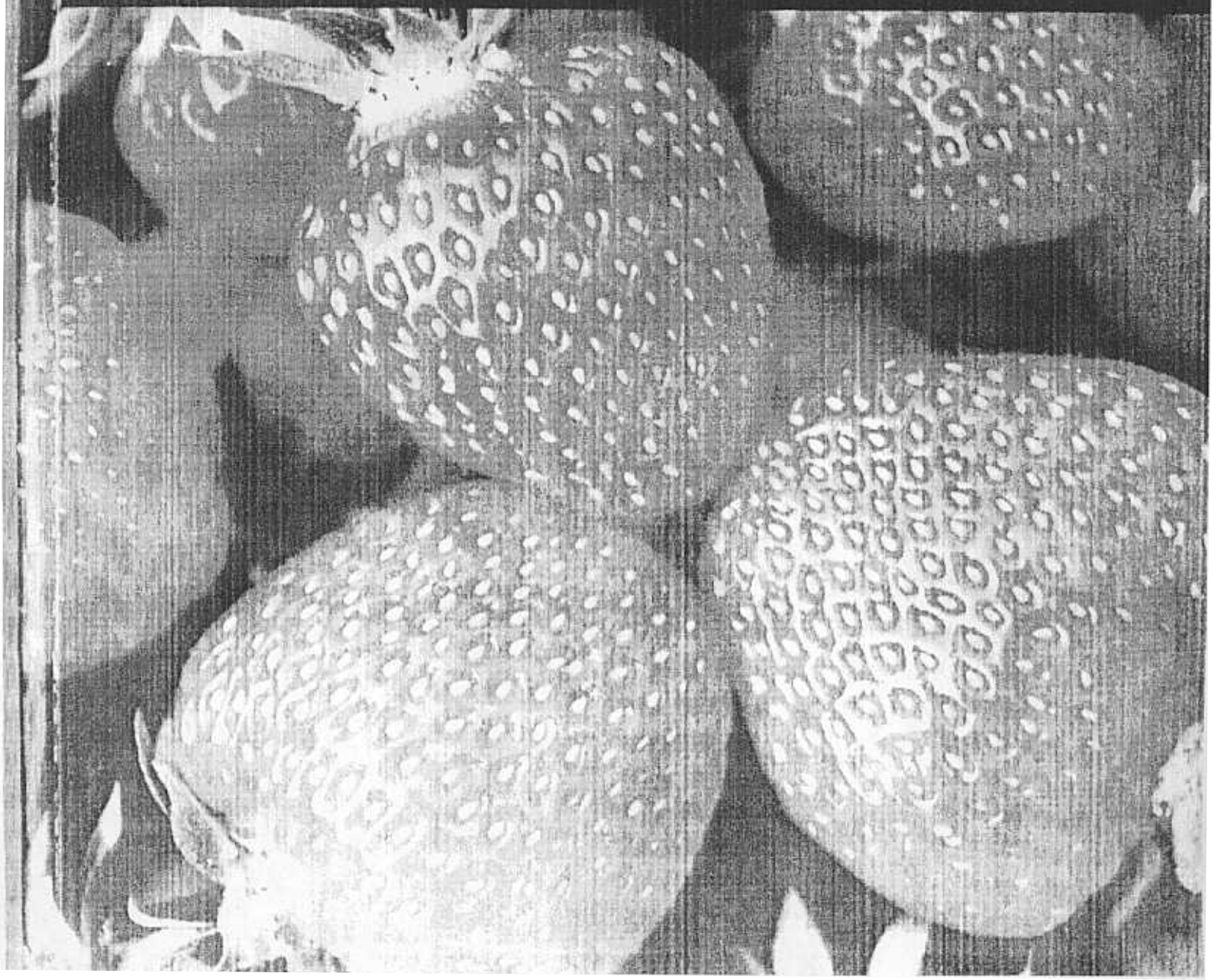
Strawberry Research to 2001



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GENETIC STABILITY OF STRAWBERRIES IN CULTURE

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ABSTRACT. The national strawberry (*Fragaria* L.) genetic resource collection is assigned to the U.S. Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository at Corvallis, Ore. This collection is preserved primarily as potted plants in a screenhouse and about 250 clones are kept as in vitro cultured plantlets. The in vitro plantlets are available for distribution to international requestors, from the primary screenhouse plants, and constitute a backup. Standard in vitro procedures involve storage of strawberry plantlets in semipermeable plastic tissue culture bags on a plant growth-regulator-free storage medium at 4 °C. These plants remain viable for several years in these conditions. We performed experiments to determine if cold storage or culture on high N^6-benzyladenine (BA) concentrations caused somaclonal variation in strawberries. No differences were detected based on randomly amplified polymorphic DNA (RAPD) markers or in observation of specific morphological traits. Our procedures produce plants from preexisting meristematic tissues and stable genotypes and avoid callus proliferation. The potential for mislabeling exists and in vitro propagation systems should include identity verification as a final step.

The USDA–ARS National Clonal Germplasm Repository at Corvallis, Ore., is assigned to preserve temperate fruit, nut, and specialty crops. The national collection of *Fragaria* L., strawberry genetic resources is stored at this facility. This collection includes about 50 species and subspecific taxa from around the world represented by about 1300 strawberry clones preserved as potted plants and more than 300 seedlots. Nearly 19% of this collection, about 250 clones, is preserved as in-vitro cultured plantlets. Additional clones will be placed in culture as time and resources permit.

IN VITRO CULTURE AT THE REPOSITORY

The goal of in vitro culture at the Repository is to preserve and retain specific genotypes. Plants are maintained in culture as a backup for the primary collection that is stored as potted plants in a screenhouse. In addition, in vitro cultures provide readily available material for distribution to domestic and foreign requestors.

The philosophy of culturing plants for genetic resource preservation is different than that for massive production for commercial purposes. We do not try to maximize plant production in conservation efforts. The Repository procedure begins with the collection of runner shoot tips from screenhouse plants. Plantlets are produced with low plant growth regulator concentrations in the medium. For storage the plantlets are planted in semipermeable plastic tissue culture bags on a plant growth regulator (PGR)-free storage medium, cold acclimatized for 1 week and placed at 4 °C. Plantlets are inventoried and rated for viability every 4 months. Strawberry plantlets can remain under these refrigerated conditions for 1 to 2 years before repropagation is required (Reed, 1991, 1999).

RESEARCH ON SOMACLONAL VARIATION IN 'POCAHONTAS' STRAWBERRY

Micropropagation of strawberries (*Fragaria* sp. is reported to lead to somaclonal

variation in some genotypes (up to 0.46% in 'Pocahontas') (Sansavini et al., 1990). Changes include leaf-color variants and dwarf plants (Cameron and Hancock, 1986; Irkaeva and Matveena, 1997; Sansavini et al., 1990; Swartz et al., 1981). Other characteristics are epigenetic in nature and disappear over time (Koruza and Jeleska, 1993). Numerous authors report that genetic changes including insertions, deletions, point mutations, and other rearrangements occur during tissue culture (Anderson et al., 1991; Kane et al., 1992) although few of the phenotypic symptoms are heritable (Karp, 1995; Sansavini et al., 1990).

Most somaclonal variations occur in plants regenerated from cultures that have gone through a dedifferentiation phase, i.e., callus. The role of PGR as agents of mutation is under study by several investigators. Arnholdt-Schmitt (1993) discovered that indole-3-acetic acid (IAA) and inositol in the growth medium resulted in DNA rearrangements and methylation changes in (*Daucus carota* L.) callus cultures. However, cropping and vegetative performance of runner progeny of micropropagated strawberry plants were not influenced by cytokinin concentration in the tissue culture medium (Beech et al., 1988).

At the NCGR we wanted to know if our in-vitro culture practices were inducing somaclonal variation. We chose to focus on the benzyladenine (BA) concentration in the medium over several passages in culture, and to look at plantlets that were stored for 4 years at 4 °C with repropagation at about 15-month intervals. 'Pocahontas' was chosen for study because of earlier reports of high levels of somaclonal variation (Sansavini et al., 1990). We tested the genetic stability of 'Pocahontas' plantlets through morphological evaluation of field-grown plants and comparisons of RAPD molecular markers in plantlets in vitro or in the field (Kumar et al., 1999).

SOURCE PLANTS

Five 'Pocahontas' plantlets originating from two potted greenhouse plants and cold stored (4 °C) on PGR-free medium for 4 years were tested. In addition five runners were collected from greenhouse plants, initiated into culture, and multiplied on 5 or 15 µM BA for 8 months before sample collection for DNA extraction. One hundred plantlets from each BA treatment and 50 cold-stored plantlets were acclimatized and field planted for morphological evaluation.

MORPHOLOGICAL OBSERVATIONS

In vitro-grown plantlets of *Fragaria ×ananassa* 'Pocahontas' on 15 µM BA medium were compact and pale green in color while those on 5 µM BA were taller and dark green. Height variation persisted in potted plants. Cold-stored plants were not morphologically different from 5 µM BA plants (Table 1). Micropropagated 'Pocahontas' plants grown on 5 and 15 mM BA medium or cold-stored on PGR-free medium for over 4 years were grown in the field to examine morphological variation. Most of the morphological characteristics did not differ for field grown plants micropropagated on 5 and 15 µM BA medium, but cold-stored plants were less vigorous both vegetatively and reproductively than BA-treated plants. Cold-stored plants had fewer trusses, lower vigor, fewer and smaller fruit and poor fruit color, but they did produce significantly more runners than nonstored plants (Table 1). Differences in morphological characteristics appeared to be epigenetic in nature, i.e., disappearing after several months growth, and were not indicative that mutations had occurred (Kumar et al., 1999).

MOLECULAR MARKERS—RAPD

Randomly amplified polymorphic DNA (RAPD) markers were used to determine if cold storage or supraoptimal levels of BA in culture medium caused genetic changes leading to somaclonal variation. No indication of mutation was detected, through gain or loss of fragments, in over 200 loci amplified by 29 of 30 Operon primers tested (Kumar et al., 1999). One primer, OPF-18 (TTCCCGGGTT), generated an inconsistent polymorphism that did not correlate with any treatments. Possible changes in methy-

lation patterns of rRNA genes of strawberries were examined using Southern blot analysis. Changes in methylation patterns were observed in one sample from plants grown on 15 mM BA medium and in one of the cold-stored plants. A RAPD length polymorphism was observed in two samples from plantlets derived from one explant.

The one variant plant arising from the BA treatments could be due to changes in gene expression, but by the end of the season that plant and its runners began to appear normal, suggesting the change was epigenetic. Sansavini et al. (1990) reported that some variations were masked by other characteristics and tended to appear in later seasons. We did not continue the study beyond one season. However, absence of polymorphism in RAPD markers and lack of major changes in DNA methylation indicated that few, if any, genetic changes were present but masked in BA-treated or cold-stored plants. The absence of polymorphisms in the specifically amplified DNA we examined does not exclude the possibility that mutations occurred in other repetitive DNA segments or coding regions (genes) of the genome. Neither BA treatment nor cold storage per se appeared to be a cause of somaclonal variation (Kumar et al., 1999).

ACTUAL PRACTICE IN THE LABORATORY

Standard culture practices at the NCGR are designed to minimize somaclonal variation. Although variant plants are rare, the staff avoids possible problems by discarding hyperhydric, and chimeral plants, and those produced from callus tissues. Multiplication medium contains 4.4 µM BA and the cold-storage medium is free of all growth regulators. Cold-stored cultures are repropagated on average at 15 months (range of 12 to 24 months). They require one or two passages in the growth room before further storage. Cultures are tested for bacterial contaminants on a regular basis (Tanprasert and Reed, 1997)

AVOIDING SOMACLONAL VARIATION

Several steps are important in avoiding somaclonal variation in micropropagation schemes. Micropropagated plants should be produced from preexisting meristems (not adventitious buds), and regenerated from young tissues and stable genotypes, while callus and protoplasts should be avoided (Jain and De Klerk, 1998). Numerous reports point to the importance of proper explant choice (Adelberg et al., 1994).

Table 1. Morphological analysis of field-grown 'Pocahontas' strawberry plants from in vitro plantlets multiplied on NCGR–FRA medium with 5 or 15 µM BA for 8 months or stored at 4 °C for 4 years on medium without N^6-benzyladenine. Data from Kumar et al., 1999.

	In-vitro growth conditions		
Characteristic	5 µM BA	15 µM BA	Cold stored
Leaf angle (mm)	107.8 a[z]	109.3 a	104.8 b
Leaf length (mm)	74.1 a	76.1 a	70.6 b
Leaf width (mm)	66.1 a	67.1 a	62.7 b
Petiole length (mm)	123.0 ab	126.4 a	119.0 b
Plant height (cm)	6.8 b	7.1 a	6.4 c
Plant spread (cm)	6.8 a	7.2 a	6.2 b
Plant vigor[y]	4.4 a	4.5 a	3.8 b
Runnering	11.4 b	11.6 b	13.6 a
Number of trusses	1.6 a	1.6 a	0.5 b
Total fruit number	13.2 a	14.3 a	5.0 b
Primary fruit weight (g)	12.2 a	11.4 a	3.5 b
Primary fruit color[x]	4.3 a	3.9 a	1.3 b
Total fruit weight (g)	33.5 a	30.2 a	7.0 b

[z] Means in a row followed by the same letter are not significantly different by t-test ($P \leq 0.05$)
[y] Plant vigor rated 1 (little growth) to 5 (very vigorous).
[x] Fruit color rated 1 (white) to 5 (dark red).

VALUE OF IN VITRO CULTURE AND STORAGE

Researchers, breeders and certification programs have an increased interest in medium-term germplasm storage for many crop plants. Improvements in storage containers, conditions, and media are needed to make in vitro culture less labor intensive and more secure as a storage system for important germplasm (Reed and Hummer, 1995). The number of accessions presently in cold storage is increasing as additional specimens are collected from around the world and cultivated genotypes are added to the germplasm core. In vitro collections will continue to play an important role in the distribution of pathogen-negative plants throughout the world. The desire for correctly identified, pathogen-negative germplasm is increasing. Some certification programs have adopted in vitro culture as standard technique in the production of pathogen-negative plants from stock collections. Virus and virus-like pathogens do not spread between in vitro cultures.

In contrast to some common industry opinions, our experience indicates that strawberry genotypes can be maintained for long-term as stable in vitro cultures. Mutation rates do not appear to be higher in culture than in vivo over the term considered in this study. However, potential for mislabeling exists and in vitro propagation systems should include identity verification as a final step. At present no single identity verification step exists and laboratories must use a combination of field evaluation and molecular techniques to check their culture systems.

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