DISSERTATION

MICROPROPAGATION TECHNIQUES AS TOOLS FOR STUDYING PLANT GROWTH, TUBERIZATION AND SPROUTING OF POTATOES

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY ABDULLA A. ALSADON ENTITLED MICROPROPAGATION TECHNIQUES AS TOOLS FOR STUDYING PLANT GROWTH, TUBERIZATION AND SPROUTING OF POTATOES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

MICROPROPAGATION TECHNIQUES AS
TOOLS FOR STUDYING PLANT GROWTH,
TUBERIZATION AND SPROUTING OF POTATOES

Several investigations were undertaken to determine if micropropagated potato cultivars of different maturity classes performed under field conditions in a manner similar to that reported for seed tuber propagated plants of the same cultivars. Plant growth analysis was carried out with special emphasis using Richards' function for evaluating growth attributes such as plant height, leaf area, and derived growth quantities such as RGR. Early, medium, and late maturing potato cultivars were grown under field, greenhouse and *in vitro* conditions to measure tuber yields. Two sprouting indices (sprouting rate index, and sprouting ratio index) were developed and used to quantify the sprouting characteristics of field, greenhouse, and *in vitro* produced tubers. Storage temperatures were 5, 10, and 20° C.

The combined field data of 1986 and 1987 indicated that plant growth, development and tuber yield were in general agreement to that of seed tuber propagated plants. The derived growth quantities (i.e. RSER, RLAER, RTGR, and RUGR) provided a useful way of comparing cultivar response and explained how the overall growth and development can be understood.

Significant correlations were reported between selected yield characteristics of field grown, greenhouse grown and *in vitro* produced tubers. Both Kennebec and Spunta had the highest yield under field, greenhouse, and *in vitro* conditions. Russet Burbank and Norland had the lowest yield while Desiree and Norgold Russet had an intermediate response.

Both sprouting indices incorporate the influence of factors such as cultivar, temperature and tuber size. Both indices also indicate that earliest sprouting occurred in Norland followed by Desiree and Russet Burbank. Field grown, and greenhouse grown tubers exhibited earlier sprouting than *in vitro* produced tubers.

Overall, these investigations illustrate the potential value of micropropagation methods in predicting yielding ability and sprouting characteristics of potato cultivars.

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

IN THE NAME OF ALLAH
THE MERCIFUL THE COMPASSIONATE

99. It is He Who sendeth down
Rain from the skies: [925]
With it We produce
Vegetation of all kinds:
From some We produce
Green (crops), out of which
We produce grain,
Heaped up (at harvest);
Out of the date-palm
And its sheaths (or spathes)
(Come) clusters of dates
Hanging low and near:
And (then there are) gardens
Of grapes, and olives,
And pomegranates,
Each similar (in kind)
Yet different (in variety): [926]
When they begin to bear fruit,
Feast your eyes with the fruit
And the ripeness thereof. [927]
Behold! in these things
There are Signs for people
Who believe. [928]

٩٩ وَهُوَ ٱلَّذِىٓ أَنزَلَ مِنَ ٱلسَّمَآءِ مَآءً فَأَخْرَجْنَا بِهِۦ
نَبَاتَ كُلِّ شَىْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُّخْرِجُ
مِنْهُ حَبًّا مُّتَرَاكِبًا وَمِنَ ٱلنَّخْلِ مِن طَلْعِهَا
قِنْوَانٌ دَانِيَةٌ وَجَنَّٰتٍ مِّنْ أَعْنَابٍ
وَٱلزَّيْتُونَ وَٱلرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَٰبِهٍ ۗ
ٱنظُرُوٓا۟ إِلَىٰ ثَمَرِهِۦٓ إِذَآ أَثْمَرَ وَيَنْعِهِۦٓ ۚ إِنَّ فِى
ذَٰلِكُمْ لَءَايَٰتٍ لِّقَوْمٍ يُؤْمِنُونَ

TABLE OF CONTENTS

	Page
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
LIST OF TABLES	ix
LIST OF FIGURES	x
INTRODUCTION	1
LITERATURE REVIEW	4
Tissue Culture of Potato	4
Growth Analysis	8
Tuberization	17
Dormancy and Sprouting	31
Summary	44
Chapter	
I. Plant Growth Analysis	48
Introduction	49
Materials and Methods	51
Results and Discussion	57
II. Tuberization of Potatoes under Field, Greenhouse and *in vitro* Conditions	79
Introduction	80
Materials and Methods	81
Results and Discussion	84
III. Evaluation of Dormancy and Sprouting Characteristics of Potatoes	94
Introduction	95
Materials and Methods	95
Results and Discussion	100
SUMMARY AND CONCLUSIONS	116
REFERENCES	118

APPENDIX 140

A.1 An Example of Richards Output 141
A.2 Parameters, Estimates, and Standard Deviation . . . 148
B Summary AOV Table of Growth Analysis Data 149
C Climatic Data During Study Periods 151

LIST OF TABLES

Page

1.1 Composition of modified MS culture media for nodal propagation, and shaken liquid tuberization *in vitro* . 52

1.2 Influence of Cultivar and Sampling Date on Plant Height Observed During Eight Serial Sampling 60

1.3 Influence of Cultivars and Sampling Date on Leaf Area Observed During Seven Serial Plant Sampling 64

1.4 Influence of Cultivar and Sampling Date on LAI Observed During Seven Serial Plant Sampling 66

1.5 Comparison of Cultivar LADs Calculated Between Day 14 and 84 after Transplanting 68

1.6 Influence of Cultivar and Sampling Date On Top Dry Weight Observed During Eight Serial Plant Samplings . 69

2.1 Tuber yield comparisons of six potato cultivars grown under field, greenhouse and *in vitro* conditions . . . 85

3.1 Comparisons of sprouting "rate" index between cultivars grown under field, greenhouse, and *in vitro* conditions 102

3.2 Effect of temperature and source of tubers on sprouting as measured by the rate index 103

3.3 Effect of Cultivar and source of tubers on sprouting as measured by the rate index 104

3.4 Comparison of sprouting "ratio" index between cultivars grown under field, greenhouse and *in vitro* conditions 106

3.5 Correlation coefficients between the two sprouting indices 107

3.6 Correlation coefficients between the two sprouting indices 108

LIST OF FIGURES

Page

1.1 Schematic diagram of the field plot layout (See text for details) 55

1.2 Top growth of micropropagated potato plantlets. Growth habit of Kennebec, Desiree, and Norland is shown in part (a). Part (b) shows the growth habit of Russet Burbank, Sangre and Kennebec 73

1.3 Time course of plant height (a) and relative stem elongation rate (b) among three potato cultivars during eight sampling dates 74

1.4 Time course of leaf area (a) and relative leaf area expansion rate (b) among three potato cultivars during seven sampling dates 75

1.5 Time course of leaf area index among three potato cultivars during seven sampling dates 76

1.6 Time course of top dry weight (a) and relative top growth rate (b) among three potato cultivars during eight sampling dates 77

1.7. Time course of tuber growth (a) and relative tuber growth rate (b) among three potato cultivars during eight sampling dates 78

2.1 Schematic diagram for the greenhouse experiment design. (See text for details) 82

2.2 Tuber yield comparison (tuber number per plant or per flask) among six potato cultivars grown under _in vitro_, greenhouse and field conditions 90

2.3 Tuber yield comparison (tuber weight in g plant^{-1} (a) or mg flask^{-1} (b) among six potato cultivars grown under _in vitro_, greenhouse and field conditions . . . 91

2.4 Yield relationships of six potato cultivars grown under field and _in vitro_ conditions 92

2.5 Yield relationships of six potato cultivars grown under field and greenhouse conditions 93

2.6 a. Microtubers produced under *in vitro* conditions prior to harvest (4 weeks) after the addition of tuber induction medial
b. Similarities in shape and color between field and *in vitro* grown tubers 94

3.1 Time course of sprout length of field grown tubers among three potato cultivars held at 5, 10 and 20°C. . 113

3.2 Time course of sprout length of greenhouse grown tubers among three potato cultivars held at 5, 10, and 20°C 114

3.2 Time course of sprout length of *in vitro* produced tubers among three potato cultivars held at 5, 10, and 20°C 115

INTRODUCTION

Importance of the Potato

The potato is one of the most important food crops worldwide. The nutritional value of the potato depends mostly on its dry matter content which averages about 20% of the tuber (106). The potato has the capacity to produce more energy and protein per unit area than any other food crop (17). Potatoes have the highest protein production per unit area and time of any major field crop (210).

Unique Features of the Potato

Potatoes are well adapted to a wide range of agroclimatic conditions. They can be grown at sea level and up to 4000 m in altitude, and from the equator to about 40° north and south (106). Potatoes have one of the richest genetic resources of any cultivated plant (210). Crossing can be made between *Solanum tuberosum* and almost all of the related wild species. Due to the long period of development and complex bud/branch relationships, potatoes show great variations between individuals. This relationship "allow much greater manipulation than in almost any other crop" (170). As a member of the Solanaceae family, it is highly responsive to many tissue culture techniques (70). There is a great potential for utilizing potatoes as a "fuel crop" for economical production of ethanol (8).

Propagation Methods

Traditionally, the potato is propagated by means of the tuber as a source of vegetative propagule. Miropropagation has become a valuable tool for producing disease-free plants. Microcultured potato plantlets are now being utilized as the starting material for the production of nuclear seed for certification purposes. They are also used for international exchange of potato germplasm.

The potato is probably the first major food crop which has responded successfully to biotechnology techniques. Biotechnology has now become a widely used term describing research dealing with the use of modern biological techniques aimed at improving crop productivity. In vitro tuberization is an example of such a technique designed to better understand potato growth, yield, dormancy and sprouting characteristics. Bajaj (17) stated that "biotechnology has literally moved the potato from the test tube to the field."

Plant growth and development usually are closely related to tuber dormancy. Studies of these correlations under in vitro and field conditions are of interest to potato physiologists, breeders and growers. An understanding of dormancy at the microtuber and minituber levels could open the door for new approaches in identifying the nature of dormancy and its mechanism. Such studies could be beneficial in developing new techniques for improving crop yield, quality and storageability.

Objectives:

This dissertation contains three chapters dealing with tissue culture techniques associated with studying potato growth and develop-

ment as well as sprouting and dormancy responses. Rapid *in vitro* clonal propagation result in the production of disease-free uniform plantlets that could be utilized in various studies on potato growth and development.

Objectives of this miropropagation research are:

(a) To compare plantlet growth performance according to previously established crop maturity classes based on conventionally produced potatoes. The Richards function will be used to fit the growth analysis data and derive growth quantities.

(b) To investigate the tuberization response of micropropagated potato cultivars of different maturity classes under field, greenhouse, and laboratory conditions.

(c) To evaluate the sprouting characteristics of the seed tuber progeny of micropropagated potato plants.

LITERATURE REVIEW

I. Tissue Culture of Potato

Background

Tissue culture techniques have attracted the attention of plant scientists since the turn of the century. There is a vast amount of literature dealing with the history of tissue culture. According to Espinoza et al. (69), G. Haberlandt was the first person to attempt to cultivate isolated plant cells *in vitro* using an artificial medium. According to Chapman (39), no practical method of potato tissue culture was reported in the literature until Barker (1953) and Barker and Page (1954) described the growth of potato nodes from sterilized sprouts *in vitro*. A number of reviews of the literature provide a detailed history of plant tissue culture (79, 178, 205, 242, 246). Miller and Lipschultz (169), and Wang and Hu (260) reviewed the literature on the types of potato tissue culture techniques.

Applications

Many researchers have focused on the importance of plant tissue culture for agriculture and industry (181, 279). They have also identified the general categories under which plant tissue culture is useful to agriculture (179, 180). These categories are:

(a) *in vitro* methods for hybridization, variety development and other genetic modifications of crop plants,

(b) establishment of specific pathogen-free plants, and

(c) enhancement of clonal propagation, and germplasm preservation.

Currently, micropropagated potato plants are being used as a starting material for the production of nuclear stock materials in seed certification programs (225, 258). They are also used in the international exchange of potato germplasm (206). Several gene banks have a collection of in vitro pathogen tested genotypes for international distribution (10). Rapid multiplication rates by in vitro techniques are now being employed by many national programs in Asia, Africa and Latin America (206). Other benefits include: maintenance of disease free clones (158) and storage of large germ plasm collections (264).

The importance of potato tissue culture has been reviewed by several investigators (11, 58, 79, 222, 260).

Tissue Culture and Conventional Potato Propagation Methods

The use of tissue culture methods as a supplement to traditional breeding methods for crop improvement has received considerable attention. Tissue culture has definite advantages in parts of the world (i.e., tropical, temperate) as a complementary method of clonal propagation (260). Wattimena et al. (263) used micropropagated plantlets as the propagule for main crops. Some techniques although showing promise for rapid propagation, are not commonly practiced (138, 207).

Tissue culture methods have the potential for a very rapid multiplication rate. Plant establishment and growth from micropropagation sources were similar to plants grown from seed potato (268). Plant vigor is higher from micropropagated plants (156). Conventional prac-

tices combined with advanced tissue culture techniques would enhance the release of new and improved crops (156).

McCown and Wattimena (156) indicated that two approaches can be used for micropropagation of potatoes:

(a) use of transplants obtained from micropropagated shoots "micro cuttings", and

(b) use of small *in vitro* tubers "microtubers" for generating transplants for direct transplanting in the field. Microtubers can be directly planted in the field provided dormancy has been broken.

Stages of *in vitro* Plant Production

According to Murashige (178), there are four stages for the production of *in vitro* plant propagule:

I. The establishment of disease free plants in an aseptic culture.

II. The multiplication of the propagules.

III. The propagation of the propagules for field or greenhouse planting.

IV. The utilization of appropriate techniques for optimum production (field performance).

There is ample literature dealing with stage I (178, 226, 258, 264) and few studies dealing with stages II and III (89, 90, 150, 264). However, almost none was found dealing with stage IV. It is, therefore, necessary to fully understand the response of *in vitro* produced plantlets to greenhouse or particularly to field conditions.

It is in stages III and IV when plants are acclimated (178). Acclimation is the ability of plants to adjust to the new environment (31). Acclimatization in tissue culture is defined as "the process in

which developing plantlets are prepared to make the adjustment from the laboratory to the greenhouse" (151). Acclimation procedures of many plants during stages III and IV gave successful results (75), nevertheless these procedures may be expensive and time consuming (51).

Crane (51) made several modifications of the in vitro environments to study their effect on plant growth and rate of water loss from detached leaves. She found that providing plants on a greenhouse bench with adequate water is enough to induce the proper level of acclimation. Bourque (30) reported that the acclimation treatments which contributed to low leaf water loss and optimum field performance also induced more heat tolerance.

Shoot-Tip Culture and nodal (stem) cuttings

This method illustrates one example of the tissue culture techniques, and it is the major one used in this project as a source of micropropagated potato plants utilized in various studies. Nodal cutting is the method of choice for potato propagation compared to other tissue culture techniques (86). Potato plants can be propagated by shoot-tip culture in petri dishes or flasks (87), subsequently rooted and transplanted into the field as cuttings. It has been reported (88, 89, 90) that yield from cuttings was between 30% and 95% of those of plants from tubers. Extensive proliferation via axillary meristem can be induced in potato shoot tips (15-20 mm) cultured in liquid media. Shoot formation can also be obtained from tuber discs (122), in vitro derived shoots (195), greenhouse grown plants (182), tuber sprouts (260) or stems of mother plants (154).

Based on his *in vitro* layering method, Wang (258) claimed that he obtained 2^{17} to 3^{17} fold multiplications per year. Other multiplication rates are 5 to 12 fold per month for potato shoot tips cultured in liquid media (89) and 10 fold per month for culturing nodes of sprouted tubers (113).

There are two sources of *in vitro* shoots; axillary shoots and adventitious shoots (262). Axillary buds are the most commonly used for shoot multiplication. Among the advantages of this method are: Faster shoot development and more genetic stability (24, 255). Plants regenerated directly from shoot-tip culture have no difference in their biochemical characteristics (57) which may indicate probable genetic stability during culture. The morphological differences, however, could be attributed to the influence of environmental factors (57).

II. Growth Analysis

Background.

The subject of potato growth and development has received wide interest since the early part of the 20th century. Several researchers (12, 19, 20, 56, 114, 177) have studied the interaction between plant physiological processes and environmental conditions. With few exceptions (20), all growth analysis of potato has been conducted under field conditions.

Plant growth processes have a close relationship with the external environmental factors surrounding it. Most of the changes occurring in rates of plant processes can be thought of as expressions of ontogenetic drift (110).

There have been several definitions of "growth", however they all tend to reach the same point. For example, growth is defined as an "irreversible change in size" (110) while Causton and Venus (38) define it as "any irreversible increase in size of an organism or of any of its parts". Ingram (114) adapted the definition of growth as "the increase in dry weight development," which refers to the combination of organ differentiation and growth coordination. Growth analysis is defined as "the study of the changes in the whole plant during its ontogeny" (170). Growth analysis plays a major role in constructing and validating plant growth models. In a typical growth analysis study, harvesting is done at intervals over the growth period (19). Plant parts are separated, and fresh and dry weights are taken. Dry weights are usually preferred in these studies because different plant parts have a different water content and plant parts lose water after harvesting and separation depending on their initial water content and the time of day.

According to Batutis (19), the general growth patterns of potato plants can be divided into three phases:

(a) Rapid stem and leaf growth with minimal tuber growth if at all. Warm temperature and high light intensity may be required to insure sufficient top growth to support tuber development later in the season.

(b) Leaf growth rate drops rapidly with linear phase of tuber growth. The net assimilation rate of the plant increases during this phase.

(c) Leaf growth declines and the weight of the tubers reaches its maximum. High temperature during this stage usually

hastens senescence of the foliage; and with long days, it tends to delay maturity.

The relative length of each phase depends mostly on cultivar (maturity) characteristics and environmental conditions (19).

According to Batutis (19), the first detailed potato model in the literature was the flow diagram by Moorby and Milthorpe (177). Some models were derived from regression analysis of potato growth and development (92, 93, 216). The POTATO model written by Ng and Loomis (183) is probably the first complete simulation model for potatoes.

Potato plant growth analysis and modeling have been carried out by several investigators for a wide array of applications. Some of these applications are listed below:

(a) Plant: pest relationship.

Studying the yield losses caused by early blight, verticillium wilt and the potato leaf hopper (123), Cultivar resistance to early blight (196), studying the nature of early dying disease (12), studying crop pest management (125);

(b) Growth and yield.

Studying the relationship between stem density and tuber yield (146), calculating the date of 50% emergence (147) and estimating dry matter assimilation and partitioning (114, 115, 147), estimating tuber bulking rates (216), predicting commercial yield (92) and graded yield (217), predicting plant growth and yield at different locations (124), predicting maximum potential potato yield (136).

Curve Fitting

Fitting primary growth data is often a common practice in plant growth analysis task. In part, Hunt (112) refers to this as the functional approach to plant growth analysis. Curve fitting may be useful for comparing different treatments, since it can remove "fluctuations in data" caused by sampling error (45). With small samples, however, curve fitting may alter the essential environmental or treatment effects, thus it may become less effective if precision is needed (200).

A common practice is to use well replicated sampling techniques at more frequent intervals throughout the growing season. The researcher must design the experiment to meet the objectives in accordance with the resources available. Hunt (111) discussed the rationale of using curve fitting. He listed 12 advantages of such practice and concluded that the main purpose is to "describe reality in a convenient way." The objectives of the study and the availability of data will determine whether or not curve fitting is needed.

The Sigmoidal function

Causton and Venus (38) stated the following:

> For any pattern of biological growth, a mathematical function giving rise to a sigmoid curve (i.e., function that is bounded by two horizontal asymptotes and having everywhere a positive first derivative) can empirically describe growth, since even indeterminate growth will cease at some stage.

Sigmoidal curves can be either symmetric or asymmetric. This depends on whether or not the inflection point is midway between the two asymptotes. A model for the sigmoidal curve is the Richards function (204) fitted as:

$$\ln Y = \ln A - \ln (1 \pm \exp (- K (t-t_0)))/N$$

where,

Y = growth attribute

A = a parameter for the asymptotic maximum

N = a parameter that describes the shape of the curve, if it has an inflection, where N = Richards M-1

t_0 = a parameter that positions the curve in relation to the time axis (t)

K = a parameter representing the rate constant

The parameters may have their biological significance. Berry et al. (22) indicated that all parameters have meanings associated with various features of physiological growth (i.e. size, time scale, rate change, and pattern).

Richards (204) indicated that the four parameters "define the growth curves completely, in a way that enables useful treatment comparisons to be made immediately of 'average' rates of growth." The general magnitudes of the standard errors of the parameters reflect the degree of variation of the data (37). In general, all parameters of the Richards function are highly correlated (37), thus testing for A and N explains to some extent the trend of t_0 and K.

The Richards function was primarily chosen in this research to provide a convenient summary of the data. Furthermore, to estimate the derived growth quantites which then help in drawing conclusive remarks about overall plant growth and yield responses under field conditions.

The Richards function is a reasonable model of a system with determinate growth (37). In plants, leaf growth is likely to have

this kind of growth pattern. However, whole plants and whole organ systems have an indeterminate growth pattern (256), therefore the Richards function may not strictly apply. In this case, the appropriate degree of polynomial functions can be used. Causton and Venus (38) provided a detailed account of the derivations and properties of the Richards Function.

Some Growth Attributes

Leaf Area

Total leaf area of any plant is generally influenced by many factors. Friend et al. (73) reported that leaf area of a wheat plant depends on the following factors:

(a) production of leaf primordia,

(b) leaf emergence,

(c) expansion of the lamina,

(d) increase in the number of meristematic tissues by branching, and

(e) loss of meristematic tissue through senescence or transformation into floral primordia.

The measurements of leaf area can be tedious and time consuming. It is difficult to report the exact leaf area under field conditions. Daughtry and Hollinger (53) indicated that the natural variability of leaf area per corn plant was about 10%. Additional sources of variation could be the measurement technique (experimental error) that might result from the nonuniformity within the plots. Leaf area can be estimated from its correlation with the length of the compound potato leaves (68). Also, a high degree of correlation (r^2 = .94)

exists between leaf area and leaf dry weights (68). It can also be estimated nondestructively by measuring total branch length. It can also be made with remote sensing technology (203). For each of the seven crops studied, a significant linear relationship was found between greenness and leaf area index.

The rate of leaf area expansion can be more important than the rate of photosynthesis itself, as explained by Wiebold and Kenworthy (265). Leaf length (or width) expansion rate can be calculated as:

$$(X_2 - X_1) / (D_2 - D_1)$$

where,

X_2 and X_1 were the measurements of the leaflet at the end and beginning of the linear expansion period.

D_2 and D_1 were the number of days after the measurements began that X_2 and X_1 were determined.

Leaf Area Index

The concept of leaf area index (LAI) was introduced by Watson (261). LAI is a widely used term to indicate the ratio of surface leaf area to the ground area in which the plant grows. Values as high as 8 are common for many crop species (215). In cultivars such as potato, the production of an extensive foliage cover early in the season is desireable (examples Desiree and Russet Burbank). this is because they intercept more photosynthetically active radiation (PAR) (4). Light interception can be maximized by proper planting density and planting time. The net assimilation rate (NAR) represents the dry matter accumulation rate per unit of leaf area per unit time (i.e. g

m^{-2} day^{-1} and it is a measure of the average photosynthetic efficiency of leaves in a crop community.

As LAI increases, crop growth rate (CGR) measured in units such as g m^{-2} (land area) day^{-1} increases up to a limit then declines as LAI increases beyond that optimum value. the relationship between LAI, NAR and CGR can be illustrated as (114):

$$CGR = NAR \cdot LAI$$

Every crop has an optimum LAI, which is considered to be between 2.5 and 5 (25). Optimum LAI refers to the leaf area at which dry matter production is maximized. The concept of optimum LAI might not be of value if used alone to explain yield diferences. It is, however, a useful indicator of some physiological development of plants. For example, optimum LAI can indicate the time at which NAR is highest and therefore the conditions at which photosynthesis is increased.

With potato, tuber bulking increases as LAI increases above the value of 1 (170). This is correlated with the increase in rate and number of tubers formed during the first few weeks following tuber initiation. Khurana and McLaren (128) reported that there is a linear positive correlation between light interception of potato canopy and LAI up to 2.25. After that, light interception increased at a slower rate. About 80% of light interception occurs with LAI of about 4 (128). A linear relationship has been reported between total or tuber dry matter production and light interception by the foliage (4). It is therefore suggested that improvement in potato production can be achieved by maximizing radiation interception. Moorby (175) found an increase in net assimilation rate when leaf area of the plant started to decline. It is suggested that this increase might be due to an

increase in the rate of photosynthesis brought about by the influence of rapidly growing tubers. Dwelle (62) reported a positive correlation between potato tuber yield and the estimated LAI. High tuber yield resulted from a high photosynthesis rate as it correlated with large leaf area (174). Significant genetic variations occur among potato genotypes (23, 82).

Leaf Area Duration

Watson (261) indicated that by integrating the area under the LAI/time curve, individual values could be summed over any time period during the growing season. This integration results in a quantity he called Leaf Area Duration (LAD). LAD is a measure of the plant's ability to produce and maintain leaf area. It is also a measure of its efficiency in photosynthesis and photosynthate utilization.

A direct linear relationship is found between tuber yield and LAD (32). The best statistical fit of this relationship was found when all leaf area indices above 3 are assumed to be 3. This is because, the efficiency of carbon fixation and utilization did not increase when LAI exceeded 3. Dwelle (62) reported a direct linear relationship between tuber yield and time when LAI was maintained at values above 3.0 (61).

Generally, the LAD is closely correlated with biological yield because longer periods of solar radiation interception generally correlate with greater total dry matter production. The relationship between LAD and yield is illustrated as follows (114).

$$\underset{(\text{weight area}^{-1})}{\text{Yield}} \approx \underset{(\text{time}^{-1})}{\text{LAD}} \cdot \underset{(\text{weight area}^{-1}\ \text{time}^{-1})}{\text{NAR}}$$

As LAD can be used to predict yield, it is only an estimate of the amount of light utilization over time. The LAD does not incorporate the amount of solar radiation available for photosynthesis, the distribution of radiation within the canopy or the efficiency of leaves in utilizing available radiation (78).

The newly developed leaves contribute substantially to carbon fixation. The "grand period of growth" as Hunt refers to it (110), begins when unfolding of new leaves and an increase in total dry weight occur continuously. Because tuber growth depends mostly on the supply of photosynthate; the rate of tuber growth and therefore final tuber yield, will reflect changes in leaf area and environmental factors.

III. Tuberization

A. *In vivo* Tuberization

Background.

There have been several reviews in the literature dealing with tuberization (52, 94, 96, 186, 191, 229). The most recent review was by Wattemina (262). He summarized those factors inducing tuberization as follows: short days, high light intensity, low night temperature, low nitrogen level, physiologically old tubers, and any combination of these factors.

Tuber Initiation:

Stolon tip enlargement is the first visible sign of tuber induction (40). Tuber formation occurs first at the base of plants or cuttings. Every leaf axil is capable of differentiating into a tuber

if provided with proper culture conditions (197). Marinos (153) studied the factors influencing stolon and tuber formation. He suggested the use of the equation:

$$\text{frequency of tuber formation} = \frac{\text{number of stolons with tubers}}{\text{total number of stolons}} \times 100$$

Gregory (91) concluded that "before tubers are initiated, the environmental requirements for tuber induction, established by the inherent properties of the plant, have to be satisfied." He suggested that tuberization could be brought about by a tuberization stimulus.

Tuber growth:

Patterns of tuber formation differ among individuals (272). Some tubers follow an approximate sigmoid growth curve (170), some with linear growth and others showing periods of growth interrupted with periods of slow or no growth (272). The slope and duration of the growth curve differs considerably between individual tubers and are not closely related to the relative time of tuber initiation (Sadler 1961 in 170). Tuber size distribution depends mainly on the range of nodes over which tubers are formed and competition of the products for photosynthesis which occur at two levels: between and within nodes (272). Tuber weight increases linearly with time (132). However, a logarithmic relationship can be found between the mean tuber weight (total weight of tubers per number of tubers) and time.

Working with 50 potato cultivars, Maity and Chatterjee (148) studied the yield contributing characteristics such as: number of shoots per plant, plant height, leaflet size, tuber number per plant and tuber weight per plant. They concluded that leaflet size, number

of tubers per plant and plant height were closely related with tuber yield.

Source: Sink relationship

Final tuber yield depends mostly on net assimilation rate (NAR) late in the season which in turn is controlled by sink demand (tuber weight and number) (49). Decreasing the source -- sink ratio of individual plants (i.e., by removing half of the leaf area) -- resulted in decreasing tuber growth rate by about 50% (66). Moorby (175) reported that tuber growth rate was greater than the growth rate of the whole plant. This may suggest the transfer of large amounts of photosynthates from the foliage to the tuber. Except for a short initial exponential phase, tuber growth rate is linear during most of the growing season and could remain so even when radiation and leaf area decrease (175). Unlike fruit or grain crops, the potato has no distinct period during which yield potential is fixed (66). Sink capacity (tuber formation) can adapt easily to changes in source supply (photosynthates) brought by changes in environmental conditions. Potato tuberization and storage of photosynthates occur simultaneously until the end of the growing period, although cell enlargement may be the major source of growth in tubers over 30 to 40 g (66).

Stem number per plant:

Tuber bulking is consistently greater with higher number of stems per plant (49). Tuber number is genetically related to the number of stems per plant (210). Each stem carries between 2.5 to 4.5 tubers. Tuber size can be influenced by the inherent cultivar characteristics (107, 108). There is a negative correlation between the number of

stems per plant and the number and size of tubers. Stem and tuber number could affect economic yield which in turn can be manipulated by controlling storage temperature and planting dates (120). Iritani et al. (118) reported a positive correlation (r^2 = 0.98) between weight of seed pieces per stem and total tuber yield in Russet Burbank.

Growing Season:

Total yield is determined by the length of the growing season and the average tuber production per unit time. Therefore, maximum yield requires high levels of daily production over a longer period (106). Burton (35) concluded that "with any one variety, the longer the growing season, the higher the yield, and the higher the dry matter content of the tuber is likely to be."

Factors Influencing tuberization

1a. Temperature

Christiansen (44) stated that "temperature is the major uncontrollable climatic factor that delimits crop production areas and limits crop yield." Potato cultivars differ widely in their response to temperature regardless of their maturity classes (131, 155). The effect of soil temperature on potato growth and development depends on the stage of growth (67). The optimal root (soil) temperature for tuber formation has been established as between 15 and 20°C by many workers (227, 276). Shading, through its effect in lowering soil temperature, improves total tuber yield (149, 167). High temperature (32/28 or 32/18°C) promotes top growth and suppresses tuber formation. While low temperature (22/18°C) has the opposite effect (159). High

temperature (35/30°C) promotes the synthesis of gibberellins in the buds which reduces tuberization (161). Temperature influences tuberization by altering the balance between endogenous gibberellins, cytokinins, and inhibitors (163).

Menzel (160) indicated that tuber formation may be controlled by at least 3 factors:

(a) a promoter, produced by the buds at cool temperature,

(b) an inhibitor, derived from the buds, which depends on warm root temperature for its formation, and

(c) a second inhibitor derived from the mature leaves and produced under warm shoot temperature.

The optimum temperature for leaf growth and stem elongation may not be optimum for total plant development and tuber growth (21). Temperature stress, accompanied with moisture stress may result in tuber malformation (211).

1b. Photoperiod

Ewing (71) used a screening technique with potato cuttings to study the effect of photoperiod on tuberization. The photoperiod that first promotes tuberization is considered the critical photoperiod (CPP). By using the cutting technique, it is possible to screen population of seedling plants into five categories of CPP. The CPP determined by the cutting technique may not predict the precise day length under which field grown plants would initiate tubers. Both high temperature and low irradiance can increase the levels of growth substances (especially gibberellins) which inhibit tuber formation (162).

1c. Carbon Dioxide

Cultural control of carbon dioxide (CO_2) at certain times during the growing season could promote tuberization by preventing stolon differentiation into leafy shoots (193). Arteca et al. (15) reported an increase in dry matter production as early as 2 days after treating the root systems of Russet Burbank for 12 hours. A substantial increase in tuberization occurred when treated plants were allowed to grow 3 to 6 weeks. The CO_2 used in photosynthesis is believed to be derived partially from CO_2 fixation via roots (14). It is therefore suggested that it might be possible to use underground CO_2 enrichment as a method of increasing productivity "possibly by means of encapsulation of CO_2" (15).

1d. Nitrogen

Tuberization can be prevented with continuous nitrogen supply (220). This consequently alters the hormonal balance within the roots and shoots. Krauss and Marschner (130) indicated that nitrogen nutrition acts with photoperiod and temperature to control tuberization by affecting the ABA:GA balance.

2. Hormones

Several workers have investigated the role of hormones in potato tuberization by analyzing endogenous hormones from induced and non-induced plants (2, 74, 135, 218). Among the endogenous hormones that have been extracted from induced or non-induced plants are: cytokinins, gibberellins and abscisic acid. Most of the use of exogenous growth regulators has been with *in vitro* rather than *in vivo* systems (262).

Okazawa (187) studied the effect of various nutrient substances and growth regulators on tuber formation. He suggested that the increase in gibberellin level might be among the principal factors counteracting tuber formation. Auxins might be indirectly responsible for tuberization. Krauss and Marschner (130) tested the hypothesis that tuberization is controlled in part by the ABA:GA ratio. They found that under conditions favoring tuberization, the ABA:GA ratio in the shoot is relatively high. Kumar and Wareing (134) reported that tuber formation can be induced by blocking the GA synthesis with Chloro Choline Chloride (CCC). When CCC is applied to the roots, early in the growing season, it accelerates the time of tuber initiation and development (80). Tuber formation can also be induced by applications of ABA to either the shoots (64) or to the stolons (130). Applications of GA, however, inhibited tuberization (94).

Many environmental conditions influence the ABA:GA ratio. For example, high night temperature may induce high GA levels which adversely affect tuberization (61). Gibberellins act as high temperature in suppressing tuberization while ABA, CCC, and low temperature have similar effects in promoting tuberization (2), These substances act "directly on the stolon tip" rather than their general influence on foliage growth (159). Growing points play a major role in the formation of the tuberization stimulus and gibberellin-like materials (188).

Palmer and Smith (191, 192) reported that cytokinins might be the specific tuberization stimulus due to a close relationship existing between tuberization and cytokinin activity. Nevertheless, it is suggested (219) that cytokinins are not primarily responsible for tuber-

ization. Harmey et al. (96) suggested that tuberization might be controlled by the interrelationship of carbohydrates supply, gibberellins, and antigibberellin substances.

Tuberization Stimulus

A tuber inducing stimulus is a transmissible factor. The stimulus mostly moves downward with little or no lateral movement (40). Kumar and Wareing (134) reported that the stimulus moves both basipetally and acropetally but tuber development occurs only at the lowest nodes of stem cuttings whether these are normally oriented or inverted. The tuberization stimulus is transmissible by grafting. Small pieces of stem from induced plants are capable of transmitting the stimulus (134). Kahn (126) suggested that each leaf in the potato plant contributes to a certain degree some tuberization stimulus. He found a positive correlation between tuberization and number of leaves and leaf area.

Physiological Age

The effect of physiological age on tuberization appears to be dependent upon the level of tuber promoting and inhibiting hormones produced in the sprouts (164). Physiologically young plants have a greater capacity for high gibberellin levels in the buds when exposed to high temperature of $32^{o}/28^{o}C$ which resulted in reduced tuberization. Abscisic acid (ABA), CCC, and lower temperature ($22/18^{o}C$) promoted tuber production.

Chapman (40) presented evidence that actively growing plants and young leaves on induced potato cutting exerted a greater ability to promote tuberization than did older leaves.

B. *In vitro* Tuberization

Background.

In vitro potato tuberization has been studied and reviewed by several workers (1, 52, 172, 198, 229, 260). Microtuber is another term used for *in vitro* produced tubers. According to Peterson et al. (199) the rapid induction of *in vitro* tubers from isolated stolon nodes was first accomplished by Barker in 1953 (18) and later by Mes and Menge in 1954 (165). The factors involved in inducing microtubers and the mechanism of *in vitro* tuberization have been reviewed recently by Wattimena (262).

In vitro tuberization is of interest to people in potato seed programs, in storage and distribution programs, and also for plant development physiologists (248). A method developed by Wang and Hu (259) for *in vitro* mass tuberization has been adapted at the International Potato Center (CIP) (9) for storage and distribution of germplasm. This method has also been adapted by McCowan and coworkers as a complementary method of clonal propagation (263). Similar methods are also reported for *in vitro* mass tuberization (209, 266).

Among the advantages of *in vitro* tubers (9, 260) are

(a) Large quantities of tubers can be produced regardless of season.

(b) Easier to handle for national programs than *in vitro* grown plantlets.

(c) Suitable for storage for several months for local use or for export (137). The export of sterile microtubers would allow the phytosanitary benefits of *in vitro* export without the technical problems of shipping green plants (70).

(d) Rate of survival of plants grown from microtubers is generally high (137).

Characteristics of Microtubers

Microtubers can be sessile or axillary given proper induction environment (260). Every leaf axil of a micropropagated shoot is able to produce microtubers. Microtubers are initiated as swollen stolons from axillary buds in about 4 days (198) or 7-18 days (189) after transfer of shoots to the tuber induction media. The physiological age of the sprouts as well as the tubers they were taken from can be an important factor with regard to the tuber formation on the stem segments (186). Cuttings of frozen meristems can be used to produce tubers (16). The method of freeze storage meristems has many practical aspects for long-term preservation of germplasm.

An interesting phenomena called "serial tuberization" occurs when microtubers are stored at lower temperatures (10°C) for longer periods of time (i.e. 6 months) (189). As many as five tubers can be produced in a serial arrangement. It is suggested that the serial tuberization system could reduce the cost of establishing disease-free potato clones.

Different methods for _in vitro_ tuberization play a major role in controlling dormancy (248). For example, when tubers were induced in total darkness, harvested, and stored at 4°C, the average natural dormancy period was 210 days. Natural dormancy was 60 days for tubers induced under 8 hours of photoperiod (1000 lux), harvested and stored at 4°C.

Wattimena (262) conducted a comparative study between the three propagation sources (tubers, microcultured shoots [microcuttings] and microtubers). He reported that total tuber yields were the same. However, microcultured plants (produced from microcuttings and microtubers) produced smaller sized but a greater number of tubers. Alsadon et al. (6) reported significant correlations between yield of microtubers and field grown tubers.

Media for <u>in vitro</u> Tuberization

1. Types

Researchers often use two types of media for <u>in vitro</u> tuberization studies; liquid media (259), or semi-solid (agar) media (113). Each type of media has its own practical importance. For example, liquid media is cheap, easy to make, and has greater purity (100). However, some cultures in liquid media need continuous agitation. Salt precipitation is more common with liquid media. Tissues grown in liquid media are usually more succulent and watery.

In a series of comparative studies, Wattimena (262) studied the role of liquid and solid media in <u>in vitro</u> tuberization. He summarized his findings as follows:

(a) No significant differences in tuber number between liquid and solid media.

(b) Tuber size and weight were significantly larger in liquid media.

(c) Tubers produced in liquid media have the highest percentage dry matter.

(d) Tubers produced in liquid media were covered with lenticels or callus while those produced in solid media were smooth.

2. Media constituents and Growth Factors

The factors influencing in vitro tuberization have been reviewed by several workers (96, 141, 189, 191, 192). Wang and Hu (259) reported that the optimum conditions for in vitro tuberization of stem cuttings were : 10 mg l^{-1} BA, 8% sucrose, 20°C and 8 hours of 100 lux incubating temperature and photoperiod respectively. Under those conditions, about 20 to 50 microtubers could be harvested from a 500 ml flask in a four-month period. Microtubers can be initiated within 4 days in media containing 12% sucrose (198).

Wattimena (262) reported that optimum levels of temperature, sugar and kinetin were 20 to 25°C, 6-8%, and 5 ppm respectively. He also reported that: (a) continuous light significantly produced more tubers than continuous dark, (b) continuous dark induced a faster tuberization than continuous light, and (c) cultivars differ significantly in their tuberization response. Faster tuber production was also reported under continuous darkness (9). Thieme and Pett (240) reported that microtuber formation can be increased by temperatures between 8°C and 10°C and photoperiods less than 12 hours. Lo et al. (144) indicated that in vitro tuberization is not a response to the osmotic concentration of the medium. They suggested that high levels of sugar enhanced the rate and completeness of tuberization.

There are different ways to alter the hormonal balance for tuberization. Some workers have changed the concentrations of cytokinins and auxins in the culture media (259) while others (248) have added anti-gibberellins such as CCC. Time of tuber initation may not be the same.

In vitro tuberization of potato sprouts can be enhanced by using ethrel at 50 ppm (77). Ethrel increased the number of tubers and produced shorter and thicker stolons and reduced root growth (5, 7, 77). Using 1000 ppm of ethrel as a dip treatment, Alsadon (5) reported that stolons produced were shorter and thicker with a minimal root growth as compared to control or 10 ppm GA treatment. Ethrel has a low degree of tuberization stimulus (236). Ethrel (25 to 500 ppm) increased tuberization when added before natural tuberization occurred (76). In contrast, Mingo-Castel et al. (172), and Van Staden and Dimalla (254) showed that ethylene inhibited *in vitro* tuberization of potato stolons.

Hammes and Nel (94) reported that tuberization occurred "as soon as" gibberellin levels drop below a "threshold level." Generally, GA is reported to inhibit tuberization, while ABA and CCC promote it (260). Claver (46) reported that ABA is not an inhibitor of the gibberellins in regard to tuberization.

Stallknecht (234) indicated that coumarin might be the potential stimulator if *in vitro* tuberization. However, Tizio and Biain (245) suggested that cytokinins might be the specific factor for tuber formation *in vitro*. Kinetin has been known for its effect on inducing *in vitro* tuberization (173, 190). High nitrogen did not affect tuberization induced by kinetin while it totally inhibited coumarin-induced tuberization (235). Media constituents and growth factors vary with cultivars and the study objectives (1).

Methods Commonly Used

The basic steps used for in vitro tuberization applied at The International Potato Center (CIP) (248) are as follows:

(a) Initial propagation through use of single node cutting.

(b) Shaken liquid culture

Whole stems (with both leaves and roots taken out) layered into a liquid media.

(c) Tuber induction stage

Sucrose, tuberization hormones added to the media.

Barker (18) discussed a method for in vitro culturing of potato tubers in White's media.

Comparison between in vitro and in vivo produced tubers

Several workers have studied the similarities and differences between in vitro and in vivo produced tubers. Duncan and Ewing (60) studied the anatomical changes associated with tuber formation on single node cuttings of Katahdin plants. Within 1 day after cutting, starch deposition and the percentage of cells in mitosis increased in the medullary region of the bud. The increase in cell size was not detected until three days later. The earliest anatomical changes associated with tuber initiation were starch deposition and mitosis. In vitro tubers appear to be morphologically similar to field produced tubers (70, 260). For example, they have well developed external and internal phloem with little primary xylem development (52). Peterson and Barker (198) provided a detailed anatomy of microtubers of Kennebec. Several internodes basipetal to stolon-apex involve radial expansion of the microtuber. Cell division may have no effect at

early stages of tuber growth. Espinosa et al. (52) indicated that the microtubers can be used to produce seed tubers with no noticeable differences from using *in vitro* plantlets. Both shape and color characteristics were similar among *in vitro* and *in vivo* produced tubers (70, 248).

Hussey and Stacey (113) indicated that the "physiological reactions of microtubers are not the same with those of field grown tubers." For example, they differ in disease susceptibility (199). These differences may be of biochemical nature rather than morphological since Peterson and Barker (198) did not notice any changes in the anatomy of *in vitro* produced tubers.

IV. Dormancy and Sprouting

A. Dormancy

Background.

Terminology is a basic problem facing researchers studying dormancy (104, 139). Rappaport and Wolf (202) addressed this point and concluded:

> . . . that there is not universal agreement on terminology is understandable, because it is difficult to characterize either induction or termination of arrested growth in unextended stems by a distinct morphological change, such as occurs during conversion from a vegetative to a reproductive form in plants.

Since the turn of the century there have been numerous reports in the literature about rest and dormancy in plants in general and in potatoes in particular (13, 29, 35, 42, 43, 48, 65, 104, 184, 202, 208, 237, 249, 250, 270, 271).

Rest Period

Rest in potato is defined as the stage, during which the buds cannot sprout as a result of endogenous causes (237, 270). However, this stage is often referred to as dormancy (184). Rest period is also defined as "the time following harvest when internal inhibition prevents tuber from sprouting (142).

The buds of a potato tuber are usually in rest "immediately" after harvest and even during the last weeks before harvest (33). In general, there seems to be no fundamental differences between rest of buds of potato tubers and that of buds of other plants (104).

Davidson (54) reported that the potato tuber has no rest period since bud growth is a continuous process after harvest. He indicated that the appearance of a readily visible sprout is not an indication of dormancy break.

To avoid problems arising from these definitions, many physiologists refer to rest period as innate, spontaneous or deep dormancy (104).

Dormant Period (Dormancy)

Emilsson (65) defined the dormant period as the time after harvest when the buds were not growing for any reason. He distinguished another state of dormancy after harvest, during which the tuber would not sprout under favorable conditions. He referred to this state as "rest period." Dormant period is also defined as the total period in which potatoes remain without sprouting within a given storage condition irrespective of their "rest period (271). Dormant period is

defined as the subsequent time when the tuber does not sprout due to unfavorable external conditions (142).

A general definition of dormancy is that it is an arrest in development of seed embryo, buds, or spores under conditions otherwise suited for growth (239). Burton (35) defined dormancy as "the absence of consistent bud growth owing to certain chemical and physical conditions in the tuber, influenced by a host of factors including the environment." Dormancy was also defined as "the state where there is no cell division when tubers or selected buds are put in an environment favorable for growth" (177). Lang et al. (139) define dormancy as "the temporary suspension of visible growth of any plant structure containing a meristem."

Dormancy as the most acceptable term, is the entire period during which the buds are unable to sprout as a result of either exogenous or endogenous factors (65). Therefore, the dormant period consists of the "rest period" and the following quiescent period.

Lindblom (142) suggested that the concept of rest and dormancy are often practical to use but theoretically, there may be no reason to distinguish between them. He concluded that "they are two parts of the same physiological process." The sum of the two periods denotes the "sprouting tendency" (142) at any storage temperature.

B. Sprouting.

Background.

Changes in "sprouting capacity" (133) can be used as an indicator of the physiological "age" of the tuber. The sequence of stages associated with tuber aging (133) are as follows: (a) the one sprout

stage, (b) the multiple sprout stage, (c) the branching stage, and (d) the small tuber formation stage. Individual tubers normally exhibit different degrees of sprouting. It is suggested (223) that seed potato tubers are not of the same physiological age although they were grown in the same season, harvested at the same time and stored under similar conditions.

Krijthe (129) considered dormancy ended when at least 80% of tubers held at 20°C developed sprouts at least 3 mm long within 3 weeks. This criterion has also been used by Emilsson (65). According to Wurr (273), total sprout length can be used as a useful measurement of sprouting. Most of the variation in total sprout length per tuber could be correlated with the differences in the time of dormancy break (274). The number of sprouts per tuber is important with regard to the purpose for which the crop is to be grown (133).

Factors influencing dormancy and sprouting

Dormancy and sprouting characteristics of potatoes are influenced by many factors such as cultivar, environmental conditions, tuber maturity at harvest and storage conditions.

a. Cultivar

Cho et al. (43) indicated that "potato cultivars are inherently different in many characteristics and it is not surprising that differences exist in length of dormancy as well as reaction to environmental conditions which influence length of dormancy." Emilsson (65) determined the length of dormancy for 51 potato cultivars stored at 5°C. He classified cultivars in regard to dormancy as follows: (a) very short (< 6 weeks), (b) short (6-9 weeks), (c) medium (9-12

weeks), (d) long (12-15 weeks), (e) very long (> 15 weeks). He found that the length of dormancy ranged from 5 to 20 weeks. He also reported variations for the same cultivar from year to year. In another study, Bogucki and Nelson (29) reported that Russet Burbank has the longest dormancy and the slowest rate of sprouting among the 10 cultivars studied.

Correlation occurs between some cultivar characteristics and length of dormancy (133). For example, cultivars in which foliage develops rapidly, or those liable to second growth and those with drought resistance often have a short dormancy. Krijthe (133) suggested that the difference between lots of the same cultivar and between growing seasons are considered to be resulting from differences in growing conditions such as soil fertility, temperature or moisture regimes. These factors affect the degree of maturity of the tubers at harvest which consequently determine the length of their dormancy.

The sprouting behavior of tubers is due mainly to cultivar characteristics which are influenced by the changes in environmental conditions during tuber development and storage (238). Tuber behavior is quite interesting in that it varies widely under different conditions. From a genetic point of view, the potatoes are heterozygous. Physiologically, each tuber has its own characteristics due to differences in time of tuber initiation, amount of starch deposited and rate of tuber bulking (117). It is, therefore, suggested that greater uniformity in behavior can be achieved by using growth regulators, allowing for more uniformity in time of tuber set and enlargement.

b. Temperature

Numerous studies have been conducted to study the effect of temperature on dormancy and sprouting responses of potato tubers (5, 29, 33, 35, 42, 65, 104, 157, 244, 270, 271). Generally, temperature affects the duration of dormancy, the length and shape of sprouts and the rate of sprouting.

Storage temperature has a major influence upon sprouting. At higher temperature (i.e. 20°C), higher degrees of sprouting is observed. This is also accompanied by high respiration and evaporative weight loss (98, 133). Simon (224) indicated that tubers stored at high temperature lose their membrane integrity, which leads to an increase in membrane permeability. This situation could be responsible for high water loss associated with high sprout weight especially after prolonged storage.

Krijthe (133) reported that differences in sprouting rate between 15 cultivars stored at temperature above 5°C are always about the same. Below 5°C sprout growth is inhibited in some varieties. He reported that tubers, from any one variety from the same field harvested at different dates showed only minor differences in sprouting rate. The major differences were found between mature or immature tubers.

Tubers held continuously at 10°C sprouted slightly more than those at 20° (29). It is suggested that an initial cold treatment of tubers could increase the number of sprouts per tuber (133, 273, 275). High temperature during the last period of tuber development markedly increase the rate of sprouting (238). In the field, extremely high temperatures could lower survival rate and induce low specific gravity (99).

c. Physiological age

Physiological age has been defined as the "physiological degeneration or loss of ability of a seed tuber to produce plants with high yield potential" (127). Toosey (247) defined it as the "physiological or biochemical state of a tuber at any given time."

Physiological age of the tuber acts primarily on the level of sprout development at planting (98). Sprout number and weight increase with increased seed tuber age (269). The state of sprouting of a tuber has a close linear relationship with the initial growth rate of the plant and can affect subsequent growth and development of the crop (98, 251). Tubers harvested immature have a longer dormancy than those harvested when more mature (213, 270).

A recent review of the literature about physiological age has been done by Sacher (213) and Van Der Zaag (277). Physiological age increases with the chronological age of the tuber and is controlled by the interacting effect of growing conditions, handling procedures, storage and sprouting conditions (28, 213, 277). It is reported (221) that sprouted and unsprouted tubers can be considered as physiologically old and young, respectively. Loss of vigor that is obvious with older seed tubers can be related to "decreased sink strength" and increased competition among multiple sprouts or shoots (129). Physiologically older seeds, on the other hand, may have a faster emergence rate than younger seed (116, 185). Some authors, however, have found no difference between them (253). Higher yield of smaller size tubers can be produced from older seed (119). Van der Zaag (277) concluded that sprouting capacity or a method derived from it should be considered as a possible measure of the physiological age or growth vigor

of seed potatoes. In general, good indicators of the physiological age (98) include -- sprout growth, duration of storage, and the sprouting capacity.

Apical Dominance

The first bud to be released from dormancy and to initiate growth is usually the apical bud (85, 176). The apical bud could have two types of effects on lateral buds (83). It can either (a) cause a partial or complete inhibition of their growth, or (b) alter their mode of growth. The subsequent growth of lateral buds depends mostly on the environmental conditions (mainly temperature). If tubers are stored at 20°C, apical dominance is established so rapidly that often only one sprout (i.e., the apical) will grow. At lower temperatures, the apical dominance is weak therefore many sprouts develop (35). Among several cultivars stored continuously at 20°C, Kennebec and Russet Burbank showed the strongest apical dominance (average of 2 sprouts per tuber) at the end of dormancy (29).

It is suggested that apical dominance is a relative rather than an absolute phenomenon. The degree of dominance depends on the cultivar, growth and storage conditions (238).

Methods of Measuring Dormancy

Cho et al. (43) compared the following methods for measuring dormancy.

(a) From planting or tuber initiation to sprouting.

(b) From harvest to sprouting.

(c) From sampling to sprouting.

(d) From stolon set to sprouting.

He concluded that planting to sprouting was the best practical field measure of dormancy due to the close correlation with tuber initiation to sprouting.

Burton (34) indicated that one must not measure dormancy from date of harvest since, in his opinion, dormancy may be over before harvest. It is suggested (34) that dormancy could be measured from the time when tuberization started until the time when elongation ceased.

Methods of measuring sprouting

A. Sprouting capacity

Krijthe (133) characterized the age of a tuber by determining its 'sprouting capacity' under controlled conditions. Branching of sprouts is a typical example of the ageing phenomenon.

Hartmans and Van Loon (98) adapted the term sprouting capacity for determining the growth vigor of seed potatoes. Sprouting capacity is simply "the sprout weight of uniform tubers expressed in g (fresh weight) sprouts per tuber, after a standard sprouting procedure whereby tubers that were stored at X°C were then desprouted and sprouted again by storing for 4 weeks at 18-20°C and approximately 80% RH in darkness."

Sprouting capacity is influenced by harvest season, temperature and length of storage period. A negative linear relationship was found between sprouting capacity and dry matter content of the sprouts (252).

B. Total sprout length

Wurr (273) indicated that total sprout length and the length of the longest sprout were the most useful measurement of sprouting. He also reported a good linear relationship between sprout dry weight and total sprout length per tuber, when tubers were stored under continuous light.

C. Day-degrees (or degree days thermal units)

Accumulated day-degrees >4°C during sprouting time (from the end of dormancy to the end of storage), can be used as a measure of sprouting characteristics of the tuber or its physiological age (3, 185). Sprout growth correlates well with physiological age of the tuber. Allen and O'Brien (3) and Gillison et al. (81) demonstrated a close relationship between emergence, leaf growth and tuber yield and number of accumulated day-degrees >4°C. Another positive linear relationship was found between length of the longest sprout in a tuber at end of storage and number of day-degrees >4°C.

D. Sprouting rate

According to Rylski et al. (212) the rate of sprouting can be determined using the method of Harrington (97), as follows:

$$\frac{N_1T_1 + N_2T_2 - - - - - - + N_nT_n}{N_1 + N_2 + N_3 - - + N_n} = \text{rate}$$

Where, N_1 equals the number of tubers sprouted at time T_1, N_2 equals the increase in number of sprouted tubers observed between T_1 and T_2 etc. This formula gives the rate of sprouting in mean days. Rate of sprouting is mainly a cultivar characteristic (157).

Methods to Prolong Dormancy

Dormancy may be controlled by an interaction between endogenous growth promoters and inhibitors (65). A decrease in inhibitor content was observed in the extract of potato tubers during dormancy and sprouting (104). Auxin induced dormancy has a different physiological basis than natural dormancy. Under natural dormancy, the growth is suppressed by the presence of relatively high levels of β inhibitors compared to that of promoters (153). Current literature continues to support the "inhibitor/promoter" hypothesis (48).

Several investigators (27, 101, 102) reported that the β inhibitor complex, found in the periderm of potato tubers is the main factor controlling the state and duration of dormancy. It was also reported that one of the main components of the β inhibitor complex is ABA and some phenolic compounds (105). Abscisin (64) inhibited bud growth of nondormant potatoes when applied to whole tubers, but was less effective when applied to isolated tuber plugs. Camptothecin, a naturally occurring plant growth regulator, inhibits sprouting by interfering with cell division in the meristematic region of the sprouts (257).

A more effective and common method is the treatment with maleic hydrazide. Paterson et al. (194) reported that treatment with a 0.25% solution 6 weeks before harvest inhibited sprouting for 8 months. Burton (36) discussed some other substances used to induce dormancy.

Methods to Break Dormancy and Enhance Sprouting

Several methods and materials have been studied for their effect in shortening dormancy.

Wounding

If dormant tubers are peeled, dormancy will be interrupted (13). Cutting tubers also tends to break their dormancy (72). This effect has not been fully understood. However, it is speculated that the wounding effect is related to ethylene production (212).

Water

Dormancy can be shortened by storing tubers under moist conditions at temperature of about 20 to 35°C (84). Tubers stored under dry conditions were dormant for at least 55 weeks under the condition of that study. It is also reported that gibberellic acid or ethylene chlorohydrin treatments were less effective than moist storage in shortening dormancy. Water, however cannot break dormancy if the tubers are buried in wet soils or vermiculite (104).

Gibberellic acid

The response of potato buds to gibberellic acid (GA) depends on the temperature at which tubers are stored and is not limited to any particular tissue of the tuber (47). The effect of GA on sprouting is conditioned by its concentration, temperature, time after harvest, and stage of tuber development (5, 7, 244).

Gibberellic acid applied as a foliage spray or as a dip treatment to potato tubers has been reported to shorten their dormancy and promote sprout elongation (5, 7, 59, 143, 201, 228, 230, 244). Harkett (95) reported that daily treatment of potato buds for 5 days after excision, with either GA or an extract of potato sprouts, shortened dormancy and promoted sprout growth. Gibberellic acid, however, did not induce sprout growth in buds with innate dormancy (249).

A five minute dip treatment in 1 ppm GA induced sprouting of freshly harvested tubers (244). Sprouting rate was greatly enhanced by temperature (201). Generally GA can enhance sprouting of newly harvested tubers by 2 to 3 weeks (201).

Mares et al. (152) indicated that GA_3 acts directly on tuber tissue and alters the coordinated growth of the tuber as a storage organ by breaking the dormancy. GA_3, therefore, induces the formation of new stolons or sprouts and creates conditions where the "primary tuber sink" can be switched off.

Rindite

Rindite vapor applied to whole tubers shortened the dormancy of all varieties tested by Lascarides (140). Rindite is a mixture of ethylene chlorohydrin, ethylene dichloride, and carbon tetrachloride (7:3:1 V/v).

Ethylene

Ethylene has a dual effect on potato tubers (212). While it markedly shortens the dormancy, it also inhibits elongation of the sprout especially during extended treatment. Tubers treated with ethylene lose their apical dominance which results in multiple sprouting (171). Sprouts usually are thickened with limited root developments. Ethylene markedly retards sprout growth and thickens them (5, 7, 109). Plant emergence is delayed when potato seed pieces were exposed to ethylene at levels above 0.05 ppm (109). These responses occurred since ethylene lowered auxin level in tuber tissue surrounding the apical bud. Swollen sprouts with enlarged lenticels and very

limited root development are common responses of potato tubers exposed to ethylene (5, 7, 243).

Cytokinins

Majestic potato tubers, known to have long dormancy, can be treated with cytokinins to break their dormancy very rapidly (103, 250). Exogenous cytokinin was effective in breaking dormancy only during short periods at specific stages in the potato life cycle, after the start and before the end of innate dormancy (249).

V. Summary

From the literature, potato tissue culture has received a great deal of attention during the last two decades. Among key people who contributed to this area of research were Bajaj (16, 17), Goodwin (86), Wang and Hu (259, 260), Wattimena (262, 263), and Wiersema, et al. (266). The International Potato Center (CIP) tissue culture research program is another example of a significant effort aimed at a better understanding of potato physiology. Such work was done by Dodds (58), Espinosa, et al. (69), Estrada, et al. (70), and Tovar, et al. (248).

Micropropagation techniques offer many advantages to scientists as well as potato growers. Among these advantages are: (a) rapid multiplication rates, (b) disease free propagules, (c) uniformity in regenerated plantlets, and (d) wide application to different genotypes.

For these and other reasons, researchers ought to be considering tissue culture methods as possibilities to quickly evaluate the vast amount of germ- plasm that is involved in most cultivar development programs. The majority of commercially important potato

characteristics such as yield, tuber quality, disease resistance, maturity and dormancy are usually identified and measured under field and conventional storage conditions -- for reasons of convenience and expediency. If tissue culture methods could provide even a portion of the many answers required to verify the potential usefulness of a seedling potato clone, a significant advancement in the search for new potatoes would result. The pursuit of such a goal is admittedly a long and difficult task since many complex questions are involved. A beginning phase of such an effort could focus on a number of different approaches. In this study, growth analysis was made on micropropagated cultivars of differing maturity classes under field conditions; a comparison of *in vitro* tuber production with greenhouse and field tuber production and also a comparison of sprouting characteristics among the same cultivars.

Interest in plant growth analysis started early this century, people such as Blackman (26) and Watson (261) laid the principles of this science. Relatively recent research done by Collins (49, 50) is a good example of applying plant growth analysis principles to potato. During the last three years, a group of researchers at Cornell University working under Dr. E. Ewing have conducted extensive studies on various aspects of potato growth analysis. Among these researchers were Batutus (19), Ben Kheder (20), and Snyder (232). Their work, however, did not deal with micropropagated potato plants.

Plant growth and development under field conditions are often influenced by the environmental conditions as well as the inherent plant characteristics such as maturity classes. Growth analysis studies play a major role in the understanding of plant performance.

The use of the Richards' function provides a valid way of evaluating plant performance. Growth attributes (i.e. plant height and leaf area) can be fitted by the Richards function and the resulting derived growth quantities (i.e. relative stem elongation rate and relative leaf area expansion rate) can be used for the evaluation of growth and yield responses. One of the best examples of utilizing the Richards' function is the work of Berry, et al. (22), and Causton, et al. (37). Hunt (111) described the advantages of Richards' function; however, there are certain limitations to its use as will be discussed in Chapter I.

Some of the key people involved in potato tuberization research are: Chapman (40), Cutter (52), Ewing (71, 72), Gregory (91), Menzel (159-164), and Moorby (175). Such classical work involved studying the influence of factors such as day length, temperature and hormonal balance on tuberization. The work of *in vitro* tuberization, however, is actually in its early stages of development. Among the investigators are Hussey and Stacey (113) and McCown and coworkers (156) at the University of Wisconsin-Madison, Dodds (58), and Estrada, et al. (70) at CIP. Wattimena (262), for his Ph.D. research, investigated the influence of many environmental and media component factors on *in vitro* tuberization. He also evaluated the yield of micropropagated plants (plantlets or microtubers grown in the field) when compared to seed tuber grown plants. The state of the art in *in vitro* tuberization is now focused on improving production (tuber number and size).

In vitro tuberization has received considerably less attention due to the relatively recent technology involved. Microtubers are morphologically similar to conventionally produced tubers; however

physiologically, there might be some difference. The relationship between microtuber yield and that of field or greenhouse grown plants may eventually be used as a basis for predicting yielding ability under field conditions.

Further work is needed to better understand the mechanism and physiology of tuberization. Some of the questions that need to be addressed on the basis of existing literature are:

- Why genotypic effects are relatively less under in vitro conditions?
- What are the effects of changing plant population (i.e. number of nodes) in the tissue culture jars?
- How does photosynthate assimilation play a role in in vitro tuberization?
- What is the role of endogenous plant hormones in in vitro tuber initiation?

There has been a vast amount of research on potato dormancy and sprouting. Bogucki and Nelson (29), Burton (34, 35), Davidson (54), Emilsson (65), and Hemberg (104) are among the famous people in this area. They evaluated the influence of factors such as genotype, tuber size, temperature and other environmental factors. The relationship between maturity class and duration of dormancy was exhibited in their work as well. The early maturing cultivars generally exhibit a short dormancy and vice versa.

The sprouting characteristics have been evaluated by people such as Krijthe (132, 133), O'Brien, et al. (185), Rylski, et al. (212), and Wurr (273). Several methods have been used to measure sprouting.

Examples are: sprouting capacity, total sprout length, degree-days and sprouting rate. The sprouting rate method was generally the most accurate since it monitored the sprout growth throughout the storage period.

Dormancy and sprouting characteristics have been further investigated on _in vitro_ produced tubers by people at CIP (69, 70 and 248) and Ewing, et al. (72). These studies are among the first efforts towards understanding of the dormancy of _in vitro_ tubers. _In vitro_ studies offer good chances for specific investigations of the biochemical growth promoter and inhibiter balance and the role they play in controlling dormancy and consequently the sprouting characteristics of _in vitro_ tubers.

CHAPTER I

PLANT GROWTH ANALYSIS

CHAPTER I. PLANT GROWTH ANALYSIS

Introduction

Much of the previous research on plant growth analysis of potatoes has been conducted with plants grown from seed tubers. Current literature reviews indicate that plant growth analysis studies have not been carried out with micropropagated potato plantlets. Micropropagation techniques offer many advantages to potato growers as well as scientists. Starting with healthy, genetically uniform plants is an important aspect in studies dealing with cultivar comparisons under field conditions.

This experiment was conducted in the field during 1986 and 1987 at the Horticultural Field Research Center. Three cultivars of differing maturity classes were used: Norland (early), Desiree (medium) and Russet Burbank (late). Biweekly plant samplings were made throughout the growing season to monitor plant development including tuber yield. The Richards function was used to fit the data from each growth attribute (i.e., top dry weight, plant height, etc.). The following derived quantities such as relative stem elongation rate (RSER), relative leaf area expansion rate (RLAER), relative top growth rate (RTGR), and relative tuber growth rate (RUGR) will be examined.

This study is one part of a project aimed at studying tuberization, dormancy and sprouting characteristics of micropropagated potato cultivars under field, greenhouse and *in vitro* conditions.

Materials and Methods

Plant Materials

Nodal cuttings were propagated to produce potato plantlets of three potato cultivars -- Norland, Desiree, and Russet Burbank; representing early, medium and late maturity when grown from seed tubers under field conditions. Furthermore, these three cultivars are known to possess short, medium and long dormancy respectively.

For some cultivars (i.e., Desiree), tubers were received from Dr. N. S. Wright in Vancouver, B.C. (Canada) on December 20, 1985 and stored at 5°C until they were transferred to room temperature to facilitate sprouting. Other cultivars had been previously propagated by nodal cuttings in the laboratory facilities of the Colorado Potato Certification Program.

Initial Propagation

Sprouts 20 to 30 mm in length were excised and sectioned into 5 mm sections, each with at least one node. Sections were surface disinfected by dipping in 70% ethanol for 20 seconds rinsed in 2% sodium hypochlorite for 2 minutes followed by rinsing in sterilized distilled water and aseptically cultured on agar media. The culture vessels were baby food jars (Ball Corporation/Gerber Products) covered with a hard plastic lid (Magenta Corporation) allowing for gas exchange.

Propogation media was a modified Murashige and Skoog (MS) media (182), as shown in Table 1.1.

The culture room was maintained at temperatures of 22 to 25°C and photoperiod of 16 hours at 2000 lux. Approximately 6 to 10 weeks

following the initial sprout explant establishment, axillary shoots and root development occurred. Fully developed plantlets were then used in subculturing.

Table 1.1. Composition of modified MS culture media for nodal propagation, and shaken liquid tuberization *in vitro*.

Compound (mg ℓ^{-1})	Single node propagation	Shaken liquid propagation	*In vitro* tuber induction
NH_4NO_3	1650	1650	1650
KNO_3	1900	1900	1900
$CaCl_2 2H_2O$	440	440	440
$MgSO_4 7H_2O$	370	370	370
KH_2PO_4	170	170	170
H_3BO_3	6.2	6.2	6.2
$MnSO_4 4H_2O$	22.3	22.3	22.3
$ZnSO_4 7H_2O$	8.6	8.6	8.6
KI	0.83	0.83	0.83
$Na_2MoO_4 2H_2O$	0.25	0.25	0.25
$CuSO_4 5H_2O$	0.025	0.025	0.025
$CaCl_2 6H_2)$	0.025	0.025	0.025
Na_2EDTA	37.3	37.3	37.3
$FeSO_4 7H_2O$	27.8	27.8	27.8
Thiamine HCL	0.4	0.4	0.4
Benzylamino purine (BAP)			5.0
Chlorocholine chloride (CCC)			500
Myo-Inositol	100	100	100
Sucrose	3%	3%	8%
Agar	0.65%		

Subculturing

When plantlets were 6 to 10 cm tall (cultivars responded differently), they were aseptically removed from the culture jars and sectioned into nodal cuttings. About 5 nodal cuttings were placed in each culture jar.

Shoots of plantlets generally attained 10-12 cm in height within two weeks.

Acclimation

About 240 two to three week old plantlets per cultivar with adequate root system were transplanted on June 4, 1986 into "cell pack" plastic trays[1] filled with Metro Mix 350[2]. Before transplanting, the soil mix was saturated with a starter fertilizer, Miracle Gro (15-30-15)[3] diluted at a rate of 1.3 cc l^{-1}. The plantlets were placed under shade cloth (50% shade) and watered every 5 min for 30 sec. from 5 a.m. to 8 p.m. Five days later Osmocote 14-14-14[4], a slow release fertilizer was applied at a rate of 1.2 g $plant^{-1}$ and the watering interval was increased to 10 minutes and later to 20 and 30 minutes.

Plantlets were placed outdoors for 4 hours on June 16 and exposed to the outdoor environment for increasing periods until 8 hour period was achieved after 14 days; watering intervals were also increased during this period.

Field Plot

The soil was a Nunn clay, with a pH of 8.1. The site was fallow for 2 years prior to planting. The plot was fertlized with 67.2 Kg ha^{-1} of nitrogen (ammonium nitrate (NH_4 NO_3)) and 168 Kg ha^{-1} of phosphorus (treble superphosphate (Ca $(H_2PO_4)_2$ and $CaHPO_4$)). Randomized block design with 4 replications of 30 plants each per cultivar was used (Figure 1.1).

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[1]American Clay Works, Denver, CO.

[2]Metro Mix is a potting soil containing the following ingredients: Canadian sphagnum, peat moss, domestic horticultural vermiculite, processed rock ash and washed granite sand. Grace Horticultural Products, W.R. Grace Co., Cambridge, MA.

[3]Stern's Miracle-Gro Products, Inc., Port Washington, N.Y.

[4]Sierra Chemical Co., Milpitas, CA.

Transplanting was done on June 24 by hand in 90 cm rows running east/west with spacing of 30 cm between plants. The average daily air temperature and solar radiation were 15°C and 27.7 MJ m^{-2} respectively. Each plantlet had at least 3 to 5 fully developed leaflets and a well developed root system. Planting depth was about 3 to 5 cm which was adequate to cover the root system of plantlets. After transplanting, about 200 cc of Miracle-Gro were applied to every plantlet. The plot was furrow irrigated the following day.

Essentially the same experiment was carried out in 1987. Transplanting to the greenhouse was on June 10 and into the field on July 2; the average daily air temperature and solar radiation were 15°C and 29.7 MJ m^{-2} respectively.

The first plant sampling involved randomly selected plantlets at transplanting. In later bi-weekly samplings, the plant on the west end of each row was selected.

In the first few samplings, plants were placed directly in plastic bags and brought to the laboratory for further measurement. Subsequent samplings were temporarily kept in cold storage at 5°C to preserve plant condition until all data collection were made; the time of storage varied from a few days to slightly more than a week.

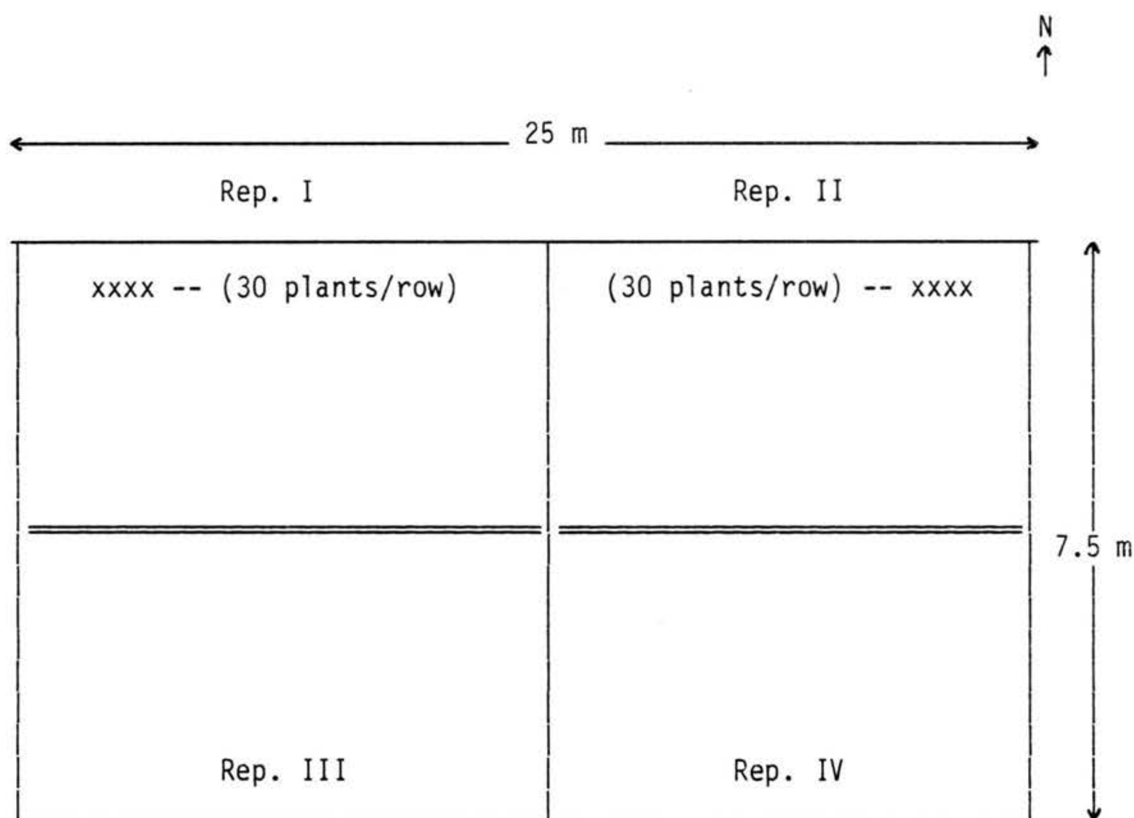


Figure 1.1. Schematic diagram of the field plot layout (See text for details).

The insecticide Sevin(R) was periodically sprayed to control Colorado Potato Beetle. Weeding was done by hand. Furrowing or "earthing up" was done three weeks after transplanting to cover the ground area near the plant base where stolons developed. At each plant sampling, the following measurements were made: plant height, leaf area, fresh weight of tubers, leaves, stems, roots and dry weight of leaves, stems, and roots. Plant samples were dried at 70°C for 3 days to ensure stability of dry weight. Richards function was used to fit the data from several growth attributes. The results from each fitted growth attribute will be shown. The statistical analysis was carried out on observed data using SPSS-X Release 2.0 (from Northwestern

University). The program was run on the Cyber 180/840 at the Colorado State University Computer Center.

Leaf area was measured with Li-Cor(R) leaf area meter (Model Ll-3100). Measurement is given in cm^2 and it indicates leaf area of one surface only. Leaves were unfolded and fed through the belt.

Leaf area index (LAI) was calculated as follows:

$$LAI = \frac{L_A}{L_p}$$

where, L_A is leaf area of a plant (cm^2)

L_p is land area in which the plant is growing (cm^2)

Leaf area durations (LAD) were calculated by integrating the LAI versus time curve. LAD has only the time dimension. The calculation was done by using Simpson's rule (program SIMPSON 2 written in APPLESOFT II BASIC) (214). The program was run on Apple II microcomputer. SIMPSON 2 provides solutions for each set of subintervals, x_i, 2, 4, 8, 16,... n and $\Delta x = n \, \Delta x_i$

The best approximation of LAD is probably:

$$\int_{t_1}^{t_2} LAI \, dt$$

which in this case is

$$LAD = \int_{t_1}^{t_2} (\beta_0 + \beta_1 t + \beta_2 t^2) \, dt$$

Since the primary data LAI_i vs.t_i were fit using quadratic model, therefore providing parameters estimates β for the integration specifically.

The following functions were used in SIMPSON 2:

For Desiree

$$LAD = \int_{14}^{84} (0.024 - 0.010t + 0.001\ t^2)\ dt$$

For Norland

$$LAD = \int_{14}^{84} (-0.104 + 0.017t - 0.0001\ t^2)\ dt$$

For Russet Burbank

$$LAD = \int_{14}^{84} (0.251 + 0028t + 0.0001\ t^2)\ dt$$

Days 14 and 84 coincided with t_1 and t_2 in the LAD equations when tuber initiation started and tuber bulking ended respectively. Therefore, a single LAD value was used to represent each cultivar per season.

Results and Discussion

General Background

A great majority of the published research on plant growth analysis of potatoes has dealt with plants grown by conventional seed tuber propagation methods. The study reported here is one of the first (if not the first) of its kind to evaluate the performance of micropropagated potato plants under field conditions by conducting plant growth analysis. The resources of personnel, time and funds required to do a complete evaluation of growth analysis of micropropagated vs. conventionally grown potatoes were beyond the scope of the research reported

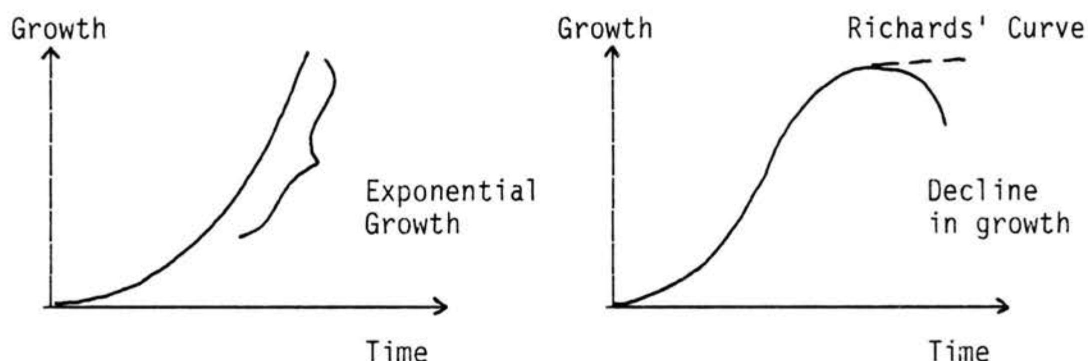
herein. Nevertheless, relevant comparisons will be included whenever the opportunity presents itself.

In the first season, nine cultivars were selected: Alpha, Desiree, Kennebec, Norgold Russet, Norland, Russet Burbank, Sangre, Spunta, and Ute Russet. Micropropagated plants were grown in the field and the growth analyses were performed. The amount of time and resources invested in collecting data in 1986 made it necessary to restrict the selection for the 1987 season to three cultivars: Norland, Desiree, Russet Burbank. The selection was based on the maturity class: early, medium, and late, respectively. The climatic data were, in general, similar for the two seasons (Appendix Table C.1); therefore, the two seasons' data sets were combined. The combined data provided additional replications which were necessary for a more precise estimate of plant growth attributes (fitted with Richards' function) and for better statistical analysis of the observed data.

The Richards Function

The Richards function, since its introduction in 1959 by F. J. Richards, has been used for modeling whole plants as well as plant parts (i.e. single leaf, fruit, seeds, roots). This function, as used in this study, offers advantages such as (a) convenient summary of the data, and (b) estimating or deriving growth quantities at any point in time. As pointed out by Williams (267), the fitted growth curves "provide a basis for interpretation without directing it." The Richards function offers flexibility in fitting plant growth data and permits the observation of trends. Derived quantities such as Relative Growth Rate (RGR) may be obtained. One negative feature of the

Richards function is its limited ability to model a time-course with no asymptote. Also data which exhibit a decline are not modeled (F. D. Moore, personal communication). Note the examples:



Data patterns which pass through the origin may not be modeled through the origin.

The predicted coordinates for growth attributes and the derived quantities were used to construct the growth curves (Figs. 1.3 to 1.7). An example of the program's output is shown in the Appendix Table A.1. Parameter estimates and their standard errors with regard to some selected growth attributes are shown in Appendix Table A.2. In Table A.1, the predicted coordinates for a growth attribute is shown as

Anti- (est. log Y), and that for the derived growth quantity, i.e. RGR as $\frac{1}{Y}\frac{dy}{dx}$. Multiple correlation coefficients and adjusted R^2 values are also given for several growth attributes as shown in Appendix Table A.2.

Statistical Analysis

Analysis of variance was conducted for every growth attribute. The findings were summarized in the Appendix Table B.1. Detailed

results were given in tables (1.2 to 1.5). Mean separation was conducted using the least significant difference (LSD) method at (P = 0.05). For the two way interaction, LSD was calculated according to the method represented by Snedecor and cochran (231).

Plant Height and Stem Elongation Rate

Significant differences in plant height were found among cultivars, sampling dates, and their interaction (Appendix Table B.1). The significant differences among cultivars observed during the eight samplings are given in Table 1.2. As plants grow and their vine structure develops, the differences become obvious and significant (Figure 1.2).

Table 1.2. Influence of Cultivar and Sampling Date on Plant Height Observed During Eight Serial Samplings.

Sampling	Plant Height (cm) [a]			LSD [b]
	Norland	Desiree	Russet Burbank	
1	9.42	12.17	9.88	5.14[2]
2	12.47	13.58	14.50	3.48
3	14.00	16.83	20.25	4.84 *
4	20.33	24.25	27.17	6.82 *
5	23.67	36.50	44.33	7.27 *
6	22.00	55.05	52.00	10.87 *
7	25.80	73.92	62.83	9.07 *
8	26.67	71.00	69.38	13.77 *

(a) Sample size (n) = 6

(b) LSD values followed with (*) indicate significant interaction between cultivars and sampling date.

About 60 days after transplng, Norland plants (example of early maturing cultivar) reached their height limits (about 25 cm) (Figure 1.3.

On the other hand, medium-late cultivars (i.e. Desiree) and late cultivars (i.e. Russet Burbank) continued to grow to greater height; 75 cm after 84 days for Desiree and about 70 cm at the final sampling date (96 days) for Russet Burbank. Russet Burbank plants exhibited a late maturity growth pattern which indicated that if the growing season had been extended, greater plant height would have resulted. In Figure 1.3, the curve representing plant height of Russet Burbank did not seem to reach an asymptote through the 96th day; indicating that a potential for more vine growth existed.

The plant height responses of micropropagated potatoes under field conditions (Fig 1.2) reflected a similar trend to that of seed tuber propagated potato plants. In a recent study by Snyder (232) on growth analysis of seed tuber propagated potatoes, a similar conclusion was reached. The late cultivars were taller than the early ones. Batutis (19) also indicated that early maturing, determinate cultivars (i.e. Norland) have shown almost total natural loss of the above ground dry matter in the field. Collins (50) indicated that the branching capacity of a given cultivar is the major factor affecting its final size. He observed that cultivars with smaller canopy, have lower branching capacity than cultivars with larger canopy.

A derived growth quantity called Relative Stem Elongation Rate (RSER) can be used to explain the differences obtained in plant height (Figure 1.3). As explained by Blackman (26), the relative growth rate (RGR) quantity is a measure of the "efficiency index of dry weight production" since more plant height will also contribute to higher plant dry weight production.

Generally, quantities such as RGR derived from Richards' function, depend upon the physiological status of the plant which reflects its ontogenetic drift and responses to environmental factors. The comparison of the RGR values alone is not recommended. Charles-Edward (41) indicated that "comparison of numerical values is not normally helpful in elucidating either physiological differences between crops grown under contrasting conditions or differences between different crops grown in the same environment." Due to the fact that all cultivars were treated similarly during propagation, acclimation, transplanting and sampling, it is to be expected that the genotypic influence probably was the major source of variation in potato plant growth and tuber yield. The genetic potential of each cultivar controlled the performance of that RGR quantity. For example, the RGR of medium (Desiree) and late (Russet Burbank) cultivars maintained a steady level at about 0.022 cm cm^{-1} day^{-1} (or 0.022 day^{-1}) for longer periods of time than did Norland (Figure 1.3). This steady level of RGR indicated that Desiree and Russet Burbank plants were exhibiting an exponential growth phase during the time from transplng up to 60 days for Russet Burbank and up to 90 days for Desiree (Figure 1.3). The lower values of RGR (0.014 day^{-1}) for Norland were steady for only 45 days. This may explain why early maturing, determinate growth cultivars have a limited RGR for plant height and for a shorter period of time compared to medium or late maturing cultivars. The rate at which RGR declined could also indicate the nature of the foliage system for each cultivar. Although Norland plants stopped their stem elongation about the 45th day, stem branching did not completely stop. This translated into slower increments of plant height (Figure 1.3). In

Russet Burbank, exponential growth was maintained longer (Figure 1.3). Stem branching continued but at a much slower rate after main stem elongation stopped. The fact that RGR values did not reach zero at the 96th day indicated that Russet Burbank exhibited a higher branching capacity resulting in a foliage increase over a longer period of time. Desiree plants, although maintaining their exponential growth stage for a longer time (Figure 1.3) had a very rapid decline in RGR at about the 88th day. Desiree (medium maturity) may have had a decreased branching capacity compared to Russet Burbank.

The rate of emergence in this study was not applicable since the plantlets were about 10 cm tall at transplng. The high (99%) survival rate, and uniformity in plant size at the beginning of the study were the result of successful acclimation procedures conducted after the plantlets were taken from culture vessels. Similar higher survival rates of these micropropagated potato plantlets were also reported by Thornton and Knutson (241).

Batutis (19) conducted a growth analysis study on potato plants grown in the field from seed tubers. He concluded that Norland is the earliest variety (among Norchip, Katahdin and Russet Burbank). Norland and Norchip emerged first and their tops grew quickly at the start of the season and declined quickly.

Leaf Area and Relative Leaf Area Expansion Rate

Significant differences were observed between cultivars, sampling dates, and their interaction (Appendix Table B.1). Significant differences between cultivars became obvious toward the second half of the growing season, see Table 1.3. The eighth sampling did not permit

leaf area measurements since frost occurred prior to leaf collection. Furthermore, interpolating or estimating leaf area at the final sampling was not appropriate because rapid plant maturity was occurring and the eighth sampling was the last one in the schedule of the season's sampling dates.

Leaf area, measured in cm^2 is shown in Figure 1.4. As might be expected, the cultivar with determinant growth habits, Norland, had a lower leaf area than Desiree or Russet Burbank. About the 48th day, Norland reached the maximum leaf area (2,000 cm^2 plant-1). While at

Table 1.3. Influence of Cultivars and Sampling Date on Leaf Area Observed During Seven Serial Plant Samplings.

	Leaf Area (cm^2)[a]			
Sampling	Norland	Desiree	Russet Burbank	LSD[b]
1	26.81	36.86	216.03	22.36[2]
2	117.38	150.31	135.83	77.21
3	345.46	743.59	880.17	459.92 *
4	1517.92	2295.62	2373.68	1066.51
5	1927.74	4840.96	6465.64	2446.50 *
6	2502.85	7447.82	9063.36	2547.15 *
7	1793.31	11596.28	7886.97	3148.31 *

[a] Sample size (n) = 6

[b] LSD values followed with (*) indicate significant interaction between cultivars and sampling date.

that time, Desiree and Russet Burbank were adding leaf area because of their extended growth habit. Total leaf area of any plant is generally influenced by many factors. Friend, et al. (73) reported that leaf area depends on factors such as production of leaf primordia, expansion of the lamina, increase in the number of meristematic

tissues by branching, and loss of meristematic tissue by senescence. Apparently, Desiree and Russet Burbank had a higher number of leaf primordia produced that was expressed as increased branching capacity. Norland, on the contrary, had fewer leaf primordia, less branching, and higher incidence of leaf senescence which contributed to smaller leaf area. In a similar study, but with tuber propagated plants, Batutis (19) reported that Norland had a relatively greater loss of the above-ground dry matter under field conditions. He also reported that early maturing cultivars have relatively low leaf area compared to medium or late maturing cultivars. These findings seem to be similar to our findings with micropropagated cultivars.

As pointed out by Wiebold and Kenworthy (265), the Rate of Leaf Area Expansion (RLAER) can be as important as the rate of photosynthesis itself. The RLAER serves as an indicator of the photosynthetic efficiency of the leaves throughout the season.

The RLAER, shown in Figure 1.4, explained the differences in leaf area between cultivars. Desiree and Russet Burbank, both having a relatively higher foliage growth capacity, were expected to have a higher leaf area. The initial higher RLAER of Desiree and Russet Burbank during the first 30 days contributed dramatically to the production of leaf area during this period (see Figure 1.4). The fact that the RLAER of those cultivars declined at a much slower rate than Norland, also explains their higher leaf area. The rapid decline of RLAER for Norland at about the 48th day was a good indication of its determinate growth habit compared to Desiree and Russet Burbank.

Leaf Area Index

Values of leaf area index (LAI) as high as 8 are common for many crop species. In general, LAI of 3 is necessary for maximum dry matter production. LAI values above 5 are not expected in potato because total biomass of plant parts is not as economically important as the underground tuber yield. The values of LAI found under the conditions of this study ranged from slightly under 1 to about 4 (see Figure 1.5). Bhagsari, et al. (23) reported similar variations among cultivars that were propagated from seed tubers. Significant differences occurred during the second half of the season, see Table 1.4. A review of Figures 1.4 and 1.5 explain the expected relationships between leaf area and LAI. Norland, with the lowest leaf area, also had the lowest LAI values. Similar relationships occurred with Desiree and Russet Burbank.

Table 1.4. Influence of Cultivar and Sampling Date on LAI Observed During Seven Serial Plant Samplings.

	LAI[a]			
Sampling	Norland	Desiree	Russet Burbank	LSD[b]
1	0.009	0.013	0.009	.006
2	0.041	0.053	0.047	0.025
3	0.122	0.263	0.311	0.159
4	0.536	0.811	0.838	0.375
5	0.681	1.709	2.284	0.868 *
6	0.884	2.631	3.201	0.898 *
7	0.633	4.096	2.786	1.115 *

[a] Sample size (n) = 6

[b] LSD values followed with (*) indicate significant interaction between cultivars and sampling date.

Optimum LAI, leaf area when dry matter production was maximized, varied among cultivars. Under the conditions of this study, it appeared that optimum LAI of Desiree, Russet Burbank, and Norland, were 3.1, 3.0, and 0.8, respectively. These values were considered optimum since they occurred at the time when tuber yield was maximized. Dwelle (62) reported positive correlations between tuber yield and estimated LAI. Higher tuber yield resulted from higher photosynthesis rate as it correlated with large leaf area (174). Canopy photosynthesis has been found to be closely associated with LAI (23). Milthorpe (170) reported that tuber bulking increases as LAI exceeds 1. This is probably correlated with tuber development during the first few weeks following tuber initiation. The relationship between LAI and tuber yields is illustrated in Figures 1.5 and 1.7. The same phenomena has been reported (149, 166, 168, 232) for potato plants grown from seed tubers. A possible explanation of the relationship between LAI and tuber yield is that higher LAI values tend to indicate increased shading of the soil, thereby reducing soil temperatures which improve tuber yield. Cultivars that attain high LAI values early in the season tend to achieve tuber yield earlier.

Leaf Area Duration

Leaf Area Duration (LAD) is essentially an indicator of photosynthetic rate and translocation and thus can be used as a measure of dry matter production efficiency. Leaf area duration is presented in units of time (days or weeks) and can be calculated between any two points in time during the growing season or extended throughout the entire length of the growing season. The values of LAD given in Table

1.5 were calculated between Day 14 and Day 84 -- during which tuber initiation started, and tuber bulking ended. Desiree plants had the highest LAD followed by Russet Burbank and Norland. The LAD values appeared to be well correlated with leaf area in a manner similar to that observed for LAI as shown in Figure 1.5. This may indicate that for cultivars with higher leaf area, the LAD was prolonged and a greater potential for higher tuber yield existed. As will be shown in

Table 1.5. Comparison of Cultivar LADs Calculated Between Day 14 and 84 after Transplanting.[a] (See text for details).

Cultivar	LAD[b] (days)
Norland	31.36
Desiree	164.36
Russet Burbank	98.14
LSD	8.21[2]

[a] Sample size (n) = 6

[b] LSD is significant at the (P = 0.05) level.

Figure 1.7, Desiree gained in tuber yield toward the end of the growing season. While Russet Burbank had a greater LAD than Norland, both cultivars produced similar tuber yields.

In studies carried out on seed tuber propagated plants (32), it was reported that a direct linear realtionship existed between tuber yield and LAD. The best statistical fit occurred when all LAI values above 3 were assumed to be 3; at this threshold value, the efficiency of photosynthesis and photosynthate utilization did not increase. Dwelle (61, 62) reported a direct linear relationship between tuber

yield and the time during which the LAI values were maintained above 3. Generally the higher the LAD, the greater the chances for light interception and utilization of photosynthesis -- this could result in greater tuber yields.

Top Dry Weight

Top dry weight included stems and leaves. Root weight was not included since it was almost impossible to obtain all the root biomass while digging the plant.

Significant differences were observed for cultivars and sampling interaction as shown in Table 1.6.

Table 1.6. Influence of Cultivar and Sampling Date On Top Dry Weight Observed During Eight Serial Plant Samplings

	Top dry weight (g)[a]			
Sampling	Norland	Desiree	Russet Burbank	LSD[b]
1	0.15	0.18	0.14	0.09
2	0.97	1.08	1.07	0.65
3	2.72	4.69	6.57	2.98 *
4	13.67	19.57	18.39	7.45
5	15.78	43.87	45.30	15.41 *
6	23.08	74.08	93.35	32.42 *
7	19.12	156.07	76.07	41.74 *
8	17.87	136.85	107.17	29.33 *

[a] Sample size (n) = 6

[b] LSD values followed with (*) indicate significant interaction between cultivars and sampling date.

Growth patterns shown in Figure 1.6 closely followed the patterns for plant height (Figure 1.3) and leaf area (Figure 1.4). Russet Burbank with greater leaf area also produced more top dry weight followed by

Desiree and Norland. The exponential leaf area expansion for Norland ended about the 45th day (Figure 1.4); a similar pattern of Relative Top Growth Rare (RTGR) is illustrated for Norland in Figure 1.6. The rapid decline of leaf area expansion coincided with the relative growth rate for tops. Rates for Desiree and Russet Burbank declined at a much slower pace. The close relationship between RLAER and top RGR suggested that top dry weight was influenced more by leaf area than stem height. Additional evidence involves stem height and stem number (data not included) which were about the same for Desiree and Russet Burbank. Soltanpour and Moore (233) reported direct linear relationship between RGR and solar radiation intercepted by the leaves.

Early in the season, when plants of the three cultivars were similar in height and leaf area, differences in top dry weight were understandably negligible. The canopy size as influenced by branching capacity had a greater influence on total top dry weight. Norland, with a small canopy size, produced the lowest top dry weight while Desiree and Russet Burbank having medium and large canopy sizes produced greater top dry weight. Similar observations on plants grown from seed tubers have been reported by Collins (49, 50). Batutis (19) and Snyder (232) observed, during growth analysis studies on seed tuber propagated plants, that late cultivars such as Russet Burbank produced more top weight. Likewise, the differences between cultivars became obvious at later sampling dates. As pointed out by Collins (50) and Batutis (19), the dry weight data can explain the differences in growth habit of potato cultivars. Batutis further suggested that "the dry matter in stems and leaves together seems to be an excellent measure of the maturity class of the variety".

Tuber Growth

Significant differences were observed between samplings (data not included). The differences between cultivars, although not significant, became more noticeable toward the second half of the season, simply because few if any tubers were formed earlier (Figure 1.7). As the growing season progressed, the early maturing cultivar produced more tubers than other cultivars. Later the tuber production seemed to stabilize while that of Desiree, for example, increased at a much higher rate. Consequently, yield for Desiree was greater than Norland at the end of the season.

Norland maintained a Relative Tuber Growth Rate (RUGR) of about 0.35 day^{-1} during the first 40 days after transplng (Figure 1.7). During the same period, Desiree had intermediate RUGR values followed by Russet Burbank. The steep decline in Norland's RUGR (Figure 1.7) explained the pattern observed in the tuber weight increase illustrated in Figure 1.7. The RUGR of Desiree declined slowly, resulting in a relatively higher tuber yield. This observation was further supported by the fact that the RUGR of Desiree never reached 0 even at the final sampling date. The RUGR of both Norland and Russet Burbank reached 0 at about 75 and 88 days, respectively.

Under the conditions of this study, leaf area development (Figure 1.4); LAI values (Figure 1.5), and top dry weight (Figure 1.6), and LAD (Table 1.5) correlated closely with final tuber yield shown in Figure 1.7. The close relationships between tuber yield and other plant growth characteristics observed in this study with micropropagated plants has also been observed for plants grown from seed tubers. Several investigators (19, 49, 50, 66, 232, 278) reported that final

tuber weight can be influenced by plant size, leaf area, RLAER, LAD, and RUGR.

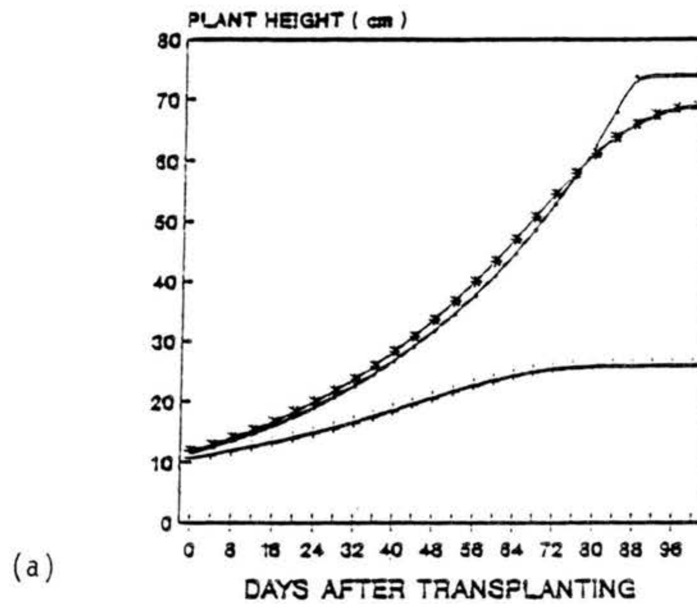
In this study, it was observed that the initial RUGR was higher for Norland with a small canopy size. However, a relatively higher proportion of assimilates were diverted to tuber development rather than top growth. Collins (50) reported similar findings in his study of cultivars with small, medium, and large canopy sizes. He also reported that net assimilation rate (NAR), measured in grams per unit leaf area per units time, followed a close pattern in changes with RUGR. A study by Bhagsari, et al. (23) indicated that genotypic variation in carbon assimilation were caused by differences in LAI among the various potato genotypes. The positive correlation between photosynthesis and LAI indicated that changes in LAI among cultivars reflected their photosynthetic rates and therefore the photosynthate supply to tubers.

A great majority of the field data collected on the three cultivars of micropropagated potatoes included in this study correctly characterized each cultivar according to its previously established maturity class. The inherent tendency to possess early, medium or late foliage growth characteristics were essentially not altered even though the seed tuber was not involved. These data strongly suggest that field growth habits of micropropagated potatoes do serve to accurately represent the true genetic potential of the test clone being evaluated.

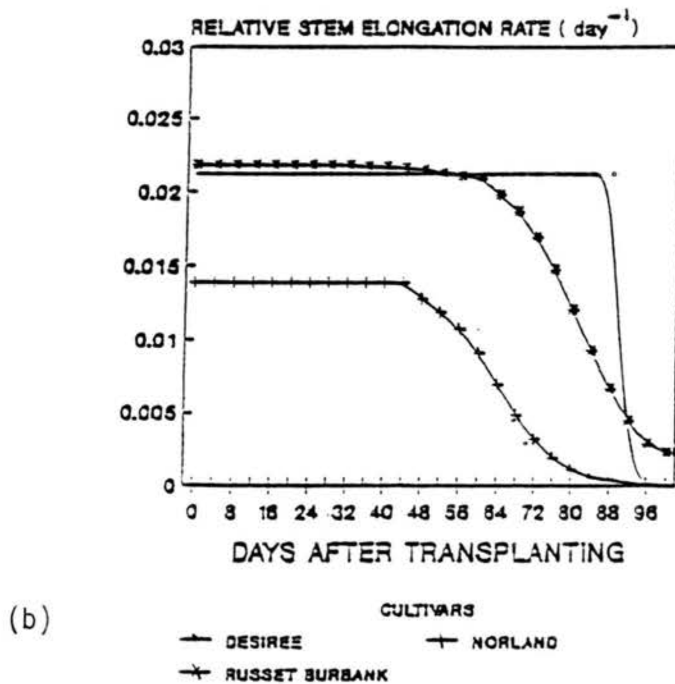
(a)

(b)

Figure 1.2. Top growth of micropropagated potato plantlets. Growth habit of Kennebec, Desiree, and Norland is shown in part (a). Part (b) shows the growth habit of Russet Burbank, Sangre and Kennebec.



(a)



(b)

Figure 1.3. Time course of plant height (a) and relative stem elongation rate (b) among three potato cultivars during eight sampling dates.

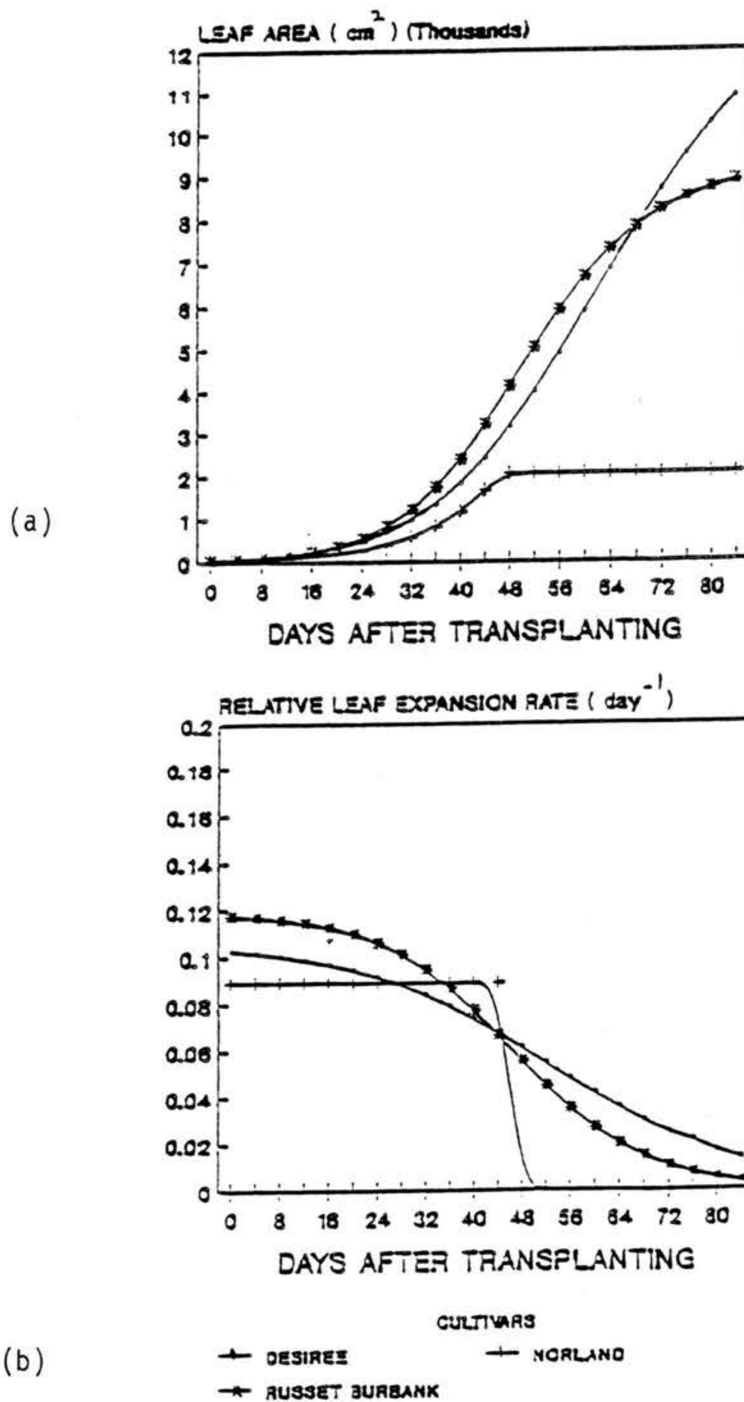


Figure 1.4. Time course of leaf area (a) and relative leaf area expansion rate (b) among three potato cultivars during seven sampling dates.

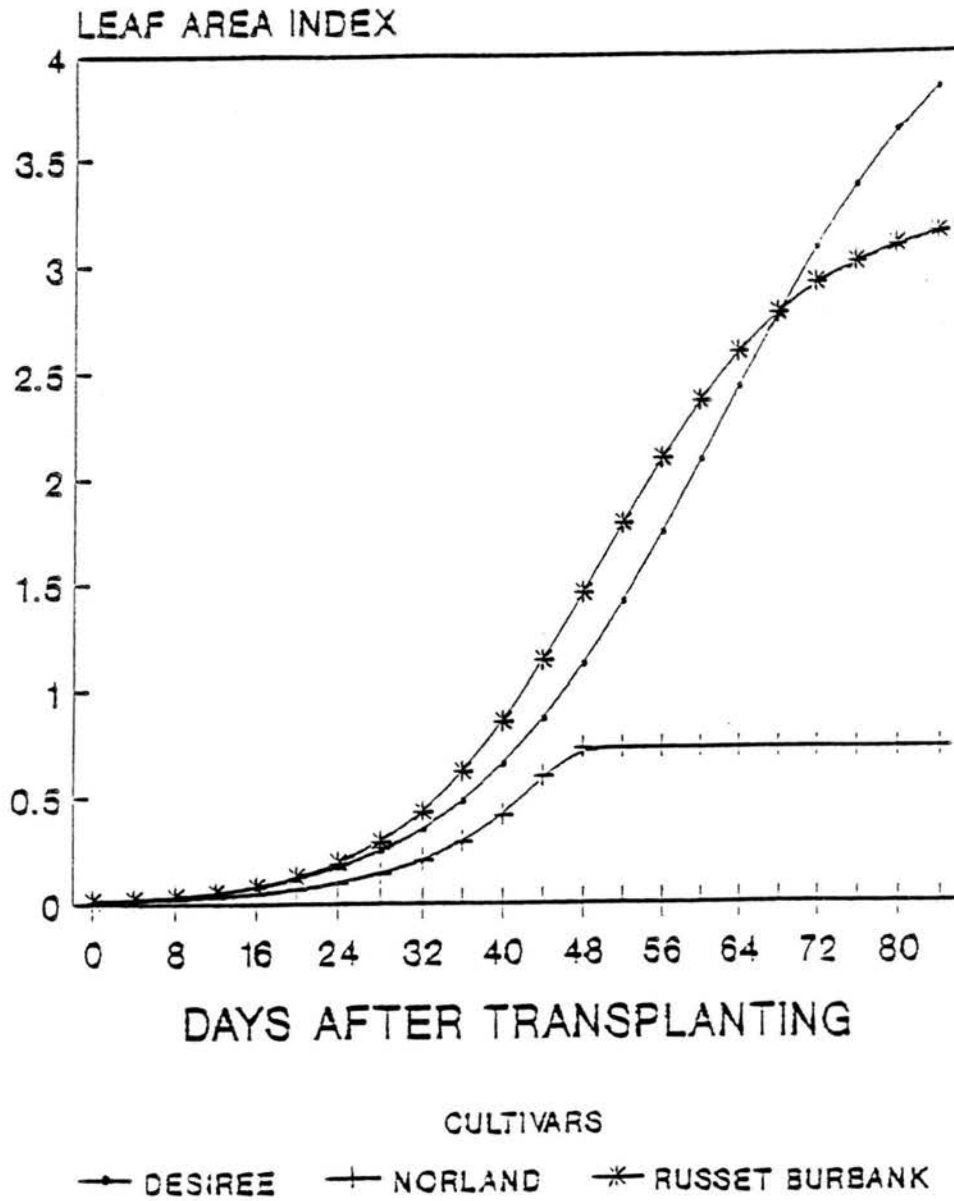


Figure 1.5. Time course of leaf area index among three potato cultivars during seven sampling dates.

(a)

(b)

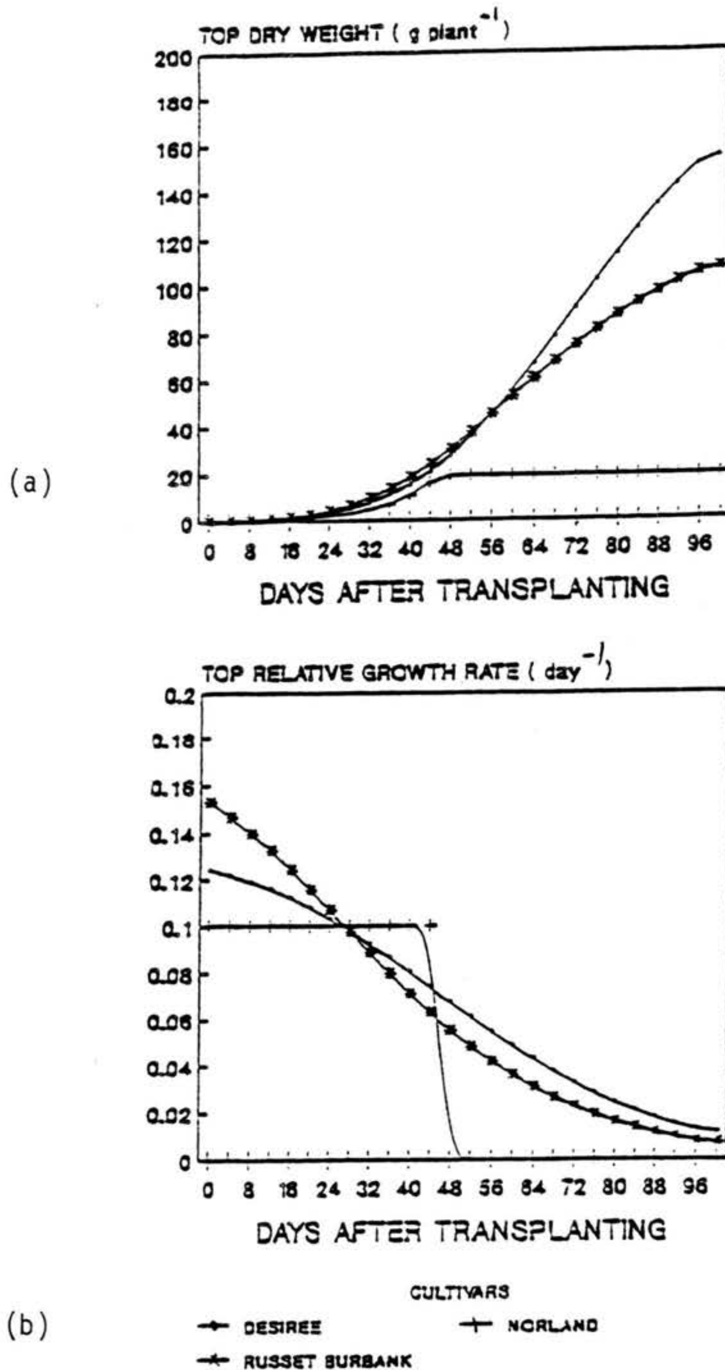


Figure 1.6. Time course of top dry weight (a) and relative top growth rate (b) among three potato cultivars during eight sampling dates.

(a)

(b)

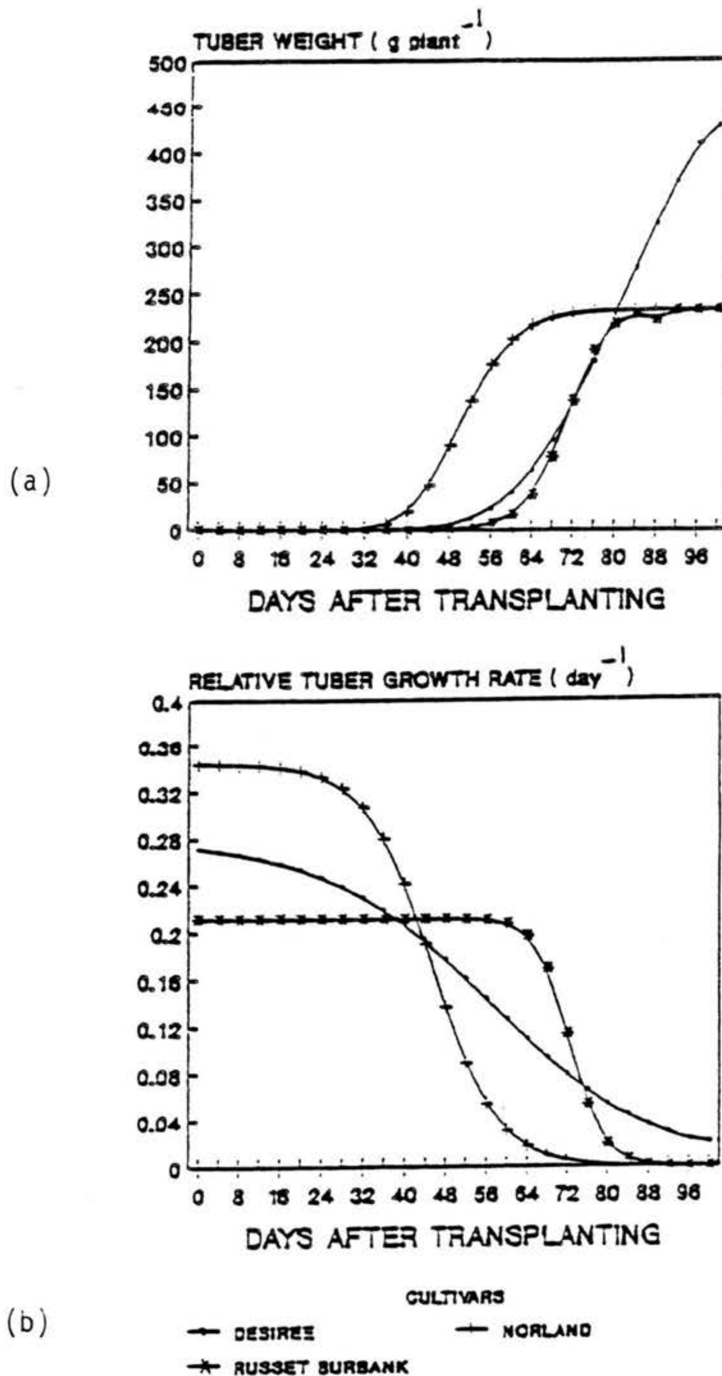


Figure 1.7. Time course of tuber growth (a) and relative tuber growth rate (b) among three potato cultivars during eight sampling dates.

CHAPTER II

TUBERIZATION OF POTATOES UNDER FIELD, GREENHOUSE AND *IN VITRO* CONDITIONS

CHAPTER II. TUBERIZATION OF POTATOES UNDER FIELD, GREENHOUSE AND *IN VITRO* CONDITIONS

Introduction

Tuberization of potatoes has been the central theme for several investigations dealing with potato growth in general. Gregory (91) stated:

> Before tubers are initiated, the environmental requirements for tuber induction, established by the inherent properties of the plant, have to be satisfied.

Tuberization has been studied under both *in vivo* and *in vitro* conditions. *In vitro* tuberization offers many advantages such as production of tubers regardless of the season, ease of handling and suitability of storage for longer periods.

In vitro produced planting stocks offer many advantages to potato growers as well as to researchers. Among these are: assurance of disease free plants; adapted to a wide range of cultivars, usually suited to growers needs and resources and maximizes likelihood of uniformity in plant establishment and growth. This study is part of a project involving plant growth analysis, dormancy and sprouting characteristics of potato cultivars. The main objectives of this study were to compare potato cultivars in their tuberization response, and explore the feasibility of estimating the yielding ability of cultivars through *in vitro* experiments.

Materials and Methods

Materials used and propagation methods followed were similar to that previously reported in Chapter I.

Plantlets from six cultivars - Desiree, Kennebec, Norgold Russet, Norland, Russet Burbank, and Spunta were acclimated and utilized for field, greenhouse minituber production and also laboratory in vitro microtubers production. The Desiree, Kennebec, and Spunta, cultures originated from tubers received from Dr. N. S. Wright in Vancouver, B.C. (Canada). Other cultivars were obtained from the Potato Certification in vitro clone bank.

Details of field tuberization methods were reported in Chapter I. The minituber and microtuber production is explained as follows.

Greenhouse Minituber Production

A randomized block design with 4 replications per cultivar as shown in Figure 2.1 was used. Plantlets were transplanted at 20 x 20 cm spacing into benches filled with Metro Mix. A border row of Sangre cv. plantlets was transplanted along the outer edges of each bench. Prior to planting, 100 cc of Miracle-Gro was applied to each plant location and shade cloth was set (50% shading).

Transplanting was carried out on September 22, 1987, and the plantlets were watered every 10 min. for 30 sec. Three days later, watering intervals changed to every 30 min. Shade cloth was removed at the end of the first week. Osmocote was applied three times at the rate of 1.2 g plant^{-1} at 10 days intervals.

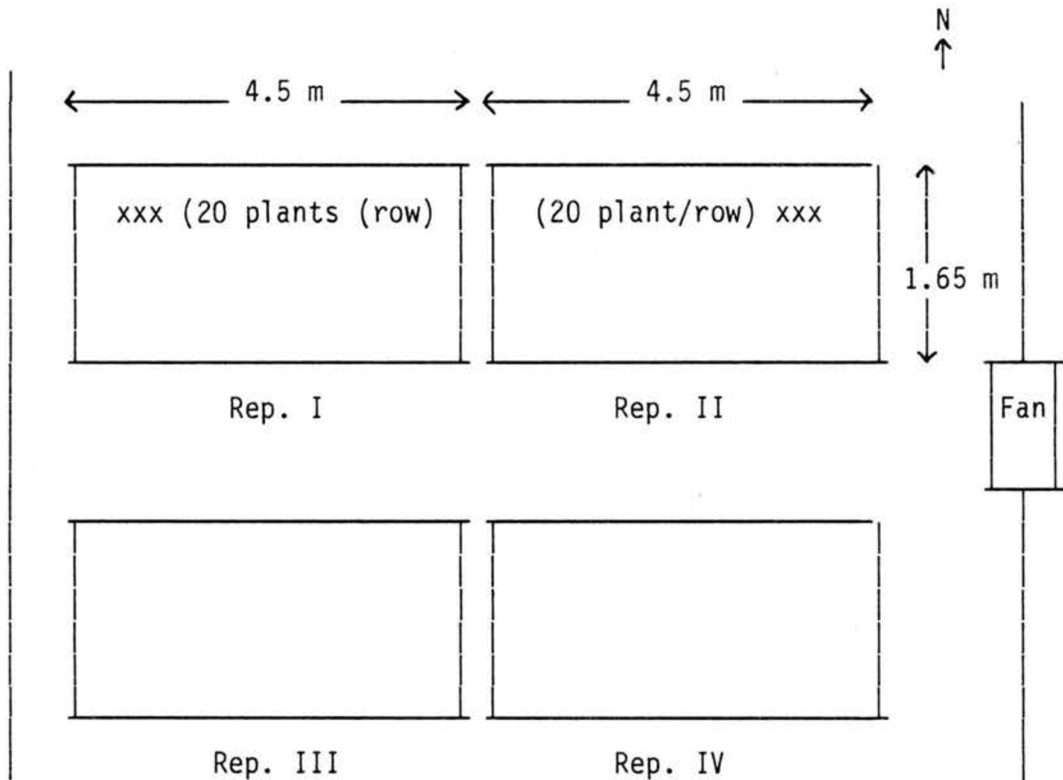


Figure 2.1. Schematic diagram for the greenhouse experiment design. (See text for details).

Harvesting was carried out on December 15, 1987 after 12 weeks of growth. Tuber number and weight were measured.

In vitro Tuberization

Four successive experiments were conducted. Four flasks per cultivar were used in each experiment. The methods utilized here were slightly modified from those reported by Pilar Tovar et al. (248) of the International Potato Center.(CIP). This method consists of three stages as follows:

A. Initial Propagation

Through single node cuttings as previously described.

B. Shaken Liquid Culture

Plantlets were aseptically sectioned into nodal cuttings (each with one leaf and its axillary bud). The root and apical portions of each stem were discarded. Fifteen nodal cuttings were placed in a 500 cc flask containing 100 cc of media (Table 1.1).

Flasks were then shaken on an orbital shaker at 70 rpm, under continuous light of 4000 to 5000 lux at 18°C. The axillary buds grew rapidly and roots developed in the first week. Two to three weeks later, plantlet growth nearly filled the flask.

C. *In vitro* Tuberization (Tuber induction)

The following components were added aseptically to the existing media: sucrose (8%), CCC (500 mg ℓ^{-1}), and BAP (5 mg ℓ^{-1}). After the addition, the flasks continued to be shaken under total darkness for four weeks.

The modifications of the method used at CIP are illustrated as follows:

A. At the shaken liquid culture stage

1. Leaves were not removed from stem segment.
2. Shaken liquid culture media did not contain C_a pantothenic acid, gibberellic acid, or Benzylamino purine (Table 1.1).
3. The flasks containing the stem segments were shaken under continuous light (4000-5000 lux) as compared to 16 h of 1000 lux.

B. At the tuber induction stage

1. Tuber induction media did not contain C_a pantothenic acid and gibberellic acid.
2. Flasks were shaken continuously under total darkness as compared to dark incubation (without shaking).

Root initiation started at about 2 days after the start of shaken liquid culture media. Shoot growth occurred following root initiation. Fourteen days after the addition of tuber induction media, stolon development was visible with some swelling of the stolon tip which was the sign for tuberization. Microtubers were formed mainly at the basal part of the shoot. In Norgold Russet, Russet Burbank and Spunta, microtubers were formed on both upper and lower portions of the shoot. At harvest, tuber number and weight were measured on a per flask basis.

Results and Discussion

While significant differences occurred between cultivars grown under field and greenhouse conditions these differences did not occur under *in vitro* conditions (Table 2.1 and Figs. 2.2 and 2.3).
A meaningful trend of differences (in tuber number per plant or tuber weight per plant) can be found in the microtuber production. This trend of variations between cultivars, under *in vitro* conditions, may provide a basis for correlation as will be discussed later.

Table 2.1 Tuber Yield Comparisons of Six Potato Cultivars Grown under Field, Greenhouse, and in vitro Conditions.[a]

Cultivars	Field		Greenhouse		In Vitro[1]	
	Number tuber $plant^{-1}$	Weight g $plant^{-1}$	Number tuber $plant^{-1}$	Weight g $plant^{-1}$	Number tuber $flask^{-1}$	Weight mg $flask^{-1}$
Desiree	12.7	751.6	8.0	94.5	7.5	472.5
Kennebec	9.8	981.5	9.3	87.9	5.7	509.7
Norgold Russet	8.6	592.7	2.8	11.1	12.0	485.3
Norland	10.7	516.2	5.3	172.7	5.8	273.2
Russet Burbank	8.7	511.1	6.0	79.7	11.8	417.4
Spunta	10.8	1006.9	7.5	107.0	6.3	550.1
LSD	2.3	273.2	3.9	30.1	NS	NS

(a) Number and weight of in vitro produced tubers are given in per flask basis rather than per plant basis.

Significant correlations ($p = 0.05$) were found between average weight of microtubers/flask and the average weight of field grown tubers per plant (Figure 2.4). Another correlation was found between average number of minitubers and the average weight of field grown tubers (Figure 2.5). These significant correlations can occur when all genotypes are planted at the same planting density which may not be optimum for individual genotypes. The yield of microtubers was reported on a per flask rather than per plant basis because it was nearly impossible to maintain separate plant identity in the culture jars. The massive root system that developed during tuber induction covered the entire lower portions of the stem cuttings. A standard

number of 15 nodes per flask were used for each cultivar. For these reasons, plus the fact that the in vitro flask microclimate is uniform for each node involved, it was decided to use tuber yield per flask as a basis for yield comparison with field and greenhouse studies.

Spunta and Kennebec both produced highest tuber weight under field or in vitro conditions (Figure 2.4). While Norland and Russet Burbank had the lowest yield under both conditions. Desiree and Norgold Russet exhibited an intermediate yield response. When comparing field and greenhouse conditions (Figure 2.5), Spunta and Kennebec produced a higher yield than Norgold Russet, Norland, or Russet Burbank. Desiree had an intermediate yield response also.

No significant correlations were found for tuber number or tuber weight per plant between greenhouse and field grown plants. Some cultivars (i.e. Desiree and Norland) produced higher numbers of small tubers under field conditions only. Cultivars with higher number of tubers may not necessarily produce higher tuber weight and vice versa. A significant correlation, however, was found between weight of field grown tubers and number of greenhouse grown tubers. This correlation suggested that tuber number rather than weight is important for reporting yield under greenhouse conditions. Greenhouse conditions may permit a valid expression of tuber number potential but limitations in soil space and possibly radiation may not permit maximum tuber size development. Maximum yield depends on a minimum number of tubers being produced to achieve an optimum top:tuber ratio.

The correlation between weight of microtubers per flask and weight of field grown tubers per plant was interesting and it may be indicative of the potential usefulness of microtuber production as an

indication of field performance. The significant correlation was reported under a given set of conditions and variables mentioned in this study. Given different conditions, the correlation may not be significant. For example, changes in number of nodes per flask and changes in component or concentration of tuber induction media or light regimes may all have contributed to the yield ability of microtubers. Further research is needed to broaden our knowledge about yield response under in vitro conditions.

To the best of our knowledge, previous work that has dealt with microtuber production (69, 70, 72, 189, 198, 248, 259, 262) did not prove any significant differences between cultivars with regard to tuber number or weight per flask. This study is no exception. The time of tuber initiation correlated well with the plant maturity class (176). Under field conditions, the "inherent properties" (91) of plants were mostly satisfied because of the obvious influence of various environmental conditions and the chances for longer growing season which allowed for expression of genetic influence. Assimilate partioning, for example, seemed to be influenced by genetics (52, 115, 229). Furthermore, tuber number was related to the number of stems per plant. In vitro conditions may not have allowed for full expression of the growth potential of cultivars. Limited growing space, shorter growing period and minimal influence of stems all seemed to influence the yield of microtubers.

A vast amount of research has been done on in vivo tuberization with conventionally grown potatoes. Differences between cultivars have also been reported. The comparative responses of conventional vs. micropropagation techniques under field conditions have been

studied by Wattimena, et al. (263). They used Norland (early maturing) and Red Pontiac (late maturing) cultivars. The propagule sources were either seed-tuber, microtuber or microshoots (plantlets). They reported that micropropagated plants had a greater number of tubers per plant than plants grown from seed tubers. At the end of the season, no differences in total tuber weights were observed among plants, produced by either method. They also reported that Norland plants grown from microshoots produced lower yield as compared with Red Pontiac plants. The research being reported herein represented similar findings. Norland plants produced lower yield (Table 2.1) while Kennebec and Spunta produced higher tuber yields.

The differences and potential similarities between yielding abilities of microtubers vs. greenhouse or field grown tubers might be explained by the physiological and anatomical features of tubers under _in vivo_ and _in vitro_ conditions. Morphologically, tubers produced under both conditions were similar (52, 70, 248) (see Figure 2.6). Phloem and xylem development and shape and color have been found identical. Tubers in both conditions, are usually formed at the lower nodes of shots or stems. Physiological differences, however, do exist (113). The influence of the mother tuber in providing nutrients and hormones for the developing plant was substituted by sucrose and other media components under _in vitro_ conditions. The tuberization stimulus of _in vivo_ grown plants may not necessarily be evident under _in vitro_ conditions because of limited foliage development.

The potential for estimating yield responses is exhibited by the _in vitro_ tuberization study. Additional work is needed to fully understand the growth responses under _in vitro_ conditions.

Understanding potato tuberization at the *in vitro* level will undoubtedly strengthen our knowledge of potato tuberization in general.

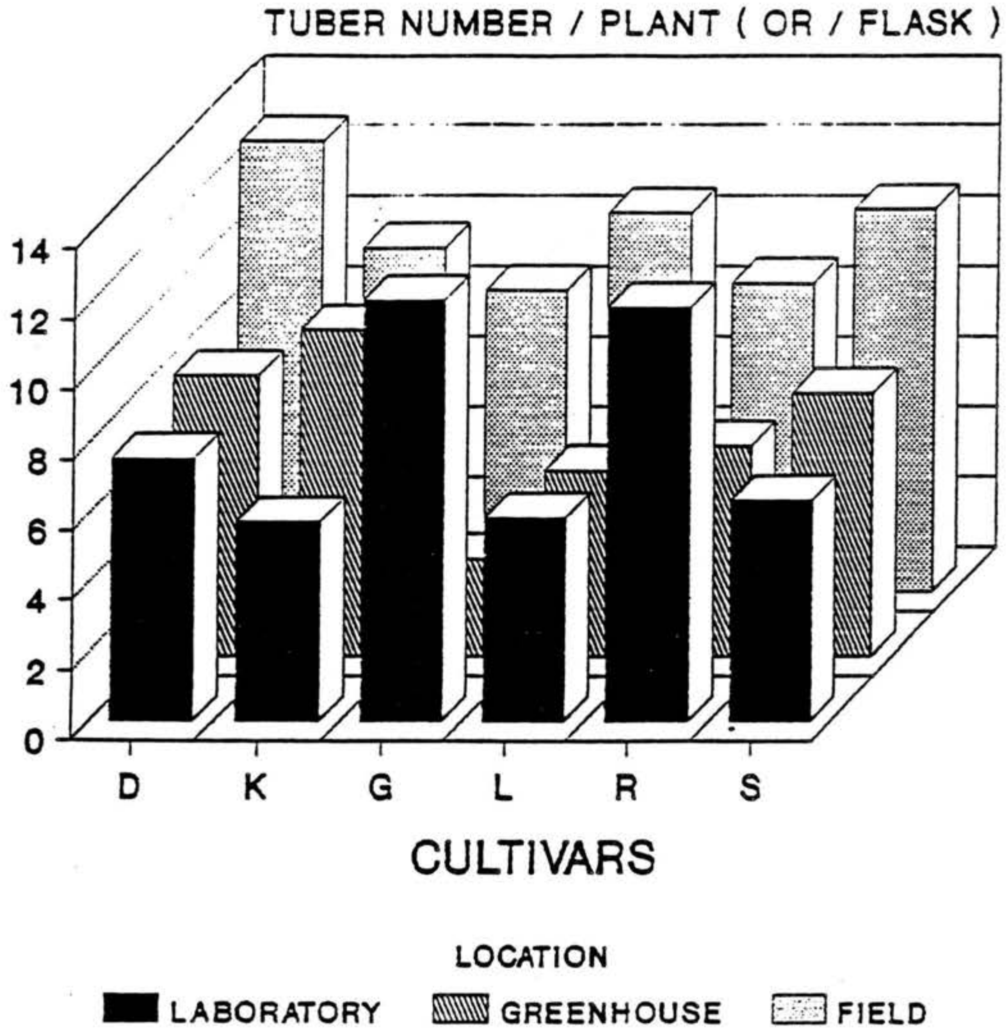
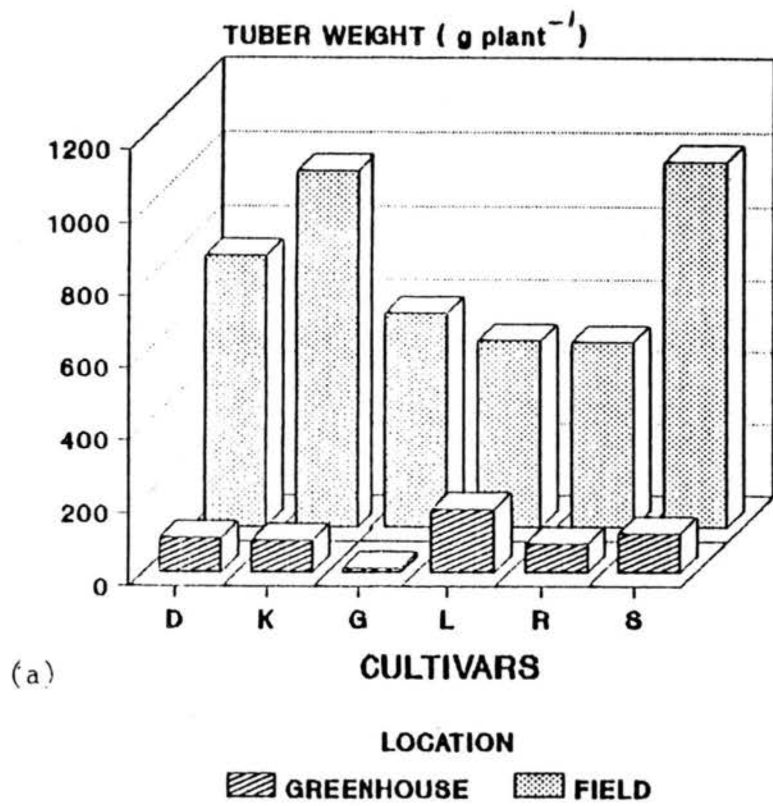


Figure 2.2. Tuber yield comparison (tuber number per plant or per flask) among six potato cultivars grown under *in vitro*, greenhouse and field conditions.



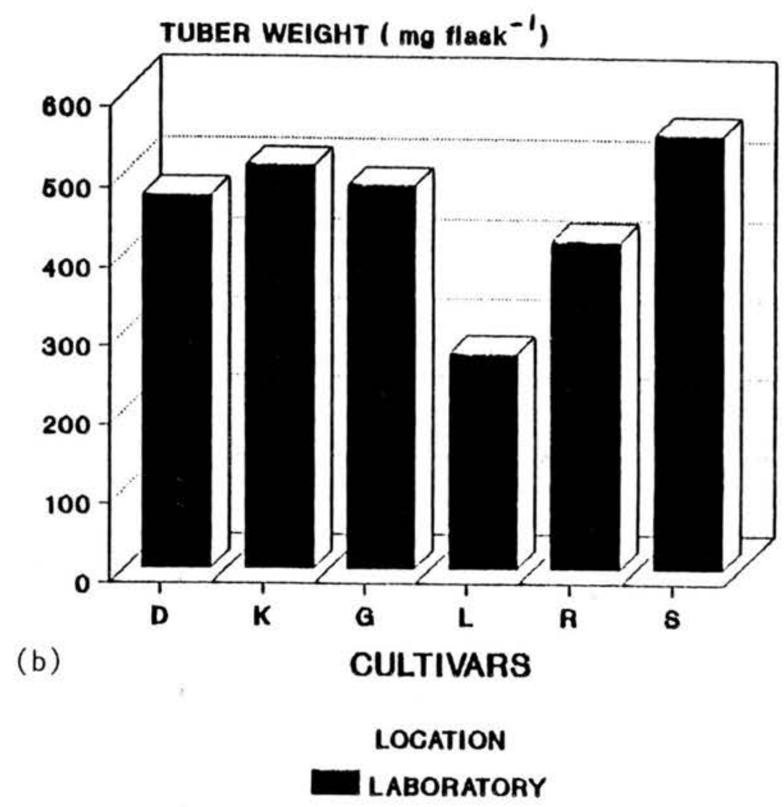
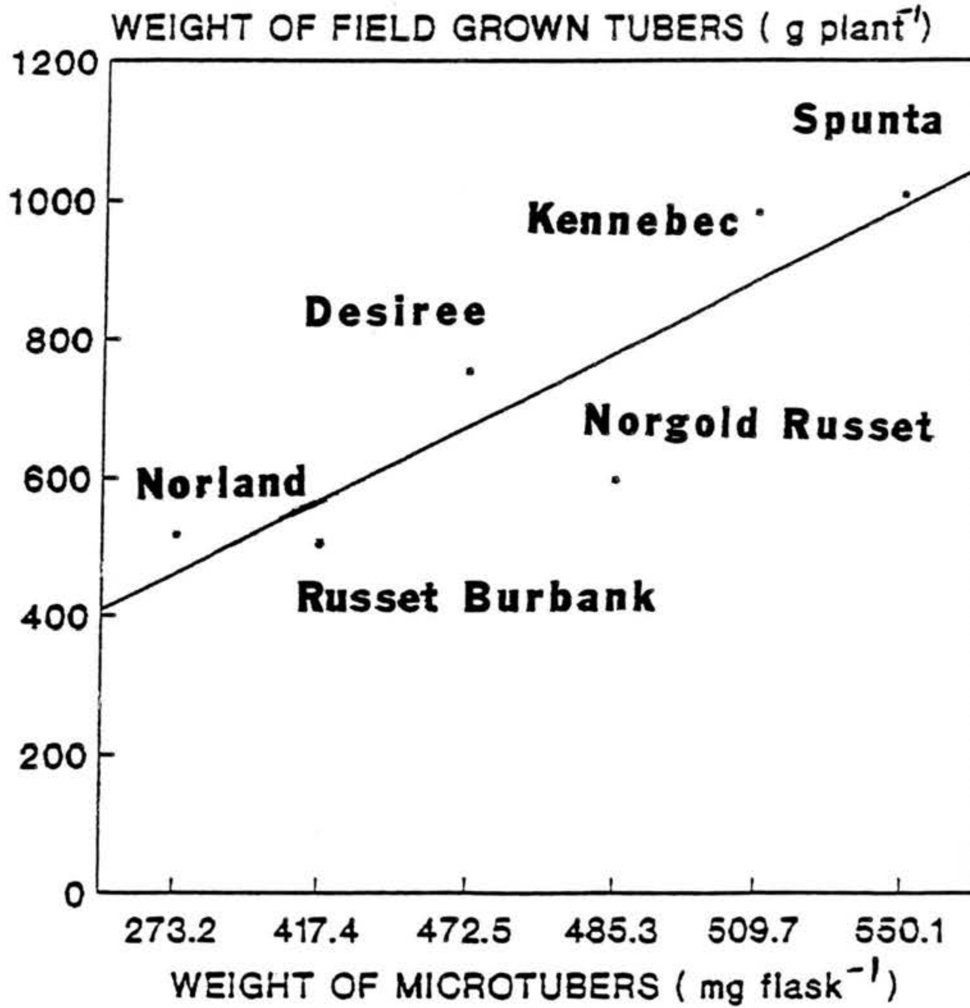
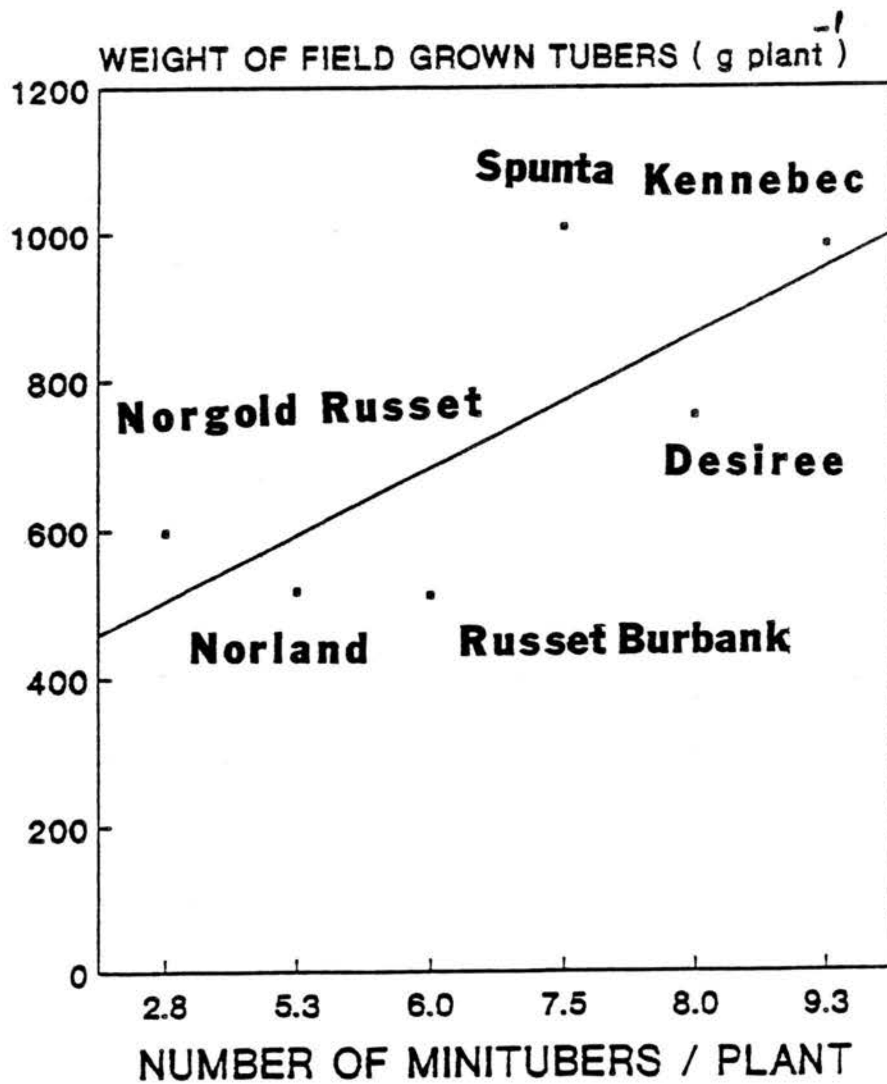


Figure 2.3. Tuber yield comparison (tuber weight in g plant^{-1} (a) or mg flask^{-1} (b) among six potato cultivars grown under *in vitro*, greenhouse and field conditions.



$Y = -63.5 + 1.75X$
$r = 0.76$
$P < 0.05$

Figure 2.4. Yield relationships of six potato cultivars grown under field and *in vitro* conditions.



$Y = 267.9 + 70.8\,X$
$r = 0.73$
$P < 0.05$

Figure 2.5. Yield relationships of six potato cultivars grown under field and greenhouse conditions.

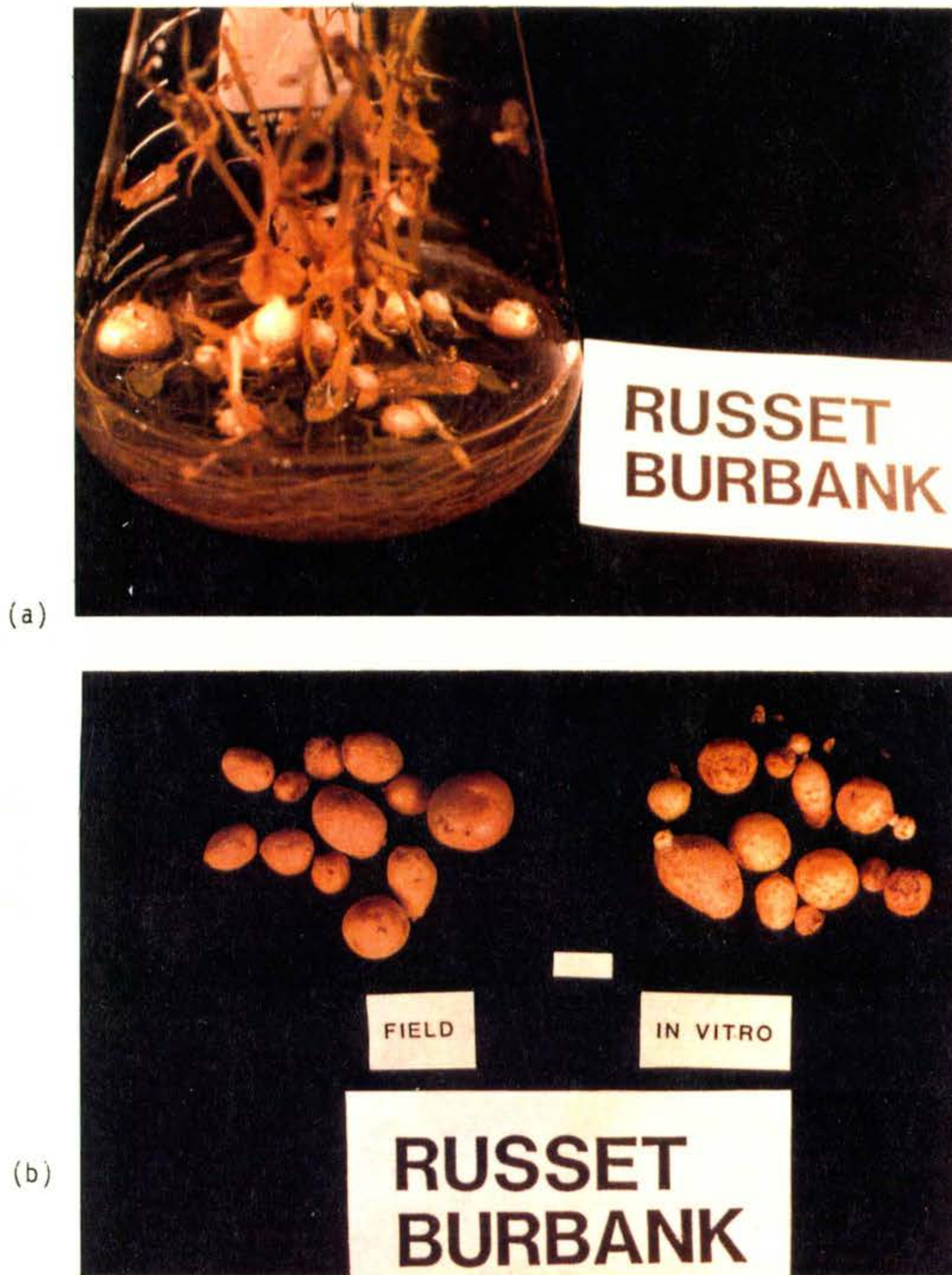


Figure 2.6. a. Microtubers produced under _in vitro_ conditions prior to harvest, after the addition of tuber induction media and with continuous darkness for four weeks at 22°C.
b. Similarities in shape and color between field and _in vitro_ grown tubers.

CHAPTER III

EVALUATION OF DORMANCY AND SPROUTING CHARACTERISTICS OF POTATOES

CHAPTER III. EVALUATION OF DORMANCY AND SPROUTING CHARACTERISTICS OF POTATOES

Introduction

Sprouting characteristics can serve as an indicator of physiological age of the tuber and the potential for overall growth and development under given sets of growing conditions (65, 98, 127, 133, 213, 269, 277). The sprouting behavior of tubers is due mainly to inherent cultivar characteristics as influenced by environmental conditions during tuber development and storage.

The bulk of previously published studies on this subject have been carried out using tubers produced on plants grown from seed tubers, while very limited research has been conducted on tubers harvested from micropropagated plants.

Two different sprouting indices were developed and evaluated for their ability to estimate sprouting behavior of microtubers (*in vitro* grown tubers), minitubers (grown in greenhouse) and field grown tubers. These indices were based on either the average "rate" of sprouting (mm/100 days) or the "ratio" of sprout weight to tuber weight (mg/g).

Materials and Methods

Post Harvest Tuber Treatment

All tubers were suberized at room temperature for 7 days. In addition, microtubers were treated with Captan (10% dust) after being

harvested to prevent fungal growth due to the presence of sucrose solution on their surface.

Size Distribution

Field grown tubers were divided into the following size categories:

a. Large diameter > 5 cm
b. Medium diameter 2.5-5 cm
c. Small (1) diameter 1-2.5 cm
d. Small (2) diameter <1 cm

Minitubers were classified into three size categories:

a. Large diameter >2.5 cm
b. Medium diameter 1-2.5 cm
c. Small diameter <1 cm

Since microtubers were smaller than either the minitubers or field growth tubers, only one size was available. It generally represented tubers with a diameter of 1 cm or less. Tuber weight was recorded prior to storage.

Storage Rooms

Three refrigerated storage temperature rooms maintained at 5, 10 and 20°C were used. The range of accuracy of temperature control was ±2°C. Each room is 2.2 x 3.3 x 2.05 m with automatic ventilation. Eight wooden shelves were built in each room. Relative humidity was maintained at about 85%. Temperature and humidity were recorded with hygrothermographs located in each room.

Of course, actual vapor pressure will increase with increasing air temperature; hence, at 20°C, the actual vapor pressure deficit,

VPD (85% RH) will be more than four times the VPD at 5°C and 85% RH. Whether the VPD difference will have a significant effect on tuber sprouting is unknown. I decided to ignore the VPD because this study is mainly involved with temperature influence rather than humidity. The higher sprouting rate occurred at 20°C might indicate that the stress of having higher VPD might be insignificant.

A split plot design was set for each room. Four replications per cultivar were used. Tuber size was randomly distributed within each replication. Each replication was randomly assigned at a given shelf so that temperature influence was uniform for all cultivars. Two tubers from each size category were placed in plastic trays randomly positioned on wooden shelves in each room.

Sprouting Measurements

Sprouting was recorded throughout the storage period. A tuber was considered sprouted if it showed a sprout of at least 3 mm in length. Sprout length was continuously recorded until it reached 100 mm.

Termination of Sprouting

Sprouting observations of field grown tubers, minitubers and microtubers were terminated after 14, 16, and 22 weeks of storage respectively; tuber and sprout weight were recorded at those times.

Sprouting indices

Two indices were developed to provde a numberical summary of sprouting characteristics which integrated some of the important fac-

tors (i.e. genotype, temperature, tuber size) influencing dormancy and sprouting responses

1. Sprouting rate index

$$\text{Rate} = \frac{L}{D_2 - D_1} \quad 100$$

where,

L = maximum total sprout length (in mm)

D_1 and D_2 were the number of days at which sprout length measurements started (D_1) and terminated (D_2)

This index indicated the sprouting rate in mm/100 days.

2. Sprouting ratio index

$$\text{Ratio} = \frac{S_w}{T_w}$$

where,

S_w = sprout weight at end of storage (in mg)

T_w = Tuber initial weight (in gms.)

This index expresses the ratio of sprout weight (mg) produced per g of tuber weight.

Both indices provide an estimate of the sprouting capacity of the tuber. The two indices have been calculated for field grown tubers, minitubers, and microtubers stored at all temperatures. Values of these indices reflect the cultivar, temperature, and tuber size influence on dormancy and sprouting. For example, large tubers from an early cultivar stored at 20°C have the highest sprouting indices.

The sprouting ratio index was developed because of the need for an easy, reliable method of estimating sprouting state. This method saves time and labor. As will be explained, sprouting ratio index closely correlated with the sprouting rate index especially for field

and greenhouse grown tubers. These findings indicated its usefulness as a complementary method that gives reliable estimate of the sprouting capacity of potatoes.

Results and Discussion

Background

The sprouting phenomena in potatoes, as it relates to dormancy and further developmental growth, has received a great deal of research attention. Several methods (3, 98, 133, 212) have been used to measure sprouting and estimate physiological age and growth vigor of the tuber. Among these methods: sprouting capacity, total sprout length, day-degree, and sprouting rate.

The two sprouting indices reported here were also attempts to develop a quantitative rather than qualitative estimate of physiological age or dormancy response of tubers. The sprouting "rate" method (mm per 100 days) was used as a standard for presenting the data. This method carefully monitored sprout growth through the storage period. The second method, sprouting "ratio" [mg sprout weight per g tuber weight] was included so that a comparison could be made and conclusions could be drawn about the feasibility and practical application of these methods.

The majority of sprouting research has been conducted with tubers produced on plants grown from seed tubers. Recently, very limited work (70, 248, 262) has been carried out with microtubers. The evaluation of sprouting responses of three types of tubers (microtubers, minitubers, and field grown tubers) produced by micropropagation

techniques has not been reported before. Therefore, this study was undertaken to address that point.

In this study, plant tissue culture, acclimation, transplanting, harvesting, and storage were all performed in a similar manner so that the genetic potential of sprouting could be clearly expressed. Dormancy was considered ended and sprouting established when the tuber developed sprouts of at least 3 mm. This arbitrary length was consistently used as a base line throughout the storage period.

The sprouting "rate" index

The data for sprouting rate index (mm sprout length/100 days) is reported in Table 3.1. As mentioned earlier, the sprouting rate index was calculated as an average rate during the duration of storage. The total sprout length per tuber (average sprout length per tuber times number of eyes per tuber) was monitored and the data was used to obtain the values for sprouting rate index.

The temperature influence on sprouting was obvious. Higher temperature enhanced sprout initiation, and further increased the rate of sprout elongation. A very obvious trend is shown in Table 3.1 .

Figs. (3.1, 3.2, 3.3) illustrate the influence of temperature on sprout growth. Total sprout length was consistently higher at 20 than at 10°C. Limited sprout growth occurred at 5°C. This response to temperature was exhibited by tubers harvested from cultivars grown under either conditions. Rate of sprout growth of field grown tubers seemed to start slow during the first 60 days of storage at 20°C (Figure 3.1), followed by a rapid increase toward the last 20 days. This trend was also shown at 10°C but with lesser magnitude. At 5°C,

minimal sprout growth occurred toward the second half of the storage period. Among cultivars, Norland exhibited the fastest sprouting response followed by Desiree at both 10 and 20°C. However, at 5°C, Desiree showed slight sprout growth increase over that of Norland. Nevertheless, the differences between them were negligible. With the exception of Norland, tubers from cultivars grown in the field exhibited a higher sprouting rate than those produced in the greenhouse; likewise, the latter had a higher rate than those propagated in the laboratory.

Table 3.1 Comparison of sprouting "rate" index between cultivars grown under field, greenhouse and *in vitro* conditions[a]

Location	Cultivar	Sprouting rate index[b] (mm/100 days) Temperature (°C) 5	10	20
Field	Desiree	18.80	51.80	72.74
	Norland	15.18	51.74	128.13
	Russet Burbank	1.14	13.10	45.77
	LSD	5.49	12.35	30.28
Green-house	Desiree	0.75	23.58	65.11
	Norland	1.92	35.00	170.15
	Russet Burbank	0	1.67	41.33
	LSD	0.97	12.24	29.27
In Vitro	Desiree	1.55	7.06	8.75
	Norland	1.42	7.11	8.93
	Russet Burbank	1.07	3.02	13.18
	LSD	0.76	1.97	4.80

[a] Sample Size (n) = 9.

[b] These values represent the average tuber size for both field and greenhouse produced tubers. For microtubers, only one tuber size was available (5-10 mm diameter).

Field produced Russet Burbank tubers clearly exhibited the features of late maturity; they were dormant during the first half of storage at 20°C, a majority of the time at 10°C, and through the entire period at 5°C. Eventually short sprouts were found. Minitubers also exhibited a similar response to temperature (Figure 3.2). Microtubers, however, had a different response pattern. The temperature influence was still obvious to induce longer sprouts but cultivars responded differently than for field grown tubers or minitubers (Figure 3.3). At 20°C, Russet Burbank exhibited higher sprout growth followed by Desiree and Norland. At 10 and 5°C, Desiree sprouts were showing faster growth than Norland.

The difference in sprout length observed between field, greenhouse, and laboratory produced tubers were obvious as mentioned above. At 20°, for example, field grown tubers produced the largest sprouts per tubers (Figure 3.1 and Tables 3.2 and 3.3).

Table 3.2 Effect of temperature and source of tubers on sprouting as measured by the rate index.[a]

Temperature(°C)	Sprouting rate index			
	Field	Greenhouse	Laboratory	LSD[b]
20	80.398	93.219	11.578	20.856*
10	35.862	21.431	5.224	7.102*
5	10.073	1.445	1.158	2.432*

[a] Sample size (n) = 12

[b] LSD values followed with (*) indicate significant differences among tuber sources

Table 3.3 Effect of Cultivar and source of tubers on sprouting as measured by the rate index.[a]

Cultivars	Sprouting rate index			
	Field	Greenhouse	Laboratory	LSD[b]
Norland	65.021	69.023	5.821	16.393*
Desiree	47.784	29.813	6.392	9.355*
Russet Burbank	20.009	14.333	5.683	4.235*

(a) Sample size (n) = 12

(b) LSD values followed with (*) indicate significant differences among tuber sources.

This response was intermediate with minitubers (Figure 3.2) and minimal with microtubers (Figure 3.3). Tuber size played an important role in the sprouting responses of tubers. The sprouting rate (Table 3.1) illustrates this fact. Higher sprouting rate index was found with field grown tubers (largest) than minitubers or microtubers which were of descending order of size. The sprouting rate of plants grown under *in vitro* conditions did not correlate with that of field or greenhouse plants. Microtuber size (avg. 0.8 mm) was much smaller than that of field grown tubers or minitubers. Consequently eye number per tuber was limited to one or at the most two. In addition, the food reserve in microtubers was limited and did not support further sprouting.

Sprouting "ratio" index

This method, expressed in (mg sprout/g tuber), provided an easy way of making a quantitative estimate of sprouting. As sprouts develop, they use some of the food reserve of the mother tuber. Consequently their weight increase and weight of mother tuber decreases.

Another source of weight loss was attributed to dehydration (water loss) especially at higher temperatures. This method of calculating the sprouting "ratio" takes into account the following considerations:

(a) Humidity in storage rooms was controlled at about 80 - 90%. Therefore, the chances that dehydration played a major role in weight change were minimized.

(b) The relative rate of water loss is usually higher from small tubers than large tubers for the same weight. This is because smaller tubers have high surface: volume ratio.

(c) Once sprouting occurs, the sprouts lose water very rapidly due to the fact that their outer surfaces are not covered with periderm layer which the tubers have.

Initial tuber weight was used as the reference figure for the calculation of sprouting ratio. Tubers with a higher initial weight usually, but not always, have a higher sprouting index. Variations are controlled mainly by genotype and temperature influence.

Table 3.4 represents the sprouting ratio values. It was obvious here, that high temperatures influenced sprouting since higher ratios were obtained. Tuber size influence was also evident. Field grown tubers exhibited a higher sprouting ratio than microtubers. Cultivar differences are also shown. Norland expressed higher sprouting ratios than Desiree. Russet Burbank exhibited lower ratios because of its longer dormancy. Much of the discussion mentioned in the section of sprouting "rate" is also applicable here.

Another feature of this method is that it is possible to determine, though indirectly, the type or shape of sprouts from sprout weight measurements. At lower temperatures (5, 10°C), apical domi-

nance was not well established; therefore, multiple sprouting occured. The greater sprout weight indicateed a greater chance of having multiple sprouts. Multiple sprouting was not adequately indicated by presenting sprout length data since such measurements were generally taken on the longest sprout.

Table 3.4 Comparison of sprouting "ratio" index between cultivars grown under field, greenhouse and *in vitro* conditions.[a]

Location	Cultivar	Sprouting ratio index[b] (mg/g) Temperature (°C) 5	10	20
Field	Desiree	3.89	26.31	31.19
	Norland	0.33	22.99	72.88
	Russet Burbank	0.08	2.91	10.22
	LSD	1.21	4.80	11.88
Green-house	Desiree	0.03	1.01	16.99
	Norland	0.06	3.88	89.21
	Russet Burbank	0	0.12	4.48
	LSD	0.06	1.63	10.44
In vitro	Desiree	0.21	8.89	9.49
	Norland	0.12	6.59	3.67
	Russet Burbank	0.41	1.21	7.92
	LSD	0.69	5.63	9.51

[a] Sample size (n) = 9.

[b] These values represent the average tuber size for both field and greenhouse produced tubers. For microtubers, only one tuber size was available (5-10 mm) diameter.

Correlation between sprouting indices

The correlation between the two methods of measuring sprouting is given in Table 3.5. Overall, these methods were highly correlated with each other for estimating sprouting under all temperatures. Fewer significant differences were obtained among cultivars or among locations. As has been discussed earlier, tuber size, maturity, class of each genotype, and preharvest growing conditions were among the factors influencing the sprouting performance. In the case of microtuber production, incidences of microbial contaminations reduced the number of observations for some cultivars. That resulted in

Table 3.5 Correlation coefficients between the two sprouting indices.[a]

Type of correlation	Correlation cefficient (r) Temperature (^{o}C) 5	10	20
Overall	0.7836**	0.8079**	0.9592**
Cultivars			
Desiree	0.998**	0.7757	0.8304
Norland	0.9701*	0.6979	0.9973**
Russet Burbank	0.6281	0.9587*	0.0424
Location			
Field	0.6199	0.9915*	1.000**
Greenhouse	0.9921**	0.8893	0.9992**
In vitro	-0.9693*	0.9533*	0.2208

**Correlation significant at 1% level

* Correlation significant at 5% level

(a) The overall correlation was based on 9 observations (3 temperatures and 3 cultivars). The correlation between cultivars or between locations was based on 3 observations only. All correlations were based on average tuber size.

uneven estimation which may have contributed to the fewer correlations found among cultivars. Further improvements in the production techniques are needed to provide better estimates of microtubers sprouting response. This will also result in improving the correlation between these two methods. The information given in Table 3.5. suggested that both methods represented an adequate measure or estimate of sprouting. This information also indicated that it was appropriate to rely only on the sprouting "ratio" method which was easier to conduct, and saved a great deal of time when monitoring sprouting. The correlation in Table 3.6 was constructed to remove the negative and lower correlation coefficient value (Table 3.5). By analyzing data from large tuber size, correlation improved. Table 3.6 shows the correlation based on the large size only from field and greenhouse grown tubers.

Table 3.6 Correlation coefficients between the two sprouting indices.[a]

Type of Correlation	Correlation coefficients (r) Temperature (°C)		
	5	10	20
Overall	0.8571 **	0.9320 **	0.8715 **
Location			
Field	0.7607	0.9824 **	0.9755 **
Greenhouse	0.9980 **	0.9071	0.9991 **

** Significant at 1% level

[a] The overall correlation was based on 9 observations (3 temperatures and 3 cultivars). The correlation between locations was based on 3 observations only. Based on sprouting data from large size field and greenhouse tubers.

Data from microtubers was not included simply because comparable large size tuber did not exist. Also, because of their nature (limited number eyes/tuber), their sprouting response would be different. Overall correlation was significant at all temperatures. Sprouting indices also correlated significantly for field and greenhouse tubers. Correlation coefficient values, if not significant, approached those values suggesting that refinements in experimental methods are needed.

Conclusions and other findings in literature

Due to the nature of tubers themselves, the various sprouting responses were expected between individuals. The genetic features play a major role in this regard. Different responses also can result from differences in growing seasons, physiological maturity, tuber size and exposures to diseases and pests. As a result, each tuber carries its own features that determine when dormancy termination occurs and how sprouting will develop. Davies (55) reported a relationship between sprout growth and nutrient availability in the mother tuber, especially calcium. Dyson and Digby (63) reported similar results. Under the conditions of this study, the effect of growing season is negligible. The effect of disease and pest is believed to be minimal. Tuber size and physiological maturity (genetically controlled) as well as temperature had the major influence on the sprouting indices reported here.

As mentioned earlier, most of the work on sprouting and dormancy has been conducted on tubers produced by the conventional propagation methods. Temperature has been found to exhibit the major influence on sprouting. The higher the storage temperature, the shorter the dormancy (5, 34, 36, 42, 65). Once sprouts were initiated, the rate of

sprout growth correlated closely with temperature (42). At 20°C, sprouting is considered optimal (36), above which a decline in sprouting rate may occur. At 5°C, sprout growth is usually very slow although some cultivars may show slight growth (132, 133). Bogucki and Nelson (29) found that Norland had a shorter dormancy period (39 days), Red Pontiac, intermediate (45 days), and Russet Burbank, the longest (62 days). Their data was for tubers stored at 20°C.

Microtuber sprouting and dormancy is influenced by the environmental conditions during the induction stage. Tovar et al. (248) reported that when tubers of ten cultivars were induced in total darkness, harvested, and stored at 4°C, the average natural dormancy period was 210 days. It was about 60 days if tubers were induced under 8 hours of photoperiod (1,000 lux), harvested and stored at 4°C. Wattimena (262) observed differences between dormancy of microtubers of six cultivars.

The influence of tuber size has been investigated by many researchers. Loomis (145) indicated that large tubers have shorter dormancy than smaller ones. Emilsson (65), working with 10 cultivars, reported similar results. On the other hand, Bogucki and Nelson (29) found very low correlation between tuber weight and length of dormancy. They concluded that tuber size did not influence the length of dormancy or sprouting at the end of dormancy. Tuber size, however, had some effect on sprouting during the two weeks following the end of dormancy.

Under each temperature, genotypic variations seemed to play a major role in determining the time and amount of sprouting. For example, Norland (early maturing) exhibited a higher sprouting rate

(Table 3.1) indicating it had a relatively short dormant period. Russet Burbank (late) had lower rates and thus exhibited less potential toward sprouting enhancement. These observations were also noted by several investigators such as Emilssen (65), Burton (36) and others. They reported that early cultivars have shorter dormancy. Under the conditions of our study, this translates to higher sprouting "rates".

Some application of the sprouting index.

In areas where production of two potato crops per year is possible, it is important that the grower use seed tubers that readily break dormancy and develop relatively large sprouts. By using these types of seed tubers and maintaining appropriate planting density, higher yield is expected, provided that other factors are controlled at their optimum levels. Moorby (176) indicated that the above criteria can be satisfied by sprouting tubers at 15°C or more. Stronger apical dominance, at higher temperatures, results in obtaining tubers with one well developed sprout.

By manipulating tuber size, storage temperature and duration and the choice of cultivars, it is possible to control the sprouting characteristic of tubers (i.e. sprout length, degree of sprouting). Selecting the optimal sprouting stage at which planting takes place will ensure further enhancement in crop growth and tuber yield.

Sprouting behavior is important since it influences the productivity of the crop. Studies reported on conventionally grown tubers (121) revealed that sprout number, and size at time of planting (as influenced by variety, tuber size, and storage temperature) can influence further plant growth and tuber yield. Iritani (116) using

seed tubers with a wide range of physiological age suggested that relative speed of sprout emergence and the degree of apical dominance can be used as measures of productivity. Planting physiologically older seed tuber results in early plant emergence, tuber initiation and bulking. Photosynthate assimilation is usually shifted toward tuber production. Therefore, the foliage development is restricted and senescence occurs early. Norland plants, under the conditions of this study and various studies of conventionally grown tubers exhibited earlier sprouting and earlier tuber bulking.

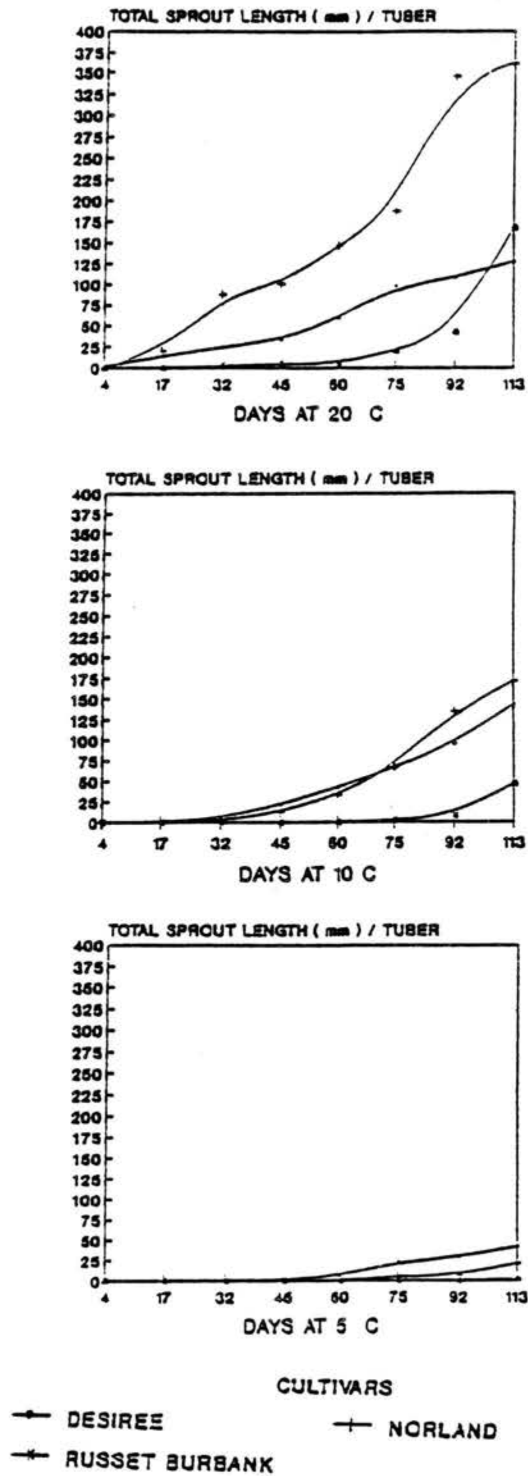


Figure 3.1 Time course of sprout length of field grown tubers among three potato cultivars held at 5, 10 and 20°C.

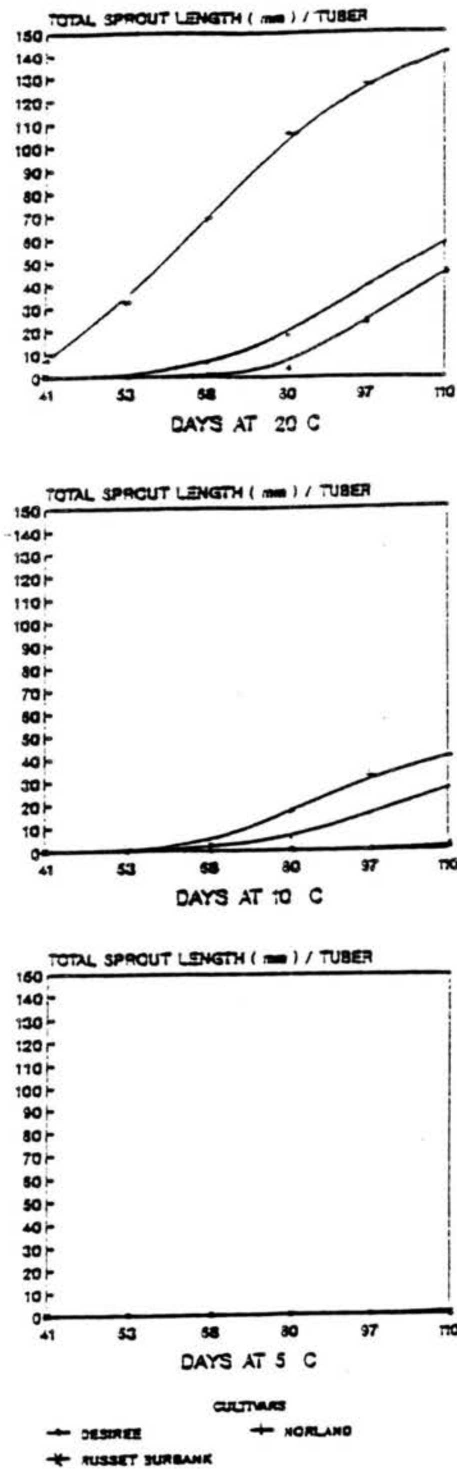


Figure 3.2 Time course of sprout length of greenhouse grown tubers among three potato cultivars held at 5, 10, and 20°C.

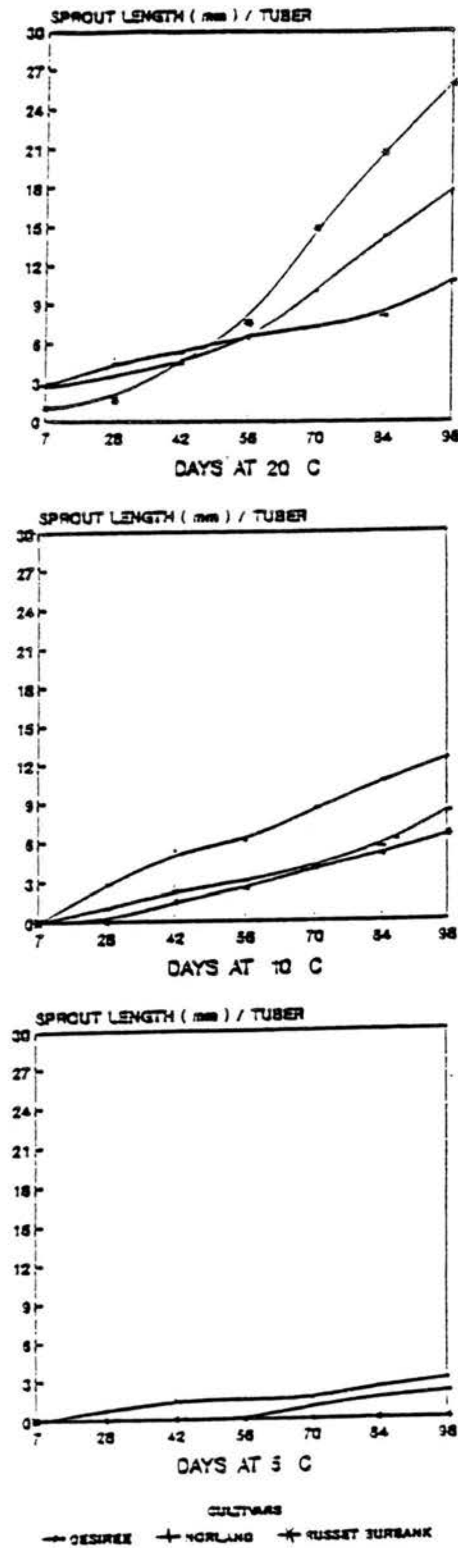


Figure 3.3 Time course of sprout length of <u>in vitro</u> produced tubers among three potato cultivars held at 5, 10, and 20°C.

SUMMARY AND CONCLUSIONS

Detailed plant growth and analysis studies of micropropagated potato plants of early, medium and late maturing cultivars grown in the field clearly indicated growth characteristics known to occur when these cultivars are propagated by seed tubers. Plant height, leaf area and leaf area index were examples illustrating these similarities. Foliage growth correlated closely with tuber initiation and development. The same correlations have been reported with seed tuber propagated plants. In the absence of the influence of the mother tuber, the inherent plant development and tuber characteristics of each cultivar were not changed.

The significant correlation reported between yield characteristics of field grown, greenhouse grown, and *in vitro* produced tubers indicate the potential value of micropropagation techniques for estimating yielding ability under field conditions. Although no significant difference (in tuber number per plant or tuber weight per plant) have been detected between cultivars under *in vitro* conditions, the trend observed was clearly indicative of differences occurring under field conditions. Limited soil volume and possibly light might not permit maximum tuber development under greenhouse condition. *In vitro* factors such as number of nodes per flask, tuberization media, and incubation conditions undoubtedly influenced the way cultivars responses. These results should encourage future research involving

yield responses of micropropagated potatoes under different growing conditions. The yield under *in vitro* conditions might be further investigated to more clearly define optimal regimes for cultivars of different maturity classes. This could provide basis for more valid prediction of tuber yield under *in vitro* conditions.

Dormancy and sprouting characteristics of micropropagated potato tubers were in general agreement with that of conventionally grown tubers. Previously reported findings on the influence of cultivar, temperature, and tuber size on sprouting have been substantiated in this study. Micropropagation techniques did not alter these characteristics. Microtubers, however, did not follow the expected pattern as per cultivar response but did respond to the temperature effect.

The sprouting indices developed in this study provided a quantitative estimate of cultivar behavior. The indices incorporate the influence of factors, such as cultivar, temperature and tuber size. Data from these indices may be helpful in scheduling planting of tubers of optimal physiological age and sprout growth which ensure optimal plant growth and tuber yield.

Overall, this study represents a beginning effort toward the goal of developing micropropagation methods that could eventually be a reliable indicator of field performance. Results indicate that field growth characteristics of micropropagated potatoes do closely represent that of tuber propagated potatoes.

A full potential and usefulness of *in vitro* methods for predictive purposes will obviously require additional research, especially on the area of tuberization.

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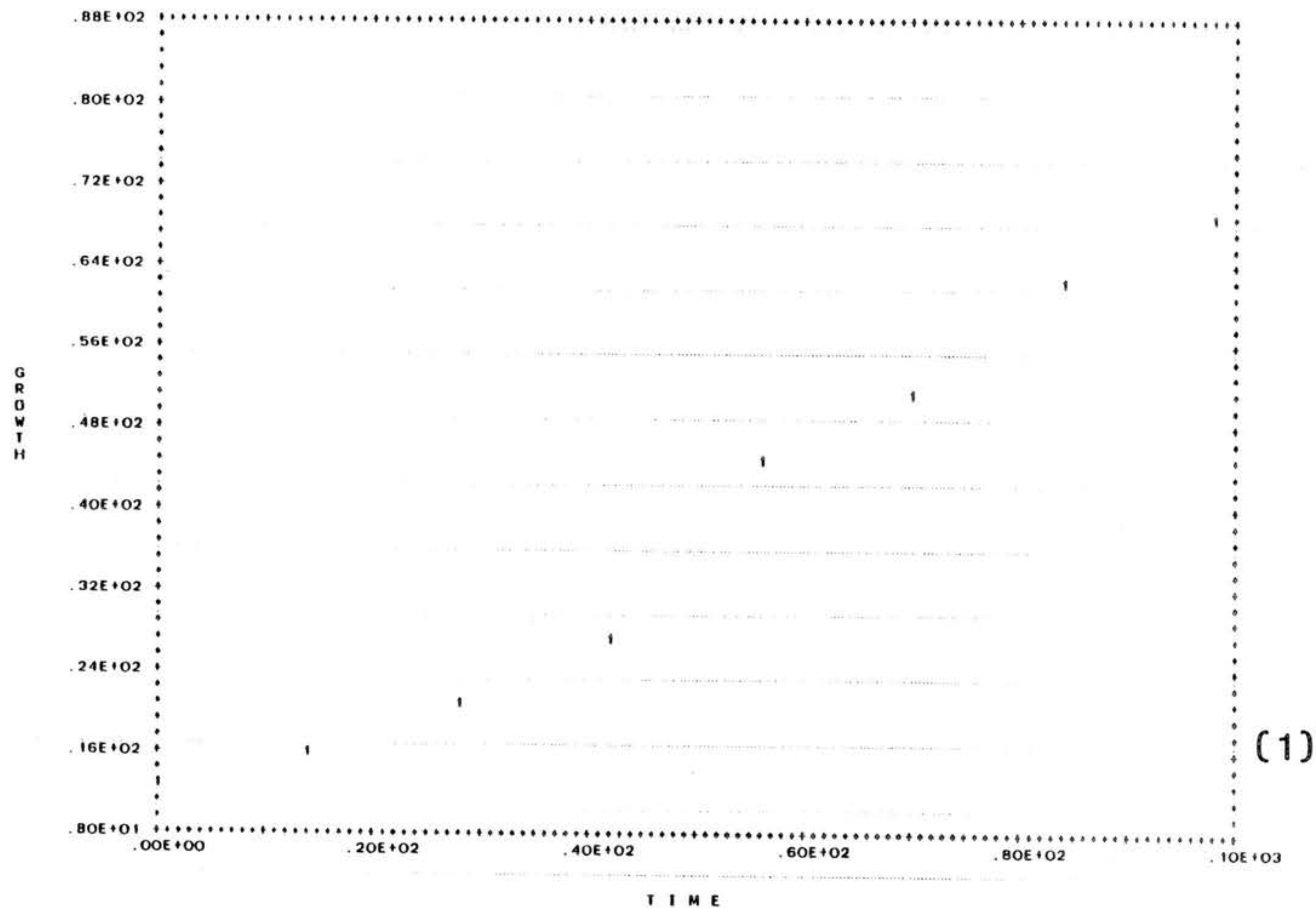
Appendix A

A.1 An Example of Richards' Output

A.2 Parameters, Estimates, and Standard Deviation

PRIMARY DATA PLOT



Y= A*(1+B*EXP (-K*X))**(-1/N) OR Y= A*(1+SGN*EXP (-K*(X-XO)))**(-1/N) (2)

FITTED AS ALOG(Y)= ALOG(A)-ALOG(1+SGN*EXP (-K*(X-XO)))/N WHERE SGN=+1 OR -1

IF N IS NEGATIVE, B OR SGN IS NEGATIVE N=RICHARDS M-1

	SQUARES OF RESIDUES	A	K	XO	N
INITIAL	.30110E+01				
CYCLE 1	.284401E-01	.70155E+02	.10946E+00	.82542E+02	.51520E+01
CYCLE 2	.281522E-01	.70078E+02	.11136E+00	.82496E+02	.52382E+01
CYCLE 3	.276622E-01	.69954E+02	.11483E+00	.82409E+02	.53942E+01
CYCLE 4	.270983E-01	.69836E+02	.11912E+00	.82298E+02	.55833E+01
CYCLE 5	.265763E-01	.69768E+02	.12328E+00	.82179E+02	.57606E+01
CYCLE 6	.261686E-01	.69767E+02	.12654E+00	.82067E+02	.58913E+01
CYCLE 7	.258974E-01	.69818E+02	.12862E+00	.81970E+02	.59654E+01
CYCLE 8	.257446E-01	.69892E+02	.12971E+00	.81893E+02	.59951E+01
CYCLE 9	.256724E-01	.69962E+02	.13020E+00	.81835E+02	.60014E+01
CYCLE 10	.256438E-01	.70014E+02	.13042E+00	.81796E+02	.60002E+01
CYCLE 11	.256345E-01	.70046E+02	.13053E+00	.81771E+02	.59983E+01
CYCLE 12	.256321E-01	.70063E+02	.13059E+00	.81756E+02	.59973E+01
CYCLE 13	.256315E-01	.70071E+02	.13062E+00	.81748E+02	.59969E+01
CYCLE 14	.256314E-01	.70074E+02	.13064E+00	.81745E+02	.59967E+01
CYCLE 15	.256314E-01	.70075E+02	.13065E+00	.81743E+02	.59967E+01
CYCLE 16	.256314E-01	.70075E+02	.13065E+00	.81743E+02	.59967E+01
STANDARD ERRORS		.97274E+01	.16824E+00	.63728E+01	.80968E+01

(3)

MULTIPLE CORRELATION COEFFICIENT= .9957

(4)

ITEM	X	LOG Y	EST LOG Y	DIFF	(5) Y	(6) ANTI-(EST LOG Y)	DIFF SQR
1	.00000	2.49114	2.46865	.02249	12.07500	11.80648	.07210
2	14.00000	2.81091	2.77364	.03726	16.62500	16.01689	.36979
3	28.00000	3.00815	3.07853	-.07038	20.25000	21.72654	2.18017
4	42.00000	3.30199	3.38277	-.08078	27.16670	29.45236	5.22426
5	56.00000	3.79174	3.68304	.10870	44.33330	39.76703	20.85086
6	70.00000	3.95124	3.96117	-.00992	52.00000	52.51858	.26893
7	84.00000	4.14049	4.15676	-.01628	62.83330	63.86434	1.06304
8	98.00000	4.23965	4.23074	.00891	69.38330	68.76776	.37889

SUM OF THE SQUARE OF RESIDUES = 30.40804

ADJUSTED R SQUARE = .98510

(7)

ITEM	DY/DX	1/Y DY/DX
1	.25722E+00	.21786E-01
2	.34891E+00	.21784E-01
3	.47293E+00	.21767E-01
4	.63813E+00	.21666E-01
5	.83740E+00	.21058E-01
6	.94125E+00	.17922E-01
7	.59386E+00	.92987E-02
8	.15999E+00	.23266E-02

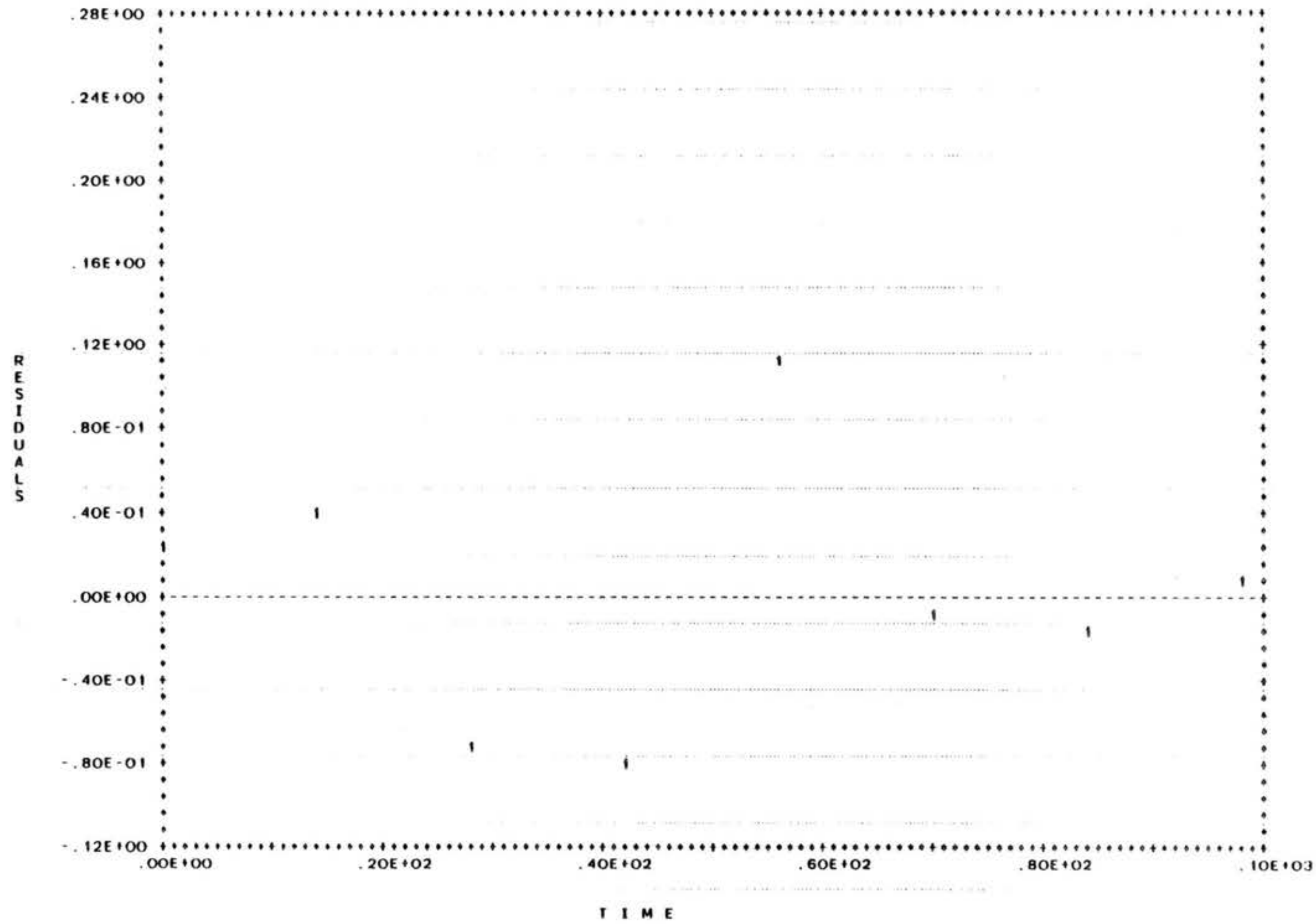
INFLECTION PT: X= .680327E+02 Y= .506606E+02 DY/DX= .945982E+00 1/Y DY/DX= .186729E-01

THERE WILL BE 98 INTERVALS COMPUTED, EACH 1.0 UNITS OF TIME APART

(6) (7)

INTERNAL	X	ANTI-(EST LOG Y)	1/Y DY/DX	SYMBOL	DY/DX	CHANGE DY/DX
1	1.00000	12.06652	.21786E-01	+	.26288E+00	.00000
2	2.00000	12.33229	.21796E-01	O	.26867E+00	.00579
3	3.00000	12.60391	.21786E-01	O	.27459E+00	.00592
4	4.00000	12.88152	.21786E-01	O	.28064E+00	.00605
5	5.00000	13.16523	.21786E-01	O	.28682E+00	.00618
6	6.00000	13.45519	.21786E-01	O	.29313E+00	.00632
7	7.00000	13.75154	.21786E-01	O	.29958E+00	.00645
8	8.00000	14.05441	.21785E-01	O	.30618E+00	.00660
9	9.00000	14.36395	.21785E-01	O	.31292E+00	.00674
10	10.00000	14.68030	.21785E-01	O	.31981E+00	.00689
11	11.00000	15.00362	.21785E-01	O	.32685E+00	.00704
12	12.00000	15.33405	.21784E-01	O	.33404E+00	.00719
13	13.00000	15.67176	.21784E-01	O	.34139E+00	.00735
14	14.00000	16.01689	.21784E-01	O	.34891E+00	.00751
15	15.00000	16.36963	.21783E-01	O	.35658E+00	.00768
16	16.00000	16.73012	.21783E-01	O	.36443E+00	.00784
17	17.00000	17.09854	.21782E-01	O	.37244E+00	.00802
18	18.00000	17.47506	.21782E-01	O	.38063E+00	.00819
19	19.00000	17.85987	.21781E-01	O	.38900E+00	.00837
20	20.00000	18.25313	.21780E-01	O	.39755E+00	.00855
21	21.00000	18.65503	.21779E-01	O	.40629E+00	.00874
22	22.00000	19.06577	.21778E-01	O	.41521E+00	.00892
23	23.00000	19.48552	.21777E-01	O	.42433E+00	.00912
24	24.00000	19.91449	.21775E-01	O	.43364E+00	.00931
25	25.00000	20.35288	.21774E-01	O	.44316E+00	.00951
26	26.00000	20.80088	.21772E-01	O	.45287E+00	.00972
27	27.00000	21.25869	.21770E-01	O	.46280E+00	.00992
28	28.00000	21.72654	.21767E-01	O	.47293E+00	.01013
29	29.00000	22.20462	.21765E-01	O	.48328E+00	.01035
30	30.00000	22.69316	.21762E-01	O	.49384E+00	.01056
31	31.00000	23.19238	.21758E-01	O	.50462E+00	.01078
32	32.00000	23.70248	.21754E-01	O	.51563E+00	.01100
33	33.00000	24.22370	.21750E-01	O	.52685E+00	.01123
34	34.00000	24.75626	.21744E-01	O	.53831E+00	.01145
35	35.00000	25.30039	.21738E-01	O	.54999E+00	.01168
36	36.00000	25.85632	.21732E-01	O	.56190E+00	.01191
37	37.00000	26.42427	.21724E-01	O	.57404E+00	.01214
38	38.00000	27.00447	.21715E-01	O	.58641E+00	.01237
39	39.00000	27.59716	.21705E-01	O	.59900E+00	.01260
40	40.00000	28.20256	.21694E-01	O	.61182E+00	.01282
41	41.00000	28.82089	.21681E-01	O	.62487E+00	.01304
42	42.00000	29.45236	.21666E-01	O	.63813E+00	.01326
43	43.00000	30.09721	.21650E-01	O	.65159E+00	.01347
44	44.00000	30.75562	.21631E-01	O	.66526E+00	.01367
45	45.00000	31.42780	.21609E-01	O	.67912E+00	.01386
46	46.00000	32.11393	.21584E-01	O	.69316E+00	.01404
47	47.00000	32.81418	.21557E-01	O	.70736E+00	.01420
48	48.00000	33.52869	.21525E-01	O	.72170E+00	.01434
49	49.00000	34.25761	.21489E-01	O	.73615E+00	.01445
50	50.00000	35.00103	.21448E-01	O	.75069E+00	.01454
51	51.00000	35.75901	.21401E-01	O	.76529E+00	.01459
52	52.00000	36.53160	.21349E-01	O	.77990E+00	.01461
53	53.00000	37.31879	.21289E-01	O	.79447E+00	.01457
54	54.00000	38.12052	.21221E-01	O	.80896E+00	.01449
55	55.00000	38.93666	.21144E-01	O	.82329E+00	.01434
56	56.00000	39.76703	.21058E-01	O	.83740E+00	.01411
57	57.00000	40.61136	.20960E-01	O	.85121E+00	.01380
58	58.00000	41.46931	.20849E-01	-	.86461E+00	.01340

59	59.00000	42.34041	.20725E-01	-	.87750E+00	.01289
60	60.00000	43.22410	.20585E-01	-	.88976E+00	.01226
61	61.00000	44.11969	.20428E-01	-	.90126E+00	.01150
62	62.00000	45.02632	.20251E-01	-	.91184E+00	.01058
63	63.00000	45.94301	.20054E-01	-	.92134E+00	.00950
64	64.00000	46.86859	.19834E-01	-	.92959E+00	.00824
65	65.00000	47.80170	.19589E-01	-	.93638E+00	.00679
66	66.00000	48.74079	.19317E-01	-	.94152E+00	.00514
67	67.00000	49.68411	.19016E-01	-	.94479E+00	.00327
68	68.00000	50.62968	.18684E-01	-	.94598E+00	.00119
69	69.00000	51.57531	.18320E-01	-	.94487E+00	-.00111
70	70.00000	52.51858	.17922E-01	-	.94125E+00	-.00362
71	71.00000	53.45690	.17489E-01	-	.93492E+00	-.00633
72	72.00000	54.38746	.17021E-01	-	.92571E+00	-.00921
73	73.00000	55.30731	.16516E-01	-	.91347E+00	-.01223
74	74.00000	56.21337	.15977E-01	-	.89812E+00	-.01536
75	75.00000	57.10248	.15404E-01	-	.87959E+00	-.01853
76	76.00000	57.97148	.14798E-01	-	.85789E+00	-.02169
77	77.00000	58.81724	.14164E-01	-	.83311E+00	-.02478
78	78.00000	59.63673	.13505E-01	-	.80539E+00	-.02772
79	79.00000	60.42711	.12825E-01	-	.77495E+00	-.03044
80	80.00000	61.18582	.12128E-01	-	.74208E+00	-.03287
81	81.00000	61.91057	.11422E-01	-	.70712E+00	-.03496
82	82.00000	62.59948	.10710E-01	-	.67047E+00	-.03665
83	83.00000	63.25108	.10001E-01	-	.63256E+00	-.03790
84	84.00000	63.86434	.92987E-02	-	.59386E+00	-.03870
85	85.00000	64.43868	.86099E-02	-	.55481E+00	-.03904
86	86.00000	64.97400	.79397E-02	-	.51588E+00	-.03894
87	87.00000	65.47061	.72928E-02	-	.47746E+00	-.03841
88	88.00000	65.92923	.66732E-02	-	.43996E+00	-.03751
89	89.00000	66.35094	.60842E-02	-	.40369E+00	-.03627
90	90.00000	66.73711	.55281E-02	-	.36893E+00	-.03476
91	91.00000	67.08937	.50066E-02	-	.33589E+00	-.03304
92	92.00000	67.40952	.45207E-02	-	.30474E+00	-.03115
93	93.00000	67.69951	.40704E-02	-	.27557E+00	-.02917
94	94.00000	67.96184	.36556E-02	-	.24844E+00	-.02713
95	95.00000	68.19706	.32752E-02	-	.22336E+00	-.02508
96	96.00000	68.40872	.29279E-02	-	.20030E+00	-.02306
97	97.00000	68.59831	.26124E-02	-	.17920E+00	-.02109
98	98.00000	68.76776	.23266E-02	-	.15999E+00	-.01921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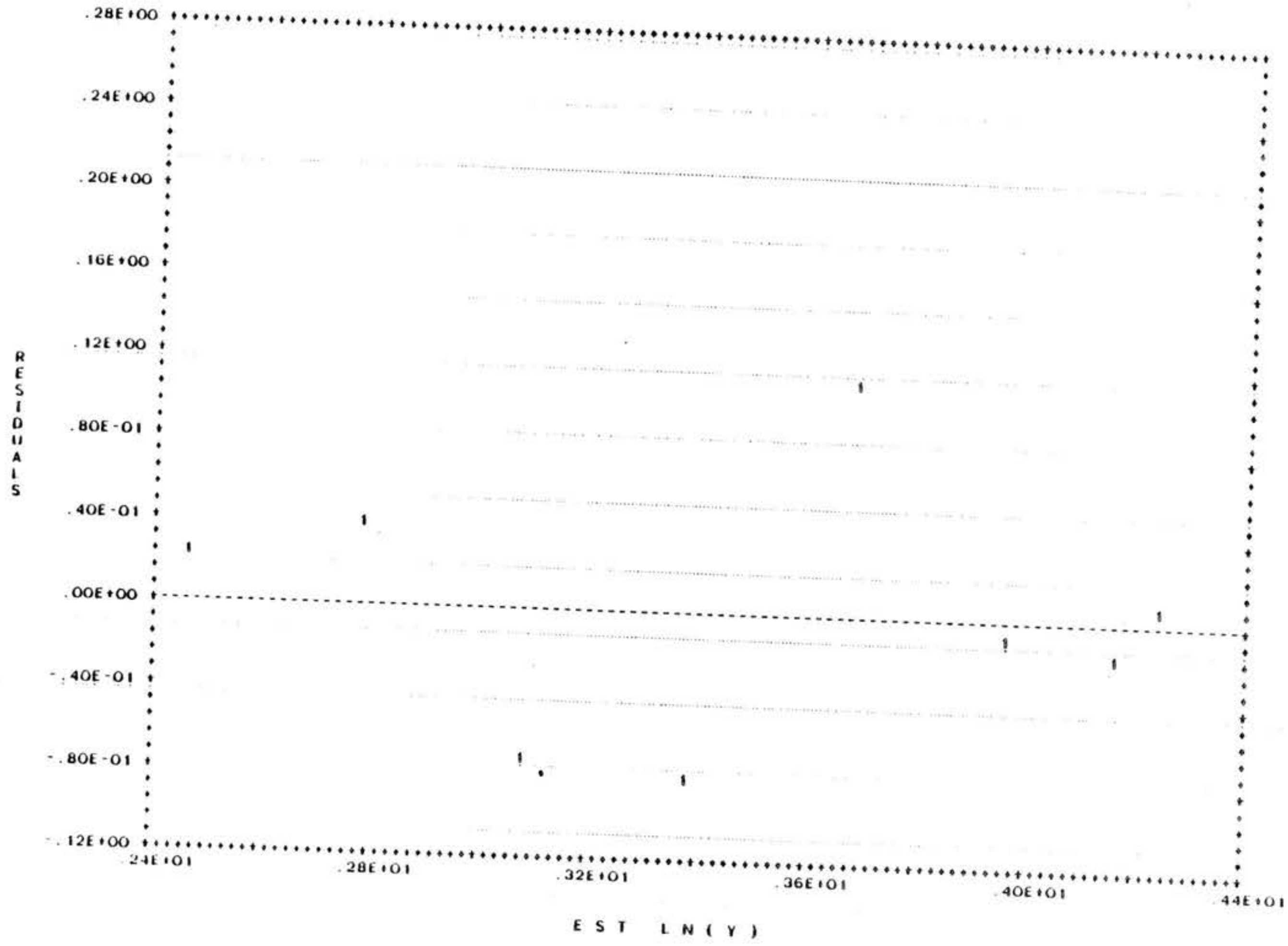
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Footnotes For A.1

1. Plot of observed growth data against time
2. Richards' function
3. Parameter's estimate
4. Parameter's standard errors
5. Observed growth data
6. Predicted growth data
7. RGR derived quantities

A.2. Parameter estimates and standard deviation of selected Richards' function output.

Growth attribute	Parameters Y and (SE)				Multiple Corr. Coef.	Adjusted R^2
	A	K	X_0	N		
Stem height/plant						
Desiree	74.00(12.95)	7.33(0.82)	88.05(10.19)	345.33(0.00)	0.9820	0.9375
Norland	25.99(1.71)	0.15(0.28)	64.18(9.39)	11.02(0.22)	0.9846	0.9466
Russet Burbank	70.08(9.73)	0.13(0.17)	81.74(6.37)	5.99(8.09)	0.9957	0.9851
Leaf area/plant						
Desiree	13542(4573)	0.063(0.028)	53.00(7.79)	0.59(0.37)	0.9989	0.9955
Norland	2053(210)	6.95(0.44)	46.29(2.22)	78.03(0.0)	0.9971	0.9886
Russet Burbank	9233(1618)	0.093(0.037)	46.66(5.09)	0.78(0.41)	0.9987	0.9946
Top dry weight/plant						
Desiree	198.11(70.89)	0.047(0.018)	47.25(14.94)	0.343(0.227)	0.9986	0.9949
Norland	18.78(2.07)	7.24(0.23)	45.49(2.25)	72.17(0.23)	0.9958	0.9855
Russet Burbank	124.63(36.56)	0.046(0.018)	28.38(24.12)	0.239(0.208)	0.9981	0.9932
Tuber weight/plant						
Desiree	603.25(784.5)	0.061(0.046)	56.80(14.87)	0.22(0.22)	0.9946	0.9812
Norland	234.52(149.33)	0.173(0.218)	45.22(6.94)	0.506(0.71)	0.9903	0.9661
Russet Burbank	234.69(189.61)	0.305(0.970)	72.46(6.64)	1.44(4.67)	0.9907	0.9677

Appendix B

Summary AOV Table of Growth Analysis Data

Table B.1. Summary AOV Table of Growth Analysis Data.[1]

Source	df	Variables				
		Plant Height (cm)	Leaf Area (cm^2)	LAI	Top dry weight (g)	Tuber weight (g)
Rep	5	NS	NS	NS	NS	NS
Cult.	2	**	**	**	**	NS
Error(1)	10					
Samplings	7	**	**	**	**	**
Sampling x cult.	14	**	**	**	**	NS
Residual	105					

Significance:

** = 1% level
NS = not significant at the 5% level

[1] Data from 1986 and 1987 seasons was combined. The statistical analysis was based on 6 replications per treatment.

Appendix C

Climatic Data During Study Periods

Table C.1. Temperature and solar radiation data collected during study periods in 1986 and 1987 at the Horticultural Field Research Center, Colorado State University, 10 miles NE of Fort Collins, Colorado.

	Temperature (°C)			Solar radiation
Date	Max	Min	Mean	$Mj\ m^{-2}$
June, 1986				
24-30	31	15	23	21.96
July, 1986				
1-7	32	14	23	24.24
8-14	31	13	22	22.63
15-21	30	14	22	20.66
22-28	31	14	23	22.77
29 - Aug. 4	30	13	22	22.91
Aug., 1986				
5-11	31	14	23	21.43
12-18	31	13	22	21.63
13-25	30	15	23	16.70
26-1	29	13	21	17.21
Sept. 1986				
2-8	24	10	17	14.93
9-15	25	6	16	14.94
16-22	23	9	16	13.44
23-29	20	7	14	14.05
30-Oct. 6	17	5	11	11.77
Oct. 1986				
7-13	17	2	10	11.73
14-20	19	1	10	NA
21-27	13	3	8	NA
July 1987				
2-8	27	9	18	24.54
9-15	25	9	17	23.08
16-22	31	10	21	26.29
23-29	32	15	24	26.86
30-Aug. 5	30	14	22	24.31
Aug. 1987				
6-12	26	13	20	21.00
13-19	27	9	18	23.69
20-26	21	11	16	11.35
27-2	25	8	17	20.67
Sept. 1987				
3-9	24	8	16	18.82
10-16	23	7	15	16.78
17-23	21	2	12	17.86
24-30	23	3	13	17.18
Oct. 1987				
1-7	22	1	12	16.70
8-14	14	-1	7	11.53

NA = Data not available