

Meristem Tip Culture and Virus Indexing of Sweet Potatoes

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ABSTRACT

Meristematic tips (0.4-0.8 mm long) of axillary shoots of 10 sweet potato cultivars developed into complete plants in 20-50 days in modified Murashige and Skoog agar medium. Several kinetin: indoleacetic acid combinations were adequate, but cultivars differed markedly in response. Of 150 plants tested, 47% did not cause virus symptoms when

grafted onto *Ipomoea setosa*. The use of small tips avoided the need for preliminary heat therapy, and the appropriate combination of growth factors allowed the use of a single medium for production of complete plants within a short time.

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The evaluation and improvement of tropical root and tuber crops often involve the transport of germplasm and, consequently, the possible distribution of pathogens in the vegetative material. Therefore, a practical method for elimination of pathogens is of special interest to personnel in plant introduction quarantine and seed certification programs.

Viruses have been eliminated from sweet potato

[*Ipomoea batatas* (L.) Lam.] by tip cuttings (5, 10), heat therapy (7, 8), shoot tip culture in agar media (12, 14, 16), and most recently a combination of the last two methods (18). Heat therapy has been insufficient sometimes (1, 13, 18), possibly because of differences in the viruses, the cultivars tested, or both. The addition of 1-naphthaleneacetic acid (NAA), 1 mg/liter to Murashige and Skoog (MS) agar medium (15) has considerably

shortened the time needed to produce complete plants from meristem tips, if the rooted tips are transferred to MS medium without NAA (4). Healthy plants were produced by this method combined with a 4- to 12-week heat therapy before excision of meristem tips (0.6 mm long) or shoot tips (2.0 mm long) (18).

We have attempted to devise a simpler method that avoids the need of heat therapy and the use of a transfer medium, but that produces healthy plants as quickly as and in equal proportion to those of the most successful method to date (18).

MATERIALS AND METHODS. — *Cultivars.* — Sweet potato cultivars Centennial, Cherokee, Hopi, Jewel, and Porto Rico were obtained from Alfred Jones,

Charleston, S. C., who kindly furnished us also with sweet potato seed. *I. setosa* cultivars Chardón, Morada, Playera, Sunny Side, and P. I. 320448 were obtained from the germplasm collection of the Mayaguez Institute of Tropical Agriculture. The last two cultivars used in previous studies, showed leaf deformities, vein clearing, stunting, and red-purple rings, respectively (1). The other cultivars showed yellow spots, vein clearing, or red-purple rings in their foliage when grown on a shaded bench with about 50% sunlight.

Media.—In preliminary experiments, the media used by Mori (14) and Nielsen (16) were used to test the response of Sunny Side and P. I. 320448. Later, the basic medium used was that of Linsmeier and Skoog's RM-

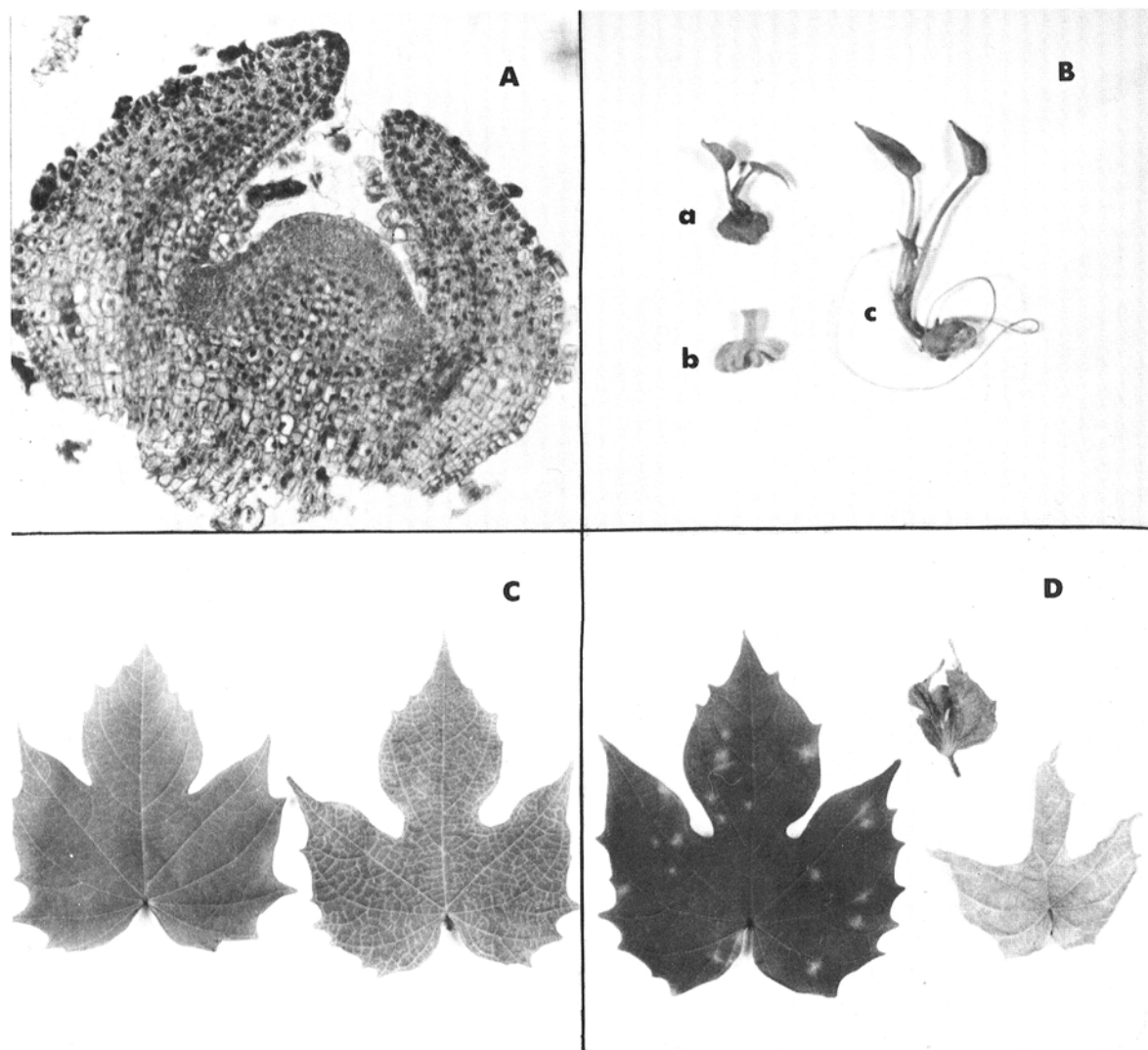


Fig. 1-(A to D). A) Meristematic shoot tip (0.7 mm) of sweet potato (*Ipomoea batatas*) cultivar Sunny Side. B) (a) Plantlet with normal shoot and well-developed callus, but no roots. (b) Abnormal plantlet with thickened leaves, no callus, and no roots. (c) Complete plantlet with well-developed callus and normal shoot. C) *Ipomoea setosa* leaves with no symptoms (left) and mild symptoms (right) of infection after grafting with tissues of symptomless plant developed from meristematic tip. D) *I. setosa* leaves with early mild symptoms of infection (left) and severe chlorosis and deformity (right), after grafts with Sunny Side as source for meristematic tips.

1964 (11) with the mineral salts as they described and the appropriate changes of indole-3-acetic acid (IAA) and kinetin as described in Results. No optional constituents of RM-1964 were used. In further testing, the basic medium of MS was used to compare the response of the 10 cultivars after changing appropriately the organic constituents based on our experiments with RM-1964. This MS basic medium was used also by Elliot with sweet potatoes (4) and contained the following (mg/liter): NH_4NO_3 , 1,650; KNO_3 , 1,900; CaCl_2 , 332; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 370; KH_2PO_4 , 170; H_3BO_3 , 6.2; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 22.3; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 8.6; KI, 0.83; $(\text{NH}_4)_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$, 0.18; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.025; CoCl_2 , 0.025; ferric sodium ethylenediaminetetraacetate, 20; thiamine hydrochloride, 2; Myoinositol, 100; sucrose, 30,000; agar, 7,000.

All the ingredients were mixed and the pH of the medium was adjusted to 5.65 before sterilization in the autoclave for 20 minutes. Glassware was washed in a cleaning solution (saturated solution of sodium dichromate, 75 ml; water, 625 ml; concentrated sulfuric acid, 1,000 ml) and rinsed several times in distilled water before use. Three milliliters of medium were placed in each glass vial (60×17 mm) with a 9-ml capacity.

Excision of shoot meristems and test environments.—Axillary shoot tips 2-3 cm long were surfaced sterilized by immersion in a 2% sodium hypochlorite solution for 5 minutes, and rinsed three times in sterile distilled water. A portion of the apex (0.4-0.8 mm long) was excised for culture (Fig. 1-A) using a straight disposable dissecting knife blade on a dissecting knife handle. Excised tips were placed on slanted medium in vials and capped and sealed with Parafilm strips. Usually 80-100 tips of each cultivar were used per trial. Contamination was kept below 5% if appropriate precautions were taken to control insects in the cultivars.

Test cultures were placed in an air-conditioned room (22-27 C) on flat wooden holders 20 cm below banks of four fluorescent lights (Sylvania, Daylight F20-T12-D) with 3,336 lux (310 ft-c) average programmed for 16 hours of light and 8 of darkness per day. Temperatures under the lights varied from 22-32 C.

Establishment in soil and virus indexing.—Plantlets were transferred when they were at least 2 cm long to soil, a mixture of soil and sand (1:3, v/v), or to sphagnum moss-perlite mixture (1:2, v/v) in clay pots. A screened

glasshouse with sunlight intensities at noon of 9,684-36,584 lux (900-3,400 ft-c) and temperatures of 20-35 C was used for the establishment of plantlets. A similar glasshouse with sunlight intensities of 30,128-86,080 lux (2,800-8,000 ft-c) at noon and temperatures of 21-45 C was used for virus indexing.

Once a plantlet was established a branch was taken from it and five nodes with axillary buds were cut starting from the place nearest the soil. They were wedge-grafted into five 3-week-old *I. setosa* seedlings just below the cotyledons, and the graft wrapped with Parafilm with the bud left exposed. In each trial were included grafts with known virus-infected Sunny Side and P.I. 320448 and nongrafted seedlings as controls. Each plantlet was tested three times during a 6-month period unless it was shown in the first test to be infected.

The susceptibility of *I. setosa* was tested in both greenhouses when seedlings had no true leaves expanded, and when they had one, two, three, and four true leaves expanded (from 1-3 weeks of germination). These seedlings were grafted with known virus-infected Sunny Side or P.I. 320448.

RESULTS.—Combination of kinetin and auxin.—In our attempts to avoid the need of a transfer from one medium to another, we found that on MS medium plus 1 mg/liter of NAA shoot tips of P.I. 320448 did not grow appreciably within 50 days and only soft white callus with roots were produced in Sunny Side, Morada, Chardón, and Centennial. Addition of 1 or 2 mg/liter of thiamine hydrochloride to the medium resulted in no changes in the response of the cultivars. Other combinations such as 10:10, 10:5, 5:10, 0:10, 5:5, 5:2, 3:1, 1:3 and 1:1 were also unsatisfactory in MS or RM-1964 media. When we changed IAA for NAA, however, we succeeded in producing more plantlets. P.I. 320448 was the least responsive, but the other cultivars responded well in a wide variety of kinetin:IAA combinations.

Plantlets were produced in modified MS medium with the following combinations tested: 5:2, 0.5:0.2, 5:3, 3:1, 4:1 and 2:1. Usually, development was better when 1 mg of folic acid was added, but not when 100 mg of yeast extract was also included in the medium. Some plantlets developed as soon as 20 days after excision, but numbers were usually low (2-3%). The rate of development varied, so that in any treatment, plants formed within 20-50 days

TABLE 1. Numbers of normal and abnormal plants formed from meristematic shoot tips of sweet potato (*Ipomoea batatas*) cultivars in 50 days in Murashiga and Skoog agar medium with various kinetin:indoleacetic acid concentrations (mg/l)

Cultivar	Plants (no.) ^a					
	5:2		0.5:0.2		2:1	
	Normal	abnormal	Normal	abnormal	Normal	abnormal
Centennial	15	14	22	7	1	0
Chardón	33	15	26	9	17	21
Cherokee	41	32	13	0	16	0
Hopi	5	21	18	2	12	0
Jewel	3	18	24	5	5	0
Morada	12	21	3	0	1	0
Playera	21	19	20	1	12	0
Porto Rico	4	12	34	3	2	0
P.I. 320448	4	0	2	0	5	0
Sunny Side	5	18	13	7	1	0

^aResults of one trial with a total of 80 excisions per cultivar for each of the growth factor combinations.

in the better combinations. This variation may have been due to the differences in size (0.4 - 0.8 mm) of the tips excised, but this idea was not tested. The response of the ten cultivars varied markedly, as can be seen in Table 1 illustrating the best combinations.

Shoot development was abnormally thick and short in several of the plantlets produced [Fig. 1-B (b)]. Even when rooted, these plantlets did not become established when transferred to the glasshouse. Fewer abnormal plants were produced in a 2:1 combination of kinetin:IAA, except for Chardón (Table 1). When shoots were normal, there was no need to wait for root formation before transfer, especially if callus was well formed [Fig. 1-B (a)]. Rootless plantlets of this type rooted well and became established in the glasshouse just as easily as those with well-developed roots [Fig. 1-B (c)].

Establishment in soil and virus indexing.—Fewer plants died when they were transferred to a mixture of soil and sand than when transferred to sphagnum moss-perlite mixture. Those that became established were usually ready for indexing 1-2 months after planting. Very few of the established plants had symptoms of infection (4 of 406), and these were only transitory foliar yellow spots. Upon indexing symptomless plants we found that 47% (70 of 150 plants tested) indexed negative for virus in *I. setosa*.

From cultivar Sunny Side, 51 plantlets tested positive for virus. None of these had shown symptoms of disease in the glasshouse, where they had been transferred to become established. When 12 of these were placed on a bench with 50% sunlight, seven plants showed yellow spots and vein clearing in their foliage 3 months later. They did not show, however, the severe symptoms shown by the source plant, and when their tissues were grafted to *I. setosa*, the symptoms expressed were mild (Fig. 1-C). The symptoms expressed by *I. setosa* were equally mild when the plants were grafted with infected tissues of other cultivars.

Many *I. setosa* plants grafted with tissues of Sunny Side, which served as the source of shoot tips, showed a severe reaction (severe chlorosis and distortion, Fig. 1-D) not observed with tissues of P.I. 320448 source plants. Under our test conditions of strong sunlight and high temperatures, we found that symptoms appeared sooner when *I. setosa* was cut back to the first two leaves at the time of grafting. Symptoms appeared in 12 days when the plant was cut and 20 when it was left uncut. Severe symptoms in grafts with Sunny Side source material usually appeared 34 days after grafting. Most grafts did not survive more than a few days, but virus transmission did not seem to require an organic union of stock and scion. There was virus transmission even in plants where the scion died within 2 days after grafting. The proportion of *I. setosa* with symptoms was usually lower (20-30%) when tissues from the P.I. 320448 source plants were used than when tissues of Sunny Side source plants were used (25-82%). Early symptoms were mild in grafts with Sunny Side source tissues, and only later (5 weeks) did severe symptoms develop.

When healthy seedlings of *I. batatas* were grafted with Sunny Side source plants, symptoms expressed were like those of the source plants. Likewise, plants of P.I. 320448 developed severe symptoms (stunting, leaf deformities) when grafted with tissues from Sunny Side source

material. Healthy sweet potato seedlings showed yellow spots, vein clearing, and red-purple rings in their foliage when grafted with tissues of P.I. 320448 known to be infected. No severe symptoms were observed in these grafts. All controls were symptomless during trials.

DISCUSSION.—The ease with which viruses may be eliminated from sweet potatoes appears to vary considerably. Failure to do so with one method may be the result of differences in the viruses present and also in the hosts. Evidence supports this assumption in other plant species (20, 21). We will be in a better position to tell if this is so in sweet potato when virus purification and comparison is accomplished. The characters known for some of those that bear the same name differ from each other (3, 6, 23), and in several cases comparison is made difficult for lack of type specimens.

The degree of assurance that a sweet potato plantlet is free of virus depends on the index host used and the test conditions. We have used *I. setosa* as have other investigators (3). Its reaction to grafts with Sunny Side source material suggests a multiple infection in this material. The mild symptoms caused by tissues of infected plantlets from shoot tips of this cultivar and previous observations of mechanical inoculations to *I. batatas* with the same source (2), also suggest that one or more pathogens were eliminated. Hollings has made similar independent observations with our Sunny Side and P.I. 320448 in grafts to *I. setosa*. When he used *Aphis craccivora* Koch and *Myzus persicae* (Sulzer) aphids, he obtained transmission from Sunny Side to *I. setosa*, but only mild symptoms appeared (*personal communication*).

Our test conditions were conducive to symptom expression, as shown by grafts with known infected material and by those from plantlets established from meristematic tips. A few plantlets (3 of 150) gave a positive reaction on *I. setosa* after two previous negative reactions. They may have been infected by insects, but also the virus content in these plantlets was possibly not sufficient in the first two tests to give a positive response on *I. setosa* upon grafting. The factors affecting symptom expression in *I. setosa* have not been studied. In our plantlets supposedly free of virus, virus development may not have reached beyond the threshold needed to show symptoms under our test conditions. We may tentatively conclude that those plants that tested negative after 6 months are free of virus. Our present limitations of available index hosts for American sweet potato viruses are apparently greater than those for the viruses found in Africa (9, 17, 19, 22). Within these limitations, however, our method supplies a practical way for rapid development of plantlets from tissue culture with a reasonable degree of assurance that they are free of viruses.

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