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UNITIZED, NONSELECTIVE CUTTING OF *IN VITRO* WATERMELON

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ABSTRACT. *Unitized, nonselective mass cutting of Stage II* Citrullus lanatus *cv. Charlee (watermelon) plant tissue cultures has been achieved with various configurations and sizes of wire cutter devices. Time studies revealed that the wire cutters increased the productivity of the cutting function over conventional scalpel and forceps by a factor of 14. Total transfer productivity, including the manual functions of removing tissue from an initial culture vessel and filling (sorting and placing cut tissue segments) new vessels, was increased by a factor of 1.8. The square grid-type cutting devices yielded from 48 to 59% as many viable bud clusters per culture vessel as hand cutting and from 65 to 95% as much tissue fresh weight. An oriented-cell configuration of wire cutter actually increased tissue fresh weight about 20% over hand cutting. The simplicity of construction and quality of material of the wire cutter render it readily autoclavable and highly flexible to function both as an aid to small operations and as an element in more sophisticated mechanical devices for larger operations.* **Keywords.** *Micropropagation, Plant tissue culture, Cutters, Mechanization.*

Regeneration of plants through the process of plant tissue culture, or micropropagation, has become widely accepted for many crops, particularly ornamentals. A capacity for rapid multiplication of virus-free plant material and new, desirable varieties is micropropagation's primary asset. The major deterrent is high production cost, much of which occurs with the intensive manual labor involved. Kurtz et al. (1991) estimated human labor to be 64% of laboratory-related production cost, with over half being technician time responsible for subculture division and transfer. Conventional tools for micropropagation transfer are scalpel and forceps. Aseptic environments must be maintained because of the sugar-rich media used as a carbon source in the *in vitro* procedure.

Commercial use of micropropagation beyond ornamentals, which generally have substantial margins per unit, depends greatly upon reducing production costs (Donnan, 1986) to be compatible with the substantially lower margins associated with field and forest crops. Repetition of tedious, manual tasks during Stage II, proliferation, should be a focus of new technology (Deleplanque et al., 1985; Anonymous, 1988). A priority of this project has been defining the hand labor components in Stage II for watermelon culture and exploring the potentials of simple mechanical devices as alternatives to scalpel and forceps for explant separation and transfer. The concept of unitized, nonselective mass cutting with a wire cutter device has been tested. Duration of culture cycle, spacing of cuts, and orientation of cut with respect to shoot tissue were explored.

LITERATURE REVIEW

Vasil (1991) stated that growth and expansion of the micropropagation industry into the vast market of vegetable, fruit, and forest species can only be achieved if drastic labor reduction is made by mechanization and automation. Mechanical dissection of multiplying shoots is greatly complicated, however, by the diverse growth habits of different cultures (Rowe, 1986). The Vitromatic System described by Levin and Vasil (1989) addressed many labor problems by growing tight meristem-like shoot bud clusters in liquid medium. This allowed mechanized transfer at appropriate times to a bioprocessor which cut, separated, and distributed propagules. The mechanical cutting of the tissue in the tight meristematic cluster was done in a nonselective fashion. The blender-type device did not provide control for the size of cuts. Some of these bud clusters developed roots and shoots under appropriate media and environmental conditions.

An automated cutting system based on image analysis, computer-controlled laser cutting and robotic tissue handling has been developed by Plant Production Systems and operated by PhytoNova, two Dutch companies (Holdgate and Zandvoort, 1992). This system provided substantial reduction in labor, but the cost of this technology limits its application to large commercial operations. A further biological limitation was imposed because only elongated shoots multiplied by nodal segments were accessible. Nonelongated propagules such as meristematic bud clusters, bulblets, protocorms, or somatic embryos would require alternative systems. A prototype blade cutter and handling device for meristematic shoot bud clusters was developed at the

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New Zealand Institute for Food Research and the New Zealand Agricultural Engineering Institute. This system was also based on a vision system with a robotic arm and computer controlled hardware (Cooper et al., 1992).

Kurata (1992) described several transplant production robots developed in Japan. Generally, robotic systems have operated too slowly (approximately 15 s/cut). At Waseda University, Dr. Y. Miwa demonstrated an automated system for lily bulb multiplication wherein manual tissue handling was replaced by a robot. Machine vision was not sufficiently sophisticated in its discrimination capabilities to enable removal of roots and separation of the bulblets. Mechanical means employing rotating disks or pressure rollers were effective. Kirin Brewery Co., Ltd. developed the TOMOCA system which used a two-dimensional grid-type blade cutter and push rods to transfer the cut material to a new agar vessel. Nodal cuttings of "bushy" axillary divisions were tested in the commercial laboratories of Twyford International, Inc., USA. Lower cost for practical application was cited as the reason for the simple cutter structure. It was integrated with mechanical handling of the agar boxes and operated at a speed of 1.7 s/unit cut.

Seedless watermelon from triploid germplasm is a value-added vegetable crop that has been hindered by difficulty in seed propagation and varietal development. Direct production of hybrid transplants for grower use would greatly reduce the time required for varietal development (Chu, 1992). A shoot bud cluster micropropagation protocol has been developed for propagation of watermelon germplasm (Adelberg and Rhodes, 1989). The apical origin of axillary divisions should allow the rapidly dividing tissue to remain true to type.

Objectives

This research was designed to develop and to test an alternative method to conventional scalpel and forceps for manipulation of tissue in the multiplication, Stage II, phase of plant micropropagation. Specific objectives were to:

- Develop an alternative cutting and separating device to conventional scalpel and forceps that would reduce hand labor.
- Evaluate the performance of a unitizing, non-selective cutter for Stage II multiplication job functions and to compare its influence on time and quality to conventional scalpel and forceps techniques.

Materials and Methods

Shoot bud clusters of *Citrullus lanatus* cv. Charlee were prepared as described in Adelberg and Rhodes (1989) by repeated subcultures of shoot apices on Murashige and Skoog (1962) medium containing 0.7% agar, 3% sucrose, 100 mg/L myo-inositol, 2 mg/L glycine, 0.2 mg/L thiamine HCl, 0.5 mg/L pyridoxine, and 0.5 mg/L nicotinic acid (pH 5.7) with 10 μM BA (benzyladenine). The cultures in these experiments had been maintained for 30 to 36 months by monthly transfers.

Cutter devices as alternatives to scalpel and forceps were qualitatively assessed by the following criteria:

- Material integrity in all current sterilization environments.
- Ease of cleaning and simplicity of maintenance.
- Quality of tissue cuts.
- Capacity as an aid to enhance human productivity.
- Potential for automation.

The unitizing cutter (fig. 1) consisted of a stainless steel wire (0.025 mm diameter) mounted in a square grid arrangement on a pivotable, aluminum handle. Cutting occurred by pressing the wire grid through the plant tissue into slots made on the upper face of the cutting block. The wire grid dimensions and slot dimensions were precisely matched. Tissues were randomly transferred by forceps to the upper face of the cutting block while the pivotal handle containing the cutting wire grid was in the open position. After being nonselectively cut, tissue segments remained on the top of the cutting block ready for transfer to new vessels. Figure 2 shows an alternative configuration of the cutting block that positioned the tissue within cylindrical cells and oriented cuts with respect to generally "uprighted" tissue clusters.

In an initial experiment using a unitizing cutter with a square grid spacing of 4.9 mm, both time studies of the

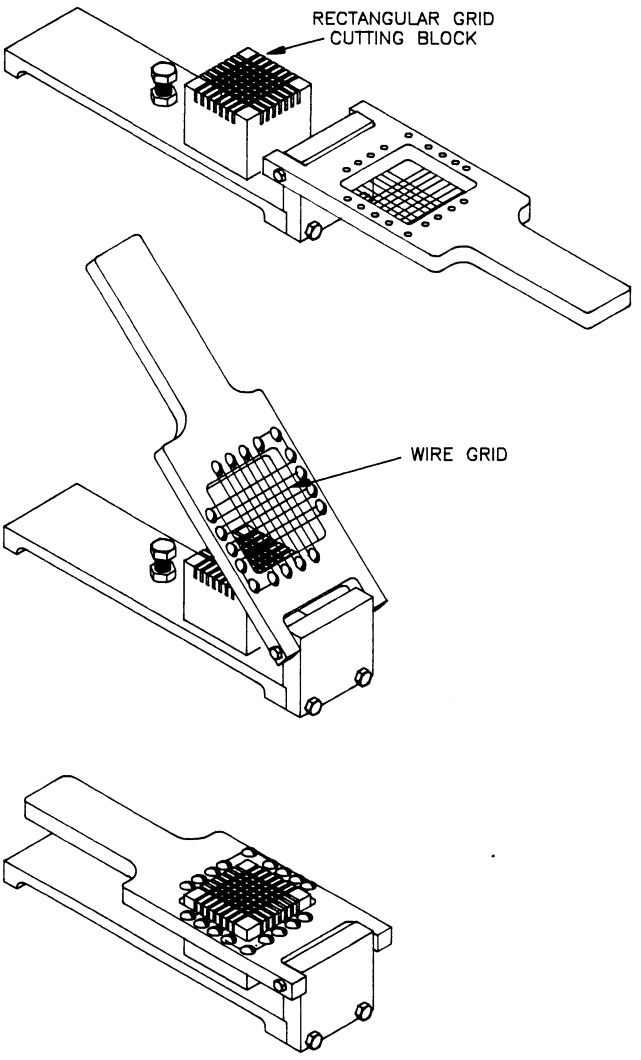


Figure 1–Unitizing wire cutter.

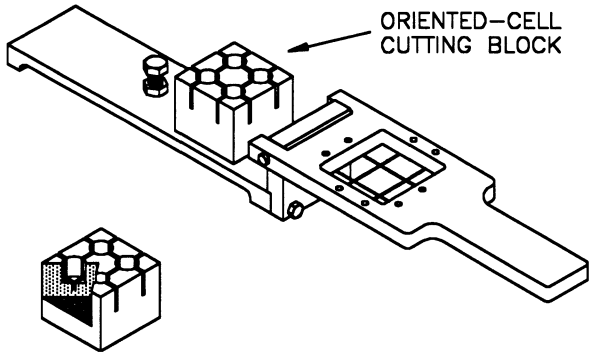


Figure 2–Oriented-cell wire cutter.

transfer processes and length of subculture growth periods were assessed. Operator functions during time studies of the transfer process under the laminar flow hoods were partitioned into three components: (1) time for *removing* tissue from the original vessel, (2) time for *cutting* tissue into bud clusters, and (3) time for *filling* new vessels with the dissected bud clusters. These parameters were compared for scalpel and forceps (hand) manipulations versus the 4.9-mm unitizing cutter. Three Magenta GA-7 vessels containing 16 bud clusters each were analyzed for both methods of cutting following each of three sequential subculture cycles. Three different growth period lengths of 14, 18, and 22 days, respectively, were used during each of the three sequential subculture cycles. Following each of the three subculture cycles, comparisons were made of tissue yields in terms of numbers of clusters containing buds and tissue fresh weights for vessels originating from hand (scalpel and forceps) cutting and from the unitizing cutter.

In a second experiment, various grid spacings and configurations of the unitizing wire cutters were compared. Three cutters were constructed with flat tops and grids of the first configuration in figure 1, and a fourth (oriented cutter) was constructed with the cylindrical cell configuration in figure 2. The three different grid spacings for the flat top cutters were 4.3, 4.9, and 5.7 mm, respectively. The cylindrical cell cutting block of the oriented cutter had four equally spaced cells of 12.8 mm diameter and 9.5 mm depth. The cylindrical cells allowed insertion first of the basal portion of the shoot bud clusters with the shoots generally upright. Each of these cells was slit to permit the cutter wire to divide plant material in the cell into four sections. The three nonselective grid and the oriented unitizing cutters were compared with hand cuts through five subculture cycles of 20 to 22 days each. Analyses were based on means from the five sequential subcultures each of which had four replications of Magenta vessels per treatment. At the initiation of each subculture cycle, Magenta vessels were inoculated with 16 approximately equally sized explants per vessel from the corresponding treatment in the previous cycle.

Results and Discussion

In the first experiment, the greatest impact of using the unitizing, nonselective 4.9-mm wire cutter instead of hand cutting with a scalpel and forceps occurred for the actual cutting time **per bud cluster** with visibly acceptable buds. This *cutting* time was reduced from 4.2 to 0.3 s, from

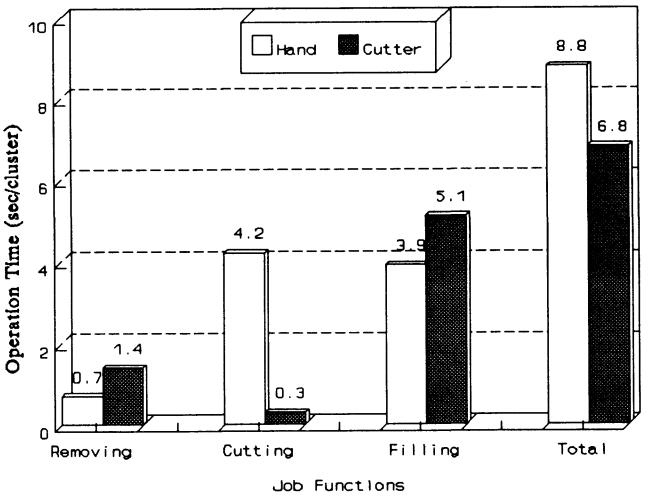


Figure 3–Relative times per bud cluster for three job functions involved in hand cutting and with the unitizing cutter.

approximately 48% of the total process time to 5% (fig. 3). The other two job functions in the transfer process—(1) *removing* and staging source tissue from the initial vessel for cutting, and (2) sorting freshly cut bud clusters for *filling* a new culture vessel—were both performed faster with hand cutting than with the unitizing cutter. The *removing* and staging function increased from 0.7 to 1.4 s, or from about 8 to 21% of the total transfer time. The *filling* time increased from 3.9 to 5.1 s, or from about 45 to 75% of the total process time. The unitizing cutter technique required 6.8 s total process time per bud cluster compared to 8.8 s per bud cluster with the hand method. The reduction in total processing time with the new device was, therefore, about 23% per acceptable bud cluster.

Table 1 summarizes the time study data as time spent under the hood **per Magenta vessel** initially inoculated with 16 explants each. Mean times were based on three replications (subcultures) with three Magenta vessels per treatment, each vessel containing 16 bud cluster explants. In contrast to figure 3, which presents these same data *per bud cluster* sorted for visibly acceptable buds, only (table 1) which presents *per vessel* data, did not sort and delete tissue segments without visibly acceptable buds. Consequently, percentages of total transfer time reductions per vessel were numerically larger than those per acceptable bud cluster. The average total transfer time per vessel was decreased from 614 s by hand to 341 s by the unitizing cutter, approximately a 44% reduction.

The incremental percentages of total process time for each of the job functions were equivalent, as should be

Table 1. Time study per Magenta vessel of the component job functions involved in the multiplication phase of plant tissue culture of watermelon

	Per Vessel with 16 Explants (s)		
Job Function	Hand $\bar{x} \pm$ S.E.	Cutter $\bar{x} \pm$ S.E.	Hand/Cutter Ratio
Removing	50 ± 2.9	69 ± 2.3	0.7
Cutting	288 ± 20.2	17 ± 1.2	16.9
Filling	276 ± 8.4	256 ± 12.5	1.1
TOTAL	614 ± 25.7	341 ± 10.7	1.8

anticipated, for the per bud cluster and the per vessel analyses. The times per vessel ratios of hand to unitizing cutter methods for the three job function categories indicated that the *removing* function was lower by hand, the actual *cutting* operation was nearly 17 times greater by hand, and the *filling* function was only slightly greater by hand, in fact, nearly equal for both methods. The *filling* function per vessel was noticeably greater by hand than by cutter, a reversal of the relationship in figure 3 where the *filling* time per acceptable bud cluster was greater by cutter than by hand. This reversal occurred because unacceptable, dissected tissues were sorted out (requiring more time) within the *filling* time per bud cluster and were not sorted in the *filling* time per vessel.

For the three sequential subcultures in the first experiment, table 2 summarizes the numbers of clusters per Magenta vessel containing visibly acceptable buds for both hand cutting and unitizing cutter methods. Similarly, table 3 summarizes tissue fresh weights per Magenta vessel. Means were based on 3 subcultures with 3 Magenta vessels per treatments, each vessel containing 16 bud cluster explants. Data were presented for 3 different growth periods—14, 18, and 22 days. Levels of statistically significant differences in yields between hand cutting and unitizing cutter treatments were indicated in the fifth column of each table. Moreover, least significant differences by the Duncan's Multiple Range test among the three growth periods are indicated by the alphameric superscripts in the second and third columns.

In terms of numbers of bud clusters (table 2), yields were significantly lower from the unitizing cutter than from the hand method, ranging from 67 to 73% among the different growth periods. In terms of tissue fresh weight (table 3), however, yields between the two cutting treatments were not significantly different. The measurement of numbers of clusters containing visibly acceptable buds was obviously a more discriminating indicator of viable yields than tissue fresh weights, which included callus and nonviable leafy tissue. For both the unitizing cutter and hand cutting, a 14-day culture period produced significantly fewer bud clusters and significantly less tissue fresh weights than 18- or 22-day cultures (tables 2 and 3). Statistically significant differences were not observed between 18- and 22-day culture periods for either yield criteria. Consequently, an 18-day culture period was desirable since it yielded more than the 14-day period and the longer 22-day period did not significantly increase yields.

Table 4 summarizes numbers and fresh weights of bud clusters derived using five cutting treatments: the three sizes of grid cutters, the oriented-cell cutter, and hand cutting. With respect to numbers of acceptable bud clusters, the three sizes of grid cutters produced between 48 to 59% of the yield for hand cutting. There were no statistical differences in numbers of clusters produced by the three grid sizes of unitizing cutters. The oriented-cell cutter produced significantly more bud clusters than the two smaller grid cutters, but not significantly different from the largest grid cutter. Hand cutting yielded significantly greater numbers of bud clusters than any of the four mechanical cutters. For yield measured as tissue fresh weight per Magenta vessel, the oriented-cell cutter produced significantly more than the grid cutters and hand cutting. Hand cutting tissue fresh weight was not significantly different from the largest grid cutter, yet was significantly greater than the two smaller grid cutters. Tissue fresh weight for the largest grid cutter (5.7 mm) was significantly greater than that for the smallest grid cutter (4.3 mm). Perhaps the larger tissue clusters from the larger grid cutter had more viable buds for regeneration. The fact that the oriented-cell cutter produced higher tissue fresh weight yield and a greater number of bud clusters than other mechanical devices merits some qualification. The tissue to be cut was oriented such that the longitudinal stem axis was generally upward, a better posture for cutting with minimal damage. Moreover, numbers of subunits were physically limited to four per cell. Consequently, each subdivision was relatively large and better oriented than those from the unitizing grid cutters. They probably experienced less shock, thereby continuing to grow more profusely and achieving the greatest tissue fresh weight of all cutting treatments, even hand cutting. For similar reasons, the percentages of usable bud clusters for the oriented-cell cutter were greater than those for the three unitizing grid cutters. The inherent physical limitation of four subdivisions per cell, no doubt, resulted in fewer total subdivisions than were possible with selective hand cutting. Moreover, it is conceivable that if an explant were improperly oriented in a cell, the increased probability of

Table 2. Yield comparison between hand cutting and unitizing, nonselective cutter in terms of number of clusters containing buds per Magenta vessel for three different culture periods

Period of Growth (Days)	Acceptable Bud Clusters (No.)			
	Hand $\bar{x}$ ± S.E.	Cutter $\bar{x}$ ± S.E.	Cutter/Hand (%)	Statistical Significance Level
14	49.8 ± 4.2[a]	36.7 ± 2.5[c]	72.4	0.01
18	73.7 ± 4.9[b]	54.0 ± 4.6[d]	73.3	0.05
22	70.6 ± 2.9[b]	47.4 ± 2.3[d]	67.2	0.05

Table 3. Yield comparison between hand cutting and unitizing, nonselective cutter in terms of tissue fresh weight per Magenta vessel for three different culture periods

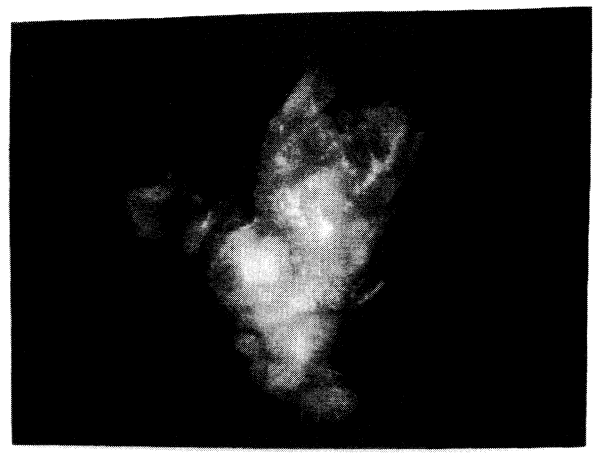
Period of Growth (Days)	Tissue Fresh Weight (g)			
	Hand $\bar{x}$ ± S.E.	Cutter $\bar{x}$ ± S.E.	Cutter/Hand (%)	Statistical Significance Level
14	5.86 ± 0.36[a]	6.21 ± 0.37[c]	106.1	N.S.
18	8.14 ± 0.75[b]	7.95 ± 0.67[d]	97.1	N.S.
22	9.03 ± 1.04[b]	7.53 ± 0.72[d]	83.4	N.S.

Table 4. Comparisons of yields measured as numbers of acceptable bud clusters and as tissue fresh weights per Magenta vessel among the three grid sizes of unitizing cutters, the oriented cell cutter, and hand cutting after 20* days of culture of watermelon

Treatments	Clusters (No.) $\bar{x}$ ± S.E.	Clusters Relative to Hand Cut (%)	Tissue Fresh Weight (g) $\bar{x}$ ± S.E.	Fresh Wt. Relative to Hand Cut (%)
a. Hand cut	67.2 ± 6.8[a]	100.0	9.2 ± 1.2[d]	100.0
b. Oriented cell	43.1 ± 2.0[b]	64.0	11.1 ± 0.7[e]	120.6
c. 4.3 mm†	36.2 ± 3.9[c]	53.8	6.0 ± 0.7[f]	65.2
d. 4.9 mm†	32.5 ± 3.9[c]	48.3	7.4 ± 1.1[f,g]	80.4
e. 5.7 mm†	39.8 ± 6.0[b,c]	59.3	8.7 ± 1.4[d,g]	94.5

* Spacing between the grid wires.
† Experiment ran 20 days instead of 18 days because of weekend.

a. Hand

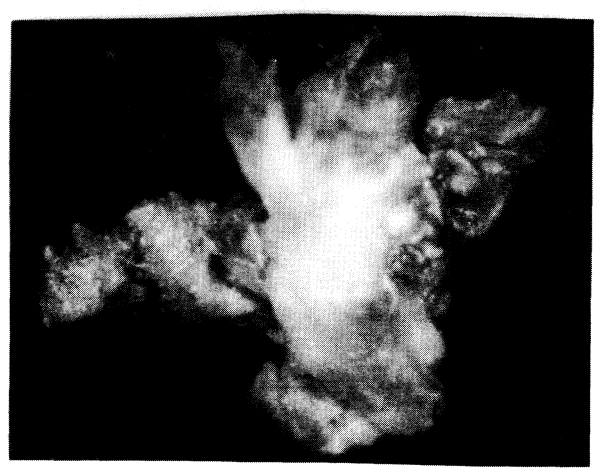


c. 4.3 mm Grid

b. Oriented-Cell

d. 4.9 mm Grid

Scale: 2 mm



e. 5.7 mm Grid

Single bud cluster's fresh weight immediately after cutting.

Treatments	Average Weight (mg) $\bar{x}$ ± S.E.	Relative to Hand-Cut (%)
a. Hand cut	67.0 ± 5.0[a]	100.0
b. Oriented cell	254.9 ± 27.1[b]	380.4
c. 4.3 mm*	46.0 ± 3.4[c]	68.6
d. 4.9 mm*	71.1 ± 7.0[a]	106.2
e. 5.7 mm*	102.9 ± 8.0[d]	153.5

Figure 4–Typical bud clusters derived from five cutting treatments: (a) hand, (b) oriented-cell wire cutter, (c) 4.3-mm, (d) 4.9-mm, and (e) 5.7-mm grid wire cutters. Table indicates tissue fresh weights and weights relative to hand-cuts for a common number of randomly selected clusters in each treatment.

randomly cutting through a viable bud and damaging it might cause some loss of numbers of usable bud clusters as compared to hand cutting.

Relative sizes of bud clusters from each cutting treatment can be discerned among the commonly scaled photographs of figure 4. Respective mean, single-bud

tissue fresh weights are quantified in the inserted table of this figure. The average weights were based on a common number of randomly selected clusters for each treatment harvested after the fifth culture cycle. Hand-cut bud clusters were approximately equivalent in size to the clusters cut by the intermediate-sized grid cutter (4.9 mm). Bud clusters were largest with the oriented-cell cutter, nearly four times the weight of the hand-cut clusters. The selectivity of hand cutting was often recognizable by the smaller area of cut surface (fig. 4a) relative to the cut surfaces with the mechanical devices (figs. 4b, c, d, e). Very clean cuts were made consistently by the wire cutters, thereby minimizing the shock possible from nonselective cutting.

SUMMARY

By applying the principle of unitization, wire grid cutting devices increased the productivity of the job function of *cutting* acceptable bud clusters in plant micropropagation by a factor of 14. Functional times for *removing* tissue from the culture vessel and placing it into the mechanical cutter and for *filling* (sorting and placing) new culture vessels with the dissected tissues were increased. Nevertheless, the total transfer productivity per vessel was increased by a factor of 1.8 (table 1). Because of their construction with stainless steel and aluminum, the wire cutters were found to be readily cleaned and repeatedly autoclaved for aseptic environments. Although their cutting was nonselective as compared to the selectivity exercised in hand cutting, the mechanical wire cutting devices yielded 48 to 64% as many viable watermelon bud clusters per culture vessel as hand cutting with a scalpel and forceps. Yield in terms of tissue fresh weight, however, ranged from 65 to 95% with the increasing grid sizes of the unitizing cutter as compared to hand cutting. The oriented-cell wire cutter actually yielded about 20% more in tissue fresh weight, i.e., biomass production, than hand cutting.

Opportunities exist for further improvements of the productivity of the wire cutter techniques by addressing also the *removing* and *filling* job functions. Methods to expedite these two job functions on either side of the *cutting* function have not been fully explored, yet these functions are now most limiting to the overall productivity of the mechanical transfer technique. Since the oriented-cell cutter showed growth advantages, growing tissues in mini-trays with the oriented-cells configuration could reduce time required to remove tissue from the original vessel and to introduce it into the cutting device. In fact, one might cut tissue directly in the mini-tray without having to move individual segments into the cutting device. Moreover, random "dumping" of the processed bud clusters into a new vessel could appreciably reduce the time required in this study to place each cluster selectively into a new vessel.

The simplicity of the mechanical wire cutter concept allows the small scale operator access and flexibility to a mechanized technique without a large investment, yet it has great potential for scale-up. The unitizing cutters should readily integrate into larger mechanized systems for handling culture vessels and for automating culturing and transferring processes in plant micropropagation. Replacing the scalpel and forceps is conceptually an important first step.

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