THESIS

EFFECT OF TISSUE CULTURE PROPAGATION ON PHENOTYPIC VARIATION OF EIGHT POTATO CULTIVARS

Submitted by

Jim R. Allen

Department of Horticulture

In partial fulfillment of the requirements

for the Degree of Master of Science

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION

BY Jim R. Allen

ENTITLED EFFECT OF TISSUE CULTURE PROPAGATION ON PHENOTYPIC VARIATION OF EIGHT POTATO CULTIVARS

BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF Master of Science

Committee on Graduate Work

H. Laun

Melton Workman

Monty D. Harrison

Kenneth W. Knutson
Adviser

K. M. Brunel
Department Head

ABSTRACT OF THESIS

EFFECT OF TISSUE CULTURE PROPAGATION ON PHENOTYPIC VARIATION OF EIGHT POTATO CULTIVARS

The effect of a rapid multiplication tissue culture propagation scheme on phenotypic variation of potato clones was studied.

Mother stock consisted of eight virus-free commonly grown potato cultivars, along with field and mutant selections of Centennial Russet and Russet Burbank cultivars. These cultivars and selections were cultured via shoot-tip culture and propagated by nodal transfers.

The experiments consisted of comparisons of plants propagated via nodal transfers for approximately one year with tuber propagated mother stock as the control. Also, the effects of different time periods in culture, cold storage and heat stress were studied. Tubers from first generation plants were harvested and replanted for second generation comparisons. The second generation comparisons provided the best analysis when all plants were grown from tubers. Much of the experimental error from juvenile growth characteristics of tissue culture plantlets was eliminated.

Observations were taken on eighteen plant characters, such as pigment on nodes. Measurements were taken on fourteen plant characters, such as terminal leaf index. There were some instances where variable readings on pigment of some plant parts were found. These instances were few in relation to the size of the experiment and were most likely

the result of environmental and physiological differences rather than genetic variation. Factors such as tuber weight and tuber dormancy that resulted in delayed emergence had more effect on plant variability than the tissue culture treatments.

Repeated nodal transfers (up to one year), cold storage or heat stress did not induce phenotypic variation in these experiments.

Jim R. Allen
Department of Horticulture
Colorado State University
Fort Collins, CO 80523
Spring 1985

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CHAPTER I

INTRODUCTION

Plant tissue culture is important in many areas of crop research and improvement. It also has economic value in certain pharmaceutical and plant propagation industries.

There are several types of plant tissue culture which may be used in many different and valuable ways. Some examples include production of pharmaceuticals, use of mutations inherent in some forms of tissue culture for the selection of desirable new varieties, and more rapid increase of desireable varieties than can be achieved by current methods. Many of these tissue culture techniques have been described and are being used in the potato industry.

Rapid clonal increase via tissue culture is used in some seed potato certification programs. Desired cultivars are being rapidly multiplied by commercial laboratories and made available for growers of seed potatoes. A small number of mother tubers, extensively tested for disease and selected for varietal purity can be rapidly increased to thousands of plantlets in a few months. There is a tremendous potential for seed improvement and disease control by the introduction of disease free nuclear stock into a seed program every year. The process consists of "in-vitro" culture using shoot-tips and rapid increase of the progeny by nodal transfers.

Some types of tissue culture, particularly cell cultures, are characterized by an increase in genetic variability. This variability can be useful, but if rapid clonal increase or preservation of a desired species is the goal, genetic stability is necessary.

The shoot-tip culture and nodal transfer scheme has not been observed to increase genetic variation. However, little research has been done on the effect of long-term propagation through repeated subculturing or procedures such as extended cold storage which would be useful in a rapid multiplication program. This study addresses some of these questions.

CHAPTER II

LITERATURE REVIEW

Plant tissue culture broadly refers to the "in-vitro" cultivation of all plant parts, whether a single cell, a tissue or an organ, under aseptic conditions (29).

The Role of Tissue Culture in Plant Research and Production

Cell cultures are used as a model to study the role of plant chemicals, organ initiation, cell and tissue differentiation, somatic cell genetics and other morphogenic processes, with a minimum influence from outside sources (18).

Plant cell and organ cultures are being utilized in commercial (including agricultural) fields. They can be raw product sources for pharmaceuticals; serve as valuable tools for plant breeding; also pathogen free seed stocks can be rapidly increased for commercial use (18). Tissue culture is also a convenient way to maintain and transport many species (1,14,21).

Plant tissue culture is being used in potato improvement research. Techniques for plant regeneration from mesophyll protoplasts have been developed (26,27,32); and virus free potatoes have been established through meristem culture coupled with heat treatment (20). Techniques have also been described for the rapid clonal increase of potato seed stocks (11,25) and the international exchange of genetic material (24).

Shoot-tip culture is the "in-vitro" culture of the meristem plus one or more leaf primordia. Methods for shoot-tip culture have been described for many ornamental, vegetable, agronomic, fruit and tree species (6). Examples include carnations (22), chrysanthemums (3), tomatoes (16), sugar beets (15), strawberries (4), and many deciduous trees (5). Shoot-tip culture for rapid clonal propagation of ferns, indoor foliage plants, woody ornamentals, bulbs and other flower crops has been developed by commercial laboratories. Food crops such as potato, pineapple and banana are also being cloned (18).

Earle and Langhans (8) list requirements that should be met before an "in-vitro" system is seriously considered as an alternative to the conventional propagation procedures. These include:

1. Cultures can be consistently established from a defined and readily identifiable plant part.
2. Cultures can produce plantlets (or other propagules) under defined conditions.
3. Plants can be successfully transplanted to greenhouse or field.
4. Repeated subculturing does not inhibit the ability of explants to reorganize and regenerate healthy plantlets.
5. Plantlets develop into plants like the parent, both initially and after repeated subculturing.
6. Rates of production are comparable to or better than rates for conventional propagation.
7. The system works for different cultivars without drastic modification.
8. Transfer and manipulation required per plantlet produced is not excessive.
9. Cultures can be stored with a minimum of care.

Steps 1, 2, 3, 4, 6, 7, and 8 have been achieved with potatoes (11,24,25). Step 5 has been studied (7,25), but little is known about the effect of repeated subculturing of different species on the genetic

stability of shoot-tip cultures. Step 9 has been achieved with cryopreservation (2,12), but little is known about the long term storage of potato plantlets at temperatures above freezing.

Variability in Plant Tissue Culture

Many types of plant tissue culture result in increased variation. Variants may be classified as follows (26):

1. Physical and morphological changes in undifferentiated callus.
2. Variability in organogenesis.
3. Changes manifested in differentiated plantlets.
4. Chromosomal changes.

Phenotypic variability can be the result of several different forms of genetic change: (a) Aneuploidy - chromosome loss or gain which influences phenotype, (b) Point mutations - possible simplex nature of some gene loci, (c) Epigenic changes - the result of differential gene expression rather than mutation (26), (d) Extra-nuclear inheritance - suggested by Genebach (10), this involves corn plants obtained from callus of a sensitive corn line resistant to the toxin of race T of *Helminthosporium maydis*.

Factors affecting genetic stability are the type, species and age of the explant, increased number of transfers and growth medium constituency (23).

These variations may serve as valuable tools in selective breeding, but if rapid clonal increase or long-term storage of crop plant cultivars is desired, plant tissue and cell cultures must be genetically stable.

Meristem and shoot-tip culture have been employed for these purposes because these techniques tend to maintain genetic stability if

callus is avoided. The important factors in maintaining genetic stability in meristem tissue seems to be (1):

1. The strict control of the sequence of DNA synthesis and mitosis which does not allow for the extra duplication of DNA which is responsible for somatic polyploidy.

2. The continuous division, which eliminates at least part of the spontaneously occuring chromosome structural changes and genetic defects impairing the reproductive integrity of the cell.

Despite the apparent genetic stability of meristem and shoot-tip culture, concern has been expressed that even here problems may occur (3). Variations have been observed to occur in carnations (13,22) and chrysanthemums (3) propagated by these methods. In carnations the "White Sim" cultivar, which is a periclinal chimera, sometimes reverts to a red flower color. The explanation seems to be that the outer layer of cells of the meristem sometimes breaks down, allowing regeneration of the outer layer of inner cells (13). The variation in chrysanthemums also seems to have occured as a result of chimeral breakdown. Thomas et al. (28) found variation in shoot-tips from protoplast derived potato clones. The suggested explanation for the problem was that chromosomal instability existed in the the original shoot or that an expression of its chimeral nature occurred.

Denton (7) compared phenotypic characters among plants of *Solanum tuberosum* from three sources:

1. Tubers from four plants regenerated from shoot-tip cultures.

2. Plants regenerated directly from shoot-tip cultures (Groups 1 and 2 originated from the same parent plant).

3. Commercial seed potatoes used as the control.

Differences were observed in flowering time, with source three flowering first, followed by sources one and two respectively. Pollen

viability and diameter showed a significant variation with decreased pollen fertility and increased diameter in groups one and two. Variation in developmental progress was reflected by an enlarged terminal leaflet in relation to lateral leaflet size in groups one and two. This juvenile character persisted throughout plant development.

The differences were suggested as being the result of "retarded" developmental physiology in regenerated plants. Tuber proteins were qualitatively the same for each group.

Twenty-six components were used in McIntosh's principle component analysis. These measurements consisted of seven leaflet characters and nineteen floral characters.

Roca et al. (25) evaluated varietal characteristics among:

1. Virus-free plants regenerated directly or via callus.
2. Original mother plants infected with potato viruses X and Y.
3. Naturally occurring virus-free plants not regenerated by culture "in-vitro".

General morphology of leaf, flower and tuber were evaluated, chromosomes were counted in root tips and an electrophoretic study of total soluble proteins and the enzyme esterase was done on two hybrid cultivars.

Leaf, flower and tuber morphology of ten other cultivars propagated "in-vitro" were compared with those of plants progagated by conventional procedures.

No qualitative differences were detected in soluble protein or the enzyme esterase patterns and chromosome numbers showed no change. Leaf, flower and tuber morphology showed no significant changes in any of the cultivars.

The variability of potato clones derived from protoplast regeneration has been studied using plant and tuber morphological characters (27,32). Characters for variability that have been found in the Russet Burbank clones are:

1. Growth habit, expressed as shortening of the internodal distance and more compact growth. Malformed leaflets and stems, altered pigment patterns and reduced vigor.
2. Tuber characteristics such as uniformity of size and degree of russetting.
3. Photoperiod requirements for flowering and ability to produce viable seed.
4. Maturity date.
5. Disease resistance.

Phenotypic variants occur naturally in potatoes through normal tuber propagation in the field, and are common in all cultivars. Types of variation include (19):

1. Bolters, characterized by greater height, stiffer and less luxuriant foliage, smaller leaflets, later maturity, greater flowering capacity, greater pigmentation and coarser tubers.
2. Wildings, characterized by a relatively large number of thin stems, numerous stolons with small tubers, shorter and rounder leaflets, absence of floral parts, and premature sprouting of tubers. (Intermediates of type 1 and 2 also occur commonly.)
3. Other foliage variations such as narrow or broad leaflets, coarse leaflets, abnormally subdivided or simplified leaflets, suppression of flowers and appearance of numerous leafy bracts on inflorescence stalks.
4. Color variations in all parts of the plant where pigment appears especially tubers, flowers and buds.

The frequency of spontaneous mutations in the potato have been estimated at between 1.5×10^{-3} and 1.2×10^{-5} depending on the character and the cultivar (27). Bolters occur frequently in some cultivars and

wildings somewhat less frequently. Foliage variations arise rarely, while pigment variations differ considerably. Tuber color changes occur fairly frequently while flower changes occur less frequently (19).

Characters Used in Describing Potato Cultivars

Descriptions of potato cultivars have been made for over 200 years. These descriptions are based on morphological features, which are numerous in the potato. These descriptions are aided by the fact that each potato cultivar is a clone and generally uniform. However, many characters used for identification are subject to considerable variation. These variations can result from the interpretation of the observer, seasonal and environmental variation and inadequate character definitions (30).

Webster (30,31) recorded characters on twenty-one potato cultivars, each for a period of two years at three locations. Characters were strictly defined and assessed as to the time of year and growth stage of the plant when observations were recorded.

In assessing the value of a particular character, two factors were of major importance:

1. The level of consistency, measured as the percentage of total individual results consistent with the mean.
2. Range of expression, which is best when alternative forms of a character are evenly balanced between varieties.

From the study many characters formally used to describe cultivars were dropped and other characters redefined and assigned a major or minor status relative to previously mentioned factors. Of the 60 or more characters adopted, 30 could be used to identify 116 potato cultivars.

Major plant, floral, tuber and stolen characters found to be useful were:

1. Plant
    a. Foliage maturity when compared with known control cultivars.
    b. Stems
        1) Pigment on internodes at the midpart of the stem.
        2) Pigment on nodes and wings, relative to pigment on internodes, at the midpart of the stem.
        3) Waved wings at midpart of the stem.
    c. Leaves
        1) Distinct or indistinct pigment on rachids and petioles.
        2) Pigment on apical rosette.
    d. Primary leaflets
        1) Distinct or indistinct pigment on petiolules, midveins and laterals.
        2) Cordate or noncordate base of terminal leaflet.
        3) Even or uneven lobes of subterminal leaflets.
        4) Waved margins.
        5) Pigment on laminae of young leaflets at apical rosettes.
        6) Mean terminal leaflet index, as leaflet breadth/ leaflet length x 100.
        7) Mean lateral leaflet index.
    e. Secondary leaflets
        1) Insertion on petiolules of laterals.
        2) Insertion on petiolules of terminals.
        3) Shape.

2. Floral

    a. Inflorescences

        1) Pigment on peduncle.

        2) Pigment on abscission ring at flowering.

        3) Pigment on pedicel at flowering.

        4) Pigment on base of bud just prior to bud opening.

        5) Pigment on calyx lobe just prior to opening.

        6) Protrusion of stigmas just prior to bud opening.

    b. Flowers

        1) Color of front of petals.

        2) Color of back of petals when front is white.

        3) Color of anthers.

        4) Normal, malformed or open anther column.

        5) Straight, kinked or curved style.

        6) Pigment in ovary interior.

        7) Flowering frequency measured as percentage of plants in flower, number of flowering inflorescences per plant and number of flowers per inflorescence.

3. Tubers

    a. Pigment of skin.

    b. Distribution of pigment.

    c. Location of pigment.

    d. Color of flesh.

    e. Mean shape index as tuber length/tuber breadth x 100.

4. Sprouts

    a. Pigment on base, midpart and tip of stolon.

Summary

Plant tissue culture is important in many aspects of plant research, improvement and production in commercial agriculture. Tissue culture is used in the potato industry for breeding, production of disease-free plants, rapid clonal increase and exchange and storage of desired species.

Cell and protoplast culture of plants are characterized by an increase in the frequency of genetic variation. The variations can be useful for breeding purposes but should not be allowed when tissue culture techniques are used for rapid clonal increase or anytime genetic stability is desired. Studies by Denton et al. and Roca et al. (7, 25) do not suggest that any increase in genetic variation occurs when meristem and shoot tip culture are used.

This study attempted to evaluate the possible effects of some rapid multiplication techniques on genetic stability of selected potato clones. The techniques included the effect of repeated nodal transfers, long term "in-vitro" propagation, cold storage and heat stress,

CHAPTER III

MATERIALS AND METHODS

Cultivar Planting Stock

Tubers of the potato *Solanum tuberosum* were selected from the following thirteen sources:

No.	Cultivar	Type
1	Burbank	Virus free
2	Russet Burbank	Virus free
3	Centennial Russet	Virus free
4	Norland	Virus free
5	Red Norland	Virus free
6	Norgold Russet	Virus free
7	Norgold Russet M	Virus free
8	Norchip	Virus free
9	Russet Burbank	Variant (pebble leaf)
10	Russet Burbank	Variant (giant hill)
11	Russet Burbank	Field selection
12	Centennial Russet	Variant (pebble leaf)
13	Centennial Russet	Field selection

Virus free tubers were obtained from Dr. N.S. Wright of Canada. Variants and field selections were obtained from certified seed potato fields located in the San Luis Valley of Colorado.

Propagation Procedures

"In-Vitro"

Tubers were treated with gibberellic acid (GA3), and placed in black plastic bags at 25°C under high humidity to break dormancy. After approximately eight weeks, sufficient sprout development occurred to permit shoot tips, (2-4mm) to be excised. Explants were surface sterilized by soaking in 70 percent alcohol for 10-15 seconds, followed by 2 percent sodium hypochlorite for two minutes and finally rinsed in sterile distilled water. One or two explants from each sprouted tuber were transferred to 220ml baby food culture jars containing a basic Murashige and Skoog medium (Table 1). After six weeks, plantlets, 5-7cm in length, regenerated from shoot tip explants were cut into nodal segments and transferred to fresh medium. Each nodal cutting consisted of a stem segment with its corresponding leaf and axillary bud. Three to five nodal cuttings per plantlet, depending on the cultivar, were transferred at ten to fourteen day intervals for the remainder of the propagative phase of the experiments.

Greenhouse

Twelve to fifteen plantlets were randomly selected from each source after each nodal transfer treatment period; removed from culture jars and transplanted to small pots containing Jiffy Mix growing medium. They were placed in a greenhouse and each plant was fertilized with one cup of liquid (7-16-19) fertilizer. A mist sprinkler system operated for thirty seconds for eight hours per day. Plantlets were covered the first three days after transplanting with nylon shading cloth (approximately 50 percent shading) and kept at a day/night temperature of 24/16°C. No supplemental light was supplied.

After ten days growth in the greenhouse, ten of the most uniform plantlets were selected and each placed in an eight inch diameter clay pot containing a 12:4:1 Jiffy mix, sand, perlite-soil mixture. Each pot was fertilized with one teaspoon of Osmocote 14-14-14 slow release fertilizer and watered with a drip nozzle system. Temperature and photoperiod were the same as previously mentioned.

Mother tubers representing each clonal source (including leaf bud tubers) were planted in eight inch clay pots and grown under the condition previously described. Leaf bud tubers will be described later on.

Field Propagation

Tubers from plants grown in the greenhouse were harvested and stored at 4.5°C. Storage time was varied for two reasons; (1) Burbank and Russet Burbank cultivars took longer to mature than other cultivars, and (2) Tubers from plants which were subjected to more nodal transfers were planted and harvested later. The actual storage periods are shown later.

Tubers were treated with rindite under dark, humid conditions to break dormancy. Following dormancy treatment, tubers were sprouted in wooden flats containing soil. On June 25, tubers were planted at the Colorado State University Horticulture Farm at Fort Collins, Colorado in a field with a clay loam soil at a pH of 7.7 and a high salt content (4.0). Preplant fertilizer at the rate of 140 lbs./acre P_2O_5 was applied to the plot site.

Weeds were controlled with a preplant application of Round-up (four tablespoons per gallon) applied at a rate of twenty gallons per acre. Cultivation and hand weeding were also used. The plants received weekly applications of Sevin throughout the growing season to

control insects. Bravo 500 was applied at weekly intervals starting August 5 and continuing until the end of the growing season to control foliar diseases.

Experiments

Two types of experiments were conducted: 1) a Cultivar Experiment and 2) a Long Term Propagation Experiment. The Cultivar Experiment was designed to investigate the effects of nodal transfer, cold storage and heat stress on plant morphology. The Long Term Propagation Experiment was designed to investigate the effect of approximately a year of tissue culture propagation by nodal transfers on plant morphology.

Two types of planting stock were used: first and second generation. First generation planting stock consisted of tissue culture propagated plantlets and the second generation planting stock consisted of the tuber progeny harvested from the first generation planting.

Cultivar Experiment

First Generation Planting: This experiment included the following treatments.

1. Each of the thirteen tuber sources subjected to three, six and nine nodal transfers (thirty-nine treatments).
2. Virus free Centennial Russet, a medium maturing cultivar, and Norgold Russet an early maturing cultivar, stored in-vitro for 8 weeks at 2°C under 1000 lux inflorescent light and a 16 hour photoperiod (2 treatments).
3. Virus free Centennial Russet and Norgold Russet cultivars stressed in-vitro by exposure to a temperature of 38°C for

72 hours in a growth chamber under 4700 lux of fluorescent/ incandescant light and a 16 hour photoperiod (2 treatments).

Plantlets receiving treatments two and three were subjected to four nodal transfers prior to cold storage or heat stress. These plantlets also were transferred to fresh culture medium for one ten day growth cycle after the treatment period ended to restore growth and vigor of the plantlets.

Plants were grown in the greenhouse as previously described in a completely rendom design with forty-three treatments and ten plants per treatment. Observations and measurements were made on plantlet foliage according to the criteria and methodology described in Tables 2 and 3. Any abnormalities in plant parts or characteristics beyond those listed in the tables also were recorded. Notes were taken at a predetermined time since plantlets did not flower and no other phenologic reference point was readily apparent. For all treatments (except those involving Burbank and Russet Burbank) observations and measurements were taken seven to eight weeks after transplanting. Burbank and Russet Burbank treatment observations and measurements were taken eight and nine weeks after transplanting respectively, because of the later maturity of these cultivars.

Data were analyzed using a two-way analysis of variance. Mean separation was accomplished using the Scott-Knot cluster analysis (9).

Second Generation Planting: The five clones of Burbank and Russet Burbank cultivars were not planted in the field with the rest of the cultivars as originally planned. They were planted later in the greenhouse because of later maturity and/or tuber dormancy requirements. Storage time for the tubers from various treatments were as follows:

	Cultivar	Treatment	Storage Time
1.	Burbank and Russet Burbank and clones	3NT	12 weeks
		6NT	7 weeks
		9NT	3 weeks
2.	Centennial Russet and clones	3NT[1]	9 weeks
		6NT	5 weeks
		9NT	1 week
3.	Norland		
4.	Red Norland		
5.	Norgold Russet		
6.	Norgold Russet M		
7.	Norchip		
8.	Centennial Russet	heat stress	1 week
9.	Norgold Russet	heat stress	1 week
10.	Centennial Russet	cold storage	2 weeks
11.	Norgold Russet	cold storage	2 weeks

The field plot experiment included tubers derived from tissue culture plantlets of the first generation planting (except the Burbank and Russet Burbank cultivars). Tubers produced by the original mother plants grown from tubers were used as the control. A randomized block design with thirty-six treatments and ten replications was used. Individual tuber weight was recorded at the time of planting.

Data were collected three to four days after flowering on characteristics of foliage and inflorescences as listed in Tables 2 and 3. Emergence and flowering dates also were recorded.

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[1]Treatments of three, six, and nine nodal transfers for cultivars three through seven received the same storage times as Centennial Russet and clones.

Tubers were harvested on October 12. Tuber yields were not measured since the late planting date did not permit meaningful tuber development on late maturing cultivars. Only the earlier maturing Norland and Red Norland cultivars produced tubers greater than one inch in diameter.

Data were analyzed using a two-way analysis of variance with replicates. Mean separation was accomplished with a Scott-Knott Cluster analysis.

The greenhouse planting included Burbank and Russet Burbank tubers harvested from the first generation planting.

During July 1982, the tubers were removed from storage and treated with rindite to break dormancy. One week later tubers were planted in eight inch pots in the greenhouse. Weights of individual tubers were recorded at planting. Growing conditions in the greenhouse were similar to the first generation planting except for seasonal changes in photoperiod and small temperature differences.

A randomized block design with twenty treatments, (five clones X three nodal transfers plus one set of control treatments) and ten replications was used. Tuber cultivars and sources included in this study were as follows:

1. Burbank (virus-free)
2. Russet Burbank (virus-free)
3. Russet Burbank variant (giant hill)
4. Russet Burbank variant (pebble leaf)
5. Russet Burbank (field selection)

Each of the five tuber sources was represented by tubers propagated normally (i.e. progeny tubers, from the original mother plants)

and tubers from tissue culture plantlets subjected to three, six and nine nodal transfers.

The plants did not flower so notes were taken three to four days after bud abscission. Dates of emergence and but abscission were recorded for all plants. Tubers were harvested fourteen weeks after emergence and stored at 4°C.

Data were analyzed using a two-way analysis of variance with replicates. Mean separation for the data was accomplished using the HSD test.

Long Term Propagation Experiment

First Generation Planting

This experiment involved nodal cutting propagation of a virus-free Centennial Russet clone for a thirteen month period and the comparison of this material with plant progeny originating from leaf bud tubers propagated from the same virus-free Centennial Russet clonal stock.

Leaf bud tubers were produced according to the method described by Lauer (17). Leaf bud cuttings consisted of stem segments with their corresponding leaf and axillary bud. They were taken from nearly mature plants and inserted into sand in a greenhouse bench so that the stem segment and axillary bud were below the surface. Small tubers one-half inch in diameter were produced in six to seven weeks. Leaf but tubers were used for the experiments because plant vigor more closely resembles tissue culture produced plantlets.

Leaf bud tubers and tissue culture plantlets were planted in a completely random design consisting of two treatments with thirty

plantlets per treatment. Methods of propagation and the data collected on plantlet characteristics were the same as described previously.

Data for the long term propagation experiment were analyzed using a one-way analysis of variance and means were separated using the LSD test.

Second Generation Planting

The tubers from the first generation planting of the long term propagation experiment were harvested and planted in the field as previously described. The tubers from both the tissue culture treatment and the leaf bud treatment were harvested at the same time, stored at 4°C for five weeks and planted in the field on June 25.

There were two treatments with thirty plants per treatment. Data were collected on plants three to four days after they flowered. Emergence and flowering dates also were recorded.

Data were analyzed using a one-way analysis of variance and the LSD test was used for mean separation.

TABLE 1

MURASHIGE AND SKOOG
TISSUE CULTURE MEDIUM

Stock Solution	Ingredients	mg/liter	grams/500ml stock solution
Nitrates	NH_4NO_3	1650.0	82.5
	KNO_3	1900.0	95.0
Sulfates	$CuSO_4.5H_2O$	.025	.00125
	$MgSO_4.7H_2O$	370.6	18.53
	$MnSO_4.H_2O$	22.3	1.115
	$AnSO_4.7H_2O$	8.6	.430
Halides	$CaCl_2.2H_2O$	439.8	22.0
	$Cocl_2.6H_2O$	.025	.00125
	KI	.830	.0415
PBMo	H_3BO_3	6.2	.31
	KH_2PO_4	170.0	8.5
	$Na_2MoO_4.2H_2O$	.25	.0125
FeNaEDTA	FeNaEDTA	36.7	1.835

Mixing Procedure

1. Add 10ml of each stock solution per liter MS medium.
2. Add organic inositol and thiamine HCl at 100.0 and 4.0mg/liter respectively.
3. Add sucrose (30 grams per litre), bring volume to 1 litre and adjust pH to 5.5.
4. Add agar (5.6 grams per litre), dissolve and autoclave.

TABLE 2

PLANT MORPHOLOGICAL CHARACTER OBSERVATIONS

Plant Part	Character	Description
Foliage		
Stem	Pigment on Internodes (above soil level)	Present or absent[1]
	Pigment on nodes (above soil level)	Present or absent
	Pigment on wings (midpart of stem)	Present or absent
	Waved wings (midpart of stem)	Present or absent
	Nodes (midpart of stem)	Swollen or straight
Leaves	Pigment on rachids (midpart of stem)	Present or absent
	Pigment on petioles (midpart of stem)	Present or absent
	Pigment on petioles and rachids (apical rosette)	Present or absent
	Openness of leaves	Open or closed[2]
	Color of foliage	Dark, medium or light compared w/ Centennial (medium)
Primary Leaflets	Pigment on petiolules (midpart of stem)	Present or absent
	Pigment on midveins (midpart of stem)	Present or absent
	Pigment on lateral veins (midpart of stem)	Present or absent

Table 2, continued

Plant Part	Character	Description
Floral		
Flower	Color of front of petal	White, pink or lavender
	Color of anthers	Lemon, yellow or orange
	Shape of anther column	Normal, twisted or malformed
	Style	Straight or bent
	Pigment in ovary interior	Present or absent
Inflorescence	Pigment on peduncle	Present or absent
	Pigment on abscission ring (at flowering)	Present or absent
	Pigment on buds (prior to bud opening)	Present or absent
Tuber		
Skin	Pigment	Absent, pink or russet
	Distribution	Wholly or partly
Eyes	Depth	Deep or shallow
	Distribution	Even or mostly on bud end

1. A description of absent for character includes indistinct pigment.

2. Open or closed leaflets indicate the number of secondary and tertiary leaflets and their size.

TABLE 3

PLANT CHARACTER MEASUREMENTS

Plant Part	Character	Method
Foliage		
Stem	Internodal distance	Length (cm) main stem/ total number of nodes
Leaves	Terminal leaf (length, width, area[1] and indices)	Average of 10 leaves midpart of stem
	First Primary leaf (length, width, area[1] and indices)	Average of 10 leaves midpart of stem
	Petiole length	Average of 10 petioles midpart of stem
Floral		
Flowers	Flowering time (maturity)	Days from emergence to flowering
	Frequency	Number of flowers per inflorescence
	Frequency	Percentage of plants to flower
	Number of flowers	Number of flowers per plant

1. Leaf area was measured on a Model LI-3000 Area Meter, Lambda Instruments Corporation; Lincoln, Nebraska.

CHAPTER IV

RESULTS

Observations and measurements were made and recorded on approximately thirty different foliage and floral characters in an attempt to determine if tissue culture propagation might elicit recognizable morphological changes. The amount of data obtained was large. A summary and condensation of the results would be desirable; however attempts to develop a more efficient way to present the data have not been successful.

Cultivar Experiment

The results of the second generation planting of the cultivar experiment, including the greenhouse planting of tubers for the Burbank and Russet Burbank cultivars, are reported. Notes were taken, but not reported on the tissue culture derived plantlets from the first generation planting because the data are misleading. The same phenomenon is illustrated in the first generation (greenhouse) phase of the Long Term Propagation Experiment. Variable planting dates (approximately one month between each treatment i.e. the three, six and nine nodal transfers) apparently resulted in changes in the growing conditions and other developmental factors, even in the greenhouse environment. The variation observed in the first generation plantings did not occur in

the second generation field grown plants. Data in Tables 4-11 represent the second generation planting of the cultivar experiment only.

Second Generation Planting

Field Planting: The observation data on plant characters are shown in Tables 4, 6 and 8, and foliage measurements are presented in Tables 5, 7 and 9.

In general, the great majority of the data recorded on more than thirty plant characters did not suggest any recognizable morphological changes associated with increasing numbers of tissue culture nodal transfers or temperature stress. There was a tendency for the pigmentation of buds and wings on the Norland and Red Norland cultivars to increase after six or nine nodal transfers (Table 4). Pigment readings in the Norgold Russet cultivar varied slightly on internodes and more noticeably on pedicels (Table 6). Pigment also varied on petiolules of Centennial Russet (Table 8). It is of interest to note that in seventy of the ninety-six sets of observations (on the presence or absence of pigment) this character was either totally present or totally absent. The pattern in the remaining twenty-six sets of observations were as follows: Sixteen sets of observations differed from the norm approximately 10 percent, which is considered normal error for these types of observations (30,31). Seven sets showed a tendency toward increased pigmentation as the number of nodal transfers increased. Three sets were mixed i.e. no discernable trend.

The "pebble leaf" variant of the Centennial Russet cultivar did not exhibit any pebbling of leaves in any of the treatments.

Thirty treatments were included in the statistical analysis of the ten plant characters measured. All data on floral characteristics and six complete treatments were not included in the analysis due to missing data. Any treatment with more than three missing observations was excluded. The treatment means for the data excluded from the analysis were recorded in the tables and denoted by x. Among the 109 sets of measurements only 3 (2.7 percent) showed statistically significant differences. These three treatments (denoted by *) did not form a meaningful pattern of treatment effects even though statistical analysis indicated a significant result. For example, in Table 5, (Red Norland) a significant increase in length of the primary leaf was found in the 6NT treatment, yet length of the primary leaf in the 3NT and 9NT treatments were less than (not significant) the control. The other two cases of statistical significance occurred in Centennial Russet (field) primary leaf indices and Centennial Russet (pebble leaf) primary leaf indices.

A somewhat erratic pattern was observed in the number of flowers per plant and flowers per inflorescence in the Centennial Russet cultivar but there was no consistent trend to indicate a treatment effect. A slight trend toward reduced percentage of plants flowering associated with increased numbers of nodal transfers was noted for the Norland (Table 5), Norgold Russet (Table 7) and Centennial Russet clones (Table 9). There was a trend toward smaller plants i.e. shorter internodal distance and petiole length, and slightly smaller leaves. This trend was more prevalent in the Norgold Russet and Centennial Russet cultivars and also in treatments which had undergone nine nodal transfers, cold storage and heat stress treatments.

Centennial Russet and Norgold Russet cultivar data did not indicate significant effects of cold storage or heat stress, but it did follow similar trends of treatments with nine nodal transfers; as explained above.

Cultivars, although similar statistically in some plant characters, were easily separated from each other on the basis of a wide range of characters. These data are shown in Tables 5, 7 and 9.

Clones of the Centennial Russet cultivar did not grow well at the experimental field site, stands were poor and there were considerable missing data. Plants were stunted, developed few flowers and expressed iron chlorosis symptoms.

TABLE 4

OBSERVATION ON SELECTED LEAF, STEM AND FLORAL CHARACTERS IN NORLAND, RED NORLAND AND NORCHIP POTATO CULTIVARS SUBJECTED TO THREE, SIX AND NINE NODAL TRANSFERS IN TISSUE CULTURE PROPAGATION COMPARED TO TUBER PROPAGATED MOTHER STOCK (CONTROL)

Type of Observation	Treatment	Percent of Plants		
		Norland V-free	Red Norland V-free	Norchip V-free
Leaves and Leaflets				
Pigment on petioles	control	100	100	0
	3NT	100	100	0
	6NT	100	100	0
	9NT	100	100	0
Pigment on rachids	control	100	100	0
	3NT	100	100	0
	6NT	100	100	0
	9NT	100	100	0
Pigment on upper petioles and rachids	control	100	100	0
	3NT	100	100	0
	6NT	100	100	0
	9NT	100	100	0
Pigment on petiolules	control	100	100	0
	3NT	100	100	0
	6NT	100	100	0
	9NT	100	100	0
Pigment on midveins	control	80	100	0
	3NT	90	80	0
	6NT	90	100	0
	9NT	100	100	0
Pigment on lateral veins	control	80	100	0
	3NT	90	70	0
	6NT	90	100	0
	9NT	100	90	0
Openness of leaflets (plants with closed leaflets)	control	100	100	100
	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100

Table 4, continued

Type of Observation	Treatment	Percent of Plants: Norland V-free	Red Norland V-free	Norchip V-free
Number of secondary leaflets	control	100	100	0
	3NT	100	100	0
	6NT	100	100	0
(plants with many)	9NT	100	100	0
Greenness of	control	100	100	0 light
(dark)	3NT	100	100	0 light
(dark, medium or light	6NT	100	100	0 light
compared w/Centennial)	9NT	100	100	0 light
(Centennial - medium)				
Stem				
Pigment on inter-	control	100	100	0
nodes	3NT	100	100	0
	6NT	100	100	0
	9NT	100	100	0
Pigment on nodes	control	100	100	0
	3NT	100	100	0
	6NT	100	100	0
	9NT	100	100	0
Pigment on wings	control	50	60	0
	3NT	60	60	0
	6NT	70	90	0
	9NT	90	80	0
Wings	control	100	100	100
	3NT	100	100	100
(w/o waved wings)	6NT	100	100	100
	9NT	100	100	100
Nodes	control	100	100	100
	3NT	100	100	100
(straight)	6NT	100	100	100
	9NT	100	100	100
Floral Notes				
Pigment on pedicel	control	100	100	0
	3NT	100	100	0
	6NT	100	100	0
	9NT	100	100	0
Pigment on	control	100	100	0
abscission ring	3NT	100	100	0
	6NT	100	100	0
	9NT	100	100	0

Table 4, continued

Type of Observation	Treatment	Percent of Plants Norland V-free	Red Norland V-free	Norchip V-free
Pigment on bud	control	70	60	0
	3NT	67	60	0
	6NT	90	100	0
	9NT	100	80	0
Flower color	control	100	100	0 white
	3NT	100	100	0 white
(plants with	6NT	100	100	0 white
lavender color)	9NT	100	100	0 white

TABLE 5

MEASUREMENTS OF SELECTED LEAF, STEM AND FLORAL CHARACTERS IN NORLAND, RED NORLAND AND NORCHIP POTATO CULTIVARS SUBJECTED TO THREE, SIX AND NINE NODAL TRANSFERS IN TISSUE CULTURE PROPAGATION COMPARED TO TUBER PROPAGATED MOTHER STOCK (CONTROL)

Type of Measurement	Treatment	Norland V-free	Red Norland V-free	Norchip V-free
Terminal Leaf				
Length		$(mm)_2$	(mm)	(mm)
	control	75.0 B	76.6 B	94.5 A
	3NT	77.2 B	75.9 B	96.2 A
	6NT	75.7 B	72.7 B	91.7 A
	9NT	74.0 B	78.5 B	95.7 A
Width		(mm)	(mm)	(mm)
	control	51.8 A	52.9 A	53.3 A
	3NT	55.3 A	54.4 A	56.3 A
	6NT	53.2 A	51.2 A	53.9 A
	9NT	54.0 A	55.7 A	55.2 A
Area		(cm^2)	(cm^2)	(cm^2)
	control	27.5 A	29.2 A	33.7 A
	3NT	31.0 A	30.0 A	36.6 A
	6NT	29.2 A	27.5 A	34.4 A
	9NT	29.4 A	31.1 A	36.3 A
Indices		(ratio w/1)	(ratio w/1)	(ratio w/1)
	control	.69 A	.68 A	.56 B
	3NT	.72 A	.72 A	.58 B
	6NT	.70 A	.71 A	.59 B
	9NT	.73 A	.66 A	.58 B
Primary Leaf				
Length		(mm)	(mm)	(mm)
	control	74.1 A	76.3 A	86.8 A
	3NT	74.9 A	75.4 A	91.1 A
	6NT	74.3 A	79.9 B*	80.1 A
	9NT	72.2 A	74.5 A	84.6 A
Width		(mm)	(mm)	(mm)
	control	45.1 A	46.8 A	46.0 A
	3NT	47.0 A	46.3 A	49.4 A
	6NT	46.2 A	42.7 A	43.8 A
	9NT	44.7 A	46.2 A	46.0 A
Area		(cm^2)	(cm^2)	(cm^2)
	control	24.8 A	27.0 A	28.2 A
	3NT	26.8 A	26.8 A	31.6 A
	6NT	26.2 A	23.0 A	25.6 A
	9NT	25.4 A	26.1 A	28.4 A

Table 5, continued

Type of Measurement	Treatment	Norland V-free	Red Norland V-free	Norchip V-free
		(ratio w/l)	(ratio w/l)	(ratio w/l)
Indices	control	.61 A	.61 A	.53 B
	3NT	.63 A	.61 A	.54 B
	6NT	.62 A	.61 A	.55 B
	9NT	.63 A	.62 A	.54 B
		(mm)	(mm)	(mm)
Petiole length	control	206 B	214 B	236 A
	3NT	211 B	218 B	242 A
	6NT	207 B	205 B	239 A
	9NT	188 B	210 B	246 A
		(cm)	(cm)	(cm)
Internodal distance	control	2.38 A	2.40 A	1.99 A
	3NT	2.34 A	2.35 A	2.01 A
	6NT	2.07 A	2.02 A	1.77 A
	9NT	1.98 A	2.29 A	1.83 A
Floral[1]				
Flowers per plant	control	19.6	19.6	19.7
	3NT	16.1	18.0	16.1
	6NT	11.4	11.4	12.3
	9NT	12.6	15.2	13.6
Flowers per inflorescence	control	15.2	15.2	12.9
	3NT	14.7	15.5	13.5
	6NT	11.4	11.4	10.8
	9NT	12.6	15.2	13.6
Percent plants flowering	control	100	100	100
	3NT	100	100	100
	6NT	90	89	100
	9NT	90	100	100
Maturity (days from emergence to flowering)	control	32.4	32.4	33.9
	3NT	34.8	34.0	37.4
	6NT	37.0	37.1	37.3
	9NT	35.9	34.7	37.1

1. Floral measurements were not included in the statistical analysis due to missing data as shown by percent of plants flowering.

2. Letter designations indicate which statistical group the mean belongs to. A different letter indicates that the mean of that treatment belongs to a different statistical group. These means are also denoted by *.

TABLE 6

OBSERVATIONS OF SELECTED LEAF, STEM AND FLORAL CHARACTERS IN NORGOLD RUSSET AND NORGOLD RUSSET M POTATO CULTIVARS SUBJECTED TO THREE, SIX AND NINE NODAL TRANSFERS IN TISSUE CULTURE PROPAGATION, COLD STORAGE AND HEAT STRESS COMPARED TO TUBER PROPAGATED MOTHER STOCK (CONTROL)

Type of Observation	Treatment	Percent of Plants Norgold Russ. V-free	Norgold Russ.(M) V-free
Leaves and Leaflets			
Pigment on petioles	control	100	100
	3NT	100	100
	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	
Pigment on rachids	control	100	100
	3NT	100	100
	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	
Pigment of upper petioles and rachids	control	100	100
	3NT	83	90
	6NT	100	90
	9NT	100	100
	cold storage	100	
	heat stress	100	
Pigment on petiolules	control	100	100
	3NT	100	90
	6NT	100	90
	9NT	100	100
	cold storage	100	
	heat stress	100	
Pigment on midveins (w/o pigment)	control	100	100
	3NT	100	100
	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	

Table 6, continued

Type of Observation	Treatment	Percent of Plants Norgold Russ. V-free	Norgold Russ.(M) V-free
Pigment on lateral veins (w/o pigment)	control	100	100
	3NT	100	100
	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	
Openness of leaflets (closed leaflets)	control	100	100
	3NT	100	100
	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	
Number of secondary leaflets (many)	control	100	100
	3NT	100	100
	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	
Greenness of foliage (dark) (dark, medium or light compared with Centennial Centennial - medium)	control	100	100
	3NT	100	100
	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	
Stem			
Pigment on internodes (w/o pigment)	control	100	90
	3NT	83	90
	6NT	90	100
	9NT	90	100
	cold storage	67	
	heat stress	90	
Pigment on nodes (w/o pigment)	control	100	90
	3NT	100	90
	6NT	100	100
	9NT	100	100
	cold storage	90	
	heat stress	100	

Table 6, continued

Type of Observation	Treatment	Percent of Plants Norgold Russ. V-free	Norgold Russ.(M) V-free
Pigment on wings	control	100	100
	3NT	100	100
(w/o pigment)	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	
Wings	control	100	100
	3NT	100	100
(waved)	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	
Nodes	control	100	100
	3NT	100	100
(straight)	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	
Floral Notes			
Pigment on pedicel	control	100	90
	3NT	57	70
(w/o pigment)	6NT	55	40
	9NT	100	0[1]
	cold storage	0	
	heat stress	43	
Pigment on	control	100	100
abscission ring	3NT	100	100
	6NT	100	100
	9NT	100	100
	cold storage	100	
	heat stress	100	
Pigment on bud	control	100	90
	3NT	100	100
(pigment prior to	6NT	100	100
bud opening)	9NT	100	100
	cold storage	100	
	heat stress	100	

Table 6, continued

Type of Observation	Treatment	Percent of Plants Norgold Russ. V-free	Norgold Russ.(M) V-free
Flower color	control	100	100
	3NT	100	100
(lavender)	6NT	100	100
	9NT	100	100
	cold storage	100	100
	heat stress	100	100

[1]Only one plant flowered in this treatment.

TABLE 7

MEASUREMENTS OF SELECTED LEAF AND FLORAL CHARACTERS OF NORGOLD RUSSET AND NORGOLD RUSSET "M" POTATO CULTIVARS SUBJECTED TO THREE, SIX AND NINE NODAL TRANSFERS IN TISSUE CULTURE PROPAGATION, COLD STORAGE, AND HEAT STRESS COMPARED TO TUBER PROPAGATED MOTHER STOCK (CONTROL)

Type of Measurement	Treatment	Norgold Russ. V-free	Norgold Russ."M" V-free
Terminal Leaf			
		(mm)	(mm)
Length	control	60.6 C[3]	59.1 C
	3NT	57.1 C x[2]	56.1 C
	6NT	56.2 C	56.7 C
	9NT	56.6 C	53.9 C x
	cold storage	58.6 C	
	heat stress	58.0 C	
		(mm)	(mm)
Width	control	39.9 B	39.4 B
	3NT	39.8 B x	37.8 B
	6NT	40.1 B	38.4 B
	9NT	40.8 B	38.4 B x
	cold storage	40.1 B	
	heat stress	40.7 B	
		(cm^2)	(cm^2)
Area	control	18.1 B	16.9 B
	3NT	17.1 B x	16.2 B
	6NT	16.8 B	16.4 B
	9NT	17.6 B	16.1 B x
	cold storage	18.1 B	
	heat stress	17.8 B	
		(ratio w/1)	(ratio w/1)
Indices	control	.66 A	.66 A
	3NT	.70 A x	.67 A
	6NT	.71 A	.68 A
	9NT	.72	.72 A x
	cold storage	.68	
	heat stress	.70 A	
Primary Leaf		(mm)	(mm)
Length	control	62.2 B	60.1 B
	3NT	59.3 B x	57.4 B
	6NT	60.5 B	58.4 B
	9NT	58.2 B	58.4 B x
	cold storage	60.4 B	
	heat stress	57.4 B	

Table 7, continued

Type of Measurement	Treatment	Norgold Russ. V-free	Norgold Russ."M" V-free
		(mm)	(mm)
Width	control	37.7 B	36.5 B
	3NT	37.3 B x	35.4 B
	6NT	37.0 B	35.6 B
	9NT	35.7 B	35.6 B
	cold storage	36.9 B	
	heat stress	35.5 B	
		(cm^2)	(cm^2)
Area	control	17.9 B	16.5 B
	3NT	17.5 B x	15.6 B
	6NT	17.4 B	16.1 B
	9NT	16.4 B	16.4 B
	cold storage	18.0 B	
	heat stress	16.3 B	
		(ratio w/l)	(ratio w/l)
Indices	control	.60 A	.61 A
	3NT	.63 A x	.62 A
	6NT	.61 A	.61 A
	9NT	.61 A	.61 A x
	cold storage	.62 A	
	heat stress	.61 A	
		(mm)	(mm)
Petiole length	control	252 A	266 A
	3NT	243 A x	248 A
	6NT	248 A	241 A
	9NT	230 A	232 A x
	cold storage	248 A	
	heat stress	252 A	
		(cm)	(cm)
Internodal distance	control	2.10 A	1.96 A
	3NT	1.80 A x	1.75 A
	6NT	1.78 A	1.68 A
	9NT	1.44 A	1.20 A x
	cold storage	1.77 A	
	heat stress	1.49 A	
Floral[1]			
Flowers per plant	control	21.0	20.0
	3NT	9.0	15.6
	6NT	12.9	13.8
	9NT	5.5	----
	cold storage	8.0	
	heat stress	10.1	

Table 7, continued

Type of Measurement	Treatment	Norgold Russ. V-free	Norgold Russ."M" V-free
Flowers per inflorescence	control	12.3	13.7
	3NT	9.0	13.3
	6NT	12.9	12.6
	9NT	5.5	----
	cold storage	8.0	
	heat stress	10.1	
Percent plants flowering	control	100	100
	3NT	100	100
	6NT	90	100
	9NT	33	----
	cold storage	33	
	heat stress	70	
Maturity (days from emergence to flowering)	control	30.0	31.6
	3NT	40.0	36.1
	6NT	33.7	40.1
	9NT	35.0	----
	cold storage	35.3	
	heat stress	35.3	

1. Floral measurements were not included in the statistical analysis due to missing data as shown by percent of plants flowering.

2. Treatments (other than floral measurements) not included in the statistical analysis due to missing data are denoted by x.

3. Letter designations indicate which statistical group the mean belongs to. A different letters indicates that the mean of that treatment belongs to a different statistical group. These means are also denoted by *.

TABLE 8

OBSERVATIONS OF SELECTED LEAF, STEM AND FLORAL CHARACTERS OF THREE CLONES OF THE CENTENNIAL RUSSET POTATO CULTIVAR SUBJECTED TO THREE, SIX AND NINE NODAL TRANSFERS IN TISSUE CULTURE PROPAGATION, COLD STORAGE AND HEAT STRESS COMPARED TO TUBER PROPAGATED MOTHER STOCK (CONTROL)

Type of Observation	Treatment	Percent of Plants Centennial V-free	Centennial Field	Centennial Pebble Leaf
Leaves and Leaflets				
Pigment on petioles	control	100	100	100
	3NT	100	100	100
(w/o pigment)	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Pigment on	control	100	100	100
rhachids	3NT	86	100	100
(w/o pigment)	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Pigment on upper	control	100	100	100
petioles and rachids	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Pigment on	control	100	78	70
petiolules	3NT	100	75	67
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Pigment on	control	100	100	100
midveins	3NT	100	100	100
(w/o pigment)	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		

Table 8, continued

Type of Observation	Treatment	Percent of Plants Centennial V-free	Centennial Field	Centennial Pebble Leaf
Pigment on lateral veins (w/o pigment)	control	100	100	100
	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Openness of leaflets (open)	control	100	100	100
	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Number of secondary leaflets (few)	control	100	100	100
	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Greenness of foliage (medium) (dark, medium or light compared with Centennial Centennial = medium)	control	100	100	100
	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Stem				
Pigment on internodes	control	100	100	100
	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Pigment on nodes	control	100	100	100
	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		

Table 8, continued

Type of Observation	Treatment	Percent of Plants Centennial V-free	Centennial Field	Centennial Pebble Leaf
Pigment on wings	control	100	90	100
	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Wings	control	100	100	100
	3NT	100	100	100
(waved)	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Nodes	control	100	100	100
	3NT	100	100	100
(straight)	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Floral Nodes				
Pigment on pedicel	control	89	89	89
	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Pigment on	control	100	89	100
abscission ring	3NT	100	100	100
	6NT	100	100	100
	9NT	100	100	100
	cold storage	100		
	heat stress	100		
Pigment on bud	control	100	100	89
	3NT	100	100	100
(pigment prior to	6NT	100	100	100
bud opening)	9NT	100	100	100
	cold storage	100		
	heat stress	100		

Table 8, continued

Type of Observation	Treatment	Percent of Plants Centennial V-free	Centennial Field	Centennial Pebble Leaf
Flower Color	control	100	100	100
	3NT	100	100	100
(plants with pink	6NT	100	100	100
color)	9NT	100	100	100
	cold storage	100		
	heat stress	100		

TABLE 9

MEASUREMENTS OF SELECTED LEAF AND FLORAL CHARACTERS OF THREE CLONES OF THE CENTENNIAL RUSSET POTATO CULTIVAR SUBJECTED TO THREE, SIX AND NINE NODAL TRANSFERS IN TISSUE CULTURE PROPAGATION, COLD STORAGE AND HEAT STRESS COMPARED TO TUBER PROPAGATED MOTHER STOCK (CONTROL)

Type of Measurement	Treatment	Centennial V-free	Centennial Field	Centennial Pebble Leaf
Terminal Leaf		(mm)	(mm)	(mm)
Length	control	72.1 B[3]	74.8 B	73.2 B
	3NT	70.3 B	71.2 B x	72.1 B x
	6NT	71.8 B	72.7 B	73.8 B
	9NT	69.6 B	68.6 B x	65.4 B
	cold storage	68.9 B		
	heat stress	66.0 B x[2]		
		(mm)	(mm)	(mm)
Width	control	43.6 B	45.4 B	44.6 B
	3NT	42.8 B	43.7 B x	44.7 B x
	6NT	44.7 B	45.4 B	44.2 B
	9NT	44.4 B	43.4 B x	41.8 B
	cold storage	42.9 B		
	heat stress	42.2 B x		
		(cm^2)	(cm^2)	(cm^2)
Area	control	21.9 B	23.8 B	23.2 B
	3NT	21.2 B	22.8 B x	23.0 B x
	6NT	22.5 B	23.3 B	23.9 B
	9NT	22.0 B	21.9 B x	20.1 B
	cold storage	20.8 B		
	heat stress	20.3 B x		
		(ratio w/1)	(ratio w/1)	(ratio w/1)
Indices	control	.60 B	.61 B	.61 B
	3NT	.60 B	.62 B x	.62 B x
	6NT	.62 B	.63 B	.62 B
	9NT	.64 B	.64 B x	.64 B
	cold storage	.62 B		
	heat stress	.64 B x		
Primary Leaf		(mm)	(mm)	(mm)
Length	control	63.2 B	67.0 B	66.0 B
	3NT	63.0 B	61.4 B x	64.2 B x
	6NT	64.1 B	62.7 B	67.6 B
	9NT	60.3 B	57.4 B x	60.3 B
	cold storage	59.5 B		
	heat stress	58.9 B x		

Table 9, continued

Type of Measurement	Treatment	Centennial V-free	Centennial Field	Centennial Pebble Leaf
		(mm)	(mm)	(mm)
Width	control	35.3 B	38.6 B	38.5 B
	3NT	35.5 B	35.1 B x	36.7 B x
	6NT	36.7 B	37.0 B	38.9 B
	9NT	33.6 B	33.4 B x	36.7 B
	cold storage	33.4 B		
	heat stress	34.3 B x		
		(cm^2)	(cm^2)	(cm^2)
Area	control	16.3 B	18.5 B	19.1 B
	3NT	16.7 B	16.4 B x	17.3 B x
	6NT	18.0 B	17.8 B	19.6 B
	9NT	15.3 B	15.2 B x	16.4 B
	cold storage	14.8 B		
	heat stress	15.5 B x		
		(ratio w/1)	(ratio w/1)	(ratio w/1)
Indices	control	.56 B	.58 B	.58 B
	3NT	.56 B	.58 B x	.57 B x
	6NT	.58 B	.60 A *	.58 B
	9NT	.56 B	.58 B x	.60 A *
	cold storage	.56 B		
	heat stress	.59 B x		
		(mm)	(mm)	(mm)
Petiole length	control	120 C	134 C	124 C
	3NT	114 C	116 C x	120 C x
	6NT	113 C	122 C	122 C
	9NT	108 C	100 C x	112 C
	cold storage	110 C		
	heat stress	97 C x		
		(cm)	(cm)	(cm)
Internodal distance	control	2.18 A	2.07 A	2.13 A
	3NT	2.09 A	2.00 A x	2.10 A x
	6NT	2.05 A	2.14 A	2.07 A
	9NT	1.89 A	1.70 A x	2.00 A
	cold storage	2.00 A		
	heat stress	1.80 A x		

Table 9, continued

Type of Measurement	Treatment	Centennial V-free	Centennial Field	Centennial Pebble Leaf
Floral[1]				
Flowers per plant	control	10.4	8.8	10.8
	3NT	6.7	6.3	8.2
	6NT	10.1	11.5	8.5
	9NT	11.4	5.0	7.3
	cold storage	2.0		
	heat stress	10.5		
Flowers per inforscence	control	7.9	8.8	9.2
	3NT	6.7	6.3	8.2
	6NT	7.6	10.0	8.5
	9NT	6.0	5.0	7.3
	cold storage	2.0		
	heat stress	7.6		
Percent plants flowering	control	89	100	99
	3NT	86	75	83
	6NT	89	70	86
	9NT	56	60	57
	cold storage	40		
	heat stress	44		
Maturity (days from emergence to flowering)	control	39.9	39.9	39.8
	3NT	42.5	39.7	39.0
	6NT	43.9	42.1	42.2
	9NT	40.4	49.0	45.8
	cold storage	43.5		
	heat stress	43.8		

1. Floral measurements were not included in the statistical analysis due to missing data as shown by percent of plants flowering.

2. Treatments (other than floral measurements) not included in the statistical analysis due to missing data are denoted by x.

3. Letter designations indicated which statistical group the mean belongs to. A different letters indicates that the mean of that treatment belongs to a different statistical group. These means are also denoted by *.

Greenhouse Planting (Burbank and Russet Burbank Cultivars)

The data from this portion of the cultivar experiment were obtained from greenhouse grown plants rather than from field plots for reasons described in Materials and Methods. The planting stock consisted of progeny tubers derived from tissue culture plantlets grown in the greenhouse. While every attempt was made to select uniform size seed tubers for planting, the data (Table 10) show that the average tuber weight in the control treatments was somewhat less than in the three, six and nine nodal transfer treatments.

TABLE 10

AVERAGE WEIGHT OF FOUR RUSSET BURBANK AND ONE BURBANK CLONES OF SEED TUBERS IN THE GREENHOUSE PLANTING OF THE CULTIVAR EXPERIMENT

		v-free (gm)	field (gm)	"p.leaf" (gm)	"g.hill" (gm)	Burb. (gm)	Ave. (gm)
Tuber wt. of planting stock	control	20.4	15.2	20.1	28.4	15.8	20.0
	3NT	35.1	40.2	39.1	15.6	20.3	30.1
	6NT	36.9	32.0	38.6	44.3	37.1	37.8
	9NT	35.4	36.2	36.4	43.8	46.5	39.7
	Ave.(gm)	32.0	30.9	33.6	33.0	29.9	

The observations on plant characters (Table 11) indicate that (as in the field planting), differences among treatments occurred only in traits involving pigment. There were 90 sets of observations and differences greater than approximately 10 percent, (considered more then normal variation) were found in 19 sets. These differences occurred on pigment readings of petioles, rhachids, midveins, lateral veins and nodes. The percent of plants differing from the norm was greater in the control than any of the nodal transfer treatments in eighteen of nineteen data set comparisons. Ten of the nineteen data sets where

differences occurred, were also on "pebble leaf" and "giant hill" variants. "Giant hill" characteristics such as taller and stiffer plant growth and coarser tubers were not observed. The "pebble leaf" clone exhibited slight pebbling of leaves on many plants, but not to the degree observed in commercial potato fields.

The plant measurement data (Table 12) show that there were significant differences among treatments in nineteen of fifty-five sets of data analyzed. Thirteen of the nineteen significant differences occurred in the "pebble leaf" and "giant hill" clones while the remaining six sets of significant effects were scattered among the other three clones. There was a trend toward smaller plants in the control compared to the plants propagated in tissue culture. This trend was generally expressed in smaller leaves, shorter petioles and shorter internodal distances. In sixteen out of the nineteen sets of measurements where significance occurred, it was between the control treatment and one of the three, six or nine nodal transfer treatments. These data indicate there was little difference between tissue culture treatments of a cultivar but the significance existing control treatments and tissue culture treatments, accounts for the trend mentioned above. The three sets where differences occurred among tissue culture treatments were: (1) Russet Burbank "pebble leaf" terminal leaf width, (2) Russet Burbank "pebble leaf" terminal leaf area and (3) Russet Burbank (field) maturity.

TABLE 11

OBSERVATIONS OF SELECTED LEAF, STEM AND TUBER CHARACTERS IN FOUR CLONES OF RUSSET BURBANK AND ONE BURBANK CLONES SUBJECTED TO THREE, SIX AND NINE NODAL TRANSFERS IN TISSUE CULTURE PROPAGATION COMPARED TO TUBER PROPAGATED MOTHER STOCK (CONTROL)

Type of Observation	Treatment	V-free	Percent of Plants: Russet Burbank Field	Russet Burbank Pebble Leaf	Russet Burbank Giant Hill	Burbank V-free
Leaves and Leaflets						
Pigment on petioles	control	70	50	0	20	50
	3NT	100	90	50	67	100
	6NT	90	100	50	100	90
	9NT	62	100	88	100	90
Pigment on rhachids	control	90	90	30	40	50
	3NT	100	90	100	80	100
	6NT	90	100	80	100	90
	9NT	88	100	75	100	90
Pigment on upper petioles and rachids	control	100	100	90	100	100
	3NT	100	100	100	100	100
	6NT	100	100	100	100	100
	9NT	100	100	88	100	100
Pigment on petiolules	control	100	100	90	90	100
	3NT	100	100	100	100	100
	6NT	100	100	100	100	100
	9NT	88	100	88	100	100
Pigment on midveins	control	90	90	60	70	80
	3NT	100	90	100	90	100
	6NT	100	100	80	100	100
	9NT	88	100	75	100	100

Table 11, continued

Type of Observation	Treatment	Percent of Plants				
		V-free	Russet Burbank Field	Russet Burbank Pebble Leaf	Russet Burbank Giant Hill	Burbank V-free
Pigment on lateral veins	control	90	90	60	70	80
	3NT	100	90	100	90	100
	6NT	100	100	80	100	100
	9NT	88	100	75	100	100
Openness of leaflets	control	100	100	100	100	100
	3NT	100	100	100	100	100
(open)	6NT	100	100	100	100	100
	9NT	100	100	100	100	100
Number of secondary leaflets	control	100	100	100	100	100
	3NT	100	100	100	100	100
	6NT	100	100	100	100	100
(few)	9NT	100	100	100	100	100
Greeness of foliage	control	100	100	100	100	100
(dark)	3NT	100	100	100	100	100
(dark, medium or light	6NT	100	100	100	100	100
compared to Centennial	9NT	100	100	100	100	100
Centennial = medium)						
Stem						
Pigment on internodes	control	100	100	100	100	100
	3NT	100	100	100	100	100
	6NT	100	100	100	100	100
	9NT	100	100	100	100	100

Table 11, continued

Type of Observation	Treatment	Percent of Plants				
		V-free	Russet Burbank Field	Burbank Pebble Leaf	Giant Hill	Burbank V-free
Pigment on nodes	control	70	70	70	50	60
	3NT	100	80	90	100	100
	6NT	90	70	90	100	90
	9NT	80	90	90	100	100
Pigment on wings	control	100	100	100	100	100
	3NT	100	100	100	100	100
	6NT	100	100	100	100	100
	9NT	100	100	100	100	100
Wings	control	100	100	100	100	100
	3NT	100	100	100	100	100
(waved)	6NT	100	100	100	100	100
	9NT	100	100	100	100	100
Nodes	control	100	100	100	100	100
	3NT	100	100	100	100	100
(straight)	6NT	100	100	100	100	100
	9NT	100	100	100	100	100
Tuber						
Skin color	control	100	100	100	100	100
	3NT	100	100	100	100	100
(russet)	6NT	100	100	100	100	100
	9NT	100	100	100	100	100
Distribution of skin color	control	100	100	100	100	0
	3NT	100	100	100	100	0
	6NT	100	100	100	100	0
(wholly distributed)	9NT	100	100	100	100	0

Table 11, continued

Type of Observation	Treatment	V-free	Percent of Plants: Russet Burbank Field	Russet Burbank Pebble Leaf	Russet Burbank Giant Hill	Burbank V-free
Depth of eyes	control	100	100	100	100	100
	3NT	100	100	100	100	100
	6NT	100	100	100	100	100
	9NT	100	100	100	100	100
Distribution of eyes	control	100	100	100	100	100
	3NT	100	100	100	100	100
(distributed mostly on bud end)	6NT	100	100	100	100	100
	9NT	100	100	100	100	100

1. Treatments of "pebble leaf" variant of Russet Burband exhibited only slight pebbling of leaves. The percentage of plants showing pebbling of leaves were: Control-60%, 3NT-60%, 6NT-90%, and 9NT-88%.

2. No plant character variations were noted on the "giant hill" variant in Russet Burbank, other than those recorded.

3. Floral observations were not taken because plants did not flower.

TABLE 12

MEASUREMENTS OF SELECTED LEAF AND STEM CHARACTERS IN FOUR CLONES OF RUSSET BURBANK AND ONE BURBANK CLONE SUBJECTED TO THREE, SIX AND NINE NODAL TRANSFERS IN TISSUE CULTURE PROPAGATION COMPARED TO TUBER PROPAGATED MOTHER STOCK (CONTROL)

Type of Measurement	Treatment	Russet Burbank V-free	Field	Pebble Leaf	Giant Hill
Terminal Leaf		(mm)	(mm)	(mm)	(mm)
Length	control	64.4	62.4	54.9 D[2]	56.8 CD
	3NT	71.1	64.2	60.0 BCD	67.4 AB
	6NT	67.0	66.3	64.0 ABCD	65.7 ABC
	9NT	66.5	67.2	69.0 AB	67.4 AB
		(mm)	(mm)	(mm)	(mm)
Width	control	39.6	37.3	34.6 EF	34.3 F
	3NT	44.9	39.3	39.1 CDEF	41.7 ABCD
	6NT	41.8	41.2	41.6 ABCD	40.8 BCDEF
	9NT	41.8	42.3	45.8 AB	42.4 ABCD
		(cm^2)	(cm^2)	(cm^2)	(cm^2)
Area	control	18.2	17.1	13.2 F	13.8 EF
	3NT	23.2	18.1	16.8 DEF	20.3 ABCD
	6NT	20.3	20.0	20.0 ABCDE	19.5 ABCDE
	9NT	20.2	20.8	22.9 ABC	20.6 ABCD
		(ratio w/1)	(ratio w/1)	(ratio w/1)	(ratio w/1)
Indices	control	.62	.60	.63	.60
	3NT	.63	.61	.65	.62
	6NT	.63	.62	.65	.62
	9NT	.63	.63	.66	.63

Table 12, continued

Type of Measurement	Treatment	Russet Burbank V-free	Field	Pebble Leaf	Giant Hill
Primary Leaf		(mm)	(mm)	(mm)	(mm)
Length	control	54.5	49.9	43.0 C	46.4 BC
	3NT	58.0	55.2	49.4 ABC	54.2 AB
	6NT	55.8	56.0	52.7 AB	56.0 A
	9NT	54.7	55.2	56.1 A	57.2 A
		(mm)	(mm)	(mm)	(mm)
Width	control	29.9	28.1	24.9 E	25.6 DE
	3NT	31.9	28.8	28.8 BCDE	30.9 ABCD
	6NT	31.3	30.8	30.8 ABCD	31.5 ABC
	9NT	29.8	34.4	34.4 AB	32.2 ABC
		(cm^2)	(cm^2)	(cm^2)	(cm^2)
Area	control	12.6	10.6	8.1 D	9.1 CD
	3NT	14.8	12.7	10.9 BCD	13.1 ABC
	6NT	13.6	13.6	12.6 ABC	13.9 AB
	9NT	12.6	13.7	15.0 AB	14.1 AB
		(ratio w/1)	(ratio w/1)	(ratio w/1)	(ratio w/1)
Indices	control	.55	.56	.58	.55
	3NT	.55	.56	.58	.57
	6NT	.56	.56	.58	.56
	9NT	.54	.58	.61	.56

Table 12, continued

Type of Measurement	Treatment	Russet Burbank V-free	Field	Pebble Leaf	Giant Hill
		(mm)	(mm)	(mm)	(mm)
Petiole length	control	130	127 BCD	104	129
	3NT	136	155 A	121	128
	6NT	142	144 ABC	127	146
	9NT	142	128 ABC	120	151
		(cm)	(cm)	(cm)	(cm)
Internodal distance	control	4.41 BCDE	4.21	4.30 DEF	4.28
	3NT	4.93 A	4.46	4.74 ABC	4.07
	6NT	4.65 ABCD	4.44	4.90 A	4.51
	9NT	4.83 AB	4.42	4.76 ABC	4.42
Maturity[1] (days from emergence to bud abscission)	control	40.1	47.7 A	45.0	40.1
	3NT	40.8	40.8 AB	42.1	42.1
	6NT	43.3	37.4 B	45.8	40.5
	9NT	46.0	47.1 A	39.7	41.2

Type of Measurement	Treatment	Burbank V-free
Terminal Leaf		(mm)
Length	control	64.7
	3NT	67.1
	6NT	71.7
	9NT	70.6

Table 12, continued

Type of Measurement	Treatment	Burbank V-free
		(mm)
Width	control	40.4 BCDEF
	3NT	44.8 ABC
	6NT	47.5 A
	9NT	45.7 ABC
		(cm^2)
Area length	control	19.0 BCDE
	3NT	21.2 ABCD
	6NT	25.7 A
	9NT	23.9 AB
		(ratio w/1)
Indices	control	.62 BCD
	3NT	.67 A
	6NT	.66 AB
	9NT	.65 ABC
Primary Leaf		(mm)
Length	control	51.1
	3NT	54.2
	6NT	57.7
	9NT	56.4

Table 12, continued

Type of Measurement	Treatment	Burbank V-free
		(mm)
Width	control	30.0
	3NT	32.7
	6NT	34.6
	9NT	32.9
Primary Leaf		(cm^2)
Area	control	11.9
	3NT	13.8
	6NT	16.2
	9NT	14.6
		(ratio w/l)
Indices	control	.59
	3NT	.60
	6NT	.60
	9NT	.58
		(mm)
Petiole length	control	122
	3NT	121
	6NT	140
	9NT	127

Table 12, continued

Type of Measurement	Treatment	Burbank V-free
		(cm)
Internodal distance	control	4.15
	3NT	3.95
	6NT	4.16
	9NT	4.10
Maturity (days from emergence to bud abscission)	control	39.1
	3NT	41.5
	6NT	39.1
	9NT	43.9

1. Floral notes were not taken since plants did not flower.

2. Means with at least one common letter are not significantly different (P=0.05) with and HSD test. This shown only in sets of measurements where significance occurred.

Long-Term Propagation Experiment - (First and Second Generation Plantings)

The observations on selected leaf, and stem characters in the first generation planting (derived from tissue culture plantlets) were compared with those on plants derived from leaf bud propagated tubers. The data from the second generation planting (progeny tubers of the first generation) included the same observations with the addition of floral notes. No treatment effects were found in either planting of the experiment (Table 13).

When leaf and stem measurements were made on plants from the first generation planting, it was found that in all measurements, except the primary leaf indices, tissue culture propagated plants were significantly different from leaf bud propagated plants (Table 14). These data were expressed as larger terminal leaves, smaller primary leaves, shorter petiole length and shorter internodal distances versus the leaf bud derived plants. Since the plants did not flower, floral observations were not taken.

Measurements of plant characters in the second generation planting were almost identical in the two treatments. Only one significant F-ratio was found, which occured in petiole length. Measurements of this trait were not significant (P=0.05) with an LSD test.

TABLE 13

OBSERVATIONS ON SELECTED LEAF, STEM AND FLORAL CHARACTERS ON FIRST AND SECOND GENERATION PLANTINGS OF THE CENTENNIAL RUSSET POTATO CULTIVAR PRODUCED BY TWO DIFFERENT METHODS OF PROPAGATION (Tissue Culture Propagation for Thirteen Months Versus Leafbud Tuber Propagation)

Type of Observation	Treatment	Percent of Plants	
		Greenhouse Phase	Field Phase
Leaves and Leaflets			
Pigment on petioles (w/o pigment)	Leaf Bud (LB)	100	100
	Tissue Culture (TC)	100	100
Pigment on rhachids (w/o pigment)	LB	100	100
	TC	100	100
Pigment on upper petioles and rhachids	LB	100	100
	TC	100	100
Pigment on petiolules	LB	100	100
	TC	100	100
Pigment on midveins (w/o pigment)	LB	100	100
	TC	100	100
Pigment on lateral veins (w/o pigment)	LB	100	100
	TC	100	100
Openness of leaflets (open)	LB	100	100
	TC	100	100
Number of secondary leaflets (few)	LB	100	100
	TC	100	100
Greenness of foliage (medium) (dark, medium or light compared with Centennial Centennial = medium)	LB	100	100
	TC	100	100
Stem			
Pigment on internodes	LB	100	100
	TC	100	100
Pigment on nodes	LB	100	100
	TC	100	100

Table 13, continued

Type of Observation	Treatment	Percent of Plants	
		Greenhouse Phase	Field Phase
Pigment on wings	LB	100	100
	TC	100	100
Wings (waved)	LB	100	100
	TC	100	100
Nodes (straight)	LB	100	100
	TC	100	100
Floral			
Pigment on pedicel	LB		100
	TC		100
Pigment on abscission ring	LB		100
	TC		100
Pigment on bud (with pigment prior to bud opening)	LB		100
	TC		100
Flower color (pink)	LB		100
	TC		100

TABLE 14

MEASUREMENTS OF SELECTED LEAF, STEM AND FLORAL CHARACTERS ON FIRST AND SECOND GENERATION PLANTINGS OF THE CENTENNIAL RUSSET POTATO CULTIVAR PRODUCED BY TWO DIFFERENT METHODS OF PROPAGATION (Tissue Culture Propagation for Thirteen Months Versus Leaf Bud Tuber Propagation)

Type of Observation	Treatment	Greenhouse Phase (1st Gen.)	Field Phase (2nd Gen.)
Terminal Leaf		(mm)	(mm)
Length	Leaf Bud (LB)	80.2	70.1
	Tissue Culture (TC)	83.0*[1]	70.5
		(mm)	(mm)
Width	LB	48.0	44.0
	TC	51.1*	44.5
		(cm^2)	(cm^2)
Area	LB	27.1	22.2
	TC	29.7*	22.6
		(ratio w/1)	(ratio w/1)
Indices	LB	.60	.63
	TC	.62*	.63
Primary Leaf		(mm)	(mm)
Length	LB	56.9	62.6
	TC	53.3*	63.1
		(mm)	(mm)
Width	LB	32.4	35.5
	TC	30.0*	36.2
		(cm^2)	(cm^2)
Area	LB	13.6	16.9
	TC	11.9*	17.4
		(ratio w/1)	(ratio w/1)
Indices	LB	.57	.57
	TC	.56	.57
		(mm)	(mm)
Petiole length	LB	116.2	124.2
	TC	98.3*	116.6

Table 14, continued

Type of Observation	Treatment	Greenhouse Phase (1st Gen.)	Field Phase (2nd Gen.)
		(cm)	(cm)
Internodal distance	LB	3.2	2.1
	TC	2.5*	2.1
Floral			
Flowering time (days)	LB		43.2
	TC		44.3
Flowers per plant	LB		8.1
	TC		9.0
Flowers per inflorescence	LB		6.9
	TC		6.9
Percent Plants flowering	LB		90.0
	TC		89.7

1. Significantly different (P=0.05; LSD test)

CHAPTER V

DISCUSSION

Cultivar Study

Second Generation Planting

Field Planting: Plant character morphology was apparently not often influenced by nodal transfer or temperature stress treatments. Pigment variations in some plant parts were probably related to seed tuber dormancy which ranged from one to nine weeks. Treatments with more nodal transfers i.e. six nodal transfers and especially nine nodal transfers received short seed tuber dormancy periods. This also was true for cold storage and heat stress treatments. The short dormancy period resulted in delayed emergence of many plants in these treatments; the emergence dates varied from July 17 to August 16. Delayed emergence would result in plants grown under different environmental conditions (temperature and photoperiod) and a shorter growing season with fewer plants flowering. The changing environmental conditions would likely result in some variation since there is a tendency for pigmentation to fade on leaves and increase on stems as the growing season progresses (30,31). This may help explain the trend for an increase in pigmentation as the number of nodal transfers was extended.

There were three cases where variation was found with no discernable trend related to treatment. The differences were found in

pedicel pigment of Norgold Russet and Norgold Russet M and lateral vein pigment of Red Norland. The occurrence of pigment in these plant parts is not a prominent character trait of the Norgold Russet cultivar. While pigmentation is a valid character to identify different cultivars it may not necessarily be a good indicator of possible genetic change in a cultivar such as Norgold Russet. Slight changes in pigmentation could easily result from changes in environmental conditions and subsequently lead to variable data in this experiment.

Measurement data of plant foliage parts within a given clone rarely revealed statistically significant differences. Of the 109 sets of measurements only 3 (2.7 percent) sets were statistically significant. Different cultivars (as might be expected) were statistically segregated from each other in terms of plant character measurements.

The Scott-Knott Cluster Analysis (9) used to analyze data from this experiment is not as sensitive as the LSD or HSD used in the other two experiments. Also the presence of five different cultivars in the experiment increased the error factor; thus minimizing its ability to detect small differences among treatments within individual clones. However, considering the large amount of data in this experiment, the influence of the Scott-Knott Cluster Analysis is not believed to be sufficient to obscure important treatment effects.

The trend (although not statistically significant) toward smaller plants and less vigorous flowering associated with increasing number of nodal transfers and temperature stress might indicate a decrease in vigor. This trend is more likely due to delayed emergence, as

previously explained. Treatments with plants emerging up to one month later had a shorter growing season resulting in smaller plants and less vigorous flowering.

Delayed emergence and resulting seed piece decay along with the poor growth of the Centennial cultivar at this location accounted for the missing data. This is evident in Tables 7 and 9 for Norgold Russet and Centennial Russet cultivars. The relatively small percent of plants flowering is an indication of shorter growing period. The lack of flowering would have made statistical analysis of floral data meaningless.

Cultivar Study (Russet Burbank and Burbank Cultivars): The observations of plant characters were similar to the experiment just discussed in that all of the treatment differences occurred in pigmentation. In 95 percent of the sets of observations the control treatment varied from the norm more than any of the nodal transfer treatments. In these cases the control exhibited less pigment than treatments subjected to nodal transfers. These results correspond to the previous experiment where in most cases pigment tended to increase with increasing number of nodal transfers or temperature stress. Plant emergence was not significantly delayed in this experiment and environmental influences were minimized. This suggests that seed tuber dormancy may influence pigment expression. Since the control treatment exhibited considerable variation from the norm, it would be difficult to conclude that the differences were the result of nodal transfers.

The amount of statistical significance in the measurements of this experiment as in the previous experiment has to be interpreted

relative to the number of comparisons in the entire experiment. Also since all five clones were very similar, thus reducing the statistical error; and an HSD was used for mean separation; consequently smaller differences were significant. Nevertheless there was considerable significance which needs to be addressed.

Most of the significance of the measurements occurred between the control treatment and one of the three, six or nine nodal transfer treatments. There were only three cases where a significant difference occurred between two of the nodal transfer treatments. The plants in the control treatments tended to have terminal and primary leaves which were shorter, narrower and consequently had a smaller area than plants in tissue culture treatments. Petiole length and internodal distance also tended to be shorter. These results suggest a trend for a larger plant and leaves associated with nodal transfer tissue culture treatments. This trend is in direct contrast to the results described in the previous experiment where smaller plants were associated with increasing number of nodal transfers. Emergence dates were fairly normal and environmental influences were minimized in the greenhouse planting. Seed tuber dormancy may have had some effect, and the reduced seed tuber size for control treatments (Table 10) could account for smaller plants and the statistically significant differences.

Long-Term Propagation Study (Both First and Second Generation Plantings): There were no treatment differences regarding observations of plant characters in either the first or second generation plantings. These data indicate no effect on plant morphology resulting from thirteen months of tissue culture propagation. In both plantings all

plants or tubers were planted at the same time and in the second generation planting all plants were produced from tubers with the same physiological age. Under these conditions, pigmentation, which accounted for all the differences in the previous experiments, was very uniform.

The measurements of the first generation planting were significantly different in all but one measurement, the primary leaf indices. The differences are exhibited as enlarged terminal leaves, smaller primary leaves and smaller plants i.e. shorter petiole length and internodal distance of tissue culture plantlets. The enlarged terminal leaf also was noted by Denton et al. (7) as a juvenile growth characteristic common in plants grown from tissue culture plantlets. The smaller primary leaves and plants are likely due to less vigorous growth of tissue culture plantlets than plants derived from leaf bud tubers.

The measurements of the second generation planting were not significantly different for any of the plant characters. Seed tubers for both treatments were virtually the same weight and physiological age. These data support the hypothesis that micropopagation by nodal transfers, even up to thirteen months does not increase the chance for permanent morphological changes in potato cultivars. It also suggests that factors such as seed tuber dormancy, environmental conditions or leaf bud versus tissue culture propagated plantlets could produce significant differences in the experiment that were merely transitory morphological changes and not the result of permanent genetic change.

Summary Comments

The accuracy of the experimental results in this study was obviously influenced by factors such as seed tuber physiology and growing environment. Also, observations and measurements of plant foliage would not detect potential physiological changes such as tuber yield or disease resistance. However, the rather comprehensive approach to observing and/or measuring thirty separate foliage characters provides an interesting and useful answer to the primary question posed at the outset; namely, does tissue culture induce morphological changes? While the data presented do not necessarily provide clear-cut or nondebatable conclusions (for reasons alluded to earlier) it is significant that the great majority of evidence supports experience of Scottish seed potato personnel (personal communication; K.W. Knutson) who report continued propagation by tissue culture has not resulted in visibly detectable changes in potato cultivars. These studies did not reveal serious reasons why Colorado seed potato growers should avoid tissue culture propagation.

Follow-up research would be desirable to evaluate more precisely the effects of seed tuber dormancy on plant morphology and thus remove some of the unavoidable ambiguity involved in this study.

Last, but not least, this study did not permit either sufficiently large population numbers or extended generations of nodal transfer propagation to separate and quantify the normal rates of spontaneous mutation from possible tissue culture induced mutation.

REFERENCES CITED

1. Amato, F.D. 1975. "The problem of genetic stability in plant tissue and cell cultures." Crop Genetic Resources for Today and Tomorrow. Cambridge University Press, p. 333-348.

2. Bajaj, Y.P.S. 1981. "Regeneration of plants from potato meristems freeze-preserved for 24 months." Euphytica 30:141-145.

3. Ben Jaacov, J. and R.W. Langhans. 1972. "Rapid multiplication of chrysanthenum plants by stem-tip proliferation." Hortscience 7(3):289-290.

4. Boxas, P. 1976. "Rapid production of virus-free strawberry by 'in-vitro' culture." Acta. Hortic. 66:35.

5. Cheng, T.Y. and T.H. Vogui-Dinh. 1979. "Clonal propagation of selected deciduous trees through tissue culture techniques." Hortscience 14(Abstr.):457.

6. Conger, B.V. 1981. Cloning Agricultural Plants via In Vitro Techniques. CRC Press, Inc. 273p.

7. Denton, I.R., R.J. Westcott and B.V. Ford-Lloyd. 1977. "Phenotypic variation of *Solanum tuberosum* L. cv. Dr. McIntosh regenerated directly from shoot-tip culture." Potato Res. 20:131-136.

8. Earle, E.D. and R.W. Langhans. 1974. "Propagation of chrysenthenum in-vitro II." J. Am. Soc. Hortic. Sci. 99:352-358.

9. Gates, C.E. and J.D. Bilbro. 1978. "Illustration of a cluster analysis method for mean separation." Agron. J. 70:462-465.

10. Gegenbach, B.G., C.E. Green and C.M. Donovan. 1977. Proc. Natl. Acad. Sci. U.S.A. 74:511.

11. Goodwin, P.B., Y.C. Kim and T.A. Adisarwanto. 1980. "Propagation of potato by shoot-tip culture. I. Shoot multiplication." Potato Res. 23:9-18.

12. Grout, B.W.W. and G.G. Henshaw. 1980. "Structural Observations on the growth of potato shoot-tip cultures after thawing from liquid nitrogen." Ann. Bot. 46:243-248.

13. Hacket, W.P. and J.M. Anderson. 1967. "Aseptic multiplication and maintenance of differentiated carnation shoot tissue derived from shoot apices." J. Am. Soc. Hortic. Sci. 90:365-369.

14. Henshaw, G.G. 1975. "Technical aspects of tissue culture storage for genetic conservation." Crop Genetic Resources for Today and Tomorrow. Cambridge University Press. p. 349.

15. Hussey, G. and A. Hepher. 1978. "Clonal propagation of sugar beets and the formation of polyploids by tissue culture." Ann. Bot. 42:477.

16. Kartha, K.K., S. Champous, O.O. Gamborb and K. Phal. 1977. "In vitro propagation of tomato by shoot apical meristem culture." J. Am. Soc. Hortic. Sci. 102:236.

17. Lauer, F.I. 1977. "Tubers from leaf-bud cuttings: A tool for seed certification and breeding programs." Am. Potato J. 54: 457-464.

18. Murashige, T. 1977. "Current status of plant cell and organ cultures." Hortscience 12(2):127-130.

19. McIntosh. T.P. 1945. "Variations in potato varieties." Scott J. Agric. 126:132.

20. Mellor, F.C. and R. Stace-Smith. 1977. "Virus-free potatoes by tissue culture." In Plant Cell, Tissue and Organ Culture, Springer-Verlag, p. 616-635.

21. Morel, G. 1975. "Meristem culture techniques for the long-term storage of cultivated plants." Crop Genetic Resources for Today and Tomorrow. Cambridge University Press, p. 327-332.

22. Phillips, G.J. 1968. "Carnation Shoot Tip Culture." Colo. State Univ. Exp. Sta. Bull. 102, 21p.

23. Reinert, J. 1968. "Control of totipotency inplant cells growing 'in-vitro'. Nature 220:1340-1341.

24. Roca, W.M., J.E. Bryan and M.R. Roca. 1979. "Tissue culture for the international transfer of potato genetic resources." Am. Potato J. 54:1-10.

25. Roca, W.M., N.O. Espinoza, M.R. Roca and J.E. Bryan. 1978. "Tissue culture method for the rapid production of potatoes." Am. Potato J. 55:691-701.

26. Skirvin, M. 1978. "Natural and induced variation in tissue culture." Euphytica 27:241-266.

27. Shephard, J.F., D. Bidney, and E. Shahin. 1980. "Potato Protoplasts in Crop Improvement." Science. 208(4439):17-24.

28. Thomas, E., S.W.F. Bright, J. Franklin, V.A. Lancaster and B.J. Miflin. 1982. "Variation amongst protoplast-derived potato plants (*Solanum tuberosum* cv. 'Maris Bard')." Theor. Appl. Genet. 62:65-68.

29. Thorpe, T.A. 1981. Plant Tissue Culture: Methods and Applications in Agriculture. Academic Press, 379p.

30. Webster, T. 1969. "Developments in the description of potato varieties (foliage)." J. Natn. Inst. Agric. Bot. 11:455-475.

31. Webster, T. 1979. "Developments in the description of potato varieties (inflorescences and tubers)." J. Natn. Inst. Agric. Bot. 12:17-45.

32. Wenzel, O.S., T. Przenwonzyn, S. Sopory and G. Melchers. 1979. "Comparison of single cell culture derived *Solanum tuberosum* L. plants and a model for their application in breeding programs." Theor. Appl. Genet. 55:49-55.