

**PRODUCTION OF VIRUS INDEXED SEED
POTATOES BASED ON TISSUE CULTURE**

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This material is a summary on the first phases of tissue culture research at the Horticultural Experiment Station, Simcoe, Ontario. The information contained within this report is intended solely for the information of the reader and is not in any way to be taken as a final recommendation on the tissue culture process or procedures.

INTRODUCTION

Currently in Ontario potatoes for elite seed are propagated via tubers. This method, while viable, is extremely time consuming. Employing this propagation technique, we are faced with years before we are able to build up sufficient stocks of a new cultivar for distribution. This gives rise to the fact that no new Canadian cultivar has gained widespread acceptance. Federal seed regulations will require that by a target date of 1984 all stock must be produced from meristem derived virus-indexed plants. This offers us a unique opportunity to couple tissue culture for the production of virus indexed stock with aseptic nodal culture of plantlets for multiplication.

Our research program has the following objectives:

1. To develop rapid multiplication techniques for seed potatoes;
2. To develop an integrated, flexible production system.

In order to meet these objectives, work is being conducted in the following areas:

1. Development of aseptic nodal culture for rapid multiplication of indexed material;
2. Development of the interface between test tube and greenhouse environment;
3. Integrating with current multiplication methods in the greenhouse.

The newer Canadian cultivars are being used for the main part of this program.

Nodal culture is the culture "in vitro" (in glass) of single or double nodal segments (i.e. a nodal cutting is comprised of a stem segment, a leaf

and an axillary bud). A potential of 5×10^{13} to 10×10^{13} plants per year from one original plant has been estimated. Theoretically, if we attained this number for direct planting into the field we could plant 1.25×10^9 to 2.50×10^9 hectares of potatoes (based on 40,000 plants/ha).

In Ontario, we plant roughly 18,000 hectares of potatoes (Ont. Pub. 20). At a planting density of 40,000 plants/ha, this works out to $18,000 \times 40,000$ or 7.2×10^8 plants. The following section illustrates the potential of a seed production system based on tissue culture.

To illustrate the rapid multiplication potential of tissue culture consider the following.

For example, if we start with one plantlet, the possibility exists to produce the following number of plantlets:

Number of Plantlets Over Time

At end of month	1	2	3	4	5	6	7
Number of Cuttings	5	25	125	625	3,125	15,625	78,125

At end of month	8	9	10	11	12
Number of Cuttings	390,625	1,953,125	9,765,625	48,828,125	344,140,625

Starting with one plantlet would save one month over starting with one nodal cutting; starting with several plantlets further increases the time saved in the cycle.

One year's production in tubes would give enough material to plant directly in the field Ontario's entire production area. Logistics and economics would likely preclude the above premise but the mathematical exercise does prove useful in illustrating the potential of the system.

Let's consider the most logical and what is at this point the most applicable alternative - that of producing enough plantlets to supply the current elite system. Again, assuming 18,000 ha at a density of 40,000 plants/ha we would need approximately $(18,000 \times 40,000)$ or 720,000,000 tubers at the certified level. Using the existing system and assuming a 10:1 production ratio, we obtain:

<u>Tubers Required to Supply the Current Elite System</u>		
	Certified	720,000,000
	Foundation	72,000,000
	Elite III	7,200,000
	Elite II	720,000
	Elite I	72,000
	Pre Elite	7,200
we need	Plantlets	720

Using the above figures and assuming only one cultivar, it would take four to five months to obtain enough plants for the Pre Elite generation. However, it remains to be seen if such production rates can be attained through the system.

The greatest potential lies in shortening the time taken to obtain planting stock. It can be seen that a few month's production in tubes would allow us to go to the field at the E II or III level. This shortens the time taken to produce seed and release an ample supply of stock to 2-3 years from 6-7 years plus reducing the incidence of virus infection. If economics dictate, the future possibility exists of reducing this time even further. This would allow for the entry of sufficient seed stock of new, promising cultivars to the market in the shortest possible time, hence increasing their chances for acceptance.

Tissue culture (nodal culture) offers us a unique tool and step forward for potato propagation. We must, however, not be lulled into a false sense of security that by starting with tissue cultured plants necessarily results in virus-free tubers all through the system. Plants will only be as disease free

as the parent material from whence they came. Agriculture Canada can provide clean stock, and if strict sanitary procedures are adhered to in each step of seed production, the resulting plants should remain clean. It will still be necessary, however, to carry out vigorous testing (indexing) for greenhouse and especially, field grown plants.

Currently we are potting plantlets from test tubes into 9 cm pots filled 2/3 full with a peat mixture and topped off with vermiculite.

The test tube plantlets are removed from the tube, the agar washed off with luke warm water and then placed into the pot. Water is added to moisten the media and then a clear plastic cup (the same diameter as the pot) is placed over the pot and taped down to keep the tender plantlet from drying out. The pot must then be placed in some type of tray where it has a constant supply of H₂O. Currently we are placing the pots into aluminum roasting pans where the pots are then watered with "Hydrosol"^{1/} at 100 ppm based on N. These pots are placed in a shaded area with fluorescent lights in the greenhouse with a temperature of 25°C (it is imperative that the temperature not be allowed to go below 20°C as this will favour tuberization) and a day length of 16 hours.

When suitable growth has been obtained (1/2 to full height of cover), the plastic cup is removed and the plant (which remains under shaded conditions) is allowed to "harden off" for three to five days before potting into 20 cm pots. If conditions are suitable, we are looking at a time frame of one, and for some conditions, 2 weeks between potting in the 9 cm pots and subsequent transfer to the 20 cm pots.

GREENHOUSE CULTURE

The next step in the production of Pre Elite tubers is culture in greenhouse

^{1/}Peters 5-26-11 soluble fertilizer with trace elements.

or controlled environment rooms. Strict sanitary precautions must be taken in either case. Greenhouses should be screened to be insect proof, and great care should be taken to prevent entry of diseased material. (e.g. contaminated pots, soil, carts or clothing of personnel).

There are several approaches to obtain tubers, once plants are potted:

1. direct tuber production;
2. production of stock plants for (i) stem cuttings to grow plants for tubers; (ii) leaf bud cuttings; (iii) transplants for field plantings.

DIRECT TUBER PRODUCTION

Plants are grown in 20 cm pots in the greenhouse for about four months. At this rate, 3 crops/year could be grown. Currently, we are evaluating fertilizer applications to find a suitable program for potatoes.

STOCK PLANTS (for stem and leaf bud cuttings)

1. Stem Cuttings

Fewer tubes of plantlets are required if stock plants are used for further multiplication. Two methods are available, stem cuttings and leaf bud cuttings. With stem cuttings, the apex is pinched to promote lateral shoot growth and at a later date terminal cuttings, about 10 cm long, are taken and rooted. The rooted cuttings are then potted up and can be used for further cuttings, or for direct tuber production. The stock plants are allowed to regrow new shoots so that further cuttings may be taken.

2. Leaf Bud Cuttings.

In this technique, single nodal cuttings are taken and rooted (under a mist system). Tubers form (in about 7 weeks) either between the stem and leaf (sessile) or on short stolons, depending on the cultivar and the day length. Several sets of cuttings can be taken from a stock plant, often over

STEM CUTTINGS



PINCH TO
BREAK
LATERAL SHOOTS

16 HOUR DAY
> 20°C
HIGH FERTILITY

STOCK PLANT



TERMINAL CUTTINGS; 10 cm. LONG
- USE ROOTING HORMONE
roots in pots

LONG DAYS
> 20°C



10 cm. POTS

→ FURTHER CUTTINGS

→ 20 cm. POTS

TO
PRODUCE
TUBERS

LEAF BUD CUTTING

STOCK PLANT,



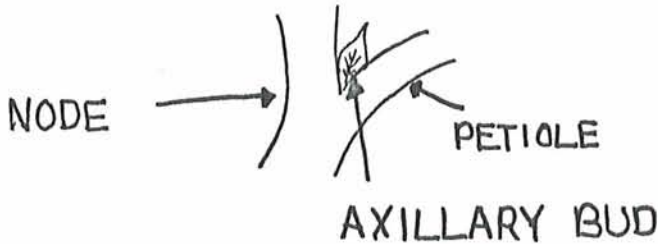
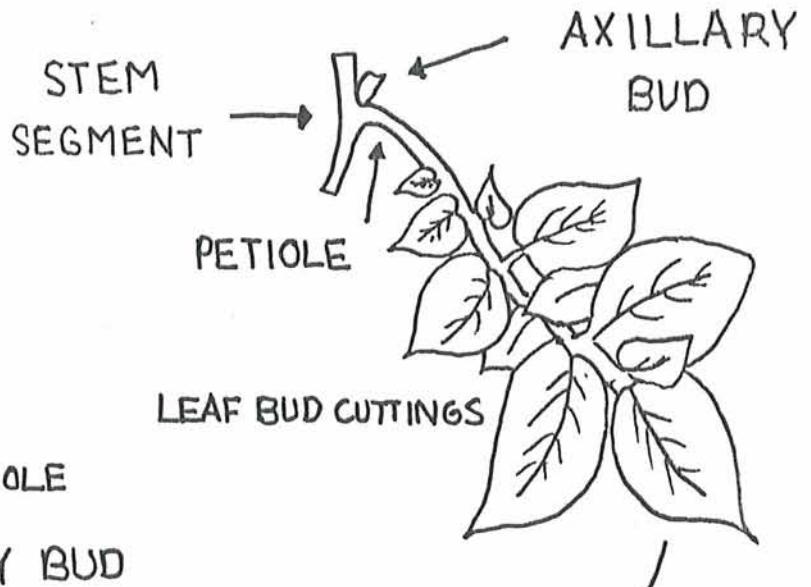
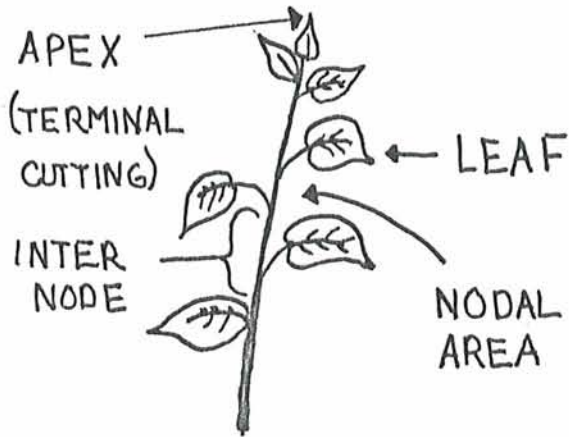
16 HR. DAY

WARM CONDITION $>20^{\circ}\text{C}$

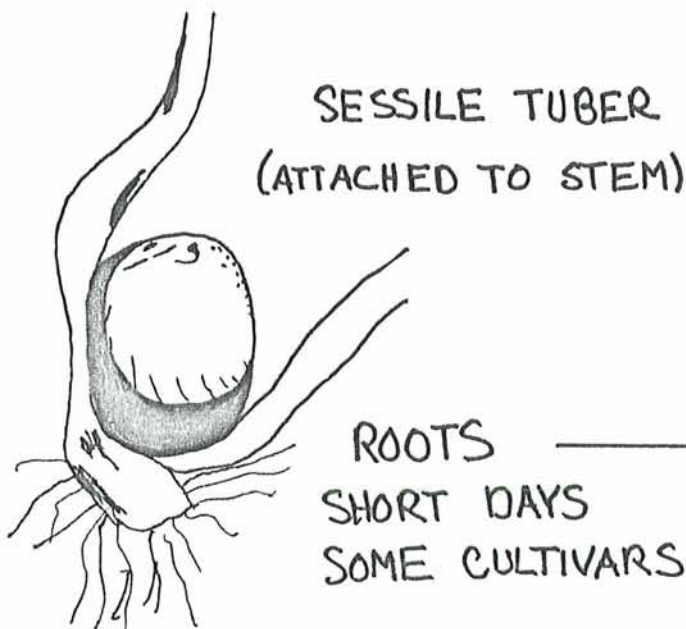
HIGH FERTILITY

CUT OFF BEFORE FLOWERING
LEAVE BASE TO REGROW

BRANCH



POTS, MIST BED,
OR PLANT TRAYS IN A MIST BED
FORMS TUBERS IN ABOUT 4-7 WKS.



SHORT DAYS
SOME CULTIVARS

