POTATO LEAF EXPLANTS AS A SPACEFLIGHT PLANT TEST SYSTEM

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The advent of regular flights of the Space Shuttle has provided an excellent opportunity for scientists to examine the effects of near weightlessness on plant growth and development. However testing during shuttle flights is faced with significant constraints. Among the more obvious of these constraints are: 1) the relatively short duration of shuttle flights, i.e., the limited time in microgravity, 2) limitations on the size of the test package, and 3) difficulties in maintaining a high degree of environmental control. The first constraint is relatively fixed, while the latter two have some degree of flexibility but are limited from a practical standpoint of cost and electrical power requirements to run experiment growth chambers; in particular, the power constraint could severely limit levels of cooling control and irradiation for photosynthesis. These impasses can be circumvented if plant materials and experiments are chosen accordingly, e.g. the seedling experiments of Cowles et al. (1984) and Brown and Chapman (1984). Unfortunately, from a perspective of developing a higher-plant "CELSS" for long duration spaceflight, it may be difficult to extrapolate results from seedling studies to mature, whole-plant phenomena such as flowering, fruiting, tuber, and tuberous root development (Wheeler and Tibbitts,

1984). Yet to evaluate and justify further ground-based testing of potential crops for spaceflight, it would be beneficial to gather information on the progression of advanced plant development under microgravity as soon as possible.

Clearly it will be impossible to grow candidate food crops through a full life cycle during Shuttle flights. However use of excised tissue or organs from Earth-grown plants may provide an alternate approach to study advanced stages of plant growth and development. For such an approach, it is important to bear in mind that initial development of tissue occurs under a 1-g environment, and these effects would have to be taken into account with appropriate controls. Whether the plant tissues sustain a "memory" of 1-g environment that affects later development is a not known and solving this likely will come only when full-term growth studies can be conducted.

The potato, Solanum tuberosum L., is one of several food crops selected for early investigation for CELSS studies (Tibbitts and Alford, 1982), and it has been known for many years that axillary buds of potato leaves have the ability to develop into a variety of stem forms (Vöchting, 1887). By excising a potato leaf and its subtended axillary bud and maintaining it in a humid environment, the bud can

be forced to develop rapidly (Gregory, 1956; Ewing, 1978). The growth form expressed by the bud appears to be directly controlled by inductive state of the mother plants (Ewing, 1978); namely, leaf cuttings from induced plants (i.e., plants that were grown in an environment promotive of tuber formation) will produce tubers at the axillary bud; in contrast, cuttings from non-induced plants tend to produce leafy upright shoots, while intermediate states of induction result in intermediate responses such as stolons (horizontal, leafless stems) or elongation tubers (Fig. 1).

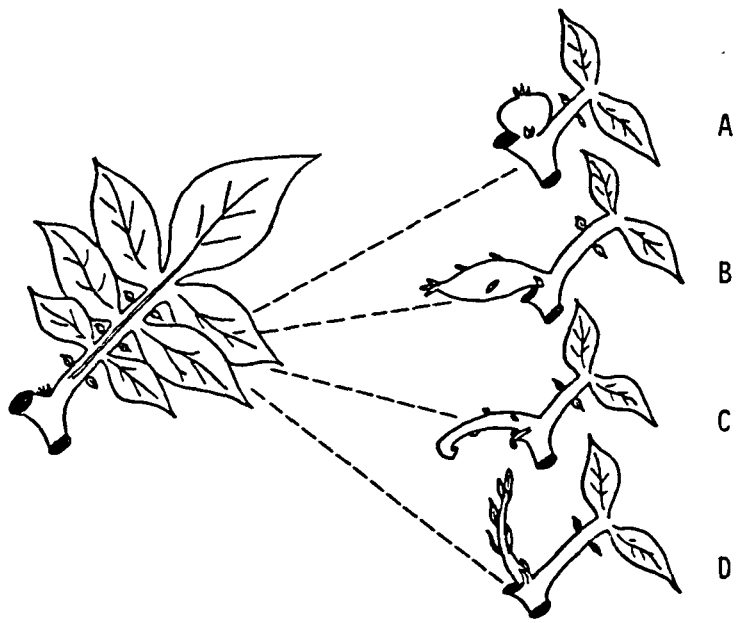

Figure 1. Drawings of axillary bud development of potato leaves 10-14 days after excision. (A) round sessile tuber on cutting taken from a plant induced to tuberize; (B) elongated tuber and (C) stolon from partially induced plants; (D) upright leafy shoot from non-induced plant.

Studies using excised potato leaves commonly involve placing the cuttings in mist beds or humidity chambers with

the basal cut end buried in a moist growing medium (Kahn et al., 1983). The buried buds develop into discernible growth forms within 4-5 days of excision, although 10 to 14-day growth cycles are frequently used to obtain full development. Nearly all leaves are capable of producing axillary bud growth after excision, but young, fully expanded are most effective (Kahn et al., 1983).

By knowing that the bud growth of leaf cuttings is controlled by the degree of tuber induction in the mother plant, one can control the leaf cutting response by controlling the growth of the mother plant. For example, tuberization of potato is known to be promoted by short photoperiods, cool temperatures, and high irradiance levels (Gregory, 1956; Ewing, 1978), thus growing plants under these conditions will lead to leaf cuttings which consistently yield tubers. Extending the photoperiod, particularly with dim light, shifts the cutting response toward leafy shoots. Similarly, increasing the temperatures (e.g. > 20^{o}C) shifts the response toward leafy shoots, particularly in combination with long photoperiods. Hence the potential exists for using potato leaf cuttings to test a variety of stem growth phenomena.

Cuttings that form sessile tubers in the leaf axils tend to produce the most biomass during 10 to 14-day growth

cycles (Kahn et al., 1983). Starch deposition and cell division increase rapidly in the buds following excision, while proteins specific to tubers can be detected within 48 h (Duncan and Ewing, 1984; Paiva et al., 1983). Thus the enlarging tubers at the leaf axils appear to be physiologically and anatomically similar to tubers formed on stolons of intact plants (Duncan and Ewing, 1984; Paiva et al. 1983).

Leaf cuttings in the PGU to study tuber growth. The size of potato leaves should pose little or no problem with regard to fitting small growth chambers such as the 1-liter PGC's of NASA's plant growth unit (PGU). Young, near-fully to fully expanded leaves can range from 10 to 20 cm length, depending on the cultivar and growing conditions. As with tuber induction, the size of potato leaves also can be controlled by environmental factors; therefore, it should be possible to selectively grow and choose leaves which maximize the available volume of the growth chambers. This may be an important consideration for maximizing total growth of the leaf cutting bud which appears to vary directly with leaf area (Kahn et al., 1983).

Typically, tuber formation is not visible until 3 to 4 days after excision but enlargement then proceeds rapidly (Fig. 2). To date, shuttle flights have averaged 7 days $\pm$ 2

days (Halstead and Dutcher, 1984) indicating approximately 0.5 g of tuber fresh mass could be obtained during an average shuttle flight (Fig. 2). The yields could be substantially increased (to 1.0 to 2.0 g) by excising leaves 4 to 5 days prior to launch thereby shifting the final harvest to 11 or 12-days-age. In this case, the rapid growth stage of bud development could be studied during microgravity.

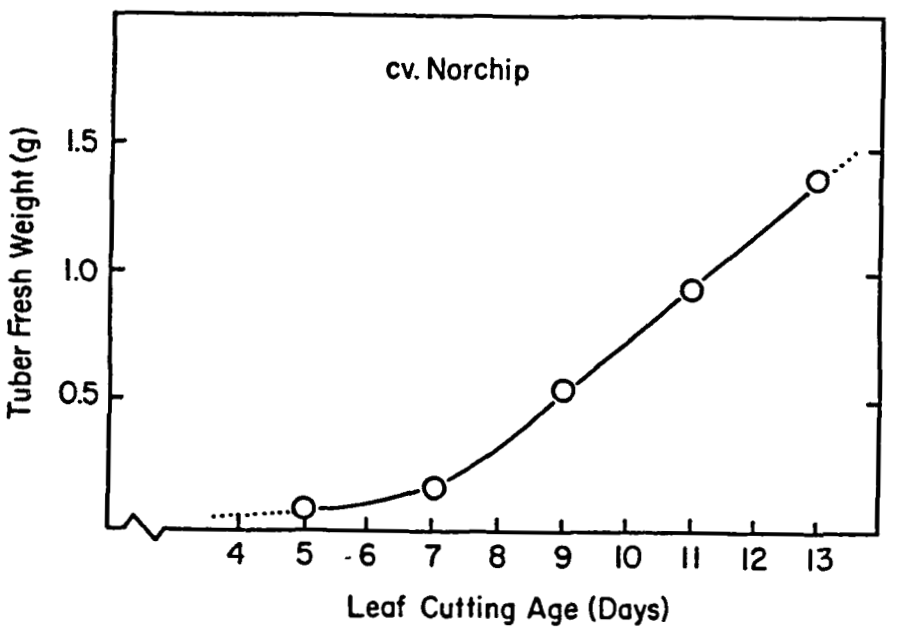

Figure 2. Growth of tubers in axils of potato leaf cuttings.

Leaf cuttings grown in humid environments for 14 days can show up to 50% increases in total fresh mass, most of which can be accounted for strictly by bud growth. This indicates that bud growth is driven by photosynthesis rather than a reallocation of existing leaf carbohydrate. Thus

irradiance levels may be a limiting factor for bud growth. To test this, cuttings from induced plants were grown under different irradiance levels obtained with varying amounts of neutral white shading. After 14 days, cuttings grown under 225 μmol $s^{-1}m^{-2}$, a level similar to that used in our past studies, produced tubers averaging 2.3 g fresh mass; in comparison, cuttings grown under 75 μmol $s^{-1}m^{-2}$, a level similar to that produced by the fluorescent lamps in the PGU, averaged nearly 2.0 g fresh mass. It appears then that bud weight gains can be enhanced by increasing the irradiance level, but good tuber growth can be obtained under 75 μmol $s^{-1}m^{-2}$. Therefore, the light levels available in a growth module such as the PGU should not be limiting for potato leaf cutting growth.

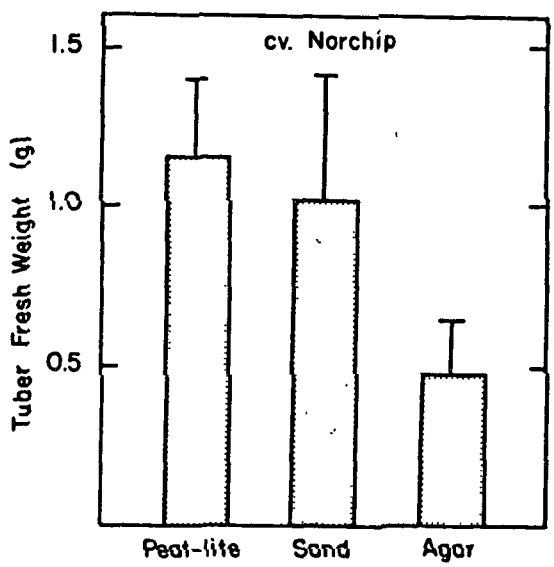

Figure 3. Comparison of tuber growth on 14-day-old potato leaf cuttings grown in peat-vermiculite (50:50 v/v), sand, and 0.6% agar. Standard deviations are shown.

Solid media such as sand or a peat-vermiculite mixture (50:50 v/v) sustain good leaf cutting growth, while a 0.6% agar medium does not (Fig. 3). The 'peat-lite' medium is light weight and has a high water holding capacity; also, this medium has been used effectively for spaceflight tests with sunflower seedlings (Brown and Chapman, 1984).

Further testing with leaf cuttings. Conceivably potato leaf buds could be grown in sterile-culture vessels on a sugar-supplemented medium thereby eliminating the need for the attached leaf and greatly reducing the overall size (Gregory, 1956; Ewing and Senesac, 1981). This approach would require complete surface sterilization of the test materials but the reduced size would permit increased sample numbers and easier accommodation to further experimental manipulation, such as spaceflight centrifugation.

The utility of potato leaf cuttings as a plant test system need not be confined strictly to the study of potato tuber (or other stem form) growth and development. The vitality and persistence of these explants makes them excellent candidates for potential photosynthesis, respiration, or other gas exchange studies as well as simplified source-sink models for carbohydrate translocation and metabolism experiments.

Summary. The use of explant tissues or organs may circumvent limitations facing whole-plant experimentation during spaceflight. In the case of potato, a crop currently being studied for application to bioregenerative life support systems, excised leaves and their subtended axillary buds can be used to test a variety of stem growth and development phases ranging from tubers through stolons (horizontal stems) to upright leafy shoots. The leaves can be fit well into small-volume test packages and sustained under relatively low irradiance levels using light-weight growing media. Tubers formed on potato leaf cuttings can yield up from 0.5 to 1.0 g fresh mass 10 days after excision and up to 2.0 g or more, 14 days from excision.

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