Proc. Fla. State Hort. Soc. 117:70-76. 2004.

REFEREED PAPER

MERISTEM-TIP CULTURE BOOSTS YIELD OF SWEETPOTATO CV. PICADITA IN SOUTH FLORIDA

DAVID Q. YING
Coral Reef Senior High School
Miami, FL 33157

ZHENTU YING AND THOMAS L. DAVENPORT[1]
University of Florida, IFAS
Tropical Research and Education Center
Homestead, FL 33031

Additional index words. Ipomoea batatas, explant size, plant vigor, tuberous roots

Abstract. **Picadita is the most important cultivar of sweetpotato [*Ipomoea batatas* (L.) Lam] grown in Miami-Dade County, Florida. Originally introduced from Cuba, it has never been "cleaned-up" by meristem-tip culture, a practice widely used to remove yield-limiting pathogens from sweetpotato cuttings. Meristem-tip culture was investigated as a means to increase the yield of this cultivar. Based on the size of the initial explants used, three groups of lines were developed and designated AD+1 (the apical dome plus one leaf primordium at <200 µm in length), AD+2 (apical dome plus two leaf primordia at 200 to 400 µm), and AD+3 (apical dome plus three leaf primordia at 500 to 1000 µm). Cultures required about 5 months from initiation to distribution of field-ready cuttings. The smaller the explants, the lower the survival rate. Only 8.5% of the AD+1 explants developed into *in vitro* plantlets whereas 64.4% of the AD+3 explants successfully developed. Total yields of the**

The authors would like to thank Mr. Severino Grandal of Rainbow Farm (Homestead, Fla.) for providing 20 storage roots of sweetpotato cv. Picadita, Mr. Manolo Hevia of M&M Farm (Miami, Fla.) for providing the space and facility to conduct field trials, and Ms. L. Wang of AgroTech (Florida City, Fla.) for funding this project. The authors also extend their appreciation to Drs. S. O'Hair, D. Seal, and Y. C. Li of TREC, IFAS, University of Florida, for their discussion and assistance for the project. This research was supported by the Florida Agricultural Experiment Station, and approved for publication as Journal Series No. R-10247.

[1]Corresponding author.

AD+1, AD+2 and AD+3 groups were increased by 146.7%, 105.5% and 46.5%, respectively, when compared with non-cultured control plants from the adjacent commercial field (control-F) in the first season. Cuttings were taken from the first season AD+1 group and planted for the second season. In the second season, the total yield of these plants was 116.2% greater than that of control-F plants. The chlorophyll content, leaf area, number of leaves, base diameter of vine, and number of lateral vines of these plants were significantly greater than those of control-F. We concluded that meristem-tip culture of the AD+1 explants significantly boosted the yield by reviving the plants to produce larger tuberous roots, presumably through efficient removal of the yield-reducing pathogens from 'Picadita'.

Native to the Americas, sweetpotato [*Ipomoea batatas* (L.) Lam] is an important crop widely cultivated in many countries in tropical and temperate regions. Due to its high yields, low-cost maintenance and rich nutrition, primarily of carbohydrates, it serves as a staple food for millions of people, especially in developing countries. It also serves as a source of forage and industrial raw materials. The sweetpotato is cultivated on nine million hectares throughout the world, yielding 121 million metric tons (FAO, 2004), making it the 7th most important crop worldwide. Along with the sweetpotato weevil (*Cylas formicarius* Fabr.), which is the most devastating insect pest of sweetpotato production worldwide, viral diseases are the most important limitations to sweetpotato production and quality. About 20 sweetpotato viruses have been reported (Salazar and Fuentes, 2000). These viruses cause severe reductions in yield and quality and increase susceptibility to pest, fungal, and bacterial attacks. Yield reductions due to viral diseases have been estimated to be 15% to 48% in China and 34% to 97% in Egypt (Salazar and Fuentes, 2000), 50% or more in Israel (Milgram et al., 1996), and up to 80% in East Africa (Wambugu, 2003).

Multiple infections with several viruses are common in sweetpotato (Carey et al., 1999; Chung et al., 1986; Clark et al, 1998; Di Feo et al., 2000). Synergistic interactions are often involved (Kreuze, 2000; Salazar and Fuentes, 2000). The most common of these disease complexes is named sweetpotato virus disease (SPVD), a syndrome caused by the dual infection and synergistic interaction of sweetpotato feather mottle potyvirus (SPFMV) and sweetpotato chlorotic stunt crinivirus (SPCSV) (Byamukama et al., 2004; Kreuze et al., 2002a, b). SPVD causes up to 98% yield loss (Mwanga et al., 2002). It is characterized by pale green chlorotic leaves or chlorosis along main leaf veins, small crinkled, strap-like or puckered leaves, severe stunting of plants, and internal cork and external cracking in tuberous roots. In addition, other endogenous pathogens, such as bacteria and fungi could also affect yield but have not yet been investigated.

'Picadita' is the most commonly grown sweetpotato cultivar in south Florida. Originally introduced from Cuba, it is a purple-skinned variety with a distinctive white or cream-colored flesh that is denser, drier, and less sweet than that of the yellow- or orange-fleshed cultivars. 'Picadita' has been grown throughout the tropical and subtropical world for centuries but has only become an important commercial crop in Florida in recent years. Over 2,000 ha of sweetpotatoes are grown annually in Miami-Dade County, with the value of the industry estimated to be $10 million, of which over 90% is 'Picadita' (M. Hevia. M & M Farm, personal communication). Little research, however, has been done on this cultivar.

All sweetpotato cultivars are propagated vegetatively and have accumulated viruses and other endogenous microorganisms such as bacteria and fungi for years. Meristem-tip culture is being utilized to eliminate endogenous pathogens and propagate disease-free cuttings for existing cultivars in the main sweetpotato growing areas of China. The availability of pathogen-free cuttings is estimated to be capable of increasing average yields of sweetpotato there by at least 30% with little or no change in inputs (Fuglie et al., 2003; Zhang and Salazar, 2000). There is currently no other practical way to control viral diseases unless virus-resistant transgenic sweetpotato plants are developed with biotechnology that is currently costly, time consuming, and only works well for a specific isolate of a virus (Okada et al., 2001). In the present paper, we report on the use of meristem-tip-culture protocols to "clean-up" the local sweetpotato cultivar 'Picadita' to increase yields for two consecutive seasons.

Materials and Methods

Plant materials and meristem-tip culture. Twenty tuberous roots of sweetpotato cv. Picadita were selected from Rainbow Farm (Homestead, Fla.). The roots were hand-washed with tap water, surface-sterilized by submerging in 0.6% NaOCl solution for 15 min, rinsed with sterile water, and planted in sterile sand in 56-L clear, covered storage boxes (Rubbermaid, Richmond, Calif.) at one to two roots per box. The shoots sprouted at 28 °C under 30 μmol s^{-1} m^{-2} fluorescent light (12-h photoperiod). They were kept vegetative by constantly cutting back the shoots when they reached the lid. Terminal segments (2 to 3 cm long) were taken from the apical portions of individual shoots, and the leaves were removed. They were dipped into 0.6% NaOCl solution with stirring for 10 min and rinsed three times with sterile water. Three groups of explants at different sizes were individually excised from the shoot apexes at each of three distances proximal to the apical meristem using a dissecting needle and scalpel with a disposable blade (Feather Stainless Steel Blade #11) under a stereoscopic zoom microscope XTL-II. They included: 1) the apical dome plus one leaf primordium at <200 μm from the tip (AD + 1), 2) the apical dome plus two leaf primordia at 200 to 400 μm from the tip (AD + 2), and 3) the apical dome plus three leaf primordia at 500 to 1000 μm from the tip (AD + 3). The explants were placed on MS medium (Murashige and Skoog, 1962) supplemented with 1.0 mg L^{-1} of 6-benzylaminopurine (6-BAP), 0.01 mg L^{-1} of α-naphthyl acetic acid (NAA), and 1.0 mg L^{-1} of gibberellic acid (GA3), and then solidified at pH 5.6 in baby food jars with 3.0 g L^{-1} of gelrite (CP Kelco, Chicago, Ill.) (Gao et al., 2000; Kuo et al., 1985). The meristem tips were cultured under fluorescent light at 30 μmol $s^{-1}m^{-2}$ at 28 °C and subcultured every 3 weeks for 3 months. Individual cultured plantlets with roots served as different lines within each of the three culture groups. They were transferred to Pro-Mix BX (Premier Horticulture, Inc., Red Hill, Pa.) that was saturated with half-strength MS salt solution in 250-mL plastic cups with four small holes on the bottom. They were placed under shade for 3 d in a 56-L clear storage box (Rubbermaid) covered with an insect-proof screen, then transferred to a half-day of sunlight for 3 d, and finally grown under full sun. After 2 weeks, vines from each line were cut into one-node segments and each planted in Pro-Mix BX saturated with half-strength MS salt solution in 15-cm plastic pots, which were again placed in the screened

clear plastic boxes for 4 weeks. The resulting vines were cut into three-node segments and planted in the field for further evaluation. The procedure of meristem-tip culture is schematically presented in Fig. 1.

Field experiments. Two consecutive field trials were conducted on Krome gravely loam soil during the first season (25 May to 21 Sept. 2002) and the second season (1 Sept. 2002 to 1 Mar. 2003) in an open field at M&M Farm, Miami, Fla. Ridges were formed 1.22 m apart according to Maynard et al. (2001).

Two ridges were planted in the first-season experiment with two other "cleaned-up" cultivars (details not described here), which served as a buffer zone on the east side of the experimental plot. A 7-m-wide strip of vacant land served as a buffer zone on the north side, to the west was a highway; and to the south was a reed windbreak. The experimental plot was, thus, spatially separated from the commercial sweetpotato production field and somewhat protected from re-infection of endemic viruses. The six to 17, three-node cuttings derived from the same lines were grouped into a block as an experimental replicate and planted at 36-cm intervals within a block with 108 cm between blocks. All cultural practices were the same as those used by M&M Farm except for one extra manual weeding in the test plots 5 weeks after planting since the 36-cm spacing between plants offered greater opportunity for weed growth than that of a commercial field. A similar batch of 68 cuttings from eight plants of 'Picadita' in a commercial field was also planted next to the east buffer zone with the same 36-cm spacing (24.5-m block). They served as the non-cultured experiment controls (control-E or Ctrl-E). A 24.5-m batch of plants in one row from the nearby commercial field was also used for a field control (control-F or Ctrl-F) where cuttings were initially planted at an average of 7.5-cm intervals. Only half of the cuttings from the nearby commercial field survived at harvest, of which only 73 of the remaining 163 plants produced commercial tuberous roots. The 73 plants in the 24.5-m block were normalized to 68 plants with 36-cm spacing in order to make valid comparison with cultured plants. Each batch (24.5 m) of the Ctrl-F and Ctrl-E was divided into five blocks and each block (4.9 m long) served as a replicate.

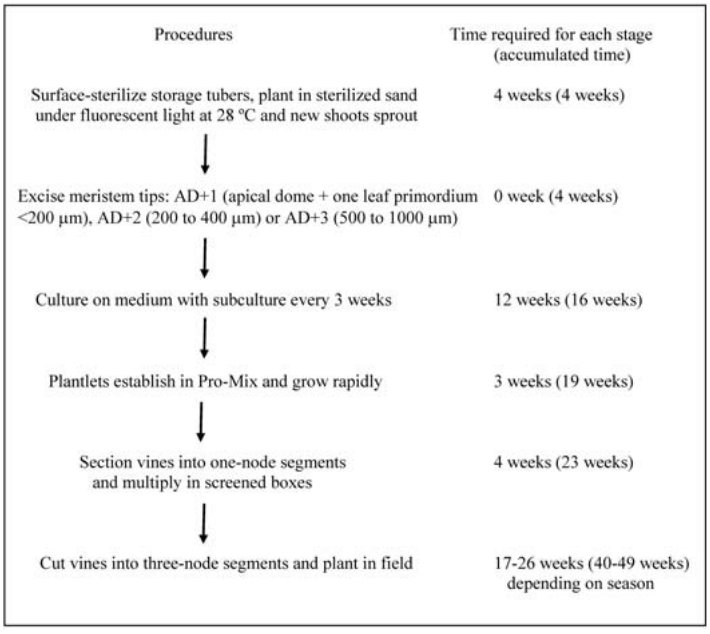

Fig. 1. Flow chart showing the procedure and time required for production of meristem-cultured 'Picadita' sweetpotato.

Plants were harvested 17 weeks after planting. If the tuberous roots were <100 g per root, they were of no commercial value and discarded. Otherwise they were counted, weighed, and graded into three categories based on individual root weight: 100 to <200 g per root, 200 to 1000 g per root, and >1000 g per root. The fresh weight of the vines in each block was also determined. All data from each repeat of this experiment were normalized as average per plant.

Cuttings from all AD+1 group plants in the above experiment were planted in another location for the second season. They were planted in four ridges (each at 500 m^2, 1.22 m × 410 m) separated by one ridge on each side planted with other 'cleaned-up' plants. Each ridge served as one replicate. The planting density and culture management were the same as those described in the first season experiment above. Plants were harvested 26 weeks after planting. Four ridges from the commercial field on the east side of the experiment plot were chosen as a control (control-F or Ctrl-F). At harvest, all roots from each ridge were collected and placed in boxes, and all boxes for each ridge were counted, of which five boxes from each ridge were randomly sampled for grading into two categories: 100 to <200 g per root and 200 g per root or more. The roots from each category for each box were then counted and weighed. The total weight for the roots of each category from each ridge was calculated as the average weight for each category of the five boxes multiplied by the total number of boxes collected from the ridge.

Measurement of leaves and vines. All measurements were taken 6 weeks after planting in the second-season experiment. Chlorophyll content of leaves at the 3rd or 4th node below the leaf that had just fully expanded on the main vine of each plant was determined by a Minolta Chlorophyll Meter SPAD-502 (Minolta Co., LTD, Japan) (Li et al., 1998). Twenty AD+1 plants from a 7.2-m block and 20 of the most vigorous plants from a control-F block with the same length were measured.

For leaf number and area measurement, all green leaves of 20 plants from each of AD+1 and control-F were counted, of which leaf blades from six plants (three from AD+1 and three from control-F) were cut off, individually pasted on letter-sized paper, and photocopied. The areas of the leaf images on the photocopy paper were carefully cut off and weighed (W_{paper}). Five blank sheets of the same photocopy paper were weighed as reference for calculation of the specific weight for the paper (131.4 $cm^2\ g^{-1}$). Based on these parameters, the leaf area of each plant could be calculated as follows:

$$A = 131.4 \times N \times W_{paper}$$

Where: A = total leaf area (cm^2) per plant;

N = number of leaves per plant;

W_{paper} = average paper weight (g) per leaf image which was 0.256 g for AD+1 plants and 0.237 g for control-F plants.

All data were averaged and the means subjected to analysis of variance (ANOVA) to determine significance followed by use of the Student's t-test or Duncan's multiple range test to determine significance between specific pairs or groups.

Table 1. Regeneration of plants produced from different sizes of meristem-tip explants.

	Plantlets *in vitro*					
	Explants of meristem tips				Plants established in soil	
Group	Size (µm)	Number	Number	%	Number	%
AD+1[z]	<200	200	17	8.5	15	88.2
AD+2[y]	200-400	100	28	28.0	26	92.9
AD+3[x]	500-1000	45	29	64.4	28	96.6

[z]Apical dome plus one-leaf primordium.
[y]Apical dome plus two-leaf primordia.
[x]Apical dome plus three-leaf primordia.

Results and Discussion

Effect of explant sizes on survival rate of cultures. The elimination of sweetpotato viruses through meristem-tip culture has proved to be very successful in many countries (Fuglie et al., 2003). It was reported that approximately 80% of plantlets derived from explants that were 200 to 400 µm in length tested virus-free, but 5% still tested positive after 2 to 3 months grown under insect-free greenhouse conditions (Kuo et al, 1985). In the current study, three different sizes of explants based on the length of meristem tips and number of leaf primordia (AD+1, AD+2, and AD+3) were excised from apical meristem tips of vigorously growing shoots. After culture initiation, meristem tips became green within 1 to 2 weeks, with the larger explants turning green first. Thereafter, the bases of the meristem tips developed into compact calli in which the tips grew into plantlets that would later produce their own roots. This usually took 2 to 3 months. A lower survival rate was observed in cultures from smaller explants; for example, only 17 plantlets out of 200 AD+1 explants were produced (8.5%) whereas 29 plantlets were obtained from the 45 original AD+3 explants (64.4%) (Table 1). The survival rate of the plantlets in soil was similar (about 90%) among AD+1, AD+2 and AD+3 groups (Table 1).

Boosting yield. In two consecutive field trials from May 2002 to March 2003, a significant increase in yield was observed in the AD+1 group, compared with control-F plants (Tables 2 and 4). In the first season from 25 May to 21 Sept. 2002, the total yield increased by 146.7%, 105.3%, and 46.5% for AD+1, AD+2, and AD+3, respectively, compared with field control-F plants in which cuttings were initially planted at an average of 7.5 cm apart (Table 2). The total yields of the three groups were also greater by 78.2%, 48.3%, and 5.8%, respectively, than control-E plants in which cuttings were taken directly from commercial field plants, but planting distance and all other cultivation were the same as that of the meristem-tip-cultured plants (Table 2). Yields of medium (200 to 1000 g) and large (>1000 g) roots of the AD+1 and AD+2 groups were significantly greater than that of both controls although the small-sized root yield was significantly lower. Furthermore, the number of roots in the medium- and large-size ranges of the AD+1 and AD+2 groups was significantly more than those from both controls although the total number of roots was not significantly different (Table 3). The number of the small (100 to <200 g) roots was significantly less than that of both controls (Table 3). Importantly, the yield of the large roots for the AD+1 group was significantly higher than all others including the AD+2 and AD+3 groups and both controls (Table 2). This indicates that the yield-reducing pathogens could be more effectively eradicated by using the AD+1 explants (<200 µm) rather than the larger explants.

The total yield of control-E plants was substantially higher (38.4%) than that of control-F plants, and the medium-sized roots solely contributed to it (Table 2). Both controls had the same source of seed vines for initial planting and all the same culture practices, but differed only in the following two aspects: 1) the planting density was 7.5 cm apart for control-F plants and 36-cm apart for control-E plants; and 2) control-F ridges were cultivated twice to control weeds and reform the beds, whereas control-E ridges were cultivated three times for the same purpose during the growing season. Preliminarily, observations indicated that the commercial plant spacing was inadequate, causing half of the cuttings to die and profitable yields to occur in only 73 plants distributed across a 24.5-

Table 2. Effect of initial cuttings derived from different sizes of explants on yield of roots in the first season.[z]

		Yield (g per plant)				Total yield increase (%) comparison with	
		Size of root by wt (g)			Total		
Group	Vine wt (g per plant)	100 to <200	200 to 1000	>1000	root wt (g)	Ctrl-F	Ctrl-E
Ctrl-F[y]	853 ab[x]	132.3 c	381.8 a	31.8 a	545.9 a	0	-27.8
Ctrl-E	794 a	114.6 bc	576.6 b	64.6 a	755.7 b	38.4	0
AD+1	1082 b	80.6 a	836.9 c	428.9 c	1346.5 d	146.7	78.2
AD+2	840 ab	79.4 a	823.7 c	217.4 b	1120.6 c	105.3	48.3
AD+3	904 ab	97.0 ab	634.1 b	68.7 a	799.8 b	46.5	5.8

[z]Planted on 25 May and harvested on 21 Sept. 2002. Tuberous roots at <100 g each were not weighed. All data were collected at harvest.
[y]Each cutting was randomly selected from a top segment of a Picadita vine in a sweetpotato production field and was planted at an average spacing of 7.5 cm. About half of plants survived at harvest time but only one from every 2 to 3 surviving cuttings could finally produce commercial tuberous roots.
[x]Mean separation within each column by Duncan's multiple range test, $P < 0.05$.

Table 3. Effect of initial cuttings derived from different sizes of explants on number of roots in the first season.[z]

Group	No. of plants	No. of roots per plants: Size of root by wt (g) 100 to <200	200 to 1000	>1000	Total root number	Total number increase (%) comparison with Ctrl-F	Ctrl-E
Ctrl-F[y]	68	1.21 c[x]	1.25 a	0.03 a	2.49 ab	0.0	-0.4
Ctrl-E	68	1.01 bc	1.43 b	0.06 a	2.50 ab	0.4	0.0
AD+1	176	0.68 a	1.70 c	0.32 c	2.70 b	8.4	8.0
AD+2	265	0.66 a	1.65 c	0.17 b	2.48 ab	-0.4	-0.8
AD+3	251	0.79 ab	1.45 b	0.06 a	2.29 a	-8.0	-8.4

[z]Planted on 25 May and harvested on 21 Sept. 2002. Tuberous roots at <100 g each were not counted.
[y]Each cutting was randomly selected from a top segment of a 'Picadita' vine in a sweetpotato production field and was planted at an average spacing of 7.5 cm. About half of plants survived at harvest time, but only one from every two to three surviving cuttings could finally produce commercial tuberous roots.
[x]Mean separation within each column by Duncan's multiple range test, $P < 0.05$.

meter distance. Calculating the average, we determined that a more appropriate spacing was 34 cm per plant. We elected, therefore, for convenience, to use a 36-cm spacing for our meristem-cultured and control-E plants. Over 99% of the control-E plants survived to harvest, and each produced medium to large roots. This demonstrates that the current planting density for sweetpotato in Miami-Dade County should be adjusted up to 30 to 40 cm apart so that the yield could be increased significantly, and the initial seed vine materials would be reduced to one-fifth of that currently required. This will save four-fifths of the labor required for cutting and planting, although this requires an extra cultivation, which can be done mechanically as usual.

Total yield of the AD+1 group was significantly greater in the second season than that of control-F plants, although the increase in total number of roots did not reach a significant level (Table 4). Further analysis showed that this yield increase resulted from a significantly greater number of medium and large roots while not significantly reducing the number of the small roots (Table 4). Compared to the first growing season, the total yield of the AD+1 group decreased 30.5% (i.e., 116.2% subtracted from 146.7%) in the second growing season whereas the total number of roots increased by 10.5% (i.e., 8.4% subtracted from 18.9%) (Tables 2-4). The size of the medium to large roots was significantly reduced in the second season (Table 5). This implies that the yield-reducing pathogens gradually re-infected the "cleaned-up" 'Picadita' plants during the growing season in an open field, a result that has also been reported for other sweetpotato cultivars (Fuglie et al., 2003; Gao and Zhang, 2000; Kuo et al., 1985).

Effect on leaf and vine. The aphid-transmitted SPFMV alone causes no foliar symptoms in East Africa sweetpotato cultivars (Gibson et al., 1997; Karyeija et al., 1998) and is difficult to detect in infected plants due to its low titer (Abad and Moyer, 1992; Esbenshade and Moyer, 1982; Gibb and Padovan, 1993). Despite this lack of overt symptoms, yields are severely reduced. The whitefly-transmitted SPCSV causes mild symptoms, such as slight stunting and purpling of lower leaves and mild chlorotic mottle in the middle leaves under appropriate conditions (Gibson et al., 1998; Winter et al., 1992). 'Picadita' sweetpotato grown in Miami-Dade County appears symptomless, and SPFMV was not detected by RT-PCR (reverse transcriptase-polymerase chain reaction) in the plant leaves from both the control-F and AD+1 group (data not shown); however, growth of the AD+1 plants was much more vigorous than that of control-F plants if grown side by side as demonstrated by the differences in plant vigor. Chlorophyll content, leaf area, number of leaves per plant, vine weight, vine base diameter, and number of lateral vines were significantly greater in

Table 4. Yield for the second season of meristem-tip cultured plants compared with that of control-F.

Group	100 to <200 g roots: No	Yield (kg)	>200 g roots: No.	Yield (kg)	Total roots: No.	Yield (kg)	Total root increase (%): No.	Yield
Ctrl-F	955[z] A[y]	139 A	566 A	194 A	1521 A	333 A	0	0
AD+1	619 A	101 A	1190 B	619 B	1809 A	720 B	18.9	116.2

[z]Planted on 1 Sept. 2002 and harvested on 1 Mar. 2003 from a 500-m^2 plot.
[y]Mean separation within each column by Student's t-test, $P < 0.01$.

Table 5. Effect of different seasons on the average size of medium to large roots.

Growing season	Average size of roots for medium to large roots: Ctrl-F (g per root)	AD+1 (g per root)
1st season (25 May - 21 Sept. 2002)	325.7 A[z]	626.7 B
2nd season (1 Sept. - 1 March 2003)	342.8 A	520.2 A

[z]Mean separation within each column by Student's t-test, $P < 0.01$.

Table 6. Difference in various parameters of leaf and vine between meristem-tip-cultured plants and control-F[z].

Vine and leaf parameter	Ctrl-F	AD+1
Chlorophyll content[y]	43.7 A[s]	54.8 B
No. of leaves per plant[x]	78.0 A	225.6 B
Leaf area per plant[x] (cm^2)	2494.4 A	7793.8 B
Length of vine[w] (cm)	81.8 B	76.7 A
Vine wt[v] (g)	86.5 A	363.2 B
Diameter of base[u] (mm)	4.9 A	5.9 B
No. of lateral vines[t]	2.2 A	4.9 B

[z]All measurements and records taken 8 weeks after planting in the second season, five best plants per repeat, total 20 plants average.
[y]Chlorophyll content measured by a chlorophyll meter SPAD-502 for each sample.
[x]All green, fully-expanded leaves on the plants were counted and measured.
[w]The main vine was measured.
[v]Vine weight included vines and leaves.
[u]Base diameter of vine was measured at the surface of soil.
[t]Lateral vines were counted within 15 cm from the base of vine.
[s]Mean separation within each row by Student's t-test, $P < 0.01$.

AD+1 plants than those of control-F plants although the vine lengths of cultured plants were shorter (Table 6). The greater vigor of the AD+1 plants implies that the meristem-tip-culture method could efficiently remove yield-reducing pathogens, including viruses and other endogenous bacteria and fungi from 'Picadita' shoots, resulting in the observed high yield. In our initial meristem-tip-culture experiment, many kinds of endogenous microorganisms (mostly bacteria) were observed in meristem tips and caused serious contamination of cultures (Data not shown). This problem was corrected by planting surface-sterilized tuberous root in sterilized sand in sealed boxes as described in materials and methods.

Investment in a facility to provide cleaned-up 'Picadita' vines for the industry is feasible. The current total estimated annual value of 'Picadita' production in south Florida is 9 million dollars. Although the initial investment for industry-level production of the pathogen-free vines may cost about a half million dollars for tissue culture labs and insect-proof growing facilities, the results here showed that the pathogen-free vines from AD-1 group could be productive for at least two growing seasons (one year) in the local field conditions, which more than doubled yield and also saved four-fifths of labor for planting. Even if only one-eighth of the current production area were to use pathogen-free vines produced from such a facility, the net profit would be half a million dollars for that acreage alone.

Conclusions

1. The AD+1 group plants derived from <200 µm explants produced the highest yields compared with the other groups derived from larger explants, presumably through efficient removal of yield-reducing pathogens, resulting in improved productivity for at least two consecutive growing seasons in an open field.
2. The yield boost produced by the meristem-tip culture was not the result of an increase in the number of tuberous roots but in the larger size of the roots that resulted from the vigorous growth of vines.
3. The current planting density of 'Picadita' in Miami-Dade County that causes about one-third yield loss due to competition at an early-growth stage should be adjusted from 7.5 cm to about 36 cm apart. Although it requires one more cultivation for control of weed growth, it saves four-fifths of the labor involved in cutting and planting seed vines.

Literature Cited

Abad, J. A. and J. W. Moyer. 1992. Detection and distribution of sweetpotato feathery mottle virus in sweetpotato by *in vitro*-transcribed RNA probes (riboprobes), membrane immunobinding assay, and direct blotting. Phytopathology 82:300-305.

Byamukama, E., R.W. Gibson, V. Aritua, and E. Adipala. 2004. Within-crop spread of sweetpotato virus disease and the population dynamics of its whitefly and aphid vectors. Crop Protection 23:109-116.

Carey, E. E., R. W. Gibson, S. Fuentes, M. Machmud, R. O. M. Mwanga, G. Turyamureba, L. Zhang, D. Ma, F. Abo El-Abbas, R. El-Bedewy, and L. F. Salazar. 1999. The causes and control of virus diseases of sweetpotato in developing countries: Is sweetpotato virus disease the main problem? p. 241-248. In CIP program report 1997-1998. International Potato Center, Lima, Peru.

Chung, M.-L., Y.-H. Hsu, M.-J. Chen, and R.-J. Chiu. 1986. Virus diseases of sweetpotato in Taiwan. Plant virus diseases of horticultural crops in the tropics and sub-tropics 33:84, FFTC Book Series. Food Fert. Technol. Ctr., Taipei, Taiwan.

Clark, C. A., M. W. Hoy, R. A. Valverde, J. M. Cannon, D. R. La Bonte, and H. Wallace. 1998. Effect of viruses on yield of sweetpotato in Louisiana, 1996-1997. Phytopathology 88:S17 (Abstr.).

Di Feo, L., S. F. Nome, E. Biderbost, S. Fuentes, and L. F. Salazar. 2000. Etiology of sweetpotato chlorotic dwarf disease in Argentina. Plant Dis. 84:35-39.

Esbenshade, P. R. and J. W. Moyer. 1982. Indexing for sweetpotato feathery mottle potyvirus in sweetpotato using enzyme-linked immunosorbent assay. Plant Dis. 66:911-913.

FAO. 2004. FAOSTAT agricultural data. http://apps.fao.org/page/collections?subset=agriculture.

Fuglie, K. O., L. Zhang, L. F. Salazar, and T. Walker. 2003. Economic impact of virus-free sweetpotato seed in Shandong province, China. http://www.eseap.cipotato.org/MF-ESEAP/Fl-Library/Eco-Imp-SP.pdf.

Gao, F., Y. Gong, and P. Zhang. 2000. Production and development of virus-free sweetpotato in China. Crop Protection 19:105-111.

Gibb, K. S. and A. C. Padovan. 1993. Detection of sweetpotato feathery mottle potyvirus in sweetpotato grown in northern Australia using an efficient and simple assay. Intl. J. Pest Management 39:223-228.

Gibson, R. W., I. Mpembe, T. Alicai, E. E. Carey, R. O. M. Mwanga, S. E. Seal, and H. F. Vetten. 1998. Symptoms, aetiology and serological analysis of sweetpotato virus disease in Uganda. Plant Pathol. 47:95-102.

Gibson, R. W., R. O. M. Mwanga, L. Kasule, I. Mpembe, and E. E. Carey. 1997. Apparent absence of viruses in most symptomless field-grown sweetpotato in Uganda. Ann. Appl. Biol. 130:481-490.

Karyeija, R. F., R. W. Gibson, and J. P. T. Valkonen. 1998. The significance of sweetpotato feathery mottle virus in subsistence sweetpotato production in Africa. Plant Dis. 82:4-15.

Kuo, C. G., B. J. Shen, M. J. Shen, S. K. Green, and D. R. Lee. 1985. virus-free sweetpotato storage roots derived from meristem-tips and leaf cuttings. Sci. Hort. 26:231-240.

Kreuze, J. 2000. Molecular studies on the sweetpotato virus disease and its two causal agents. PhD Diss., Swedish Univ. of Agric. Sci., Uppsala.

Kreuze, J. F., R. F. Karyeija, R. W. Gibson, and J. P. T. Valkonen. 2002a. Mechanisms by which the sweetpotato virus disease (SPVD) is caused following a co-infection of sweetpotato feathery mottle virus and sweetpotato chlorotic stunt virus in sweetpotato plants. African Potato Assoc. Conf. Proc. 5:409-413.

Kreuze, J. F., E. I. Savenkov, and J. P. T. Valkonen. 2002b. Complete sequence and analyses of the subgenomic RNAs of sweet potato chlorotic stunt virus reveal several new features for the genus *Crinivirus*. J. Virol. 76:9260-9270.

Li, L. C., A. K. Alva, D. V. Calvert, and M. Zhang. 1998. A rapid nondestructive technique to predict leaf nitrogen status of grapefruit tree with various nitrogen fertilization practices. HortTechnology 8:81-86.

Maynard, D. N., G. J. Hochmuth, M. L. Lamberts, C. S. Vavrina, W. M. Stall, T. A. Kucharek, P. A. Stansly, and S. E. Webb. 2001. Sweetpotato production in Florida. EDIS HS738. Univ. Fla., Gainesville. http://edis.ifas.ufl.edu/BODY_CV136.

Milgram, M., J. Cohen, and G. Loebenstein. 1996. Effects of sweetpotato feathery mottle virus and sweetpotato sunken vein virus on sweetpotato yields and rates of reinfection of virus-free planting material in Israel. Phytoparasitica 24:189-193.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497.
Mwanga, R. O. M., J. W. Moyer, D. P. Zhang, E. E. Carey, and G. C. Yencho. 2002. Nature of resistance of sweetpotato to sweetpotato disease. Acta Hort. 583:113-119.
Okada, Y., A. Saito, M. Nishiguchi, K. Kimura, M. Mori, K. Hanada, J. Sakai, C. Miyazaki, Y. Matsuda, and T. Murata. 2001. Virus resistance in transgenic sweetpotato feathery mottle virus. Theor. Appl. Genet. 103:743-751.
Salazar, L. F. and S. Fuentes. 2000. Current knowledge on major virus diseases of sweetpotatoes, p. 14-19. In: Y. Nakazawa and K. Ishiguro (eds.). Proc. Intl. Wrkshp. Sweetpotato Cultivar Decline Study. 8-9 Sept. 2000, Miyakonojo, Japan. http://ss.mykz.affrc.go.jp/Workshop/WS2000/proceedings/pdf/p014_salazar.pdf
Wambugu, F. M. 2003. Development and transfer of genetically modified virus-resistant sweetpotato for subsistence farmers in Kenya. Nutrition Rev. 61:S110-S113.
Winter, S., A. Purac, F. Leggett, E. A. Frison, H. W. Rossel, and R. I. Hamilton. 1992. Partial characterization and molecular cloning of a Closterovirus from sweetpotato infected with sweetpotato virus disease complex from Nigeria. Phytopathology 82:896-875.
Zhang, D. and L. Salazar. 2000. CIP's strategy for controlling sweetpotato virus diseases in developing countries. In: Y. Nakazawa and K. Ishiguro (eds.). Proc. Intl. Wrkshp. Sweetpotato Cultivar Decline Study. 8-9 Sept. 2000, Miyakonojo, Japan (Abstr.). http://ss.mykz.affrc.go.jp/Workshop/WS2000/workshop/pdf/s3_dapeng.pdf.