1994

Mass Handling of Watermelon Microcuttings

Jeffrey Adelberg
Clemson University, jadlbrg@clemson.edu

Y Alper

R E. Young

B B. Rhodes

MASS HANDLING OF WATERMELON MICROCUTTINGS

Y. Alper, R. E. Young, J. W. Adelberg, B. B. Rhodes

ABSTRACT. *Modifications were made in the configurations of the unitizing, nonselective wire cutters used by Alper et al. (1992) for mass cuttings of Stage II* Citrullus lanatus *cv. Charlee (watermelon) plant tissue cultures to further enhance productivity. Mounting the cutter in an inverted position over the receiving vessel eliminated time required for filling. This cut-and-dump technique became 4.8 times more productive for the total transfer process than the conventional scalpel and forceps technique when both time and yield of cut segments with visible buds were considered. A concept for growing fewer, larger tissue clusters per vessel in mini-trays with orienting cells and cutting with correspondingly sized oriented cell wire cutters yielded as much tissue fresh weight as conventional agar vessels and afforded the potential to reduce time required for the removal job function with the cut-and-dump technique.* **Keywords.** *Micropropagation, Plant tissue culture, Cutters, Mechanization.*

Micropropagation has become an important method to multiply rapidly virus-free varieties of crops that are difficult to propagate by conventional methods, e.g., seeds, cuttings, and divisions. The major factor limiting the cost competitiveness of micropropagation is the expense of labor inherent in the intense manual handling currently required (Kurtz et al., 1991; Chu, 1992). Most of this labor is dedicated to Stage II proliferation. Expansion of the micropropagation industry into the vast market of vegetable, fruit, and forest species can only be achieved by mechanization and automation of the micropropagation process (Vasil, 1991).

Tests conducted in this study represent an extension of the work of Alper et al. (1992) with unitized, nonselective mass cutting of *in vitro* watermelon. The former studies compared various designs of wire cutting devices as alternatives to conventional scalpel and forceps cutting through time studies and product characteristics. The scope of this study includes modification of the wire cutter device to enhance the transfer to new vessels as well as the cutting process, exploration of the influence of agar concentration on yield of nonselectively cut watermelon tissue, and investigation of the potential of trays with cell space restrictions to influence physical properties of wire-cut tissue.

Article was submitted for publication in August 1993; reviewed and approved for publication by the Emerging Technologies Div. of ASAE in January 1994. Presented as ASAE Paper No. 93-1019.

Use of specific company name is for identification purposes only and does not indicate endorsement by Clemson University over other brands of similar functional equipment.

The authors are **Yekutiel Alper**, Visiting Scientist, Agricultural and Biological Engineering, **Roy E. Young**, ***ASAE Member Engineer***, Professor, Agricultural and Biological Engineering, **Jeff W. Adelberg**, Research Associate, Horticulture Dept., **Bill B. Rhodes**, Professor, Horticulture, Clemson University, Clemson, S.C.

LITERATURE REVIEW

Mechanized handling of *in vitro* plant material has received the attention of several investigators. It tends to assume formats nearly as variable as the highly diverse growth habits of different plant species and culture explants. Techniques and tools vary from the conventional hand scalpel and forceps to liquid pumping systems to robots.

Somatic embryogenesis has stimulated considerable interest in the plant tissue culture world because of its potential for mass cloning of very large numbers of somatic embryos (Lutz et al., 1985). Conceptually, production in liquid bioreactors (Styer, 1985), automated quality detection systems through computer vision analyses (Grand d'Esnon et al., 1989) and fluid transport of "artificial seeds" (Redenbaugh et al., 1987; Gautz et al., 1989) of somatic embryos are all attractive and logical visions for automated micropropagation. The primary limitations, however, for realizing commercially the perceived potentials of somatic embryogenesis are (1) difficulties in reproducibly inducing embryogenesis and insuring appropriate embryo development, and (2) the fact that developed embryos must still be delivered to an environment for further plantlet development, i.e., organogenesis (Payne et al., 1992).

Organogenic multiplication systems, although perhaps less efficient than embryogenic systems, are amenable to a large variety of species and are documented to produce phenotypically and genetically stable plantlets. Consequently, automation of organogenic systems is thoroughly justifiable. In fact, plantlets produced from embryogenesis may be very feasibly handled by techniques developed for automating organogenic systems.

Assuming conventional agar-based plant tissue culture, PhytoNova, a commercial company in the Netherlands, introduced a sophisticated, automated machine utilizing robotic tissue handling, image analysis, and computer-controlled laser cutting (Holdgate and Zandvoort, 1992; Brown, 1992). Their system used a high-powered laser beam to cut nodal sections at a rate of about one explant

per 3 s. Laser cutting minimized demands for sterilizing the cutting device. The system provided substantial manual labor reductions by automating cutting, transporting, planting, and record-keeping functions, yet it required increased maintenance and supervisory personnel. Major limitations of this technology included specificity to stem section explants, relatively low production rate, and high capital investment attractive only to larger commercial operations. Nonelongated propagules such as meristematic bud clusters, bulblets, protocorms, or somatic embryos would need alternative systems. A less commercially developed blade cutter prototype cutting and handling device for meristematic shoot bud clusters has been under development at the New Zealand Institute for Food Research and the New Zealand Agricultural Engineering Institute (Cooper et al., 1992). This system also utilizes a computer vision system with a robotic arm and a tri-sectioned blade cutter device.

Kurata (1992) described five micropropagation transplanting prototypes being developed in Japan: the TOMOCA system from Kirin Brewery Co., Ltd.; the KOMATSU system from the Ministry of Agriculture, Forestry and Fisheries and Komatsu, Ltd.; the MIWA system for lily bulblets from Waseda University; the MIWA system for chrysanthemums; and the TOSHIBA system from Toshiba Corporation. All these systems are robotic and designed for agar-based culturing. Typical times of operation for recognizing, cutting, and planting a node section explant have been 15 s; less than 5 s is viewed as the target for feasibility. Iwasaki's (1991) TOMOCA system used a two-dimensional grid, blade-type cutter head that dissected the tissue from above into 36 cubical sections and selectively removed cubes with push rods to transfer the cut material to four fresh agar vessels. "Bushy" plants like *Ficus benjamina* L. from the commercial laboratories of Twyford International, Inc., USA, were separated with this system at rates of about 1.7 s per unit cut. The simple multiple-units blade cutter was relatively low cost and suitable for practical applications. The KOMATSU system incorporated a horizontal cutter that dissected stem sections in different vertical planes as the culture vessel was moved past the cutting blade. Miwa's (1991) robotic system for lily bulb micropropagation automated the processes of removing roots from the bulbs, separating individual bulb scales, and transferring and transplanting separated bulb scales. Although machine vision was not suitable for separating bulblets and removing their roots, they did utilize image processing to select and to transplant appropriate dissected explants of stem sections from their robotic chrysanthemum micropropagation system. Rotating disks functioned as the cutter devices for the chrysanthemums. The TOSHIBA system incorporated both a sensing robot and a cutting robot and automated the processes of loading trays, recognizing plantlets, cutting internodes, and transferring explants to new vessels. The cutter device was a scissors mechanism.

The Vitromatic system of Levin and Vasil (1989) cultured meristematic clusters in liquid medium in a bioreactor. It allowed mechanized transfer at appropriate times to a bioprocessor which separated and sized meristems with a homogenizer and distributed propagules into a multi-cell plastic matrix. The matrices provided contact with the nutrient medium in culture vessels to grow plantlets rapidly. It was used primarily with meristematic-type explants.

The unitizing, nonselective cutter (Alper et al., 1992) reduced cutting time per propagule by a factor of 14 compared with the scalpel and forceps method. Because of its simplicity and low cost, this device can be applied readily by small and medium scale operators as an operator's aid. It can be used with a wide range of plant types and in both agar and liquid culture systems. This report addresses simple modifications of the originally reported cutter configuration and of accompanying processes and protocols to further enhance its labor efficiency and product quality.

Objectives

This research was designed to develop and to test equipment and processes to enhance productive efficiency of handling the multiplication phase, Stage II, of plant micropropagation. Specific objectives were to:

- Study the impact of concentration of agar in nutrient media on growth of dissected tissue.
- Develop and test alternative cut-and-transfer protocols to enhance the productivity of the unitizing, nonselective cutting device.
- Test the influence of explant orientation in multi-cell growing trays on handling, yield, and quality of tissue cut with a unitizing, nonselective wire cutter.

Methods and Materials

Explants for these studies were shoot bud clusters of *Citrullus lanatus* cv. Charlee. They originated from repeated subcultures of shoot apices on medium with 10 μM BA as described in Adelberg and Rhodes (1989). Starting cultures for these experiments had been maintained for 30 to 36 months by monthly transfers on agar-based media.

Cut-and-Dump and Agar Concentration

The initial experiment compared cutting with the 4.9-mm-square grid, unitizing wire cutter (Alper et al., 1992) and randomly dumping watermelon shoot buds onto fresh agar with the conventional practice of dissection by scalpel and forceps (hand cut) and selective placement onto fresh agar. Both treatments were transferred to new vessels with media containing conventional concentrations of agar of 7 g/L. Scalpel and forceps manipulation was the control treatment. The study was initiated from hand-cut stock cultures that started with 16 clusters of tissue per vessel. Data were collected over 4 sequential culture cycles of approximately 18 days each. For each culture cycle, replications were made in four vessels for each of the two treatments. After each sequential culture cycle, tissue from one of the four hand-cut vessels was divided into four equal parts which were cut, respectively, by the wire cutter and dumped into individual vessels with new media. These four vessels became the cut-and-dump treatment for the next culture cycle. In addition, 16 new clusters from hand-cut tissue were placed in each of four vessels for continuation of the control treatment through the next culture cycle. At the conclusion of each culture cycle, data

were collected by selective scalpel and forceps dissection of the four cut-and-dump vessels and the three remaining hand-cut vessels (after the one referred to earlier was used for the next cycle's cut-and-dump stock material) to make comparisons of fresh weights and numbers of cut segments with visible buds.

The "dumped" tissue in the initial experiment visually appeared to have less surface contact with the agar medium than tissue selectively placed on the medium with forceps. Therefore, a second experiment was conducted using the cut-and-dump technique and four treatments of agar concentrations—4, 5, 6, and 7 g/L—to vary the "softness" of the medium. Seven grams per liter was the control. Each concentration level was replicated four times in Magenta GA-7 vessels containing 50 mL of medium. Four bud clusters from hand cut stock material were prepared with an inverted 5.7-mm square grid, unitizing wire cutter (fig. 1) and dumped into each treatment vessel. The larger 5.7-mm grid was selected because of greater biomass production in the earlier study by Alper et al. (1992). After a 21-day culture cycle, fresh weights of tissue from each vessel were recorded. The tissue was then cut again by the same unitizing cutter. Numbers of tissue segments containing visible buds as judged subjectively by the same tissue culturist were counted for each vessel of each treatment. Sixteen new dissected tissue segments of equivalent size from each treatment vessel were dumped into each of four new vessels for replications of the same agar concentration. Data were collected over three culture cycles. Relative dry matter content (dry weight/fresh weight) was determined from tissue collected after the third (final) cycle. First, fresh tissue from each vessel were weighed and recorded. Then this tissue was dried for 72 h at 62° C and respective dry weights were recorded. Finally, the appropriate ratios of dry weight to fresh weight were tabulated.

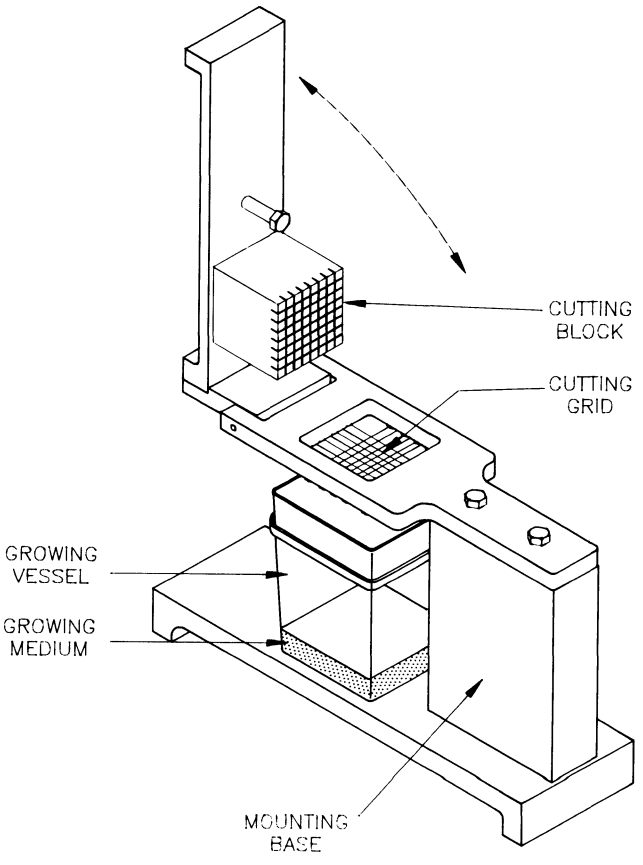

Figure 1–Unitizing cutter mounted on base for cut-and-dump operation.

The unitizing cutter in figure 1 consisted of a rectangular stainless steel wire (0.025 mm diameter) mounted in a square grid arrangement on an open-faced aluminum plate. A cutting block with slots made in one face was mounted to an aluminum handle attached to pivot into the plate holding the wire grid. These two parts were inverted and mounted to an aluminum support base which allowed direct cutting and dumping into an open Magenta vessel placed under the square grid. Clusters of plant tissue were placed on the upper surface of the wire grid and then pressed through the wire grid by the slotted cutting block. The wire grid spacing and slot dimensions were matched with precision. Cut tissue segments dropped directly into the open Magenta vessel below.

Cut-Sort-and-Dump vs. Cut-and-Dump

A third experiment was conducted to compare time efficiency and shoot bud growth among three cutting and handling, or transfer, treatments:

A. Hand cutting and transferring using conventional scalpel and forceps manipulations.
B. Cut-and-dump using the unitizing, nonselective wire cutter.
C. Cut-sort-and-dump using the same wire cutter, but collecting the cut tissue in a sterile petri dish and sorting out only pieces containing visibly viable buds and subsequently dumping the buds manually into new vessels.

The unitizing cutter with a 5.7-mm square grid was also used for these experiments. In each of three 18-day culture cycles, six replications of each transfer treatment were made in Magenta vessels containing 50 mL of media with a 5.5 g/L agar concentration for treatments B and C and 7 g/L agar for the hand cut treatment A. The 5.5 g/L agar concentration for these treatments was selected based on observed tissue performance and physical limitations from the previous agar concentration experiments.

The stock tissue for each new vessel in the next culture cycle was four bud clusters taken from a vessel of the same treatment in the previous culture cycle. For the conventional scalpel and forceps technique (the control treatment A), job functions were partitioned into three timed categories: (1) removing the tissue from original vessels, (2) cutting, and (3) sorting and placing the selected segments into new vessels. For treatment B, cut-and-dump, the job functions were partitioned into two timed categories: (1) removing the tissue from original vessels and (2) cutting and dumping tissue into new vessels. Treatment C, cut-sort-and-dump, was partitioned into four job functions: (1) removing the tissue from original vessels, (2) cutting, (3) sorting, and (4) dumping the selected cuts into new vessels. The same operator, who had extensive experience with conventional tissue culture of watermelon and had been pretrained with the wire cutters, was the subject for all time studies. Plant parameters measured were tissue fresh weight and number of cut segments with visible buds. After the third (final) culture

only, fresh weights were recorded also for individual cut segments of tissue from the various treatments.

Orienting Cells and Mini-Trays

A fourth experiment was conducted to study growth and "oriented" cutting of watermelon shoot buds restricted to grow in a cellular space. Polycarbonate blocks (50 × 50 × 13 mm; 2 × 2 × 1/2 in.), drilled with three treatment cell diameters of 9.5, 12.7, and 15.9 mm (3/8 × 1/2 × 5/8 in.), respectively, were placed inside the rigid frames of polypropylene Sigma membrane raft squares (Sigma Chemical Company, St. Louis, Mo.) with the membranes removed (fig. 2). The combination was designated as a "mini-tray." Cell diameters were selected after previous experience with cells smaller than 9.5 mm diameter showed restrictions to growth of watermelon buds. Magenta GA-7 vessels were filled with 40 mL of medium at an agar concentration of 7 g/L. The mini-trays were placed in the Magenta vessel while the agar was molten. Six vessels served as replications for each cell size treatment and a control treatment where the buds grew conventionally on agar without any structure. The three cell size (mini-tray) treatments coupled with oriented cell cutting were compared with conventional agar vessels and hand cutting with scalpel and forceps.

The experiment was run through three culture cycles of 20 to 22 days each. Three of the six treatments (vessels) with the plug trays were cut by hand and used for subculture and data collection. The other three vessels were cut by the oriented cutter (fig. 3) and counted for data collection. Half of the vessels grown in the normal way were cut by hand with a scalpel and used for subculture and data collection, and the other half were cut by the 5.7-mm square grid wire cutter (fig. 1) and counted for data collection. Tissue fresh weight and the number of cut segments with visible buds were recorded at each subculture; sizes of cut segments were measured after the third (final) subculture.

The oriented cutter in figure 3 contained four cylindrical cells of varying diameters equally spaced in its cutting block. All cells were 9.5 mm (3/8 in.) deep. Each cell was slit to allow the cutter wire to divide plant material inserted in the cell into four segments. The direction of the cut was parallel to the stem axis of the shoot bud which was oriented apically upward. Tissue cultured in the various

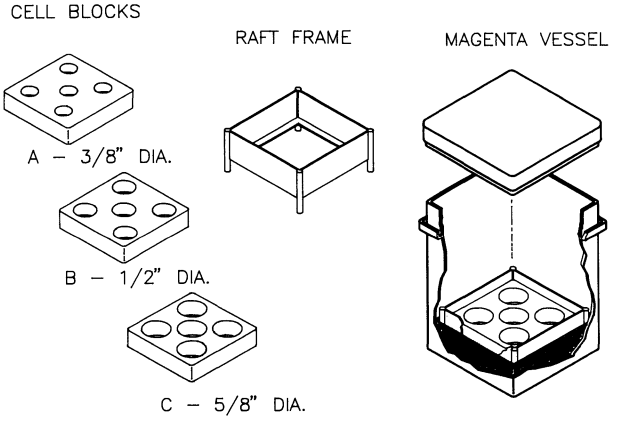

Figure 2–Mini-tray with the restricted growing cells.

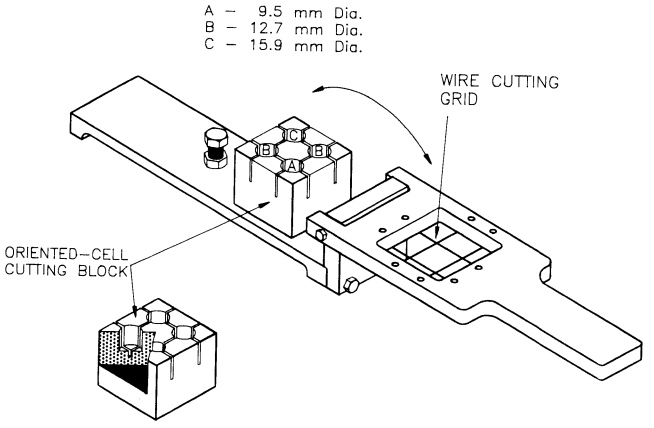

Figure 3–Oriented cell cutter.

sized mini-trays were cut in the correspondingly sized cell in the oriented wire cutter.

Results and Discussion

Cut-and-Dump

Quick subculture transfers were achieved in our initial cut-and-dump experiment by dumping cut tissue segments directly through the wire surface of the inverted unitizing, nonselective cutter into a vessel of fresh media. Table 1 indicates that tissue fresh weights and numbers of visible bud segments from the cut-and-dump procedure were around 90% as great as those from conventional hand cutting and placing protocols. The mean values were based on four culture cycles with 3 to 4 vessels with 16 explants each in each treatment. It was visually apparent, however, that tissue falling freely onto the surface of the medium with 7 g/L agar concentration failed to penetrate to a depth comparable to that of tissue "pushed" into the surface of the agar by conventional hand transfer. This observation raised the concern that contact area of transferred tissue with media was not sufficient for the cut-and-dump tissue.

Table 2 shows the influence of using "softer" media derived by reducing the agar concentrations from the conventional 7 g/L (considered the control) to 4, 5, and 6 g/L. With the cut-and-dump technique, all three lower agar concentrations yielded significantly greater tissue fresh weights at the 5% level than on 7 g/L concentration, yet differences among themselves were not statistically significant. The differences in fresh weights were the results of tissue growth as indicated by the comparable dry weight/fresh weight ratios for all agar concentrations. In other words, higher tissue water content was not

Table 1. Comparisons of tissue fresh weights and number of cut segments with visible buds per vessel for unitizing cut-and-dump and hand cut processes

	Fresh Weight		Number of Segments	
Treatments	(g) ± S.E.	Unitizing/Hand (%)	No. ± S.E.	Unitizing/Hand (%)
Unitizing Cut-and-Dump	6.98 ± 0.44	91.7	61.7 ± 4.63	89.3
Hand Cut	7.61 ± 0.42	100.0	69.1 ± 3.60	100.0

Table 2. Fresh weight and number of cut segments with visible buds per vessel of four different agar concentrations (g/L) in the medium, derived by the cut-and-dump technique ($P \leq 0.05$)

Treatment (g/L of agar)	Fresh Weight per Vessel (g) ± S.E.	No. of Segments No. ± S.E.	Relative to 7 g/L of Agar* Fr. Wt (%)	Relative to 7 g/L of Agar* No. (%)	Dry Wt./ Fr. Wt (%)
4	7.39 ± 0.64[a]	32.8 ± 3.33[cd]	150	117	7.9
5	6.92 ± 0.53[a]	34.0 ± 2.32[cd]	140	121	8.6
6	6.76 ± 0.27[a]	39.2 ± 3.01[c]	138	140	7.4
7	4.91 ± 0.24[b]	28.0 ± 1.87[d]	100	100	7.7

* 7 g/L agar is the common rate of agar concentration used in the growing medium.

responsible for variability in fresh weights. Although not statistically significant, the mean number of scalpel-and-forceps-cut segments were greater with all agar concentrations less than 7 g/L. Six g/L agar concentration yielded the greatest number of cut segments with visible buds. On the other hand, 4 g/L agar concentration was not sufficient to gel the media adequately. Consequently, we judiciously chose 5.5 g/L for the third experiment. Since a wire cutter with larger grid spacings (5.7 mm) was used in the agar concentration study than in the initial cut-and-dump study (4.9 mm), direct comparisons must be treated cautiously between tables 1 and 2. Perhaps the lesser fresh weight with the 5.7-mm cutter in table 2 than with the 4.9-mm cutter in table 1 for a 7 g/L agar concentration occurred because of less direct contact of larger tissue segments (5.7-mm cutter) with the medium and, subsequently, less growth. The reason is certainly not intuitive.

Cut-Sort-and-Dump vs. Cut-and-Dump

Table 3 summarizes the time studies comparing appropriate job functions involved in conventional hand (scalpel and forceps), cut-and-dump, and cut-sort-and-dump transfer procedures per four bud clusters used for establishing each new vessel. The mean total transfer time spent per four bud clusters were 160.0, 21.2, and 60.8 s, respectively, for the hand, cut-and-dump, and cut-sort-and-dump processes. Consequently, the cut-and-dump procedure consumed 13.3% as much time as hand transfer, and the cut-sort-and-dump procedure 38.0% as much. In other words, cut-and-dump reduced the total processing time by a factor of 7.5, and cut-sort-and-dump reduced it by a factor of 2.6.

The times per four bud clusters spent taking the tissue from the original vessel and placing it for dissection (removing) were similar for all three techniques. Cutting times were reduced from 76.2 s manually to 5.2 s by cut-sort-and-dump and to 5.8 s by cut-and-dump, factors of reduction of 14.7 and 13.1, respectively. These reductions of times for the cutting function are very comparable to the 14:1 reduction factor cited by Alper et al. (1992).

Sorting and placing hand cut segments from the four bud clusters required 72.5 s per new vessel filled, while sorting plus dumping functions in the cut-sort-and-dump procedure required 43.5 s per vessel. On a per cut segment with visible buds basis, these respective refilling activities consumed nearly equivalent times of 4.09 s for hand cut and 4.00 s for the cut-sort-and-dump techniques. None of these job functions associated with refilling new vessels were present in the cut-and-dump technique. Herein lies a very significant advantage of cut-and-dump over hand cutting and cut-sort-and-dump.

The mean number (with standard errors) of cut segments with visible buds for the time studies in table 3 were 17.3 ± 0.80 for hand cutting and 11.3 ± 0.58 for cut-and-dump and cut-sort-and-dump. Since cut segments from the cut-and-dump technique were placed directly into the new vessel, there was no opportunity to count them. Therefore, they were assumed to equal the number of segments actually counted for the cut-sort-and-dump technique. Production rates (segments/s) could be calculated by dividing the number of segments by the total transfer times in table 3 for the corresponding transfer techniques. Therefore, the production rates were 0.11, 0.53, and 0.19 segments/s for hand cut, cut-and-dump, and cut-sort-and-dump, respectively. From the standpoint of relative productivity ratios, the cut-and-dump technique was 4.8 times more productive than the hand cut technique, and the cut-sort-and-dump technique was 1.7 times more productive than hand cut. The cut-and-dump technique was also 2.8 times more productive than the cut-sort-and-dump technique.

At the end of each growth cycle of vessels initiated with four bud clusters, yields in terms of tissue fresh weight per vessel and fresh weight per cut segment with visible buds

Table 3. Time studies using groups of four bud clusters to compare job functions for three Stage II watermelon tissue culture transfer techniques: hand cut, cut-and-dump, and cut-sort-and-dump

	Hand Cut (s) ± S.E.	Hand Cut (%)	Cut-and-Dump (s) ± S.E.	Cut-and-Dump (%)	Cut-Sort-and-Dump (s) ± S.E.	Cut-Sort-and-Dump (%)
(1) Removing	11.3 ± 0.55	7.15	15.4 ± 0.64	72.6	12.1 ± 0.61	19.9
(2) Cutting	76.2 ± 4.91	47.6	—	—	5.2 ± 0.28	8.6
(3) Sorting & Placing	72.5 ± 3.36	45.3	—	—	—	—
(2,3) Cut & Dump	—	—	5.8 ± 0.32	27.4	—	—
(2) Sorting	—	—	—	—	34.3 ± 1.73	56.4
(3) Dumping	—	—	—	—	9.2 ± 0.53	15.1
Total Transfer	160.0 ± 7.37	100.0	21.2 ± 0.70	100.0	60.8 ± 1.91	100.0
Unitizing/Hand (%)	—	—	13.3	—	38.0	—

Table 4. Yields as fresh weight per vessel, fresh weight per cut segment with visible buds, and number of cut segments with visible buds per vessel for three transfer techniques: hand cut, unitizing cut-and-dump, and unitizing cut-sort-and-dump*

Treatments	Fresh Weight Weight Gain (g) ± S.E.	Fresh Weight Unitizing/ Hand (%)	Fresh Weight Fresh Weight per Segment (mg) ± S.E.	Number of Segments No. ± S.E.	Number of Segments Unitizing/ Hand (%)
Unitizing Cut-and-Dump	3.37 ± 0.27[a]	62.5	68.6 ± 3.87[e]	23.0 ± 0.39[c]	41.3
Unitizing Cut-Sort-and-Dump	2.77 ± 0.36[a]	51.4	68.3 ± 6.21[e]	26.4 ± 2.28[c]	47.4
Hand Cut	5.39 ± 0.38[b]	100	84.1 ± 7.14[f]	55.7 ± 3.40[d]	100

* These data were recorded at the end of each culture cycle, not at the beginning as was the time study data in table 3.

and number of cut segments from hand-cut, unitizing cut-and-dump and unitizing cut-sort-and-dump are shown in table 4. Tissue fresh weight gain per vessel was significantly greater at the 5% level for the hand cut technique than for the two unitizing cutter techniques. In fact, fresh weight yields with the cut-sort-and-dump technique were 51.4% as great as those of the hand-cut treatment, and those of the cut-and-dump technique were 62.5% as great as hand cut. Similarly, the numbers of cut segments with visible buds for cut-and-dump were 41.3% as many as with hand cut, and those with cut-sort-and-dump were 47.4% as many as hand cut. Measurements of the fresh weights of each cut segment with visible buds immediately after cutting at termination of the third (final) culture cycle in table 4 indicated that the unitizing cutter segments were slightly smaller than hand cut segments, about 81% as large. This difference was only significant at the 10% level. Nevertheless, with between 41.3% and 47.4% as many cut segments per vessel for cut-and-dump and cut-sort-and-dump, respectively, as for hand cut, the cut-and-dump technique yielded 62.5% as much fresh weight per vessel after a 20-day culture cycle as hand cut, and cut-sort-and-dump yielded 51.4% as much. Apparently bud segments cut by the unitizing cutter experienced greater growth rates than those cut by hand. Perhaps they were less shocked.

It is inappropriate to compare yield data in tables 1 and 4 because two different sizes of unitizing cutters were used in the two distinctly different experiments. A 4.9-mm grid spacing cutter was used for the experiment summarized in table 1, and a 5.7-mm grid spacing cutter was used to derive data in table 4.

Orienting Mini-trays and Wire Cutter

Observing from the previous experiment that the largest job function with the cut-and-dump technique remained the removal of tissue from the initial vessel, 72.6% of the total process time (table 3), we envisioned that reducing the number of bud clusters to be lifted by forceps might further improve the efficiency of this technique. The mini-trays with five cells each permitted fewer forceps movements per vessel. Tissue growth, however, was confined to the restrictive cell spaces and forced to grow more vertically upward than in agar with no structures (conventional).

Yield data from tissue grown in these mini-trays and prepared with hand cutting, a unitizing wire cutter, and an oriented wire cutter are recorded in table 5. Tissue fresh weight per vessel increased with increasing cell diameters of the mini-trays. The least fresh weight in the smallest cell diameter of 9.5 mm was 85.5% as great as that in the conventional vessel with no restricting structure. Statistically, mean fresh weight yields in the largest diameter cells were at least as great as in the conventional vessels. The smallest diameter cells did significantly restrict growth at the 5% level as compared with the largest diameter cells.

Table 6 indicates that fresh weights per segment cut with the corresponding 15.9-mm oriented wire cutter cell were also significantly greater at the 10% level than other tissue cut in correspondingly sized oriented cutter cells or with the 5.7-mm unitizing cutter for the tissue from the conventional vessels. All tissue segments in the oriented cutter column of table 6 were significantly heavier than segments in the hand-cut column at the 1% level. Standard errors for tissues cut by the oriented cutter were also noticeably large, indicating wide variability in sizes of tissue clusters divided into four quadrants. Perhaps some variability was induced by more tissue growing toward one quadrant space than another or uneven division of more vertically grown tissue by the downward movement of the cutting wires. The oriented cutter yielded between 71 to 85% as many cut segments as hand cutting among the various diameter cell sizes (table 5). On the other hand, the unitizing, nonoriented 5.7-mm cutter yielded only 60% as many cut segments as hand cutting. Consequently, the orientations of growth and cutting of tissue increased the percentage of segments yielded. This response should positively influence the productive efficiency of the unitizing cutting technique. The mini-tray concept reduces the number of tissue clusters to be removed from a single vessel; therefore, it reduces time required to remove tissue from the culture vessel. This circumstance should further enhance the productive efficiency of the unitized cutting concept over hand cutting. Time studies have not been conducted at this time. Further research on these aspects needs to be conducted.

Summary

By inverting the unitizing wire cutter to permit cut tissue to drop directly into new vessels, productive efficiency of the unitizing cutter can be enhanced. In fact, the cut-and-dump technique became 4.8 times more productive than the conventional hand cut technique. The oriented growth and cutting concepts have potential to improve productive efficiency further by offsetting yield

Table 5. Fresh weight and number of cut segments with visible buds per vessel for watermelon tissue culture grown on agar with no structure added and on agar with structures having three diameters of restricted cells and cut by hand, by a unitizing cutter and by the comparable oriented cell cutter ($P \leq 0.05$)

	Fresh Weight		Number of Segments			
Cell Diameter Treatments	Weight per Vessel (g) ± S.E.	Cell/No. Structure (%)	Unitized Cutter No. ± S.E.	Hand Cut No. ± S.E.	Oriented Cutter No. ± S.E.	Oriented/ Hand (%)
9.5 mm	2.18 ± 0.14^{b}	85.5	—	19.5 ± 1.12^{ef}	16.5 ± 1.12^{cd}	84.6
12.7 mm	2.44 ± 0.18^{ab}	95.7	—	21.6 ± 1.52^{e}	15.3 ± 0.71^{d}	70.8
15.9 mm	2.81 ± 0.24^{a}	110.0	—	22.7 ± 1.55^{e}	17.4 ± 0.99^{cd}	76.6
Conventional	2.55 ± 0.13^{ab}	100.0	17.3 ± 1.53^{cd}	28.9 ± 1.75^{f}	—	59.8

Table 6. Fresh weights per cut segment with visible buds immediately after cutting tissue from the third (final) culture cycles by hand and with the appropriate oriented cutter cell ($P \leq 0.10$)

Cell Diamter Treatments	Final Fresh Weight per Segment (mg) ± S.E.	
	Hand Cut	Oriented Cutter
9.5 mm	53.5 ± 4.2^{a}	104.3 ± 22.5^{c}
12.7 mm	37.5 ± 2.5^{b}	126.1 ± 20.7^{c}
15.9 mm	61.3 ± 8.2^{a}	177.6 ± 17.6^{d}
Conventional	52.3 ± 5.5^{a}	95.4 ± 12.1^{c}*

* Cut with the 5.7-mm unitizing wire cutter

reduction typically incurred by unitizing, nonselective cutters. The mini-tray cell concept potentially reduces the number of bud clusters to be removed from a vessel for transfer. Thus, the mini-trays aid to reduce the removal job function, which is the largest fraction job function in the cut-and-dump technique.

Unitizing, nonselective wire cutters and mini-trays are both simple concepts with potential to enable efficient scale-up of plant micropropagation. Because of their simplicity, they can be incorporated into typical manual operations as operator aids and potentially be more cost effective than sophisticated robotic or automated concepts.

ACKNOWLEDGMENT. Acknowledgment is extended to the Binational Agricultural Research and Development (BARD) Fund for financial support through Project US-1678-89.

REFERENCES

Adelberg, J. W. and B. B. Rhodes. 1989. Micropropagation from zygote tissues of watermelon. In *Proc. of Cucurbitacea '89*, ed. C. E. Thomas. Charleston, S.C.: U.S. Vegetable Laboratory.

Alper, Y., J. Adelberg, R. E. Young and B. B. Rhodes. 1992. Unitized, non-selective cutting of *in vitro* watermelon. ASAE Paper No. 92-7502. St Joseph, Mich.: ASAE.

Brown, F. R. 1992. Robotics and image analysis applied to micropropagation. In *Proc. of the Int. Symp. on Transplant Production Systems*, eds. K. Kurata and T. Kozai, 283-296. Dodrecht, Netherlands: Kluwer Academic Publishers.

Chu, I. Y. E. 1992. Perspective of micropropagation industry. In *Proc. of the Int. Symp. on Transplant Production Systems*, eds. K. Kurata and T. Kozai, 137-150. Dodrecht, Netherlands: Kluwer Academic Publishers.

Cooper, P. A., J. E. Grant, L. Kerr and G. English. 1992. Development of a prototype automated cutting system for in vitro multiplication. *Proc. of the Int. Symp. on Transplant Production Systems*, Abstract, 146. Yokohama, Japan.

Gautz, L. D., S. K. Upadhyaya and R. E. Garrett. 1989. Hydro-pneumatic singulation of gel-encapsulated propagules. *Transactions of the ASAE* 32(3):791-794.

Grand d'Esnon, A., R. Chee, R. C. Harrell and D. J. Cantliffe. 1989. Qualitative and quantitative evaluation of liquid tissue cultures by artificial vision. *Le Technoscope de Biofutur* 26:3-9.

Holdgate, D. P. and E. A. Zandvoort. 1992. Automated micropropagation and the application of a laser beam for cutting. In *Proc. of the Int. Symp. on Transplant Production Systems*, eds. K. Kurata and T. Kozai, 297-311. Dodrecht, Netherlands: Kluwer Academic Publishers.

Iwasaki, A. 1991. Development of a Robot for plant tissue culture. *Proc. of 2nd Nat. Symposium on Recent Development of Transplant Production Systems*, 61-66. Organizing Committee of Int. Symp. on Transplanting Production Systems, Tokyo.

Kurata, K. 1992. Transplant production robots in Japan. In *Proc. of the Int. Symp. on Transplant Production Systems*, eds. K. Kurata and T. Kozai, 313-329, Dodrecht, Netherlands: Kluwer Academic Publishers.

Kurtz, S. L., P. D. Hartman and I. Y. E. Chu. 1991. Current methods of commercial micropropagation. In *Scale-Up and Automation in Plant Propagation*, ed. I. K. Vasil, 7-13. San Diego, Calif.: Academic Press Inc.

Levin, R. and I. K. Vasil. 1989. Progress in reducing the cost of micropropagation. *IAPTC Newsletter* 59:2-12.

Lutz, J. D., J. R. Wong, J. Rowe, D. M. Tricoli and R. H. Lawrence. 1985. Somatic embryogenesis for mass cloning of crop plants. In *Tissue Culture in Forestry and Agriculture*, eds. R. R. Henke, K. W. Hughes, M. J. Constantine and A. Hollaender, 105-116. New York: Plenum Press.

Miwa, Y. 1991. Micropropagation robot for lily bulb. *Proc. of 2nd Nat. Symp. on Recent Development of Transplant Production Systems, Organizing Committee of Int. Symp. on Transplant Production Systems*, 74-79. Tokyo, Japan.

Payne, G., V. Bringi, C. Prince and M. Shuler. 1992. *Plant Cell and Tissue Culture in Liquid Systems*. Munich, Germany: Hanser Publishers.

Redenbaugh, K., D. Slade, P. Viss and J. A. Fujii. 1987. Encapsulation of somatic embryos in synthetic seed coats. *HortSci.* 22:803-809.

Styer, D. J. 1985. Bioreactor technology for plant propagation. In *Tissue Culture in Forestry and Agriculture*, eds. R. R. Henke, K. W. Hughes, M. J. Constantin and A. Hollaender, 117-130. New York: Plenum Press.